Indian Internet Journal of Forensic Medicine and Toxicology 2023;21(1): Print ISSN: 0973-1970, **Online ISSN:** 0974-4487

Original Research Paper

VARIATION IN VITREOUS BIOCHEMICAL CONSTITUENT LEVELS WITH LENGTHENING TIME SINCE DEATH AND CORRELATION WITH THEIR BLOOD LEVELS

RESEARCH PAPER

Authors

Tushar Saini[1*], Vijay Arora[2], Susheel Sharma[3], RS Yadav[4],

1 Medical Officer (Specialist), Civil Hospital, Nurpur,Distt. Kangra (H.P.)

2 Professor & Head,Department of Forensic Medicine and Toxicology, DRPGMC, Kangra at Tanda

3 Professor, Department of Forensic Medicine and Toxicology, DRPGMC, Kangra at Tanda

4 Professor &Head, Department of Biochemistry, DRPGMC, Kangra at Tanda

Corresponding author: Dr Tushar Saini, drtusharsaini@gmail.com, +919816046243

Abstract

*Background:*The Vitreous Humor (VH) is a transparent, gel-like substance that fills the space between the lens and the retina in the eye. It helps maintain the eye's shape, provides support to the retina, and allows light to pass through to the retina where images are formed. The major function of this fluid is to support the shape of the eye and transmitting light to the retina. In this article, the effect of VH and its biochemical constituents since the time of death has been analysed. This research also demonstrates the changes within biochemical constituents of VH as time increases after the death of a person. Besides that, this research is significantly beneficial for future researchers for evaluating changes in levels of VH after death and correlation with blood levels. *Objective:* To determine Post Mortem Interval from Potassium (K+) concentration in Vitreous, correlation of biochemical constituents with both eyes and additional objective is the correlation of levels of Biochemical Constituents i.e. Na+ , K+ , Cl- and Urea in Vitreous and Blood. *Material and Method:* This study was conducted on the human dead bodies brought for medico-legal autopsy to the mortuary wing of Department of Forensic Medicine & Toxicology, Dr. RPGMC, Kangra at Tanda over a length of one year after obtaining informed consent. "Vitreous Humor" samples were collected from

both eyes first from right eye and then from left eye after external examination of the dead body at the time of post-mortem examination. Collecting blood directly from the heart and analysing ocular fluids can be crucial for accurate assessments. For instance, VH is often analysed in forensic investigations because its composition is more stable compared to blood by post-mortem changes, making it a reliable source for various measurements. Collected samples of both eyes of deceased individuals tended to be analysed for "Na+, K+, Cl^- by "Medica Easylyte Sodium or potassium analyser" and Urea is analysed by "XL- 300". *Results:* There is a direct relationship between the K^+ (Potassium) level in VH levels and Time Since Death (TSD), but there is no exact linear correlation between increasing levels of potassium with TSD and there is no statistically significant difference between the biochemical parameters in left and right eye and also blood has a positive correlation with the vitreous. Regression equation derived from our study for estimation of TSD from potassium levels in VH is: 0.049+1.57 X K^+ ± 4.4. *Conclusion:* This study highlighted the increase in the Potassium levels with increasing post mortem interval (PMI) but also shows that there is no mathematical linear correlation between PMI and TSD.

Keywords: Vitreous Humor (VH), Time since death (TSD), Post Mortem Interval (PMI), Biochemical constituents

Received: Dec 01, 2022. Received Revised: Jan 31, 2023, Accepted: Jan 31, 2023. Available Online: Jul 01, 2023.

Introduction:

"The term "Post Mortem Interval (PMI)" refers to the time span following a person's death, which is utilized to estimate both the time and cause of death. Accurately determining the PMI has posed a significant challenge in the field of Forensic Medicine.Despite the difficulty in determining PMI through a single method, a combination of chemical and physical methods creates a new path in the medical world[1].During investigating a crime, it is crucial to determine "time since death". Different bodily fluids, including the vitreous humor of the eye, exhibit notable chemical alterations right after a person's death. The changes in the biochemical components within the vitreous humor post-mortem, such as potassium and sodium chloride levels, as well as glucose concentrations, can provide significant insights, especially considering the rate of change of each biochemical constituent over time.

Determination of chemical changes occurring within vitreous humor leads to asserting the "time since death" of a person by a Forensic Pathologist.

VH is utilized in place of human blood for post-mortem analyses aimed at estimating the post-mortem interval (PMI), as it is believed that VH is less prone to autolytic changes. This quality helps reduce post-mortem contamination from the diffusion of drugs, which tend to be present at high concentrations in the thoracic or abdominal regions at the time of death. Only fewer studies are available in the past to find the correlation between vitreous biochemical constituents with blood and difference between the biochemical constituents of both eyes simultaneously. Therefore, the present study consists of multiple aims to determine any mathematical correlation of rise of potassium with time and direct effect of blood biochemical constituents on vitreous biochemical constituents as well as to see any difference of biochemical constituents in both eyes.

Materials and Methods:

The current research was carried out within the department of Forensic Medicine and Toxicology at Dr. Rajendra Prasad Government Medical College and Hospital in Kangra, Tanda (HP), and spanning duration of one year from January 2020 to January 2021. In the present study, 96 cases were taken. Out of them, there were 29 females and 67 males. Information regarding "time since death" for dead individuals that have been brought for medico-legal autopsy has been gathered from police as well as hospital records. Furthermore, more information about deceased individuals has been collected from friends, relatives, and eyewitnesses.

Vitreous humor samples were obtained from both eyes, starting with the right eye followed by the left, following an external examination of the deceased during the postmortem procedure. Blood samples were drawn directly from the heart after the thoracic cavity was opened. The "vitreous fluid" samples were collected by puncturing the sclera and aspirating the gelatinous material from the back chamber (Posterior) of the eye using a 10 ml syringe and 20-gauge needles. Materials such as “syringes” as well as “rubber stopper glass vials” that were cleaned with "double distilled water" and dried in a hot air oven, have been used in order to collect “vitreous samples” of dead bodies. "1.5 to 2.0 ml" of vitreous fluid has been "aspirated slowly" along with "guarded suction" that has been taken from "center of the eyeball" with a help of a 10 ml syringe with 20-gauge needle by puncturing of about “5-6 mm away” from the “limbus temporally”. In order to avoid retraction of the eyeball liquid paraffin

has been replaced within eyeball for "cosmetic purposes"[2].

Some cases were excluded from the study like cases of injury to eyes, burns cases, cases with age group less that 1year and age group more than 60 years and turbid or blood containing vitreous.

Statistical Analysis:

The data was inputted into Microsoft Excel, and statistical analysis was conducted using Epi-Info version 7.1. The quantitative numeric variables were expressed as means and standard deviations (SD), while the qualitative categorical variables were represented in terms of frequency and percentages. A correlation coefficient and regression equation were derived to estimate the time since death based on potassium levels for each eye through linear regression analysis. ANOVA was employed to compare the means.

Results:

During this period, a total of 113 cases were reviewed, of which 17 were excluded from the study based on the established exclusion criteria. The recorded actual postmortem interval varied, with minimum and maximum times noted at 2.55 hours and 42.40 hours, respectively.

The table 1 below illustrates that the largest number of cases fell within the age group of 16-30 years, accounting for 38 out of 96 cases (39.6%). This was followed by the 31-45 years age group, which comprised 33 cases (34.4%). Additionally, there were only 3 cases (3.1%) in the age group of under 15 years (Table 1.1).

Table 1 Distribution of cases according to age group

AGE GROUP	NUMBER	PERCENTAGE
<15 YEARS	3	3.1
16-30 YEARS	38	39.6
31-45 YEARS	33	34.4
>46 YEARS	22	22.9
TOTAL	96	100

There were 50 (52.1%) cases recorded as hospital death and rest i.e.46 (47.9%) deaths were as non-hospital deaths. The mean time after which the inquest was done was 14.79±5.8 hours in hospital death while in non-hospital deaths, it was 24.3516.94 hours. Minimum time of inquest was 3 hours and maximum were 72 hours (Table 2).

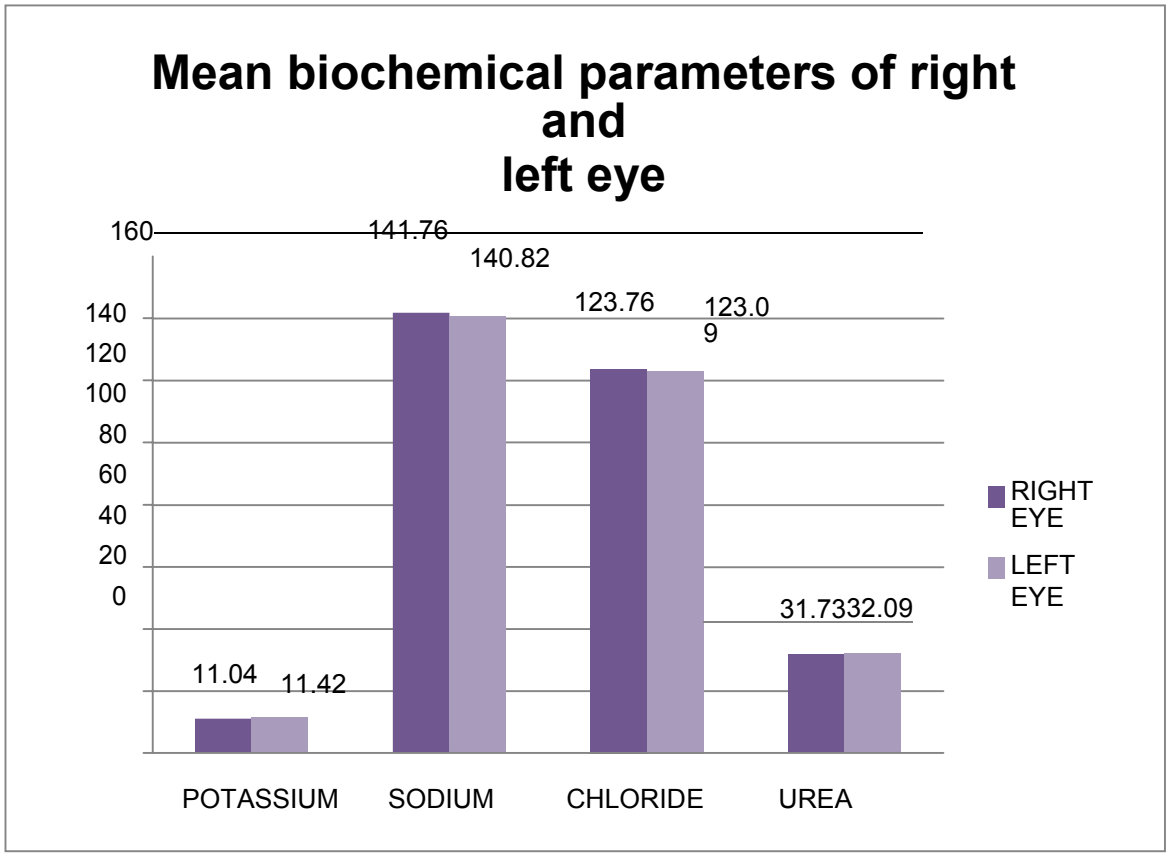
Table 2 Distribution of cases according to place of death

PLACE OF DEATH	NUMBER	PERCENTAGE	MEAN TIME±SD	RANGE
HOSPITAL DEATH	50	52.1	14.79±5.8	3-28
NON-HOSPITAL DEATH	46	47.9	24.35±16.94	4-72
TOTAL	96	100	19.37±13.28	3-72

Eyes difference:

In this study, the average levels of potassium and urea were greater in the left eye, whereas sodium and chloride levels were elevated in the right eye. Nevertheless, the differences in biochemical parameters between the left and right eyes were not statistically significant (p value <0.001) (Fig 1).

Comparison of mean biochemical parameters of right and left eye (Fig 1)



Blood correlation with vitreous biochemical constituents:

All the biochemical parameters of eye were statistically significantly associated (<0.001) and

showed positive association with blood parameters. Pearson correlation coefficient (r) was ranging from 0.295 to 0.513 (Table 3).

Table 3 showing Pearson correlation coefficient (r) of Ptassium, Sodium, Chloride in VH

	CORRELATION COEFFICIENT (r)	P VALUE
POTASSIUM	0.513	<0.001
SODIUM	0.295	<0.001
CHLORIDE	0.375	<0.001
UREA	0.394	<0.001

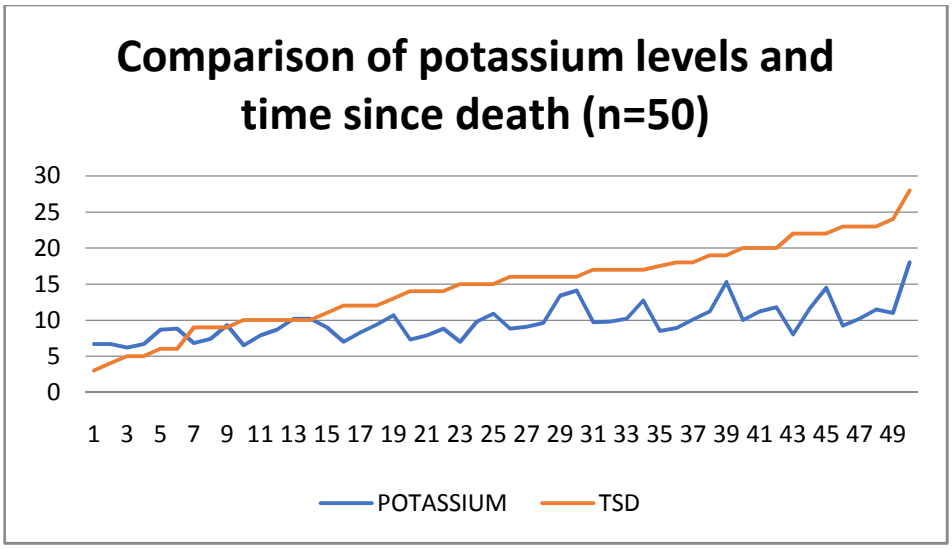
Correlation of values with other formulas:

From linear regression between time since death as per hospital records and time calculated from potassium levels in eye, it was observed that there was a correlation of 0.659 between the both. While when compared with time since death calculated as per formula by Hessing, Sturner and James, the correlation was 0.57. On comparing time since death calculated by the equation derived from potassium levels in left eye with time as per Hessing, Sturner and James formula, the correlation was 0.85. However, all the correlations were statistically significantly associated (P value <0.001).

Comparison of potassium levels and time since death:

There is no discernible relationship between potassium levels and the time since death. When the times are arranged in ascending order, it's evident that potassium levels do not exhibit a consistent pattern, indicating a lack of linear correlation with the post-mortem interval.

Comparison of potassium levels and time since death (n=50) (Fig 2).



Regression equation obtained from the study:

Regression equation for estimation of time since death from potassium levels in vitreous humor:

TIME SINCE DEATH= 0.049+1.57 * K^+ ± 4.4

DISCUSSION:

Since the 20th century, forensic specialists have endeavored to get the precise time of death, or post-mortem interval (PMI). It was found that the time since death (TSD) is not always precise in 100% of cases; however, as the PMI extends, the confidence interval also widens, making it increasingly challenging to accurately pinpoint the exact time of death when it is believed to have occurred.

Determining the TSD is crucial in medico-legal autopsies from various factors, including body cooling, ocular changes, post-mortem lividity, rigor mortis, decomposition alterations, and the contents of the stomach, intestines, and urinary bladder, there are also numerous chemical changes that commence following death. These changes occur in a relatively systematic manner until the body breaks down, potentially providing valuable insights into this question.[3].

The vitreous humor is a fluid that remains comparatively resistant to degradation and contamination after death. Because of this post-mortem stability, it proves to be highly

beneficial in forensic pathology. Precisely determining the post-mortem interval (PMI) holds significant importance in criminal investigations and legal proceedings. The concentrations of chemical constituents in human vitreous humor fluctuate over time following death, which can assist in estimating the PMI.[4].

Numerous studies have investigated the concentration levels of electrolytes in the VH to find the post-mortem interval (PMI). The current research indicates a considerable increase in potassium levels within the VH as PMI extends, aligning with findings from previous studies[5-9]. The increase in potassium levels is attributed to the vascular choroids and retinal cells autolysis in the eye.

In this study, we found a weak relation of potassium levels in vitreous with TSD which can be attributed to the lower TSD in our study. We observed mean TSD in cases with precise TSD was 14.79±5.8 ranging from 3 to 27hours.According to Mohamed, it is observed that potassium level in vitreous significantly increases with increased PMI; however, it is also stated that potassium shows a significant negative correlation with PMI. This referenced idea depicts accurate results as conducted under this study[10].

A positive association of these biochemical parameters has been observed with blood parameters from evaluating the correlation coefficient of considered biochemical parameters. As observed by Savini, the correlation of blood and vitreous humor is a significantly essential indication that serves in with effective studies and research based on Forensic studies[11]. Therefore, association of biochemical parameters of vitreous with blood can be confirmed effectively.

The linear regression correlation between vitreous potassium and TSD demonstrated a highly significant relationship (R, 0.049; P<0.0001). Pounder observed notable differences in vitreous potassium levels between the two eyes of the same person. However, the findings of the current study do not corroborate their conclusion regarding discrepancies in vitreous potassium between eyes. A key factor contributing to the differing reports on between-eye variations at the same PMI could be discrepancies in research methodologies and potential sample handling prior to analysis.[4]

We noted a weak correlation between potassium levels in the vitreous humor and the time since death (TSD), which may be due to the relatively low TSD observed in our research. The average TSD for cases with accurately known times of death was 14.79±5.8 hours, with a range of 3 to 27 hours. Chandrakanth HV[12] conducted a study that included cases with well-

defined times of death, spanning from 0 to 36 hours since death, and did not find a substantial link between vitreous chemistry and the post-mortem interval. Moreover, Singh D et al[13] discovered that the relationship between increasing vitreous potassium levels and post-mortem interval was not linear, but rather logarithmic. Bortolotti F[14] pointed out that the percentage error in PMI calculations diminishes with longer PMIs, becoming reasonable for practical purposes after 24 hours post-mortem. Sturner[15], Madea et al.[6], and James et al[16] utilized a PMI range of 4.5 to 84.3 hours to construct their regression equations, with a mean around 30 hours. This significant range in PMI employed in the study provides adequate data for analysis.

In certain research, the PMI was estimated by multiple compound analysis, wherein a reasonably precise equation was utilized to determine the PMI. Researchers use numerous chemical analyses and integrate all data to ensure accurate estimation in order to get the precise PMI.[17-21] that the involvement and influence of regression model in this study could be ineffective towards determination of TSD of deceased individuals taken under study. Results have stated that sodium, chloride and urea levels and concentrations in vitreous liquid has no significant relation with PMI and TSD determination and is observed to decrease in concentration. Experimental demonstrations conducted in this study have confirmed that age, sex, cause of death and refrigeration of dead bodies imposes no significant impact on potassium levels of vitreous humor.

CONCLUSION:

There is a rise in potassium levels with a growing post mortem interval, according to research on potassium in the vitreous humour, however there is no mathematical relationship between PMI and TSD. It is clearly clear from the study's results that there is no conclusive correlation between an individual's period since death and rises in potassium levels. The fact that the study's cases are hospital fatalities with established causes of death and brief time intervals between deaths may account for the non-linear post-mortem increase in vitreous potassium.

In hospitalized cases, treatments/medications are given which affects the serum levels of K^+ and has strong correlation with vitreous K^+ levels detected in this study.To improve the accuracy of PMI estimation equations, setting up a scoring system for multiple constituent simultaneous analyses to evaluate the results of each single component and consider various causes of death, states of illness, and influencing factors can be helpful for estimating PMI. These findings show that calculation of TSD from K^+ level in vitreous have not so much significance and other parameters need to be taken along with this parameter.

Ultimately, in line with the objectives of our research, we deduce that elevated potassium levels and TSD have a direct association, but no mathematical link. Based on potassium levels in vitreous humour, our investigation yielded the following regression equation for time since death: TIME SINCE DEATH= 0.049+1.57 X K+ ± 4.4. Additionally, the biochemical parameters in the left and right eyes did not differ statistically significantly; however, blood has a positive correlation with all of the biochemical elements in vitreous humor, including K+, Na+, Cl-, and urea.

Conflict of Interest: Nil

Ethical Issues: Throughout the sampling process, there was no "drug trial" and no living human beings or animals were involved in the study.

Financial Disclosure: Samples of vitreous fluid as well as samples of blood from deceased individuals which are preserved exist in the "Department of Forensic Medicine and Toxicology". Furthermore, analysis of samples based on "biochemical constituents" such as "level of potassium, sodium, chloride and urea", is done using facilities that are available in "Department of Biochemistry". Above experiment as well as a collection of samples from dead bodies are not funded by any agency that result in enhancing overallbudget.

References:

1.Agarwal RL, Gupta PC, Bhasin S, Nagar CK. Determination of the time of death by estimating potassium level in the cadaver vitreous humor. Ind J Ophthalmol. 1983;31(5):528-531.

2.Garg V, Oberoi SS, Gorea RK., Kaur K. Changes in the levels of vitreous potassium with increasing time since death. J Ind Acad Forensic Med. 2004;26(4):136-9.

3.Mulla A.Role of vitreous humor biochemistry in Forensic Pathology. [MS Thesis]. Saskatchewan, Canada: University of Saskatchewan. 2005.

4. Pounder DJ, Carson DO, Johnston K, Orihara Y. Electrolyte concentration differences between

left and right vitreous humor samples. J Forensic Sci. 1998;43(3):604-7.
5. Jaffe FA. Chemical post-mortem changes in the intraocular fluid. J Forensic Sci. 1962;7:231-7.
6.Madea B, Henssge C, Honig W, Gerbracht A. References for determining the time of death by potassium in vitreous humor. Forensic Sci Int. 1989;40(3):231-43.
7. Coe JI. Post-mortem chemistries on human vitreous humor. Am J Clin Pathol. 1969; 51(6):741-50.
8. Farmer JG, Benomran F, Watson AA, Harland WA. Magnesium, potassium, sodium and calcium in post-mortem vitreous humor from humans. Forensic Sci Int. 1985;27(1):1-13.
9. Prasad BK, Choudhary A, Sinha JN. A study of correlation between vitreous potassium level and post mortem interval. Kathmandu Univ Med J. 2003;1(2):132-4.
10. Mohamed SA, Radwan R.A, Salama E. Studying Histopathological changes in suprarenal gland versus vitreous humor potassium level in estimation of postmortem intervals: experimental study in rabbits. Egyptian J Forensic Sci Applied Toxicol. 2020;20(2):61-72.
11.Savini F, Tartaglia A, Coccia L, Palestini D, D'Ovidio C, De Grazia U et al. Ethanol determination in post-mortem samples: correlation between blood and vitreous humor concentration. Molecules. 2020;25(12):2724.
12.Chandrakanth HV, Kanchan T, Balaraj BM, Virupaksha HS, Chandrashekhar TN. Post-mortem vitreous chemistry- An evaluation of sodium, potassium and chloride levels in estimation of time since death (during the first 36 hours after death). J Forensic Legal Med. 2013;20(4):211-6.
14. Singh D, Prashad R., Sharma, S.K., Pandey AN. Double logarithmic, linear relationship between postmortem vitreous sodium/potassium electrolytes concentration ratio and time since death in subjects of Chandigarh zone of northwest. India. J Indian Acad Forensic Med. 2005;27:159-65.
15. Bortolotti F, Pascali JP, Davis GG, Smith FP, Brissie RM, Tagliaro F. Study of vitreous potassium correlation with time since death in the post-mortem range from 2 to 110 hours using capillary ion analysis. Med Sci Law. 2011;51(1):20-3.
16. Sturner WQ. The vitreous humor: post-mortem potassium changes. Lancet 1963;1:807-8.
17. James RA, Hoadley PA, Sampson BG. Determination of post-mortem interval by sampling vitreous humour. Am J Forensic Med Pathol. 1997;18:158-62.
18. Lange N, Swearer S, Sturner WQ. Human postmortem interval estimation from vitreous potassium: an analysis of original data from six different studies. Forensic Sci Int. 1994; 66:159-74.
19.Jashnani KD, Kale SA, Rupani AB. Vitreous humor: Biochemical constituents in estimation

of postmortem interval. J Forensic Sci. 2010; 55:152-37.

20.Thierauf A, Musshoff F, Madea B. Post-mortem biochemical investigations of vitreous humor. Forensic Sci Int. 2009; 192:78-82.

21.Garg V, Oberoi SS, Gorea RK, Kiranjeet K. Changes in the levels of vitreous potassium with increasing time since death. J Indian Acad Forensic Med. 2004; 26:136-9.