



Estimation of Post-Mortem Interval from the Changes in Vitreous Humour Potassium (K⁺) and Sodium (Na⁺) Level

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Abstract

Introduction: The accurate estimation of time since death is of great value in medico-legal investigations of serious crimes. It was reported by several workers that measurement of the levels of potassium in the vitreous humour had a critical role in accurate prediction of time since death (within two hours). The present study was aimed to know the relation between sodium and potassium levels of vitreous humour and time since death.

Materials and Methods: A hospital based cross sectional observational study was carried out on 200 medico-legal cases at a tertiary care teaching hospital in western Rajasthan from 1st January 2016 to 31st December 2016. Vitreous fluid was collected from posterior chamber of eye by needle aspiration and detailed about the deceased i.e. age, sex, cause of death, exact time of death, time of sampling and corresponding Na⁺, and K⁺ level was recorded in a predesigned proforma.

Results: The present study showed linear correlation between rise in vitreous potassium (K⁺) ion concentration with increasing post-mortem interval ($r=0.831$). Rate of rise of vitreous potassium concentration was 0.29 meq/l/hr (Coefficient of regression=3.46 meq/l/hr). Sodium ion concentration in vitreous humour shows decline with increasing post-mortem interval but this decrease was statistically not significant ($P>0.05$).

Conclusion: Vitreous potassium (K⁺) ion concentration had a linear correlation with time since death. So, it can be used where estimation of time since death is difficult to know by other means in medico-legal cases.

Keywords: Medico-legal cases, post-mortem interval, vitreous humour, sodium and potassium level.

Introduction

The interval between death and the time of post-mortem examination is called 'post-mortem interval' which is very important in criminal cases as it helps the investigators to track the suspected person and to save the innocent ones. An approximate estimate of post-mortem interval can be estimated by routine methods like. cooling of

body, post-mortem staining, rigor mortis, changes in eye, decomposition changes, contents of stomach and bowels, contents of urinary bladder and circumstantial evidences.^[1]

Various body fluids like blood, spinal fluid, aqueous humour and vitreous humour of eye show chemical changes immediately or shortly after death. These changes progress in a fairly orderly

fashion until the body disintegrates. Each change has its own time factor or rate. Thus determination of these chemical changes could help the forensic pathologists to ascertain time since death more precisely.^[2]

Although no single measurement gives a completely reliable estimate of the post-mortem interval, combinations of chemical determinations can be useful adjuncts in cases of un-witness death.^[3] Amongst these vitreous humour of eye is relatively stable, less susceptible than other body fluids to rapid chemical changes and contamination, easily accessible and its composition is quite similar to that of aqueous fluid, cerebrospinal fluid and serum; thus it is suitable for many analyses to estimate post-mortem interval.^[4]

The determinations that may prove valuable include the potassium, sodium, ascorbic acid, non-protein nitrogen, lactic acid and chloride and magnesium, phosphate and bicarbonate content of the vitreous humour. Although no single measurement gives a completely reliable estimate of the post-mortem interval, combinations of chemical determinations can be useful adjuncts in cases of un-witness death.^[3,5-6]

Materials and Methods

It was a hospital based, cross sectional, observational study, carried out at Mahatma Gandhi and Mathura Das Mathur Hospitals, associated with Dr. S. N. Medical College, Jodhpur (Rajasthan) from 1st January 2016 to 31st December 2016. After taking approval from institutional ethics committee, all the medico-legal autopsies reported to forensic medicine department were included in the study after taking consent from first degree relatives. Cases where the time of death was unknown, body was in advanced stage of decomposition, case of ocular disorder, extracted sample become spoiled or amount of aspirated vitreous humour was less than 0.5 ml was excluded from the study.

Relevant information about the deceased i.e. age, sex, cause of death, exact time of death, time of

sampling and corresponding Na⁺ and K⁺ level was recorded in a pre-designed, pre-tested proforma. Vitreous fluid was collected from posterior chamber of eye by needle aspiration through a puncture made 5-6 mm away from limbus using a 10 ml sterile syringe and 20 gauge needle. Rubber stopper glass vials washed with deionized distilled water and dried in hot air oven was used for sampling. Gelatins or water was placed in posterior chamber for cosmetic purpose. The sample taken was immediately sent to Department of Biochemistry Dr. S. N. Medical College, Jodhpur for biochemistry using electrolyte analyser.

Results

200 cases died in hospital and brought to the mortuary were selected for estimation of K⁺ & Na⁺ in vitreous humour of both right & left eyes. Out of these 127 (63.5%) were males and 73 (36.5%) were females with male to female ratio of 1.739:1. The age range was 8 years to 90 years. Majority of cases were in age group 21-30 years (42.5%) of life followed by age group 31-40 years (19.5%), age group 11-20 years (17.50%), age group 41-50 years (11.00%) and age group > 51 years (7.50%). Figure-1 shows the 4 broad groups divided according to cause of death; trauma (43%), poisoning (28%), Burn (26%) and natural death (3%). The overall range of time since death was 0 to 72 hours. Maximum number of cases were in the range between 12.1 to 24 hours (37.50%) followed by 6.1 to 12 hours (29.50%), 3.1 to 6 hours (19.5%) and 1.1 to 3 hours (9.5%). The 86.5% studied cases were in range of 3.1 to 24 hrs.

The overall range of vitreous potassium ion concentration was 3.1 to 24 meq/l. Table-1 shows that there was an increase in level of vitreous potassium (K⁺) as the Post-mortem interval advances. Coefficient of Correlation was calculated using INDOSTAT software. The data showed that the coefficient of correlation for potassium (K⁺) ion concentration in the vitreous humour was 0.831. This indicates that there was

high degree of correlation between post-mortem interval and potassium (K^+) ion concentration of vitreous humour. Therefore post-mortem interval can be calculated, if vitreous potassium (K^+) ion concentration is known. The value of coefficient of regression was 3.46 meq/l/hr. It appears that 1meq/l potassium (K^+) ion concentration of vitreous increases in 3.46 hours of post-mortem interval.

Table-2 shows mean potassium ion concentration in vitreous humour of right & left eye in relation to various causes of death. The results indicate that there was no statistically significant association of vitreous potassium ion concentration in right & left eye in relation to various causes of death.

No significant correlation between vitreous sodium ion concentrations with increasing post-

mortem interval was observed (Table-3). The linear correlation of the vitreous sodium (Na^+) ion concentration was found statistically insignificant ($r=0.045$) therefore the coefficient of regression could not be derived. Table-4 shows mean sodium ion concentration in vitreous humour of right & left eye in relation to various causes of death. The results indicate that there was no statistically significant association of vitreous sodium ion concentration in right & left eye in relation to various causes of death.

The regression equation for each variable was established as under:

- Post-mortem interval = $-16.22 + 3.75 \times K^+$
- Post-mortem interval = $165.70 + 1.15 \times Na^+$

Where:

K^+ = Potassium ion concentration in Vitreous

Na^+ = Sodium ion concentration in Vitreous

Table-1: Vitreous potassium (K^+) ion concentration vis-a-vis cause of death

Cause of death	Trauma	Burn	Poisoning	Natural death
No. of eyes	172	106	112	10
Time since death (in hours)	K^+ concentration in meq/l			
0-1	4.10	-	-	-
1.1-3	4.30	4.40	4.20	-
3.1-6	5.40	5.70	4.90	-
6.1-12	7.10	7.00	7.10	7.30
12.1-24	8.50	8.80	8.40	8.10
24.1-36	11.90	12.20	-	-
36.1-48	-	13.90	-	14.30
48.1-72	16.80	-	16.60	-

($r=0.831$, Coefficient of regression = 3.46 meq/l/hr)

Table-2: Vitreous potassium (K^+) ions concentration in right and left eye vis-a-vis cause of death

S. No.	Cause of death	Mean+SD		Range	
		Right	Left	Right	Left
1.	Trauma	7.0±2.02	7.0±2.06	3.9-16.4	3.8-17.1
2.	Burn	7.5±2.31	7.5±2.31	3.9-15.2	3.9-15.1
3.	Poisoning	7.3±2.01	7.3±2.02	3.9-16.5	3.8-16.7
4.	Natural Death	8.7±2.73	8.8±2.81	7.1-14.2	6.9-14.4

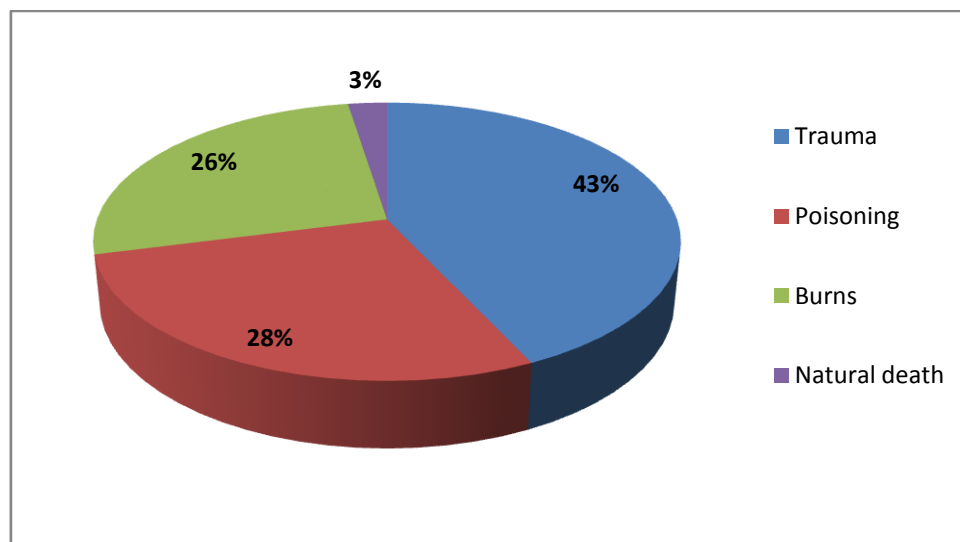
Table-3: Vitreous sodium (Na^+) ion concentration vis-a-vis causes of death

Cause of death	Trauma	Burn	Poisoning	Natural Death
No. of eyes	172	106	112	10
Time since death (in hours)	Na^+ concentration in meq/l			
0-1	143.00	-	-	-
1.1-3	140.50	143.50	143.40	-
3.1-6	138.20	137.50	137.10	-
6.1-12	134.80	135.60	134.80	135.90
12.1-24	129.50	127.50	130.70	133.20
24.1-36	125.40	127.50	-	-
36.1-48	-	122.00	-	121.50
48.1-72	119.60	-	119.50	-

($r=0.045$)

Table-4: Vitreous sodium (Na^+) ions concentration in right and left eye vis-a-vis cause of death

S. No.	Cause of death	Mean \pm SD		Range	
		Right	Left	Right	Left
1.	Trauma	134.4 \pm 5.68	134.3 \pm 5.83	178.2-145.9	120.4-146.1
2.	Burn	133.3 \pm 6.60	133.1 \pm 6.91	120.1-147.0	119.2-146.8
3.	Poisoning	133.3 \pm 5.19	133.4 \pm 5.08	120.0-143.6	119.0-143.1
4.	Natural Death	132.5 \pm 5.45	132.7 \pm 6.13	122.1-136.8	120.9-137.8

**Figure-1:** Distribution of cases according to cause of death

Discussion

Total 400 samples of the vitreous humour from 200 dead bodies died in MGH & MDM Hospitals Jodhpur (Rajasthan) with known time of death were collected for measurement of potassium & sodium concentration in vitreous humour to find out the simple and accurate method of estimation of post-mortem interval.

In the present study maximum numbers of cases studied were died due to road traffic accident and males were more than females. Maximum numbers of cases were in the age group of 20-40 years and maximum number of cases fall in range of 12-24 hours of time since death.

It was observed that there was considerable rise in the vitreous potassium level with increasing post-mortem interval. The rise of vitreous potassium ion concentration varied from 3.85 to 16.70 meq/l. The linear relation of the increase in vitreous potassium (K^+) concentration with increase in post-mortem interval was both arithmetic and as well as logarithmic. So potassium (K^+) ion level in vitreous humour is one of the most accurate method of estimating post-mortem interval.

This was consistent with observations by many workers including Sturner et al (1964)^[6], Lie (1967)^[7], Madea et al (1989)^[8], James et al (1997)^[9], Munoz Basus et al (2002)^[10], Jashnani et al (2010)^[11] and Ahi et al (2011)^[12]. This course of rise in potassium level in vitreous is due to the autolysis of the vascular choroids and retinal cells of the eye which release substantial amount of potassium into vitreous humour (Lie, 1967)^[7]. Hughes (1965)^[3], Coe (1969)^[13] and Mckoy et al (1983)^[14] found that rise of vitreous potassium concentration with post-mortem interval is biphasic in which the slope of the first few hours after death is steeper than for more prolonged times after death which was not observed in this study.

In the present study linear rise of vitreous potassium concentration up to 57 hours was noticed. Potassium concentration beyond 57 hours was not estimated because none of the case was reported after 57 hours of post-mortem interval.

On the basis of INDOSTAT software we have derived followings:

- Coefficient of Correlation:** Positive correlation was found between vitreous potassium concentration and post-mortem interval. The coefficient of correlation for potassium ion concentration in the vitreous humour was 0.831. This indicates that there is high degree of correlation between potassium concentration and post-mortem interval. The confidence limit was 7.6.
- Coefficient of Regression:** The value of coefficient of regression was 3.46 meq/l/hr that means the rate of rise of vitreous potassium concentration was 0.29meq/l/hr.
- Regression Equation:** After calculating the coefficient of correlation and coefficient of regression, the regression equation was derived. The post-mortem interval can be calculated by regression equation.

$$\text{Post-mortem interval} = - 16.22 + 3.75 \times K^+$$

Table-5: Comparative evaluation of PMI vis-a-vis vitreous potassium (K^+) of already established regression equations by previous workers

S. No.	Mean value of Vitreous Potassium (K^+) ion conc. with known post mortem interval (PMI)	6.9 meq/l (6-12 hours)	8.56 meq/l (12-24 hours)	12.1 meq/l (24-36 hours)	13.98 meq/l (36-48 hours)
1.	Sturner et al (1963)^[6] PMI = 7.14 x K^+ conc. - 39.1	10.16±4.7 hours	22.01±4.7 hours	47.29±4.7 hours	60.71±4.7 hours
2.	Madea et al (1989)^[8] PMI = 5.26 x K^+ conc. - 30.9	5.39±20 hours	14.12±20 hours	32.74±20 hours	42.63±20 hours
3.	Present study PMI = - 16.22 + 3.75 x K^+	9.65± 7.6 hours	15.88±7.6 hours	29.15±7.6 hours	36.2±7.6 hours

Table-6: Statistical correlation of vitreous potassium (K^+) ion concentration reported by various workers

Workers	Statistical Parameters			
	Coefficient of correlation between (K^+) ion conc. and Post-mortem Interval	Rise of Vitreous Potassium (K^+) conc. Up to	Rate of rise of vitreous Potassium (K^+) ion conc. per hour	Confidence limit in hours
Sturner et al (1964)^[6]	0.987	100 hours	Very Slow	± 4.7 hours
Agarwal et al (1983)^[2]	0.985	24 hours	0.52 meq/l	± 3.39 hours
Govekar et al (1990)^[15]	0.855	60 hours	0.15 meq/l	± 5.70 hours
Present Study	0.831	57hours	0.29 meq/l	± 7.6 hours

On the other hand the vitreous sodium (Na^+) ion concentration decreases with increasing post-mortem interval in present study. However this decline was not statistically significant. The observation in our study was not consistent with Jaffe (1962)^[5] who observed vitreous sodium constant for at least 30 hours after death. The findings of present study were also not consistent with Lie (1967)^[7] who observed small and variable changes in sodium concentration bearing no relation to post-mortem interval.

The present study showed that there was no statistically significant difference in levels of vitreous potassium & sodium concentration between the two eyes of body. Regarding

potassium our observations were similar to the other workers such as Sturner et al (1964)^[6], Hughes (1965)^[3] and Coe (1989)^[13]. Our findings were not consistent with the report Madea et al (1989)^[8] who found relevant differences between the two eyes.

Conclusion

It was concluded from the present study that:

- The linear correlation of the increase in vitreous potassium ion concentration with increasing post-mortem interval was both arithmetic as well as logarithmic (statistically significant).

2. Vitreous sodium ion concentration showed decline in its level with increasing post-mortem interval but we could not correlate this decrease with post-mortem interval (statistically not significant).

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Conflict of Interest: Nil

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