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Thanatochemistry: Study of vitreous humor potassium

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Abstract This study has been carried out to determine the death interval from the biochemical parameter of vitreous potassium. In 308 medicolegal cases vitreous humor was taken and analyzed for potassium with known time of death. There was a linear rise in potassium concentration with increasing death interval. Regression equation was calculated for the same. The study indicates that potassium levels in vitreous for determining death interval are useful and can afford a good method of determining the death interval along with other traditional methods. Also the previously established formulae for estimating death interval from vitreous potassium were also studied.

1. Introduction

Biochemical analysis of different body fluids in relation to their death interval has become a useful supplementary procedure to the other traditional signs such as postmortem lividity and rigor mortis in death interval estimation.

After the initial studies done by Naumann and Sturner, numerous studies have established the potential utility of vitreous humour in estimating the time of death.[1–8] Few substances particularly vitreous potassium has received most attention. It is known that vitreous potassium approximates the serum levels in many experimental animals. The eyeball is well protected anatomically and is much less subjected to contamination or putrefactive changes than with blood, serum, or CSF.[3] Also, it has a large volume, is easily obtainable, usually free from contamination and the changes in its biochemical parameters take place more gradually. Several investigators have drawn different regression equations with vitreous potassium and death interval (Table 1). The present study was undertaken to show the relation of vitreous potassium with increasing death interval and to establish regression equation. In addition, the present study aimed to test the different published formulae on vitreous potassium studies.

2. Material and methods

The cases that were admitted in our hospital and brought for medicolegal autopsies with known death interval are selected. Only cases where the treating physician certified the precise time of death and cases without any major metabolic disorders

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Table 1 Traditional formulae for estimating death interval.

Authors – years	Equation obtained	Formula proposed
Sturner et al. (1963)	$Y = 0.14X + 5.6$ ($r = 0.987$)	Death interval = 7.14 × Potassium − 39.1
Adelson et al. (1963)[30]	$Y = 0.17X + 5.36$	–
Coe (1969)[31]	$Y = 0.332X + 4.99$ ($X < 6$ h)	–
Coe (1969)[31]	$Y = 0.162X + 6.19$ ($X > 6$ h)	–
Madea et al. (1987)	$Y = 0.19X + 5.88$ ($r = 0.86$)	Death interval = 5.26 × Potassium − 30.9
James et al. (1997)[27]	$Y = 0.23X + 4.2$ ($r = 0.54$)	Death interval = 4.32 × Potassium − 18.35
Madea et al. (2001)[10]	$Y = 0.16X + 7.35$	–
Salam et al. (2012)[32]	$Y = 0.72X - 6.57$ ($r = 0.61$)	Death interval = 1.337 × Potassium + 9.050
Mihailovic et al. (2012)[33]	$Y = 0.36X + 4.35$ ($r = 0.927$)	Death interval = 2.749 × Potassium − 11.978

Y = Potassium (mEq/l) and X = Death interval.

were taken. Total 308 cases were examined and studied with respect to different age and sex at different death intervals. The details regarding the age, sex, date and time of death, the circumstances, and the history are elicited from the inquest papers, medicolegal case reports, and death certificate issued from the hospital. The ambient temperature ranged 20–30 °C before the samples were taken.

By using a sterilized 20 gauge hypodermic needle 1.5–2 ml crystal clear vitreous humor is aspirated without exerting much pressure from the outer canthus of each eye, the tip of the needle is near the center of the eye ball. Water is injected for cosmetic restoration of the eyeball after aspiration of vitreous fluid.

Analysis is done immediately after the vitreous humor was aspirated. Prior to the analysis the sample fluids are centrifuged at 3500 rpm for 10 min and then the supernatant are used for analysis. No other method for homogenization for vitreous humor was used. The samples for vitreous humor potassium were analyzed on Medica's Easylyte Plus Na/K/Cl Analyser by the Ion selective method. The reagents used were from Teco Diagnostic, USA.

The statistical analyses for the data were carried out using the statistical software data analysis pack within Microsoft excel office 2007. Linear regression analysis, ANOVA and Pearson correlation were used for statistical analysis. The cases were divided into five groups according to the death interval.

3. Results

The postmortem interval varied from 01.45 to 35.18 h. Table 2 shows descriptive statistical values of vitreous potassium in the postmortem state.

The values of vitreous potassium and death interval were significantly correlated ($r = 0.526$) (see Fig. 1). The intercept of the regression line on the y axis for the potassium scatter plot was 7.43 mmol/L. The slope of the regression line (or the rate of rise of concentration postmortem) calculated from the potassium data was 0.368 mmol/L per hour. From these data the following equation was constructed –

$$\text{Death interval} = 2.71 \times \text{Potassium} - 20.19. \quad (1)$$

The standard error of estimate was ±2.9 h and 95% confidence interval of the regression being ±5.8 h. When the data were applied to different equations previously published the equation of Sturner yielded a mean overestimation of 33.52 h with SD of 24.7 h; Adelson equation showed mean overestimation of 33.9 h with SD of 20.4 h; Coe (< 6 h) equation showed

Table 2 Shows descriptive statistic values of vitreous potassium in the postmortem state.

Regression statistics	
SUMMARY OUTPUT	
Multiple *R*	0.526
R square	0.277
Adjusted *r* square	0.274
Standard error	2.953
Observations	308

	df	*SS*	*MS*	*F*	*Significance F*
ANOVA					
Regression	1	1024.02	1024.02	117.43	2.25E−23
Residual	306	2668.39	8.72		
Total	307	3692.41			

	Coefficients	Standard error	*t* Stat	*P*-Value	Lower 95%	Upper 95%
Intercept	7.434	0.380	19.53	9.9E−56	6.685	8.183
X variable 1	0.368	0.033	10.83	2.25E−23	0.301	0.435

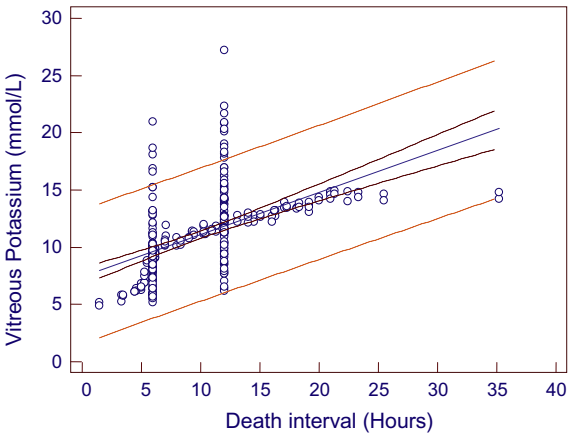

Figure 1 Correlation of vitreous potassium with death interval.

mean overestimation of 18.3 h with SD of 10.6 h; Coe (>6 h) equation showed mean overestimation of 30.5 h with SD of 21.4 h; Madea (1987) showed mean overestimation of 27.6 h with SD of 15.0 h; Madea (2001) showed mean overestimation of 23.6 h with SD of 21.6 h; James showed mean overestimation of 23.6 h with SD of 21.6 h; Salam (2012) showed mean overestimation of 24.3 h with SD of 4.7 h; Mihailovic (2012) showed mean overestimation of 18.6 h with SD of 9.5 h (Table 3).

4. Discussion

Numerous hypotheses have been promulgated for the rise in potassium with time. One suggestion was that the vascular choroid and the retinal lining cells might be the potential sources of this ion influx.[4–8] Naumann[2] has suggested that it may be due to an influx of this ion into the vitreous from the autolysis of cell membranes. According to Madea et al.[9,10] with postmortem breakdown of metabolite mainly anaerobic glycolysis active membrane transport stops and the loss of selective membrane permeability and diffusion of ions and other parameters according to their concentration gradients starts. Furthermore, analytical concentrations are affected by postmortem changes like redistribution/hemoconcentration. The fact that the intracellular concentration of potassium is much higher than extracellular concentration would tend to support such a source.

Various studies conducted until now have well established that the analysis of vitreous humor is time-honored.[4–29] In different studies, the rise in vitreous potassium after death appears to be a constant phenomenon. Almost all equations presented in the literature show a linear dependence between the death interval and potassium values. In the present study intercept in the equation for all bodies is 7.43 mmol/L. The intercept values are more or less similar to Sturner,[4] Adelson,[30] Coe,[31] Madea et al.,[9,10] James,[27] Salam,[32] Mihailovic.[33]

Table 4 Regression statistics at various death intervals in our study.

	Intercept (mmol/L)	Slope (mmol/L per hour)	Correlation (r)
0–6 h	1.2	1.35	0.36
6–12 h	6.19	0.55	0.3
12–18 h	9.13	0.242	0.68
18–24 h	8.65	0.26	0.76
>24 h	13.95	0.014	0.23

In our equation, the slope of regression line was 0.36 mmol/L per hour. Because the slope is slightly higher compared with Sturner,[4] Adelson,[30] Coe,[31] Madea et al.,[9,10] and James,[27] we tested it in different death intervals (Table 4). The slope is higher in the first 6 h than in the latter intervals. Also in first 12 h, there is weak correlation between vitreous potassium and death interval, which becomes significantly stronger after that. This may be due to biphasic nature of potassium concentration, which rises more rapidly in the first few hours after death.

In our study correlation coefficient between vitreous potassium and death interval was somewhat low compared to other investigations (r = 0.526). According to Eisner[34] in man and some apes the vitreous is heterogeneous with a dense cortex and a center of low density. In vitro studies of Kinsey and Reddy[35] have shown that there is net accumulation of potassium across the anterior surface of the lens and a corresponding leak of potassium from the posterior surface of the lens into the vitreous body, which is balanced by a loss of potassium from the vitreous through the retina into the circulating blood. Gradients of potassium ion concentrations differences in anterior segment, center and posterior segment of vitreous body were studied by Bito with higher gradient found in anterior and posterior segment than the center of the vitreous body.[36] This may influence correlation coefficient between vitreous potassium and time since death compared to other investigations.

Almost all the studies reported that there exist significant positive correlation of potassium concentration in relation to death interval and formula was evolved. However, in European countries owing to cold climate the bodies are well preserved and less subjected to rapid deterioration than the bodies found in hot tropical countries like India, causing variation in results. In the present study, the analysis of vitreous potassium was done on ion selective electrode, which according to most clinical chemists and physician is the method of choice and is the best solution to mitigate errors while analyzing fluid by flame photometry.[37] Thus, results obtained by these methods are less likely to have technical errors than other methods adopted till date.

Table 3 Difference between actual and expected values of death interval from vitreous potassium applied on different equations.

	Sturner (1963)	Adelson (1963)[30]	Coe (<6 h) (1969)[31]	Coe (> 6 h) (1969)[31]	Madea (1987)	Madea (2001)[10]	James (1997)[27]	Salam (2012)[32]	Mihailovic (2012)[33]
Mean	39.5	33.9	18.3	30.5	27.6	23.6	23.6	24.3	18.6
SD	24.7	20.4	10.6	21.4	15.0	21.6	21.67	4.7	9.53

Some investigators may consider this method unusable in practice. However, though sometimes it is still far from ideal, we consider that the vitreous potassium in any case can give some indication of the approximate time since death. However, there is little doubt that the combination of the vitreous potassium method and the time tested signs of physical changes after death like rigor mortis, lividity, and deep rectal temperature should enable the forensic faculty to improve accuracy in estimating the death interval in unwitnessed deaths.

Conflict of interest

None declared.

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