Original Research Paper

# Role of Vitreous Humor and Synovial Fluid Potassium Levels in Estimating Postmortem Interval: A Study

**Rangaiah Y.K.C[1], Mahesh Mandala[2], Harish Kumar Poola[3], Surekha V[4], Kattamreddy Ananth Rupesh[5], Shankar R[6], Padma Vijayasree A[7]**

**[1]Associate Professor, [2,3]Assistant Professor, Department of Forensic Medicine and Toxicology, Kurnool Medical College, Kurnool, Andhra Pradesh, [4]Assistant Professor of Forensic Medicine, and Toxicology, Santhiram Medical College, Nandyal, Andhra Pradesh, [5]Assistant Professor of Forensic Medicine and Toxicology, ACSR Government Medical College, Nellore, Andhra Pradesh, [6]Professor of Forensic Medicine and Toxicology, Government Medical College, Anantapur, Andhra Pradesh, [7]Professor of Biochemistry, Kurnool Medical College, Kurnool, Andhra Pradesh.**

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**Abstract**

Determining the time since death is a crucial aspect of a forensic autopsy as it helps establish the sequence of events leading to death, narrow down the list of potential suspects, and aid in legal investigations. The post-mortem interval can be estimated using various indicators such as body temperature, rigor mortis, and biochemical changes in bodily fluids, and this information can provide valuable insights into the circumstances surrounding the death. This study aimed to investigate the correlation between post-mortem interval and the levels of sodium and potassium in vitreous humor and synovial fluid. The study included 100 cases, with 71% males and 29% females, and poisoning cases were the most prevalent. The results indicated a linear correlation between time since death and potassium levels in both vitreous humor and synovial fluid, suggesting that potassium concentration values could be useful for estimating time since death. However, sodium levels showed a negative correlation, indicating they are not significant for this purpose. These findings could be valuable for forensic investigations and the determination of the time since death. Additionally, the study found that alcohol could be detected from vitreous humor, further highlighting its potential as a forensic tool.

**Keywords:** Vitreous humor, Time Since Death, Thanato-chemistry, Post-mortem Interval, Synovial Fluid.

## Introduction

Estimating the post-mortem interval (PMI) is a critical task in forensic investigations and is one of the objectives of a medico-legal autopsy. There are several gross, histomorphological, thanato-chemical, and molecular methods to estimate time since death in the immediate, early, and late post-mortem period[1]. One important method used to estimate PMI with accuracy during the first 24 hours is to measure the concentration of potassium in bodily fluids such as vitreous humor and synovial fluid. Potassium is an electrolyte that is found in high concentrations in these fluids, and its concentration changes after death

**Corresponding Author: Surekha V,** Assistant Professor, Department of Forensic Medicine, and Toxicology, Santhiram Medical College, Nandyal, Andhra Pradesh.

**E-mail:** drsurekha1712@gmail.com

**Mobile:** +91 91219 80288

due to numerous factors such as the breakdown of cellular membranes and the release of intracellular potassium. By measuring the potassium levels in these fluids, forensic investigators can estimate the time since death.

The estimation of post-mortem potassium concentration from body fluids such as Cerebro Spinal Fluid (CSF), aqueous humour, vitreous humour, blood, serum, and synovial fluid has been employed in practice to evaluate times since death (TSD). Vitreous humour (VH) is a transparent jelly-like tissue that fills the eyeball behind the lens, composed of collagen fibres and hyaluronan. VH contains electrolytes such as $Na^+$, $K^+$, $Cl^-$, $Ca^{++}$, and $Mg^{++}$, and the average potassium level in VH is about 3.8 mmol/l. Synovial fluid, which lubricates and cushions joints, contains components such as hyaluronic acid, proteinases, collagenases, lubricin, $Na^+$, $K^+$, $Ca^{++}$, and glucose. TSD estimation from VH and synovial fluid is accurate, with studies showing the latter to be the most precise.[2,3]

The aim of this study was to examine the utility of vitreous humor and synovial fluid potassium levels in estimating PMI and understand the feasibility of utilizing it on a routine basis in our casework. Furthermore, an attempt was made to know if vitreous humor chemistry can of help in knowing the cause of death in poisoning cases.

## Materials and Methods

The study was conducted at the Department of Forensic Medicine and Toxicology, Kurnool Medical College from 2019 to 2021. Cases were sorted using a selective convenient sampling technique. The study population comprised a total of 100 cases in which the exact time of death was known. The study sample was selected from medico-legal cases that underwent autopsy at the centre, and had a treatment history at Kurnool General Hospital, Kurnool, where clinicians recorded the time of death.

Vitreous humor was collected from both the eyes using a sterile 20-gauge needle and 5 ml syringe, 5-6mm away from sclero-corneal junction. Similarly, synovial fluid was collected from both knee joints using a large bore needle. The samples were forwarded to the department of Clinical Biochemistry, Kurnool General Hospital, Andhra Pradesh, and were analysed by a fully Automated Beckman Coulter Au 480 Analyzer. In addition to this, vitreous humor samples from 33 cases (23 poisonings & 10 alcohol intoxications) were sent to Regional Forensic Science Laboratory, Kurnool for detecting the poisons using analytical chemistry techniques.

To reduce the errors in the results, cases with injuries to the orbit and knee, aspirations that yielded turbid sample, cases with history of renal disease and any other eye or orbit diseases were carefully excluded from the study.

The sodium and potassium electrolyte concentrations of all the samples were calculated and data analysis conducted. Sturner's regression formula was used to verify the accuracy of the method in estimating time since death after taking the average of potassium concentration in four different samples in each case.

## Results

In the 100 cases, 47 cases had a Post-mortem interval (PMI) within 12 hours, 44 cases had a PMI between 12.1 to 24 hours, and 9 cases had a PMI exceeding 24 hours. The sex distribution of the study sample is 71% male and 29% female. The profile of causes of death in the study sample is as follows, 23 due to poisonings, 21 due to head injury, 14 due to snake bite, 13 due to burns, 10 due to alcohol intoxications, 5 due to hanging, 4 each due to electrocution, road traffic accidents, drowning and 2 due to blunt injury chest.

Alcohol was detected in 10 cases during the toxicological analysis of vitreous humor, while in the 23 cases of poisoning, the poison was not detected. The descriptive statistics of right sided and left sided samples are summarised in Tables 1 and 2 respectively. The correlation among TSD and right sided and left sided VH and SF electrolyte concentrations are summarised in Table 3 and Table 4 respectively. The Table 5 summarizes the results of the T-test applied to the case data with a TSD (time since onset of symptoms to diagnosis) of less than 12 hours, while Table 6 presents the results for the case data with a TSD between 12-24 hours.

The regression value obtained from right VH $K^+$ data is 0.704 with the constant and potassium

concentration as predictors. Similarly for right SF $K^+$ data the R value is 0.763, for the left VH $K^+$ the R value is 0.737 and the left SF $K^+$ the R value is 0.797. We also analysed the relationship between the dependent variable "Time since death" and the predictor variable "Right K Vitreous humour" using a linear regression model. The model included unstandardized and standardized coefficients. The unstandardized coefficient for the constant was -13.203 and the unstandardized coefficient for Right K Vitreous humour was 3.412. The standardized coefficient for Right K Vitreous humour was 0.704, indicating that a one-standard deviation increase in Right K Vitreous humour was associated with an increase of 0.704 standard deviations in the dependent variable. Both coefficients were statistically significant ($p < 0.001$), suggesting that the relationship between Right K Vitreous humour and Time since death was unlikely to be due to chance. Similarly, the standardised coefficient beta value with predictor variable right SF $K^+$ is 0.763 and is statistically significant. The beta value for the same test on left VH and left SF $K^+$ are 0.737 and 0.797, respectively. These tests are also statistically significant.

**Table 1: Descriptive Statistics of VH and SF Samples on the Right Side**

| | p value | TSD | N | Mean | SD | SE | 95% CI | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | lower bound | upper bound | | |
| Rt eyeNa | P<0.001 | <12 hrs | 47 | 138.9 | 8.005 | 1.1676 | 136.592 | 141.293 | 126.5 | 152.6 |
| | | 12- 24 hrs | 44 | 143.8 | 7.522 | 1.134 | 141.552 | 146.126 | 123.4 | 154.1 |
| | | >24 hrs | 9 | 137.8 | 5.998 | 1.9993 | 133.145 | 142.366 | 124.8 | 144.6 |
| | | Total | 100 | 141 | 7.988 | 0.7988 | 139.405 | 142.575 | 123.4 | 154.1 |
| Rt eye K | P<0.001 | <12 hrs | 47 | 6.864 | 1.742 | 0.2541 | 6.3524 | 7.3753 | 3.2 | 13.3 |
| | | 12-24 hrs | 44 | 8.915 | 1.868 | 0.2816 | 8.3466 | 9.4825 | 5.5 | 15.1 |
| | | >24 hrs | 9 | 11.13 | 3.164 | 1.0546 | 8.7014 | 13.5653 | 7.1 | 18.4 |
| | | Total | 100 | 8.15 | 2.364 | 0.2364 | 7.6813 | 8.6195 | 3.2 | 18.4 |
| Rt. KneeNa | P<0.001 | <12 hrs | 47 | 138.8 | 7.156 | 1.0438 | 136.663 | 140.865 | 126.8 | 151.4 |
| | | 12-24 hrs | 44 | 143.8 | 7.895 | 1.1901 | 141.402 | 146.202 | 123.2 | 158.4 |
| | | >24 hrs | 9 | 137.6 | 6.231 | 2.077 | 132.766 | 142.345 | 125.2 | 145.4 |
| | | Total | 100 | 140.9 | 7.80 | 0.780 | 139.323 | 142.421 | 123.2 | 158.4 |
| Rt. Knee K | P<0.001 | <12 hrs | 47 | 6.643 | 1.482 | 0.216 | 6.207 | 7.078 | 3.1 | 11.3 |
| | | 12-24 hrs | 44 | 8.139 | 1.080 | 0.162 | 7.810 | 8.467 | 5.6 | 10.8 |
| | | >24 hrs | 9 | 10.811 | 2.658 | 0.886 | 8.767 | 12.855 | 7.0 | 16.3 |
| | | Total | 100 | 7.676 | 1.896 | 0.189 | 7.300 | 8.052 | 3.1 | 16.3 |

**Table 2: Descriptive Statistics of VH and SF Samples on the Left Side**

| | P value | TSD | N | Mean | SD | SE | 95% CI | | Min | Max |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Lower Bound | Upper Bound | | |
| Lt Eye Na | | <12 hrs | 47 | 139.35 | 8.1104 | 1.183 | 136.97 | 141.73 | 126.5 | 153 |
| | P<0.001 | 12-24 hrs | 44 | 143.9 | 7.5449 | 1.1374 | 141.61 | 146.2 | 124.5 | 155 |
| | | >24 hrs | 9 | 137.8 | 6.3906 | 2.1302 | 132.89 | 142.71 | 124.6 | 145 |
| | | Total | 100 | 141.28 | 8.0315 | 0.8031 | 139.62 | 142.81 | 124.5 | 155 |
| Lt Eye K | | <12 hrs | 47 | 6.717 | 1.4021 | 0.2045 | 6.305 | 7.129 | 3.5 | 10.5 |
| | P<0.001 | 12-24 hrs | 44 | 8.909 | 1.7979 | 0.271 | 8.362 | 9.456 | 5.7 | 14.8 |
| | | >24 hrs | 9 | 11.133 | 3.1028 | 1.0343 | 8.748 | 13.518 | 7.1 | 17.9 |
| | | Total | 100 | 8.079 | 2.265 | 0.2265 | 7.63 | 8.528 | 3.5 | 17.9 |
| Lt Knee Na | P<0.001 | <12 hrs | 47 | 139.93 | 7.387 | 1.0775 | 136.76 | 141.1 | 126.5 | 152 |
| | | 12-24 hrs | 44 | 144.28 | 7.703 | 1.1613 | 141.94 | 146.62 | 124.2 | 153 |
| | | >24 hrs | 9 | 137.44 | 6.8369 | 2.279 | 132.19 | 142.7 | 123.2 | 145 |
| | | Total | 100 | 141.15 | 7.9278 | 0.7928 | 139.58 | 142.72 | 123.2 | 153 |
| Lt Knee K | P<0.001 | <12 hrs | 47 | 6.662 | 1.6078 | 0.2345 | 6.19 | 7.134 | 3.6 | 12.1 |
| | | 12-24 hrs | 44 | 8.052 | 1.113 | 0.1678 | 7.714 | 8.391 | 5.8 | 10.6 |
| | | >24 hrs | 9 | 11.167 | 3.3548 | 1.1183 | 8.588 | 13.745 | 7.2 | 18.9 |
| | | Total | 100 | 7.679 | 2.0755 | 0.2076 | 7.267 | 8.091 | 3.6 | 18.9 |

**Table 3: Correlation among TSD and Right Sided VH and SF Electrolyte Concentrations.**

| | | Right Na VH | Right K VH | Right Na SF | Right K SF |
|---|---|---|---|---|---|
| TSD | Pearson Correlation | -0.045 | 0.704 | -0.050 | 0.763 |
| | P Value | 0.659 | 0.000 | 0.622 | 0.000 |
| | N | 100 | 100 | 100 | 100 |

**Table 4: Correlation among TSD and Left Sided VH and SF Electrolyte Concentrations.**

| | | Left VH Na | Left VH K | Left SF Na | Left SF K |
|---|---|---|---|---|---|
| TSD | Pearson Correlation | -0.61 | 0.737 | -0.062 | 0.797 |
| | P Value | 0.544 | 0.000 | 0.537 | 0.000 |
| | N | 100 | 100 | 100 | 100 |

**Table 5: T Test for the Case Data in which TSD was Less Than 12 Hours**

| Group Statistics | | | | | | |
|---|---|---|---|---|---|---|
| | P Value | side | N | Mean | SD | Std.Error Mean |
| Na Vitreous humor | p<0.05 | Right | 47 | 138.943 | 8.0045 | 1.1676 |
| | | Left | 47 | 139.353 | 8.1104 | 1.1830 |
| K Vitreous humor | p<0.05 | Right | 47 | 6.8638 | 1.74187 | 0.25408 |
| | | Left | 47 | 6.717 | 1.4021 | 0.2045 |
| Na Synovial Fluid | p<0.05 | Right | 47 | 138.764 | 7.1560 | 1.0438 |
| | | Left | 47 | 138.928 | 7.3870 | 1.0775 |
| K Synovial Fluid | p<0.05 | Right | 47 | 6.643 | 1.4822 | 0.2162 |
| | | Left | 47 | 6.662 | 1.6078 | 0.2345 |

**Table 6: T Test for the Case Data in which TSD is Between 12-24 Hours**

| Group Statistics | | | | | | |
|---|---|---|---|---|---|---|
| | **P Value** | **side** | **N** | **Mean** | **SD** | **SE** |
| Na Vitreous humor | p<0.05 | Right | 44 | 143.839 | 7.5220 | 1.1340 |
| | | Left | 44 | 143.902 | 7.5449 | 1.1374 |
| K Vitreous humor | p<0.05 | Right | 44 | 8.9145 | 1.86807 | 0.28162 |
| | | Left | 44 | 8.909 | 1.7979 | 0.2710 |
| Na Synovial Fluid | p<0.05 | Right | 44 | 143.802 | 7.8945 | 1.1901 |
| | | Left | 44 | 144.280 | 7.7030 | 1.1613 |
| K Synovial Fluid | p<0.05 | Right | 44 | 8.139 | 1.0805 | 0.1629 |
| | | Left | 44 | 8.052 | 1.1130 | 0.1678 |

## Discussion

The results demonstrate a strong utility of potassium levels of body fluids in estimating post-mortem interval. The time since death and sex distribution of our cases were like studies by Rama and Amith Mulla.[4,5] Several researchers used VH for identifying poisons and drugs responsible for death of the individuals[6-10]. However, we were successful in only establishing alcohol intoxication using vitreous humor samples. The lack of standardisation of methods used for the purpose could be a reason for not being able to identify other poisonings whereas other researchers achieved this[8]. The Tables 1 and 2 indicate that with increasing post-mortem interval there is a linear rise in potassium levels and fall in sodium levels in both VH and SF. These results are also statistically significant. These results are concordant with previous studies in total.[3] The regression analysis in the study indicate that the values of potassium concentration in VH/SF and post-mortem interval correlate with each other and exhibited statistical strength.

Table 3 displays the Pearson correlation coefficients for potassium concentration in both the right side vitreous and synovial fluid samples. The coefficients for the right side samples were 0.704 and 0.763, indicating a positive correlation between potassium concentration and time since death (TSD). Similarly, Table 4 shows the Pearson correlation coefficients for potassium concentration in the left side vitreous and synovial fluid samples. The coefficients for the left side samples were 0.737 and 0.797, respectively, also indicating a positive correlation between potassium concentration and TSD. These results suggest that the concentration of potassium in both right and left side vitreous and synovial fluids can serve as reliable indicators of TSD. The group statistics in Table 5 and 6 suggest that there are no significant differences in the mean concentrations of Na and K between the right and left side samples of both vitreous humor and synovial fluid.

Vitreous humor and synovial fluid are two body fluids that can be useful in forensic pathology to aid in the determination of the cause and manner of death. Both fluids can provide information about the presence of drugs and alcohol, unknown substances, and the diagnosis of diseases. They can also be used for genomic analysis. Further research could focus on refining the methods for using these fluids to estimate post-mortem interval accurately, detecting specific types of drugs and alcohol, and developing new methods for identifying unknown substances. The potential of these fluids for diagnosing diseases and for genomic analysis could also be explored. There is enough research about VH and SF for post-mortem interval estimation. It is time to explore further areas of research like applying OMICS technologies to cells in these body fluids.[11-15]Studying the thanato-omics of these body fluids can give us some insight into finding thanato-markers for different cause of death in future. The role of vitreous calcium and magnesium levels in estimating time since death were studied and found not to be useful in practice. Hence, potassium concentration remains the important tool even now with some researchers even attempting to go further for age and temperature standardisations.[16-18]

## Conclusion

The potassium concentrations in vitreous humor and synovial fluid are a valuable tool in estimating post-mortem interval and can be taken up for practice as a routine method in our day-to-day medico legal practice. There is also a strong need to ramp up the expertise in using VH and SF for the purpose of chemical analysis in poisoning cases.

**Limitations:**

The study had a small sample size of 100, which may limit the reliability of the results. Larger sample sizes are generally preferred as they increase the precision and generalizability of the findings because of narrow confidence intervals.

**Competing interests:** None to declare

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