



## Variation In Vitreous Biochemical Constituent Levels With Lengthening Time Since Death And Correlation With Their Blood Levels

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### ARTICLE INFO

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### ABSTRACT

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 transmit light to the retina. In this article, the effect of VH and its biochemical constituents since the time of death has been analyzed. 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 to evaluate changes in levels of VH after death and correlation with blood levels.

**Objective:** To determine Post Mortem Interval from Potassium (K<sup>+</sup>) concentration in Vitreous, correlation of biochemical constituents with both eyes, and an additional objective is the correlation of levels of Biochemical Constituents i.e. Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, 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 the right eye and then from the left eye after external examination of the dead body at the time of post-mortem examination. Collecting blood directly from the heart and analyzing ocular fluids can be crucial for accurate assessments. For instance, VH is often analyzed in forensic investigations

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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 were analyzed for “Na+, K+, Cl-” by “Medica Easylyte Sodium or potassium analyzer,” and Urea is analyzed by “XL- 300.”

**Results:** There is a direct relationship between the K+ (Potassium) level in VH 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 the left and right eye, and also blood has a positive correlation with the vitreous. The regression equation derived from our study for the estimation of TSD from potassium levels in VH is:  $0.049 + 1.57 \times K^+ \pm 4.4$ .

**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.

## Introduction

“The term “Post Mortem Interval (PMI)” refers to the time span following a person’s death, which is used 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. During the investigation of 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 a few studies are available in the past to find the correlation between vitreous biochemical constituents with blood and the difference between the biochemical constituents of both eyes simultaneously. Therefore, the present study consists of multiple aims to determine any mathematical correlation of the rise of potassium with time

and the direct effect of blood biochemical constituents on vitreous biochemical constituents, as well as to see any difference in the 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), spanning a 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 the “time since death” for deceased individuals brought in for medico-legal autopsy has been gathered from police and 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 and rubber stopper glass vials that were cleaned with double distilled water and dried in a hot air oven have been used to collect vitreous samples from dead bodies. “1.5 to 2.0 ml” of vitreous fluid has been slowly aspirated along with guarded suction taken from the center of the eyeball with the help of a 10 ml syringe with a 20-gauge needle by puncturing about “5-6 mm away” from the limbus temporally. In order to avoid retraction of the eyeball, liquid paraffin has been replaced within the eyeball for cosmetic purposes.

Some cases were excluded from the study, such as cases of injury to the eyes, burn cases, cases with an age group less than 1 year and an age group more than 60 years, and turbid or blood-containing vitreous.

## Statistical Analysis

The data was input 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 the minimum and maximum times noted at 2.55 hours and 42.40 hours, respectively.

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 deaths, and the remaining 46 (47.9%) deaths were non-hospital deaths. The mean time after which the inquest was conducted was  $14.79 \pm 5.8$  hours for hospital deaths, while for non-hospital deaths, it was  $24.35 \pm 16.94$  hours. The minimum time of inquest was 3 hours, and the maximum was 72 hours (Table 2).

### 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).

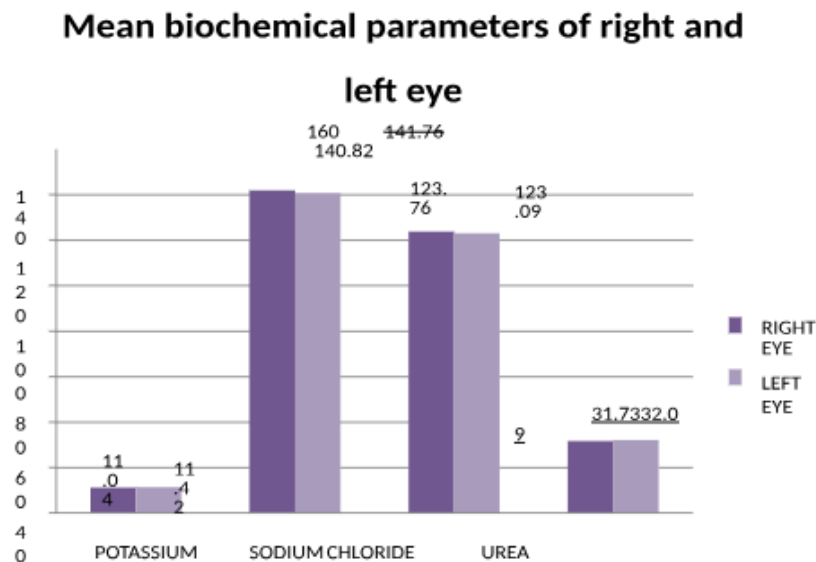
### Blood correlation with vitreous biochemical constituents

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.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

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



**Table 3:** showing Pearson correlation coefficient (r) of Potassium, 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 the eye, it was observed that there was a correlation of 0.659 between the two. When compared with time since death calculated as per the formula by Hessing, Sturmer, and James, the correlation was 0.57. When comparing time since death calculated by the equation derived from potassium levels in the left eye with time as per the Hessing, Sturmer, 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.**

### 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**

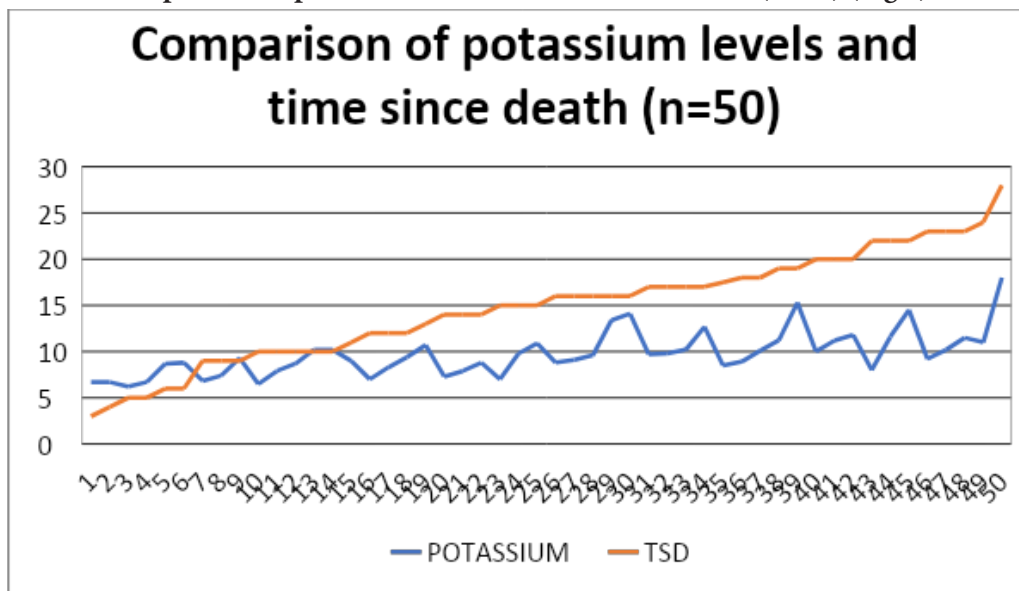
### Discussion

Since the 20th century, forensic specialists have endeavored to determine the precise time of death, or post-mortem interval (PMI). It has been found that the time since death (TSD) is not always precise in 100% of cases. However, as the PMI increases, the confidence interval also widens, making it increasingly challenging to accurately pinpoint the exact time of death.

Determining the TSD is crucial in medico-legal autopsies due to 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 occur following death. These changes occur in a relatively systematic manner until the body breaks down, potentially providing valuable insights into this question.

The vitreous humor is a fluid that remains relatively stable after death, making it 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

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



Numerous studies have investigated the concentration levels of electrolytes in the vitreous humor to determine the post-mortem interval (PMI). Current research indicates a significant increase in potassium levels within the vitreous humor as the PMI increases, aligning with findings from previous studies. The increase in potassium levels is attributed to the autolysis of vascular choroids and retinal cells in the eye.

In this study, we found a weak relationship between potassium levels in the vitreous humor and TSD, which can be attributed to the lower TSD in our study. We observed a mean TSD in cases with a precise TSD of  $14.79 \pm 5.8$  hours, ranging from 3 to 27 hours. According to Mohamed, it has been observed that potassium levels in the vitreous humor significantly increase with an increase in 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.

A positive association of these biochemical parameters has been observed with blood parameters by evaluating the correlation coefficient of the considered biochemical parameters. As observed by Savini, the correlation between blood and vitreous humor is a significantly essential indication that serves in effective studies and research in Forensic studies. Therefore, the association of biochemical parameters of vitreous humor with blood can be confirmed effectively. The linear regression correlation between vitreous potassium and TSD demonstrates 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.<sup>4</sup>

We note 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 is  $14.79 \pm 5.8$  hours, with a range of 3 to 27 hours. Chandrakanth HV<sup>12</sup> 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<sup>13</sup> discovered that the relationship between increasing vitreous potassium levels and post-mortem interval is not linear, but rather logarithmic. Bortolotti F<sup>14</sup> pointed out that the percentage error in PMI calculations diminishes with longer PMIs, becoming

reasonable for practical purposes after 24 hours post-mortem. Sturmer<sup>15</sup>, Madea et al.<sup>6</sup>, and James et al<sup>16</sup> 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.<sup>17-21</sup> The involvement and influence of the regression model in this study may be ineffective towards the determination of TSD of deceased individuals taken under study. Results state that sodium, chloride, and urea levels and concentrations in vitreous liquid have no significant relation with PMI and TSD determination and are observed to decrease in concentration. Experimental demonstrations conducted in this study have confirmed that age, sex, cause of death, and refrigeration of dead bodies impose no significant impact on potassium levels of vitreous humor.

## Conclusion

There is a rise in potassium levels with a growing postmortem interval, according to research on potassium in the vitreous humor. However, there is no mathematical relationship between PMI and TSD. It is 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 postmortem increase in vitreous potassium.

In hospitalized cases, treatments/medications are given which affect the serum levels of  $K^+$  and have a 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 the calculation of TSD from  $K^+$  levels in vitreous does not have much significance, and other parameters need to be taken into account 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 humor, our investigation

yielded the following regression equation for time since death:  $\text{TIME SINCE DEATH} = 0.049 + 1.57 \times \text{K}^+ \pm 4.4$ . 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  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Cl}^-$ , 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, are preserved in the “Department of Forensic Medicine and Toxicology.” Furthermore, analysis of samples based on biochemical constituents, such as the levels of potassium, sodium, chloride, and urea, is done using facilities available in the “Department of Biochemistry.” The above experiment, as well as the collection of samples from dead bodies, is not funded by any agency, resulting in a lack of overall budget enhancement..

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