

Estimation of time since death from potassium ion concentration in vitreous humour

¹Dr. Pallavi P. Kadwe(Chintalwar), ²Dr. Shreya Nigoskar,

³Dr. Rajesh Chintalwar

1Medical biochemistry Phd student Index Medical College Indore

2Professor and Head Index Medical College Indore

3Associate professor DMMC Nagpur

Corresponding Author

Dr. Rajesh Chintalwar

Associate professor DMMC Nagpur

Abstract: Introduction: Time since death (TSD) also called, as post-mortem interval is an important element in investigation of crime and court trial. The large volume, easy accessibility, resistance to bacterial degradation and relatively inert nature makes the vitreous humour to be the most suitable for chemical analysis. Lactic acid, hypoxanthine and potassium are the common parameters studied in vitreous humour. We aimed to study the change in concentration of potassium in the vitreous humour for estimation of time since death. **Material and Methods:** This is a cross-sectional, hospital based study conducted in the Index medical college and hospital, Malwanchal University, Indore, from April 2019 to August 2023. All deceased cases with known time of death which were brought to mortuary were included in the current study. Body with previous ocular injury or surgery, bodies with extreme decomposition, posterior segment diseases and paediatric cases <less than 12 years of age were excluded. A total of 120 cases could be included. The collected data was tabulated in MS excel 2020 and analysed with SPSS software, version 20. **Results:** Mean age of deceased cases was observed 38.71 ± 23.24 years with range from 13-77 years. Male: Female ratio in this study was 2.53:1. The most important cause of death among them was found to be 'unclassified death' which was followed by trauma. Mean time since death calculated from potassium levels in vitreous humour was found to be 12.7 ± 7.62 hours and that from police or hospital records was 12.0 ± 8.10 hours and the difference between two was non-significant. Also, a positive significant correlation was found between TSD using potassium level and TSD as per police or hospital records. In this study, a linear relation was found between potassium level and time since death. **Conclusion:** our study concludes that

biochemistry of vitreous humour is important in post-mortem analysis and measuring potassium level in vitreous humour is an important tool to determine time since death.

Keywords: Time since death, Potassium, Vitreous humour, Post-mortem interval

Introduction: Death is inevitable in human being and it cannot be predicted. Time since death also called as post-mortem interval (PMI) is the time period between death of a person and its post-mortem examination. Any death which is not due to an underlying disease condition can be thought to be an unnatural death which is actually a combination of multiple factors and estimating time since death very difficult in these cases.[1, 2] A forensic expert has to help the investigating agencies regarding cause of death and time since death. [3] In autopsy, cause of death and time since death (TSD) can be studied by many methods. Its an important element in court's proceedings as it helps in investigating suicidal, homicidal and sudden unnatural death cases.[4] It helps the investigating officer to reach the person who are thought to be accountable for the criminal activity and leave the innocent people.[5] In civil cases, it helps in property dispute as well. [6]

The exact evaluation of time since death is a challenging and difficult task. Time since death depends upon many factors like presence of underlying disease condition, humidity & temperature of the environment and thermodynamics of end terminal action etc. In these situations, chemical evaluation of different body fluids helps a lot in determination of TSD. [7, 8] Many researches have been done to evaluate the time since death through a range of physiological, metabolic & biochemical alterations from different fluids of body like CSF (cerebrospinal fluid), blood and VH (vitreous humour). [9] As described by Sturner in 1963, post-mortem vitreous humour is the more apt fluid for biochemical examination than CSF & blood due to its resistance to bacterial degradation and microbial contamination and its presence in physically protected environment.[10, 11] That's why it is comparatively inert and not affected by abrupt fluctuation in chemistry of blood. The other reasons are its large volume and it is easily obtainable.

The common parameters studied in vitreous humour are lactic acid, hypoxanthine and potassium. [12, 13] After death, the concentration of potassium increases in the vitreous humour due to retinal cells and vascular choroidal autolysis. [13] Considering this we aimed to study the change in level of K (potassium) in vitreous humour for estimation of time since death.

Material and Methods: This is an observational, hospital based, cross-sectional study done in the biochemistry department in co-ordination with forensic medicine department of Index medical college and hospital, Malwanchal University, Indore, from April 2019 to August 2023. Ethical clearance was obtained from institutional ethics committee. All deaths which were brought to mortuary with known time of death were part of the study. Death in the hospitals and deaths outside, both were included. Body with previous ocular injury or surgery, bodies with extreme decomposition, posterior segment diseases and paediatric cases <less than 12 years of age were excluded. A total of 120 cases could be included in the said study period on the basis of convenience sampling method. Vitreous humour sample was collected from posterior chamber of right eye during autopsy using 10ml syringe with 20G needle. Procedure was done slowly to avoid injury of loose fragments of tissues by a puncture done 5-6mm from the limbus and sample was collected in a rubber stopper vial and immediately sent to laboratory for estimation of vitreous K concentration by automated electrolyte analyzer (Siemens ADVIA1800).

Exact death time was recorded from record of police, record of hospital, family, friends, relatives or known of the deceased. Relevant informations of the dead i.e. age, residential address, gender, death reason, time of death, humidity, temperature, time of each sampling and related K levels were completely noted in the proforma. Sturner and Gantner's formula (1964) [14] was applied to calculate time since death as follows:

“TSD (hrs) = 7.14 K⁺ (mEq/L) - 39.1”, K⁺ is vitreous humour K level.

The collected data was entered in MS excel 2020 and analysed using SPSS software, version 20. “Continuous variables were used as mean±SD” while comparative data were used as ratio & proportions. Unpaired t- test was done to see if there is any difference in time calculated from potassium level in vitreous humour and that from police or hospital record.

Spearmann's correlation was applied to see the correlation between TSD calculated from body signs, police records and that from potassium levels. For Spearmann's correlation “p-value <0.01 was considered to be statistically significant”.

Results:

Our research comprised a total of 120 deceased cases and potassium level in the vitreous humour of them was studied to determine post mortem interval. Table 1 illustrates the basic characters of the cases. Mean age of cases was 38.71±23.24years ranging from 13-77years.

Out of total 120 cases, 31 (25.8%) fall in 13-30years age group, 68 (56.7%) were in 31-60 years age group and remaining 21 (17.5%) were >60 years of age. Males constituted 71.6% of the study population and females were 28.4% with Male: Female ratio of 2.53:1. Of all the deceased cases, 71 (59.2%) were from the rural area and remaining 49 (40.8%) were from urban area. The most communal reason of death among them was found to be unspecified death affecting 62 cases (51.7%) followed by trauma including road traffic accident (RTA) affecting 37 cases (30%). Asphyxia including hanging and strangulation constituted 11 cases (9.2%) and poisoning was the cause of death in 10 deceased (8.3%).

Table 1: Basic parameters of the deceased subjects

Parameters	Category	Frequency	Percentage
Age	13-30	31	25.8
	31-60	68	56.7
	>60	21	17.5
Gender	Male	86	71.6
	Female	34	28.4
Address	Rural	71	59.2
	Urban	49	40.8
Cause of death	Poisoning	10	8.3
	Trauma	37	30.8
	Asphyxia	11	9.2
	Unclassified	62	51.7

As can be seen in table 2, the mean time since death calculated from potassium levels in vitreous humour was found to be 12.7±7.62 hours with median 12.0 hours and interquartile range (IQR) 7.2-20.4 hours. As per police or hospital records the mean time since death was 12.0±8.10 hours with median value 13.3 hours and IQR 6.5-19.6 hours. The difference in the mean time since death between the two groups was observed as non-significant (p-value 0.491).

Table 2: Data of Time since death using potassium level and as per police/hospital records

Parameters	TSD using potassium value (in hours)	TSD using police/hospital records (in hours)	p-value
Mean ±SD	12.7±7.62	12.0±8.10	0.491
Median	14.0	13.3	
IQR (Interquartile range)	7.2-20.4	6.5-19.6	

Spearman’s correlation was applied to see the correlation between TSD using potassium level and TSD as per police or hospital records. A positive significant correlation was found between two with p-value<0.1 and correlation co-efficient 0.937. So we can state that evaluation of TSD by K level in vitreous humour in deceased cases is a very good method as no significant difference was found between mean TSD calculated by potassium in vitreous humour and that from police/hospital records.

Table 3: Comparison of TSD values estimated using potassium in the vitreous humour & Police records

Spearman’s correlation		TSD using potassium value	TSD using police/hospital records
TSD using potassium value	Correlation-coefficient	1.000	0.937
	Significance (two-tailed)	-	0.000
	Number	120	120
TSD using police records	Correlation-coefficient	0.937	1.000
	Significance (two-tailed)	0.000	-
	Number	120	120

Discussion: One of the essential components in all autopsy cases is to calculate time since death as it has great values in criminal investigation and court trial. Vitreous humour is a fluid in the body which is well protected from bacterial contamination and is somewhat inert and not changed by sudden alteration in blood chemistry. This makes vitreous humour an ideal fluid for estimation of post-mortem chemicals. The levels of chemicals in VH of eye change over time after death, which help investigating officer to calculate time since death. [15, 16] Many of the studies have documented the use of vitreous humour potassium level to determine time since death. In the present study we studied potassium levels in vitreous humour of 120 deceased cases to know the time since death. In current research, the mean age of deceased was noted as 38.71±23.24 years with age range of 13-78 years. Study by Prince M. Paul found slightly higher mean age i.e. 40.27±17.91 years with age range 3-80 years. [17] Sparks et al (1989) also took cases with age range of 1 to 84 years in their study. [18] In our study we excluded deceased cases aged ≤ 12 years accounting the less amount of VH fluid in them. In this study, out of 120 deceased cases 86 (71.6%) were males while 34 (28.4%) were females. Amith Mulla et al. (2005) did study with 613 subjects with male to female ratio of 3:1. [19]

We found time since death calculated by K concentration in VH to be comparable with the time since death from police or medical records. There was no any significant difference

between two (p-value=0.491). In current research, a linear relation was seen among potassium range and time since death. The potassium concentration was seen to be increased as time since death increases. Spear mann's correlation was observed as significant with Correlation coefficient 0.937 & p-value <0.001. This was supported by studies of Jashnani et al., Cordeiro et al., Madea et al. and Farmer et al. [20, 21, 22, 23] In contrast to above study Singh D et al found that the relationship of increasing level of vitreous potassium with increasing PMI was not linear but it was logarithmic.[24]

Other studies depicted use of other electrolytes for determination of time since death. James et al. showed that hypoxanthine in the vitreous humour can also be used to calculate time since death while Cordeiro et al. recommended use of urea and hypoxanthine in vitreous humour to calculate time since death. [25, 21]

In our study age, sex and cause of death was not found to be having effect on vitreous humour potassium which is in accordance with Jashnani et al. along with Ahi & Garg, who said that other factors like age of deceased and gender had no bearing on the K concentration in the VH. [20, 26]

Conclusion: Mean time since death in the current study was 12.7 ± 7.62 hours. Our study showed that vitreous potassium level is a sensitive indicator of time since death. There is a linear correlation between potassium level in vitreous humour and time since death. Also, time since death calculated by vitreous humour potassium level and actual time since death as per police or medical record are correlated significantly. So over all we can conclude that biochemistry of vitreous humour is important in post-mortem analysis and measuring potassium level in vitreous humour is an important tool to determine time since death.

Source of funding: NIL

Conflict of interest: NIL

References:

1. Gautam G: Review of Forensic Medicine and Toxicology (Including Clinical & Pathological Aspects) . Jaypee Brothers Medical Publisher (P) Ltd., New Delhi, India; 2015.
2. Ignatius PC: Forensic Medicine and Toxicology . Elsevier, India; 2018.
3. Awan NR. Principles of Forensic Medicine and Toxicology. Lahore, Pakistan 2004
4. Aase S: Autopsy--still the gold standard?. Tidsskr Nor Laegeforen. 2013, 133:730-1. 10.4045/tidsskr.13.0293

5. Parikh CK. Parikh's Text Book of Medical Jurisprudence, Forensic Medicine and Toxicology. 6th ed. Delhi, India: CBS Publishers and Distributors; 2000. p.1.
6. Vij K. Textbook of Forensic Medicine and Toxicology. 2nd ed. New Delhi, India: BI Churchill Livingstone Pvt Ltd; 2002. p. 144.
7. Madea B: Forensic Medicine: Findings, Reconstruction, Assessment (Book in German). Springer Nature, Switzerland; 2015.
<https://link.springer.com/book/10.1007/978-3-662-43500-7>
8. Dell'Aquila M, De Matteis A, Scatena A, Costantino A, Camporeale MC, De Filippis A: Estimation of the time of death: where we are now?. Clin Ter. 2021, 172:109-12. 10.7417/CT.2021.2294
9. Bévalot, F., Cartiser, N., Bottinelli, C., Fanton, L. & Guitton, J. Vitreous humor analysis for the detection of xenobiotics in forensic toxicology: a review. Forensic toxicology 34, 12–40, doi:10.1007/s11419-015-0294-5 (2015).
10. Sturner, W. Q. The vitreous humour: postmortem potassium changes. Lancet 1, 807–808, doi:10.1016/S0140-6736(63)91509-5 (1963).
11. Mihailovic, Z., Atanasijevic, T., Popovic, V. & Milosevic, M. B. Could lactates in vitreous humour be used to estimate the time since death? Medicine Science & the Law 51, 156–160 (2011).
12. Zilg B, Alkass K, Kronstrand R, Berg S, Druid H: A rapid method for postmortem vitreous chemistry/deadside analysis. Biomolecules. 2021, 12:32. 10.3390/biom12010032
13. Go A, Shim G, Park J, Hwang J, Nam M, Jeong H, Chung H: Analysis of hypoxanthine and lactic acid levels in vitreous humor for the estimation of post-mortem interval (PMI) using LC-MS/MS. Forensic Sci Int. 2019, 299:135-41. 10.1016/j.forsciint.2019.03.024
14. Sturner WQ, Gantner GE: The postmortem interval: a study of potassium in the vitreous humour. Am J Clin Pathol. 1964, 42:137-44. 10.1093/ajcp/42.2.137
15. Chen YQ, Cai JF, Wen JF. Advances in the studies of postmortem interval estimation by the levels of chemical components in human vitreous humor after death. Fa Yi Xue Za Zhi. 2009;25(1):53-6.
16. Pounder DJ, Carson DO, Johnston K, Orihara Y. Electrolyte concentration differences between left and right vitreous humor samples. J Forensic Sci. 1998 May;43(3):604-7. PMID: 9608696.
17. Paul, Prince & Sneha, S. & Pradhan, Priyadarshini & Kumar, P.. (2021). Estimation of post-mortem interval from vitreous potassium: An autopsy based study. Journal of Indian Academy of Forensic Medicine. 43. 370-373. 10.5958/0974-0848.2021.00094.4.
18. Sparks DL, Oeltgen PR, Kryscio RJ and Hunsaker JC. Comparison of chemical methods for determining postmortem interval. J Forensic Sci. 1989;34(1):197-206.
19. Amith Mulla, et al. Role Of Vitreous Humor Biochemistry In Forensic Pathology-July 2005, University of Saskatchewan
20. Jashnani KD, Kale SA, Rupani AB: Vitreous humor: biochemical constituents in estimation of postmortem interval. J Forensic Sci. 2010, 55:1523-7. 10.1111/j.1556-4029.2010.01501.x
21. Cordeiro C, Ordóñez-Mayán L, Lendoiro E, Febrero-Bande M, Vieira DN, Muñoz-Barús JI: A reliable method for estimating the postmortem interval from the

- biochemistry of the vitreous humor, temperature and body weight. *Forensic Sci Int.* 2019, 295:157-68. 10.1016/j.forsciint.2018.12.007
22. Madea B, Henssge C, Hönig W, Gerbracht A: References for determining the time of death by potassium in vitreous humor. *Forensic Sci Int.* 1989, 40:231-43. 10.1016/0379-0738(89)90181-3
23. Farmer JG, Benomran F, Watson AA, Harland WA: Magnesium, potassium, sodium and calcium in postmortem vitreous humour from humans. *Forensic Sci Int.* 1985, 27:1-13. 10.1016/0379-0738(85)90099-4
24. Singh D, Prasad R, Sharma SK, Pandey AN. Double logarithmic: Linear relationship between postmortem vitreous sodium/potassium electrolytes concentration ratio and time since death in subjects of Chandigarh zone of North West India. *Journal of Indian Academy of Forensic Medicine.* 2005;27(3):159-68.
25. James RA, Hoadley PA, Sampson BG: Determination of postmortem interval by sampling vitreous humour. *Am J Forensic Med Pathol.* 1997, 18:158-62. 10.1097/00000433-199706000-00010
26. Ahi RS, Garg V: Role of vitreous potassium level in estimating postmortem interval and the factors affecting it. *J Clin Diagn Res.* 2011, 5:13-5.