

Comparative Study on Post Mortem Analysis of Sodium and Potassium Levels of Vitreous Humour and Synovial Fluid in Determining Time Since Death

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Abstract

Background: Time since death (TSD) is the period between the death and time of examination of a body. Numerous studies conducted on analysis of potassium in vitreous humour have shown that the vitreous potassium levels increase with rise in TSD. Studies done on postmortem biochemistry of synovial fluid have proposed a positive linear relationship between potassium and TSD. But, very few authors have compared findings of these two fluids. So, this study was undertaken to compare the accuracy of determining post-mortem interval from Sodium and Potassium levels of vitreous humour and synovial fluid.

Methods: A total of 140 cases were taken with certified time of death. These were divided into 7 groups i.e. 0-6 hours, 6-12 hours, 12-18 hours, 18-24 hours, 24-30 hours, 30-36 hours and above 36 hours. Vitreous humour and Synovial fluid from knee joint were aspirated from both sides of body and were analyzed using Medica Easylyte Na/K Analyser. Results were obtained using SPSS 24 software.

Conclusions: Potassium has a strong positive correlation with TSD in both fluids. Sodium has weak negative correlation. There is no difference between values of both sides. There is no statistically significant difference between values of Vitreous humour and Synovial Fluid. Hence, both can be used alternatively.

Keywords: Potassium; Synovial Fluid; Thanatochemistry; Time since death; Vitreous Humour.

Introduction

Time since death (TSD) is the period between the death and time of examination of a body. One of the most important objectives of post mortem examination is determination of time since death (TSD), also known as post mortem interval.¹ It is important to know when the crime was committed and helps in establishing the corpus delicti of a crime. Forensic experts being an 'expert witness'

have a great responsibility as their opinion guides the course of investigation and judicial proceedings. Thanatology is the scientific study of death in all aspects. Changes in body after death are traditionally divided into immediate, early and late changes.¹ These are helpful in estimation of TSD. The changes like cooling of body (algor mortis), eye changes, post-mortem staining (livor mortis), stiffening of body (rigor mortis), condition of stomach contents,

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bladder and bowel contents, decomposition changes and circumstantial evidence can sometimes yield reasonable results.¹ But, the estimates of post mortem interval using above changes are variable because of various internal bodily and external environmental factors and also prone to observer bias. Hence, Thanatochemistry has gained importance. It is described as the changes that occur in the chemical composition of body fluids such as blood, vitreous humour, synovial fluid and cerebrospinal fluid immediately after death.²

Blood and body fluids like cerebrospinal fluid were commonly used but since 1960's other compartmental body fluids like synovial fluid and vitreous humour are being investigated upon as they are topographically isolated and more protected.³

Intracellular concentration of potassium is 2-40 times higher than that in plasma.² After death, as the pumping mechanism of cell becomes inactive and cell wall becomes semi permeable, potassium leaks through it to reach equilibrium.⁴ Vitreous fluid is a fragile, transparent, inert, hydrogel that is present between the lens and retina and occupies 80% of eye's volume.⁵

The underpinning principle in analysing vitreous humour is that it is a closed compartment which is separated from the rest of the body. The composition of the vitreous is closely related to that of serum, aqueous humour and cerebrospinal fluid.⁶ Numerous studies have been conducted on biochemical analysis of vitreous humour. These studies have shown that the vitreous potassium level increases with increase in postmortem interval.^{2,6,7-11}

Similarly, synovial fluid is also isolated and protected from injury and decomposition. Synovial fluid can be analysed in cases of severe trauma to eye where the integrity of the eye globe is lost. It is also available in ample amounts as there are multiple sites of sample collection. Studies done on postmortem biochemistry of synovial fluid have proposed a positive linear relationship between potassium and TSD.^{2,4,12-14} The most commonly studied stable biochemical markers which can be analysed using simple techniques and minimal laboratory setup accessible for universal use have not been studied by all authors. Very few authors have pursued the comparison of these two fluids. So, this study

had been undertaken to compare the accuracy of determining post-mortem interval from Sodium and Potassium levels of vitreous humour and synovial fluid.

Material and Methods

This study was conducted at Mortuary of Department of Forensic Medicine in association with Central laboratory of the Department of Biochemistry, SMS Medical College, Jaipur. The study samples were collected between December, 2020 and August, 2021. Only cases with confirmed time of death were included in the study. The cases were recruited for the study only after obtaining consent and procedure of sample collection was done at the time of post-mortem examination. Inadequate samples and turbid or blood mixed samples were discarded and fresh case was allocated to compensate for such cases.

Inclusion criteria:

1. All the cases admitted in hospital and whose exact time of death is known and certified by treating physician.
2. All the cases without any major metabolic derangement prior to death.
3. All the cases in which guardians give consent for study.

Exclusion criteria:

1. Cases with previous history of eye or orbital injury or surgery, posterior segment diseases.
2. Cases with previous history of joint diseases, previous injury or surgery.
3. All brought dead cases at SMS Hospital emergency.

Cases were selected and organized according to known time since death in 7 groups namely between 0-6 hours, 6-12 hours, 12-18 hours, 18-24 hours, 24-30 hours, 30-36 hours and above 36 hours. 20 cases were included in each sub-group. Samples were collected from both sides of vitreous and synovial cavity.

Collection of sample of Vitreous Humour:

Vitreous humour was collected from the posterior chamber by aspirating gradually through a puncture 5-6 mm away from the limbus using a sterile 20 gauge

hypodermic needle. After sample collection, water was injected to restore cosmetic appearance.

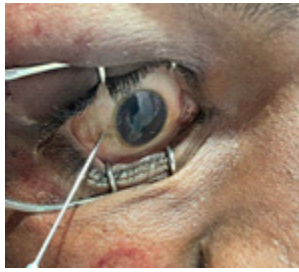


Image 1: Correlation between TSD and K⁺ levels of Right Vitreous humour

Collection of sample of Synovial fluid:

The synovial fluid was aspirated using a sterilized 16 gauge needle by puncturing the suprapatellar pouch from lateral side, just below the patella pushing it directly backwards.



Image 2: Correlation between TSD and K⁺ levels of Right Synovial Fluid

Analysis was done within 1 hour after the synovial fluid and vitreous humour were aspirated on working days. All sample fluids were centrifuged at 3500 r.p.m. for 10 min and then the supernatant was used for analysis. On non-working days or emergency hours, the samples were centrifuged and

supernatant was stored at 4⁰C and subsequently analyzed. The samples were analyzed for sodium and potassium levels in the Department of Biochemistry section of Central Laboratory on Easylyte Plus Na/K Analyser by Ion selective method.

Prior to initiation of study, pilot samples (ten each) of vitreous humour and synovial fluid were analysed to assess efficacy of working with the assumption that being clear fluids, they could be analysed similar to serum.

Collected data was entered into Microsoft excel data sheet which was tabulated for data interpretation. This was then subjected to statistical analysis using with appropriate statistical tests (Linear regression, ANOVA and T Test) and results were obtained. Statistical analysis was performed using SPSS software 24th version.

Results

20 cases were included in each of the seven sub-groups, making a total of 140 cases in which each sub-group contributed 14.28% of cases of total cases included in the study. Cases included in the present study ranged from minimum two hours and maximum one hundred fourteen hours post mortem interval.

Amongst the study subjects, there were 36 females (25.71%) and 104 males (74.29%). There were 20, 68, 41, 9 and 2 cases in age groups of less than 20 years, 20 to 40 years, 40 to 60 years, 60 to 80 years and above 80 years respectively.

According to the cause of death, there were 63 cases (45%) of mechanical injuries, 13 cases (9.29%) of thermal injuries, 17 cases (12.14%) of asphyxia, 26 cases (18.57%) of poisoning and 21 cases (15%) of deaths due to pathology as revealed on autopsy.

Table 1: Descriptive Statistics TSD and K⁺ levels of Right Vitreous humour

Descriptive Statistics			
	Mean	Std. Deviation	N
Time Since Death (in hours)	24.26	18.658	140
Vitreous Humor (R) Potassium (mEq/L)	9.365	3.0450	140

Table 2: Correlation between TSD and K⁺ levels of Right Vitreous humour

		Time Since Death (in hours)	Vitreous Humor (R) Potassium (mEq/L)
Time Since Death (in hours)	Pearson Correlation	1	.823**
	Sig. (2-tailed)		.000
	N	140	140
Vitreous Humor (R) Potassium (mEq/L)	Pearson Correlation	.823**	1
	Sig. (2-tailed)	.000	
	N	140	140

As per table 2, Correlation is significant at the 0.01 level (2-tailed). R value of .823 indicates that there is strong positive correlation between two variables. It concludes that there is increase in levels of potassium concentration of vitreous humour of right eye as the post mortem interval increases. R² value of .678 indicated that 67.8% of total variation in Time Since Death (in hours) could be explained by potassium levels of right vitreous humour.

Similarly, Descriptive statistics, Pearson Correlation coefficient, Regression results were obtained for Sodium and Potassium levels of Vitreous Humour and Synovial Fluid of both sides. Graphical representation of correlation of K levels with TSD of right side are shown in figure 1-2. Left side graphs were also prepared.

Table 3: R values of Na⁺ and K⁺ levels of Right and Left vitreous humour and synovial fluid

	Group	R value
Vitreous Na ⁺	Right	-.343
	Left	-.448
Vitreous K ⁺	Right	.823
	Left	.827
Synovial Na ⁺	Right	-.519
	Left	-.518
Synovial K ⁺	Right	.863
	Left	.867

As per table 3, we found that potassium has strong positive correlation with TSD in both vitreous humour and synovial fluid whereas sodium had weak negative correlation with TSD.

Further, on application of T test for comparison between right and left sides, P value was >0.05. Hence, it is concluded that there is no difference among levels of sodium and potassium in left and right side of vitreous and synovial fluid.

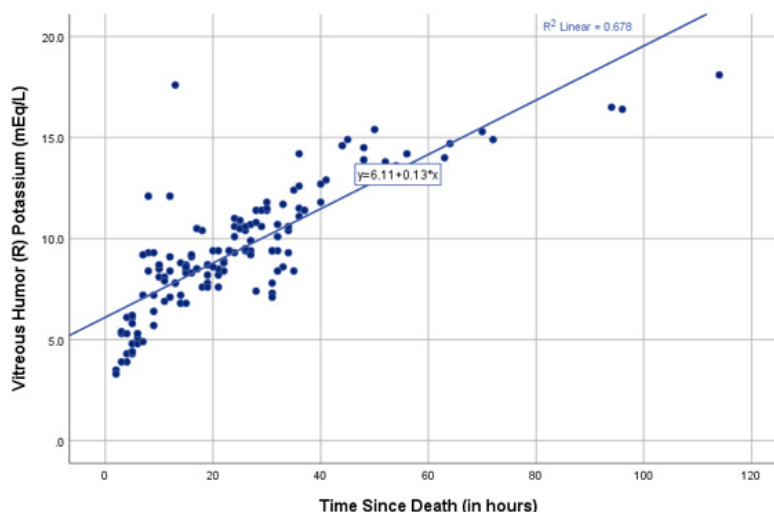


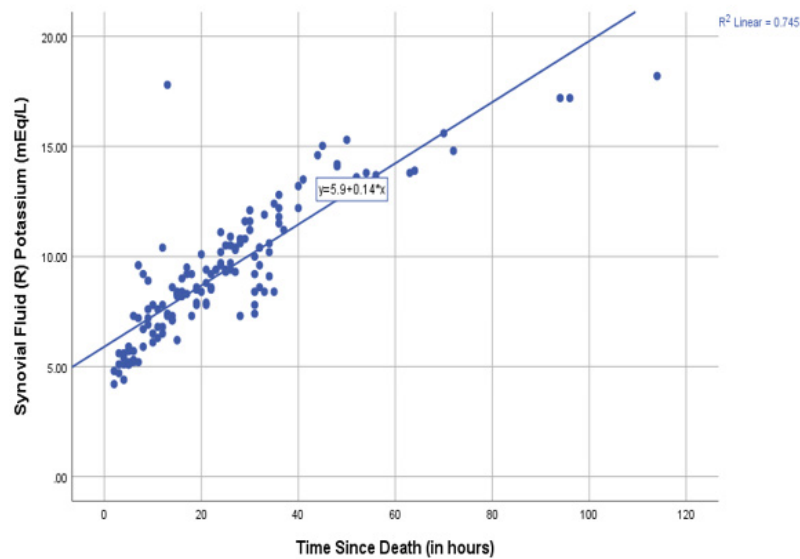
Figure 1: Correlation between TSD and K⁺ levels of Right Vitreous humour

Table 4: T- test for comparison between vitreous humour and synovial fluid

Group Statistics	Group	N	Mean	Std. Deviation	t value	p value
Right	Vitreous Na ⁺	140	140.6	12.65	-0.700	-0.484
	Synovial Na ⁺	140	141.44	6.48		
	Vitreous K ⁺	140	9.37	3.04	0.26	0.796
	Synovial K ⁺	140	9.27	3.00		
Left	Vitreous Na ⁺	140	141.5	6.66	0.05	0.935
	Synovial Na ⁺	140	141.43	6.45		
	Vitreous K ⁺	140	9.34	3.04	0.14	0.885
	Synovial K ⁺	140	9.28	2.99		

As per Table 4, P value is >0.05 when vitreous and synovial fluid are compared. The result is not significant at $p < 0.05$. Hence, it is concluded that there is no difference among levels of sodium and

potassium in vitreous and synovial fluid i.e. while determining time of death, synovial fluid reveals as accurate results as that in vitreous humour or vice versa.

Figure 2: Correlation between TSD and K⁺ levels of Right Synovial Fluid

Discussion

The magnitude of study population of the present study was quite comparable to most other studies, rather higher than most of them. Other studies were conducted on 154 cases²; 100 cases⁶ and 35 cases.¹⁵

The present study classified the subject collection in seven groups and included twenty cases in each sub-group, contributing to 14.28% cases in each group. One study² included five groups of 0-6 hours, 6-12 hours, 12-18 hours, 18-24 hours and 24-36 hours. Other study⁶ had divided into three groups of less than 12 hours, 12-24 hours and more than 24 hours.

The time since death of our study subjects ranged from 02 hours to 114 hours which is much wider than other similar studies - 1.45 hours to 35.18 hours² and 2.17 hours to 7.92 hours¹⁵; but similar to that done in Chennai.(1 to 102 hours).⁶

The cadavers were from various age groups. The youngest subject was 3 years of age and oldest 85 years. There were 104 males (74.29%) and 36 females (25.71%) in the present study. Age and gender of cadavers included in various studies has not been specified in most other studies. The mean age of study subjects in the present study was 37.17±16.19 years.

The present study included 119 cases of unnatural deaths and 21 cases of natural deaths. One study done at Germany¹¹ included 42 cases of sudden natural deaths and 32 cases of sudden unnatural deaths.

In the present study, statistical analysis for electrolyte concentration in relation to time since death was studied using cumulative means of their concentrations which has also been done similarly by other study.¹¹

The present study revealed a statistically significant linear correlation of potassium levels of both vitreous humour and synovial fluid with time since death which is also reported by other authors.^{2,6,10,11,15}

There is no difference between values of right and left side. Similar inference was drawn by other authors^{6,7,15} who analyzed both right and left side samples.

From the present study, it is concluded that vitreous humour, well documented as a reliable fluid to study postmortem biochemistry can be replaced with synovial fluid and vice versa. Thus synovial fluid can be used as an alternative to vitreous humour which has also been concluded by other authors.^{6,11,15} However, the conclusion is variable to other study² which concluded that synovial fluid potassium values can be more confirmatory than vitreous humour potassium for determination of time since death.

Conclusions

There is a statistically significant linear correlation of potassium levels of both vitreous humour and synovial fluid with time since death. The samples from right and left eyes and knee joints did not reveal a significant difference in these two electrolyte biochemistry results, which implies that any side samples may be used. Synovial fluid and vitreous humour samples are equally reliable for analysis of post mortem potassium electrolyte concentrations in relation to post-mortem interval and can be used an alternate to each other.

Conflict of Interest: None

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Ethical Clearance: Taken

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