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Thanatochemistry: Study of synovial fluid potassium

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Abstract Death interval estimation is one of the major attributes obtained from thanatochemistry. Body fluids like vitreous humour have been consistently used to estimate death interval from regression equations, but these may be useless when dealing with eye trauma, ocular disorders or in mutilated remains. Until recently, there was no consistent assessment of the reliability of measurements of the synovial fluid for death interval estimation. The purpose of this paper is to test previously developed regression formulae for estimating death interval based on synovial fluid potassium and to assess its reliability in estimating death interval. Synovial fluid potassium was measured on a sample of 308 individuals. Death interval was regressed on synovial fluid potassium and a regression formula was obtained. The regression model provided a 95% confidence interval of ±3.0 h and a correlation coefficient of 0.739. Compared to other studies, regression formulae based on the synovial fluid potassium in the present study provided considerably less standard errors.

1. Introduction

In manypast years, a variety of chemical methods have been employed to determine death interval.[1] Thanatochemistry is used to describe changes that occur in the chemical composition of various body fluids immediately after death.[2] Several investigators have tried to find the relation between death interval and postmortem biochemical changes in various body fluids such as blood, serum, cerebrospinal, vitreous, and synovial fluid.[3] Various studies conducted till now have well established that the analysis of vitreous humour is time-honoured. 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.[4–18] Until now, synovial fluid has been investigated for determining alcohol concentration, drug distribution into synovial fluid and postmortem chemistry regarding the cause of death.[19,20] Very few studies on cadaveric synovial fluid and death interval have been carried out.[21–23] These studies revealed that synovial fluid could be used as a postmortem examination tool compared to the vitreous humour and the spread of values was comparable in all examined electrolytes.[21] Different regression equations with the cadaveric synovial fluid potassium and death interval have been drawn by several investigators (Table 1).[21–23]

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Table 1 Different studies showing formula for determining death interval from cadaveric synovial fluid.

Author–year	Equation obtained
Madea (2001)	Death interval = −45 + 7.69 × potassium
Sahoo (1997)	Death interval = 2.94 + 0.113 × potassium
Sheikh (2007)	Death interval = 5.28 + 2.20 × potassium

Potassium is the most commonly used component in postmortem biochemistry. Intracellular concentration of potassium is as high as 2–40 times the concentration of potassium within plasma. After death, as the pumping mechanism of cell becomes inactive and the cell wall becomes semi-permeable, potassium leaks through it to approach the equilibrium.[2,3] Measurements of biochemical levels in the synovial fluid have some advantages over those of blood and CSF. The most obvious is easy sampling. Similar to the vitreous humour, the synovial fluid is isolated and well protected anatomically and is usually well preserved at postmortem and is less subjected to contamination and putrefactive changes like that of blood or CSF. In addition, chemical changes occur slowly in the synovial fluid than in blood or CSF, extending the period of time during which the synovial fluid may be used to estimate death interval. The purpose of this paper was to evaluate the use of postmortem synovial potassium for estimating the postmortem interval and to assess its reliability with previous studies on synovial fluid potassium.

2. Material and methods

The cases that were admitted in our hospital and brought for medicolegal autopsies with known death intervals are selected. Only cases without any major metabolic disorders were taken and the precise time of death was certified by the treating physician. A total of 308 cases were examined and studied with respect to different age and sex at different death intervals. The details relating to age, sex, date and time of death, the circumstances, and the history are elicited from the inquest papers, medicolegal case reports, and death certificate are issued from the hospital. The ambient temperature ranged from 20–30 °C before the samples were taken. In supine position, the synovial fluid is aspirated with a sterilized 18 gauge needle with 10 ml syringes by puncturing the suprapatellar pouch from the lateral side, just below the patella and pushed directly backwards. Cases of knee injury, infection of the knee, or the synovial fluid that was found to be discoloured and haemorrhagic during extraction were excluded. Analysis is done immediately after the synovial fluid was aspirated. Prior to the analysis the sample fluids are centrifuged at 3500*g* for 10 min and then the supernatant is used for analysis. No other method for homogenization of synovial fluid was used. Samples for synovial potassium were analysed 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 of 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 utilized 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 statistic values of the constituents of the synovial fluid in postmortem state and serum as control samples from different postmortem intervals. The value of the synovial fluid potassium and death interval was significantly correlated ($r = 0.739$). The intercept of the regression line on the y axis for the potassium scatter plot was 5.44 mmol/L. The slope of the regression line (or the rate of rise of concentration postmortem) calculated from the potassium data was 0.353 mmol/L per hour (Fig 1). From these data the following equation was constructed:

$$\text{Death interval} = 2.83 \times \text{Potassium} - 15.41$$

The standard error of estimate was ±1.5 h and 95% confidence interval of the regression being ±3 h. When data were applied to different equations previously published the equation of Madea et al. yielded a mean underestimation of 14.0 h with an SD of 14.9 h; Sahoo's et al. showed a mean overestimation of 6.08 h with an SD of 4.7 h while Sheikh et al. showed a mean underestimation of 15.0 h with an SD of 3.6 h.

4. Discussion

The postmortem changes in biochemical electrolytes particularly potassium occur with postmortem breakdown of the metabolite mainly due to anaerobic glycolysis due to which active membrane transport stops and loss of selective membrane permeability and diffusion of ions and other parameters according to their concentration gradients starts.[2] Furthermore, analytical concentrations are influenced by postmortem changes like redistribution/haemoconcentration. The fact that the intracellular concentration of potassium is much higher than the extracellular concentration would tend to support such a source. Rise of potassium in the synovial fluid can be attributed probably to autolysis of the cell membrane of the synovium. The synovial compartment having a homogeneous distribution of cations like potassium throughout showed a stronger correlation coefficient with time since death.[24,25]

Till now, various studies conducted have well established that the analysis of the vitreous humour is time-honoured.[2] Promising results have been obtained especially in the development of potassium concentration over time in the synovial fluid and were comparable with those of vitreous humour.[21–23] This is of importance particularly in cases of trauma to the eye or congenital absence of the eyeball or due to some other reasons. In our study, there was a rise in potassium levels with increasing postmortem interval similar to other studies.[21–23]

In earlier studies, synovial fluid was examined for determining the cause of death, drug distribution, and alcohol level estimation and in rheumatological investigations.[19,20] Yielding found potassium values in normal and diseased joints to be quite variable.[19] More found that biochemical electrolyte modifications were related more directly to duration of the pathological process that leads to death than with the natural process.[20] In different studies, the rise in synovial potassium after death appears to be a constant phenomenon.[21–24] Almost all equations presented in the literature show a linear

Table 2 Descriptive statistic values of the potassium of synovial fluid in postmortem state.

SUMMARY OUTPUT						
Regression statistics						
Multiple *R*	0.739					
R square	0.546					
Adjusted *R* square	0.545					
Standard error of estimate	1.596					
Observations	308					
ANOVA						
	df	SS	MS	*F*	Significance F	
Regression	1	940.673	940.673	369.132	1.6126E-54	
Residual	306	779.790	2.548			
Total	307	1720.464				
	Coefficients	Standard Error	*t* Stat	*P*-value	Lower 95.0%	Upper 95.0%
Intercept	5.44	0.205	26.451	4.73447E-81	5.0376	5.847
X Variable 1	0.352	0.018	19.212	1.6126E-54	0.316	0.389

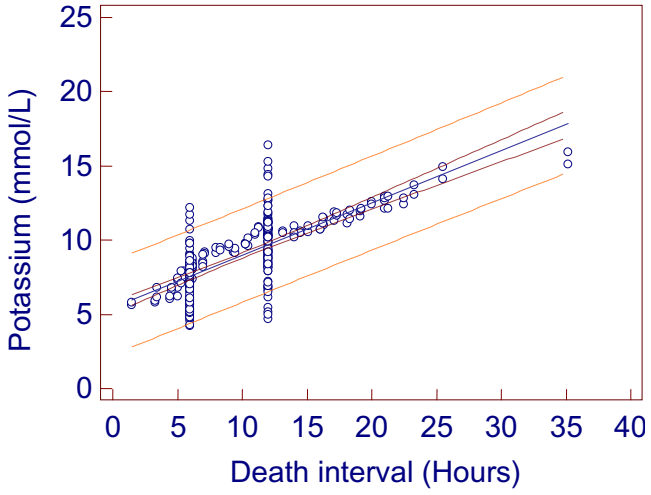

Figure 1 Correlation of synovial fluid with death interval.

dependence between the death interval and potassium values.[21–23] In the present study the intercept in the equation for all bodies is 5.44 mmol/L. The intercept values are more or less similar to Madea et al.[21] and Sheikh et al..[23] However, Sahoo et al.[22] showed an extreme intercept of 2.94 mmol/L. This can be explained by the fact that the variation in experimental methods and sample characteristics may account for the difference noted with slope. In our equation, the slope of the regression line was 0.35 mmol/L per hour. Because the slope is slightly higher compared with Madea et al.[21], Sahoo et al.[22] and Sheikh et al.[23], we tested it in different death intervals (Table 3).

The slope is highest in the first 6 h than in the latter intervals. Madea et al.[21] reported a low slope of 0.13 mmol/L per hour that made the slope of regression flatter which tends to underestimate the death interval from the obtained regression. Also in the first 12 h there is a weak correlation between synovial potassium and death interval which becomes significantly stronger after that. This may be due to the biphasic nature of potassium concentration which rises more rapidly in the first few hours after death. Madea et al.[21] Sahoo et al.[22], Sheikh et al.[23] reported that there exists a significant positive correlation of potassium concentration in relation to death interval and a formula was evolved. Sahoo et al.[22] observed a somewhat erratic rise of postmortem potassium while Sheikh[23] showed a steady rise of potassium concentration up to maximum 48 h of death and had a positive correlation with time lapse. In Indian studies the analysis was performed in a flame photometer which is prone to have an erratic result. In the present study, the analysis of synovial potassium was done on an ion selective electrode which according to most clinical chemists and physicians is the method of choice and is the best solution to mitigate errors while analysing fluid by flame photometry.[25,26] Thus, results obtained by these methods are less likely to have technical errors than other methods adopted till date.

In present study, the ambient temperature in which the bodies were kept ranged from 20 to 30 °C before the samples were taken. In temperate countries owing to cold weather, the bodies are well preserved and less subjected to rapid

Table 3 Regression statistics at various death intervals in our study.

Death interval	Slope (mEq/l per hour)	Intercept (mEq/l per hour)	Pearson correlation
0–6 h	0.36	5.06	0.190
6–12 h	0.24	6.97	0.24
12–18 h	0.30	6.21	0.835
18–24 h	0.31	5.9	0.844
>24 h	0.10	11.91	0.754
Overall total hours	0.352	5.44	0.739

deterioration than the bodies found in hot tropical countries like India. The environmental temperature has a definite influence on the synovial fluid potassium with increasing death interval. The variation in temperature at which the bodies were kept in different studies might have caused differences to the regression lines compared to our study.

In the present study there was a very small 95% confidence interval of ±3 h and small standard deviations for death time estimation which are in contradiction to Madea et al.[21] who stated: ''Especially the development of potassium concentrations over time in synovial fluid is nearly the same as in vitreous humor''. The probable reason for such results may be due to the fact that the synovial compartment has a homogeneous distribution of cations like potassium throughout while the vitreous body has a heterogenous distribution of gradients of potassium in the anterior segment, centre and posterior segment with a higher gradient found in anterior and posterior segments than the centre of the vitreous body.[2,21]

The present study had established that there is a rise in potassium with increasing death interval. Regression equations can be formulated from the synovial fluid potassium. Synovial potassium has good correlation with death interval and can be used for estimating death interval. Thus, our study concludes that the synovial fluid potassium has good reliability in estimating death interval.

Conflict of interest

None declare.

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