

An Evaluation of Time Since Death, from Potassium Level of Vitreous Humour in the Eyes of Deceased, Brought for Autopsy at Department of Forensic Medicine and Toxicology in a Government Medical College of Garhwal Region, Uttarakhand: A Cross Sectional Study

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How to cite this article: Pankaj Sahu, Niranjana Kumar Gunjan, Kailash Gairola et. al. An Evaluation of Time Since Death, from Potassium Level of Vitreous Humour in the Eyes of Deceased, Brought for Autopsy at Department of Forensic Medicine and Toxicology in a Government Medical College of Garhwal Region, Uttarakhand: A Cross Sectional Study. Indian Journal of Forensic Medicine and Toxicology / Volume 18 No. 3, July - September 2024.

Abstract

Background: The Post-mortem interval is the time between death and the examination of a corpse. It is important to know when the crime was committed. It helps the police to start their investigations with the information available and to process cases more efficiently.

Materials and Methods: The sample of vitreous humour was collected from the posterior chamber of both eyes by aspirating gradually and slowly through a puncture 5-6 mm away from the limbus using a sterile 20-gauge needle taking care to avoid tearing of any loose tissue fragments surrounding the vitreous chamber.

Results: In the present study there were 80 subjects, out of these subjects' the maximum subjects were from 26-35 years and the minimum were from 66-75 years of age group.

Conclusion: Our study found that an appreciable pattern of increased potassium concentration in vitreous humour was observed every 12 hours of post-mortem interval, with an approximate 2mmol/l increase.

Keywords: Time since death, vitreous humour, medicolegal post-mortem examination.

Introduction

Not a day goes by without news of unnatural deaths such as murders, suicides, or accidents. In all of these cases, the time elapsed since death is an important key point for the investigative team to

begin an investigation. In most cases, the apparent cause of death is always given by the investigating officer, which is consistent with the doctor's opinion. The issue, however, is the time elapsed since death and the time of the event, which form the starting point of the investigation¹.

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Submission date: January 20, 2024

Revision date: February 7, 2024

Published date: July 17, 2024

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The Post-mortem interval is the time between death and the examination of a corpse. It is important to know when the crime was committed. It helps the police to start their investigations with the information available and to process cases more efficiently. It also helps in the inclusion and exclusion of suspects and criminals and in verifying the statements of the suspect. Estimated time since death, useful in the event of a dispute like property inheritance, insurance claims, etc².

Changes that occur in the chemical composition of bodily fluids such as blood, vitreous humour, synovial fluid, and cerebrospinal fluid immediately after death are explained by tanto-chemistry. After death, electrolytes and chemicals are redistributed, cellular integrity is lost, and energy-dependent transmembrane transport does not occur. Therefore, it will be difficult to interpret post-mortem blood samples. Only stable analytes can be predicted in blood samples due to impaired active membrane transport and rapid metabolic degradation after death³.

Among various body fluids such as blood, serum, cerebrospinal fluid, aqueous humour, synovial fluid and vitreous humour, the determination of vitreous potassium concentration is the most widely used method⁴.

The vitreous fluid, a crucial supporting structure for the eyeball's optical function, is an acellular, transparent, inert, colourless, hydrophilic viscous fluid that lies between the lens and retina. Its volume is roughly 4 cc, and its weight is about 4 grams. With soluble proteins, amino acids, low molecular weight components, glucose, type II collagen, hyaluronic acid, inorganic salts, and ascorbic acid, it is made up of 99% water^{5,6}.

There is a close relationship between the vitreous' composition and that of serum, aqueous humour, and cerebrospinal fluid. It is easily accessible, comparatively stable, less prone to abrupt chemical changes, and well-guarded against contamination and decomposition. Because of this, it is a better fluid to analyze when determining the time since death than other fluids⁷.

Even in cases of severe head injuries and burns, the vitreous humour and eye are well protected. This was often described as a "miraculous Escape"⁸.

Numerous studies have demonstrated that as the post-mortem period lengthens, the vitreous potassium level rises. An increase in the post-mortem interval was correlated with a linear rise in potassium concentration⁹.

Potassium increases along with the post-mortem interval, while there is no correlation with calcium or sodium and a negative correlation with sodium¹⁰.

The sodium concentration in the normal body is 136-145 mEq/l. The potassium concentration in the normal body is 50-55 mEq/kg body weight, or 160 mmol/l. At the same time, 98% of potassium is inside the cell. Its extracellular concentration is 3.5-5.5 mmol/l. Normal vitreous sodium is 118-124 mmol/l and vitreous potassium is 2.6-4.2 mmol/l. Potassium is actively transported from the ciliary body into anterior and posterior chambers. Lens also contributes².

AIMS & OBJECTIVES

The study will be conducted to:

1. To assess the use of vitreous humour in the finding of postmortem interval.
2. To compare the level of potassium in both eyes after death.

Methods/ Methodology

- **Study design:** A cross-sectional study
- **Study population:** All dead bodies brought for postmortem examination to the Department of Forensic Medicine & Toxicology in VCSGGIMS & R,Srikot,Srinagar,Pauri Garhwal,UTTARAKHAND
- **Place of Study:** Department of Forensic Medicine & Toxicology, VCSGGIMS & R,Srinagar, Pauri Garhwal, Uttarakhand.
- **Sampling Technique:** Continuous Sampling method.
- **Period of study:** 1st June 2023 to 31st December 2023
- **Inclusion criteria:** all dead bodies brought for medico-legal postmortem examination to the Department of Forensic Medicine & Toxicology, whose exact times of death are known were selected for the study.
- **Exclusion criteria:** (a) Cases whose exact time of death is not known and with previous

history of eye or orbital injury or surgery, posterior segment disease.

- (b) Decomposed and charred bodies.

- **Method of Collection of Data:**

1. The sample of vitreous humour was collected from the posterior chamber of both eyes by aspirating gradually and slowly through a puncture 5-6 mm away from the limbus using a sterile 20-gauge needle taking care to avoid tearing any loose tissue fragments surrounding the vitreous chamber.
2. The sample was kept in plain sterile vial. Then the samples were properly labeled and sent to the biochemistry laboratory.
3. The samples were centrifuged at 3000 rpm for 10 minutes by LAB LINE AUTOMATED ANALYZER.
4. The sample was analyzed in PSR ST-PRO electrolyte analyzer by the Ion selective electrode (ISE) method.
5. All the biochemical analysis was carried out immediately post extraction. The sample was analyzed for the potassium content of vitreous humour. Finally, the data was analyzed and various statistical tests were performed on it.
6. The information regarding the exact time of death was gathered from the police inquest report, death certificate, death slip, and clinical details from hospital records, correlated and checked by relatives, friends of the deceased, and concerned investigating officers.
7. Only the clear vitreous humour was analyzed. Turbid or blood-stained samples were discarded.

- **Statistical Analysis:**

1. The collected data was compiled in a MS Excel version 21.
2. Normal data was presented as mean SD± while non-normal data was presented as median IQR.
3. All the statistical analysis was done on IBM SPSS version 20. P value <0.05 were taken as statistically significant for all the measures of association.

- **Statistical tests:** The one-way ANOVA test and regression line formula were used for comparison and show linear regression.

Results

Table 1: Distribution of cases based on age (N=80)

Age (in years)	No. of cases	Percentage (%)
04-15	04	5
16-25	15	18.7
26-35	27	33.7
36-45	14	17.5
46-55	12	15
56-65	06	7.5
66-75	02	2.5
Total	80	100

In the present study, there were 80 subjects, out of these subjects' maximum subjects were from 26-35 years, and the minimum were from 66-75 years of age group (Table 1).

Table 2: Distribution of cases based on gender (N=80)

Gender	No. of cases	Percentage (%)
Male	53	66.2
Female	27	33.7
Total	80	100

In the present study, there were 80 subjects, out of these subjects' maximum subjects were Males 66.2% and the minimum were females 33.7 years of age group (Table 2).

Table 3: Distribution of cases based on post-mortem interval time

(N=80)

Post-mortem Interval Time (PMI)	No. of cases	Percentage (%)
0-12 hours	18	22.5
12-24 hours	38	47.5
24-36 hours	15	18.7
36-48 hours	09	11.2

In the present study distribution of cases based on post-mortem interval time the maximum subjects were in between 12-24 hours and the minimum subjects were 36-48 hours (Table 3).

Table 4: Potassium concentration level (mmol/l) of both eyes

Eye	Mean	Median	SD	Min	Max	P value
Right	7.87	7.75	1.47	5.9	12.1	0.601
Left	7.63	7.5	1.25	5.7	11.9	

The potassium ion concentration in both as we observed in the present study had a p value of 0.601. The concentration of potassium ions in the two eyes therefore not significantly different. In the present study, maximum potassium ion concentration (right

eye 12.1 mmol/l & left eye 11.9mmol/l) was observed in the subject with postmortem interval of 36-48 hours and least concentration (right eye 5.9 mmol/l & left eye 5.7 mmol/l) was observed in the subject with postmortem interval 0-12 hours (Table-4).

Table 5: Concentration of potassium in vitreous humour with post-mortem interval

Eye		Post-mortem interval {hours}				
		0-12	12-24	24-36	36-48	
R	Mean	6.60	7.78	9.23	8.52	One way ANOVA test. F = 0.41
	SD	1.24	1.19	1.21	1.47	
L	Mean	6.37	7.52	8.96	9.52	P Value = 0.54
	SD	1.26	1.21	1.22	1.25	

Model	R	R Square	Adjusted R Square	S. E
1	0.512 a	0.262	0.252	10.054

Table 6: Linear regression for the right eye potassium level

a. Predictors: (Constant), Right eye potassium level

b. Dependent Variable: Actual TSD

Model	Unstandardized Coefficients B Std. Error		Standardized Coefficients Beta	t	Sig.
Constant	-5.907	6.034	0.512	-0.979	0.331
Right eye K	3.963	0.753		5.260	0.000

Table 7: Linear regression for the left eye potassium level

Model	R	R Square	Adjusted R Square	S.E
2	0.836 a	0.698	0.694	6.428

Model	Unstandardized Coefficients B Std. Error		Standardized Coefficients Beta	t	Sig.
Constant Left eye K	-34.988	4.543	0.836	-7.702	0.000
	7.766	0.578		13.435	0.000

We used SPSS software to conduct the following linear regression to determine the regression line from 80 autopsy cases where the time since death was less than 48 hours. Regression analysis has been conducted on the potassium levels in the left and right eyes.

The constant for the linear regression formula for Table 6 is -5.907 and at last the regression formula for right eye potassium level is $Y = (3.963 \times X) - 5.907$.

The constant for the linear regression formula for Table 7 is -34.988 and at last the regression formula

for left eye potassium level is $Y = (7.766 \times X) - 34.988$.

Where, X = potassium concentration and Y = time since death.

Discussion

- During the study period a total of 80 medico legal autopsies were performed at Department of Forensic Medicine & Toxicology, VCSGGIMS & R, Srinagar, Pauri Garhwal, Uttarakhand, during the period of 1st June 2023 to 31st December 2023. In our study, maximum number of autopsy cases were in the age group of 26-35 years i.e., 27 (33.7%), followed by 16-25 years i.e., 15 cases (18.7%), (table 1). These findings are in consistency with findings of Patel UP et al¹.
- The present study majority of victims were male 53(66.2%) as compared to female 27 (33.7%). Similar findings were seen in studies done of Kurup SS et al.¹¹[Table-2]
- In the present study, in most cases samples were collected within 12-24 hours of death followed by 0-12 hours and 24-36 hours of death. And few samples were collected with 36-48 hours post mortem interval. [Table-3]
- The observed potassium ion concentration in both eyes in the current study had a p-value of 0.601. As a result, [Table 4] shows that the concentration of potassium ions in the two eyes is not significantly different. Numerous investigations also reveal that the concentration of potassium ions in the two eyes is the same.
- In the present study, the subjects with post-mortem intervals of 38-48 hours had the highest concentration of potassium ions (right eye 12.1 mmol/l and left eye 11.9 mmol/l), while the subjects with post-mortem intervals of 0-12 hours had the lowest concentration (right eye 5.9 mmol/l and left eye 5.7 mmol/l) [Table 4].
- The concentration of potassium ions in the vitreous humour increases linearly with increasing post-mortem intervals, as Tables 5 and 6 & 7 show. This shows a direct correlation between the increasing post-mortem interval and the rise in potassium ion concentration.
- We observed, following the application of the regression formula, that the potassium concentration in the vitreous humour increased steadily over 12 hours. The concentration of potassium in the vitreous humour was found to increase significantly every 12 hours after the death, at a rate of about 2 mmol/l. Thus, measuring the time since death based on variations in vitreous humour potassium content was far more beneficial.
- In an effort to determine the post-mortem interval, a number of studies have been conducted on the electrolyte concentration level in vitreous humour.
- Nauman et al¹³ done a study on 211 post-mortem cases in 1959. Which is showed an increase in the vitreous K⁺ values; however, no attempt was made to correlate these values with the post-mortem interval. Additionally, an average concentration of 7.2 mg/dl was found, corresponding to an average post-mortem interval of 9 hours.
- Jaffe et al¹⁴ examined 31 cases (none of them had uremia or electrolyte imbalance) in 1962, and the K⁺ concentration in the vitreous was connected to the post-mortem period. He discovered a steady increase in K⁺ levels that begins soon after death and lasts for 125 hours. Bodies kept at room temperature and those refrigerated did not significantly differ from one another.
- Sturner et al¹⁵, Gantner et al¹⁶ Conducted a more thorough analysis in 1963 and 1964 (involving 54 coroner and 37 hospital cases). The average difference between the two eyes was 0.1 meq/L in 15 of these cases where vitreous humour was extracted from both eyes at the same time. The post-mortem interval and the K⁺ values, which were determined by flame photometry, showed a linear relationship in the 54 coroner cases.
- Coe et al¹⁷ in the year of 1969 found a biphasic rise in vitreous K⁺ levels although there was a linear rise with increasing post-mortem interval up to 100 hours. In the initial hours following death, there was a more rapid rise, with a 95% confidence interval of about ± 12 hours.

- Gregora et al¹⁸ in the year of 1978, calculated the amount of K⁺ in the vitreous humour of 47 deceased people using the technique of atomic absorption spectroscopy. Research revealed that the quantity of both increased linearly with the lapse of time since death.
- In 2003, Prasad et al¹⁹ conducted a study on the relationship between the vitreous K⁺ level and the post-mortem interval. The results indicated a strong positive association between the rise in K⁺ level following death and the post-mortem interval.
- Choo-Kang E et al²⁰ discovered a linear correlation between the vitreous K⁺ concentration and the post-mortem interval after analyzing 105 instances. In the early post-mortem period, the rise was biphasic, with a steeper slope than in the later hours.
- In the current study, the potassium ion concentration for both eyes across age groups was found to be non-significant ($p = 0.601$) (Table 4). Similar findings were seen in studies done by Rathinam RD et al²¹.
- 349 samples out of 269 instances were used. By using flame photometry, the K⁺ levels of the two eyes were found to be similar, in studies done by Adelson et al²².
- Mull A et al²³ proposed in his research that the concentration of vitreous biochemical components varies at the same rate in the same pair of eyes, and that this time-dependent variation can be used to precisely estimate the post-mortem period. Potassium levels increased linearly from 7.04 mEq/L to 15.81 mEq/L, which is comparable to the values reported by Govekar G et al²⁴.

Conclusion

The study conducted at the Department of Forensic Medicine & Toxicology, VCSGGIMS &R, Srinagar, Pauri Garhwal, Uttarakhand, to know the Time since Death, From Potassium Level of Vitreous Humour in the Eyes of the Deceased during a period from 1st June 2023 to 31st December 2023, comprising a total of 80 medico-legal autopsies. Our study found that an appreciable pattern of increased potassium concentration in vitreous humour was observed every 12 hours of post-mortem interval, with an approximate 2mmol/l increase. It

corresponds to the rule of 12. Significantly raising potassium levels with time after death is beneficial for law enforcement. The findings of this study research will improve time-of-death estimation and help law enforcement agencies solve crimes more quickly.

Financial support and sponsorship: Nil

Conflicts of interest: There are no conflicts of interest

Ethical Clearance: Obtained from Institutional Ethical Committee Veer Chandra Singh Garhwali Govt Institute of Medical Sciences and Research Srinagar Pauri Garhwal Uttarakhand with reference number MC/IEC/2022-23/80 on date 8.7.2023.

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