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Original communication

Exploring time of death from potassium, sodium, chloride, glucose & calcium analysis of postmortem synovial fluid in semi arid climate

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ABSTRACT

Estimation of time of death (TOD) with fair accuracy from postmortem changes still remains an important but difficult task to be performed by every autopsy surgeon under different climatic conditions. The environment plays an important role in the process of decomposition and thereby affecting the levels of electrolytes and other biochemical parameters in the postmortem samples. Since, there is limited information available on the levels of these biochemical parameters from semi arid environment, the present study was aimed to explore time of death by analyzing electrolyte, glucose and calcium levels of postmortem synovial fluid collected from samples under such climatic conditions. The synovial fluid samples from two hundred and ten bodies brought to University College of Medical Sciences and associated Guru Teg Bahadur Hospital Delhi for medico-legal postmortem examination, during the period of November 2010 to April 2012, were analyzed for potassium, sodium, chloride, glucose and calcium. Univariate regression analysis of electrolyte concentrations of synovial fluid showed significant positive relationship between time of death and potassium ($r = 0.840$, $p = 0.000$). However, there was negative relationship between time of death and sodium ($r = -0.175$, $p = 0.011$) & glucose ($r = -0.427$, $p = 0.000$) and no significant relationship was found between time of death and calcium ($r = 0.099$, $p = 0.152$) & chloride ($r = 0.082$, $p = 0.24$) among the samples analyzed.

1. Introduction

The main objectives of conducting medico-legal autopsies are to establish the identity of the deceased, to determine the cause of death and to estimate the time of death.[1] Estimation of the time of death (TOD) is one of the most important aspects of forensic medicine. Time of death is important in both criminal cases as well as civil cases.[2] Apart from its obvious legal importance; its solution has been so elusive as to provide a constant intellectual challenge to workers in science.

Evidence for estimating the time of death may come from three sources i.e. Corporal, Environmental and Anamnestic evidence. All three sources of evidence should be explored and assessed before offering an opinion on when death or a fatal injury occurred.[3]

Numerous models have been proposed in the last 60 years for the determination of time of death by chemical means. Many of them were reviewed by Schleyer in his monograph on the determination of the time of death by means of thanato-chemistry about 50 years ago (1963) and none of these early methods has gained any practical value since they do not meet the demands in practice like being precise, reliable, or giving an immediate result.[4] In recent years most of the work has been concentrated on the biochemical changes that occur in the different body fluids such as blood, cerebrospinal fluid, vitreous humor, pericardial fluid and synovial fluid.[5] These fluids have been studied not only for the estimation of time of death but also to determine the cause of death, manner of death and the condition of the deceased at the time of death.

Synovial fluid is isolated and well protected by the bursa sac with firm tissue structures and is much less subject to putrefactive changes due to bacterial propagation.[6] Synovial fluid is a well-investigated fluid compartment in rheumatology. It is useful for certain biochemical tests such as electrolyte concentration, urea, creatinine, glucose, lactose and alcohol. Synovial fluid is one of the available biological specimens for the prediction of Blood alcohol

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concentration or Urine alcohol concentration within a range in autopsy cases in which suitable blood and/or urine specimens cannot be obtained. It has been stated that electrolyte concentrations are significantly influenced by the environmental factors.[7] Hence, a relatively unexplored postmortem synovial fluid biochemistry was studied in the samples from semi arid climatic condition of Delhi, India.

1.1. Study design

Cross Sectional Observational Study.

2. Material and methods

Two hundred and ten medico-legal cases (36 Females and 174 Males) brought to the mortuary for postmortem examination at University College of Medical Sciences and Guru Teg Bahadur Hospital Delhi, during the period November 2010 to April 2012 were included in the study. All the samples were recovered from donors in a comparable semi arid climate.

The cases were divided into different groups based on time of death and each group contained a minimum of 30 cases to make each group statistically significant. The time interval in each group was 12 h i.e. 0–12 h, 12+ to 24 h, 24+ to 36 h, 36+ to 48 h, 48+ to 60 h, 60+ to 72 h and 72+ h onwards. Hence a total of 210 cases were included in the study.

Only the cases with known time of death were included in the study. Cases with injury to knee joint or obvious joint pathology, hemorrhagic samples, samples from cases with electrolyte disturbances (Burns, Dehydration, Renal Failure, etc.) and highly decomposed dead bodies with insect infestation were excluded from the study.

3. Procedure

In supine position, the synovial fluid was aspirated with an 18 gauge needle attached to 10 ml syringe by puncturing the suprapatellar pouch from lateral side, just below the patella and pushed directly backwards and about 1–1.5 ml of the fluid was aspirated.[8] Analysis was done with out delay for electrolyte, sugar and calcium estimation in routine biochemistry laboratory at our hospital, except for chloride levels which was estimated by kit method. Samples were stored for chloride estimation for batch analysis. If same day analysis was not possible due to technical problems in the laboratory, samples were stored at −80 °C. (No effect of pre analytical error due to low temperature is noted in animal studies so far).[9]

Prior to the analysis the sample was centrifuged at 3500 rpm for 10 min and then the supernatant fluid was used for analysis. Synovial fluid sample was analyzed for sodium, potassium, chloride, calcium and glucose. Analytical methods used for estimation in mentioned in Table 1.

Table 1
Analytical methods used for estimation.

Parameter	Analytic method
Sodium, Potassium	Ion selective electrodes[a,10]
Chloride	Thiocyanate[11]
Calcium	O-Cresol phenolphthalein[12]
Glucose	GOD POD (glucose oxidase peroxidase)[13]

[a] Estimation of Na and K was done on AVL 9181 based upon ion selective electrode technique. The range of K estimation in this machine is 1.5–15 mmol/L (AVL 9181 Roche), beyond which linearity is not maintained. So, for estimation of K within above range synovial fluid was diluted accordingly. Dilution was made in the ratio upto 1:4 using distilled water depending upon the sample reading.

Relationship between concentrations of all the parameters in synovial fluid and time of death was established using SPSS 17.0 for windows 7 software. The results of the analysis were obtained in the form of tables consisting of mean, Pearson correlation, regression, ANOVA, correlation coefficients, collinearity diagnostics, case wise diagnostics and residual statistics. p-value less than 0.05 was considered as statistically significant.

4. Results

The values along with different parameters of electrolyte, calcium and glucose in synovial fluid measured are mentioned in Table 2.

Univariate regression analysis of electrolyte concentrations of synovial fluid showed significant positive relationship between time of death and potassium ($r = 0.840$, $p = 0.000$). However, there was a negative relationship between time of death and sodium ($r = -0.175$, $p = 0.011$) & glucose ($r = -0.427$, $p = 0.000$). Whereas, no significant relationship could be established between time of death and calcium ($r = 0.099$, $p = 0.152$) & chloride ($r = 0.082$, $p = 0.24$). (Fig. 1).

The corresponding regression equations for estimation of time of death from synovial fluid sodium, potassium and glucose are as follows:

$$\text{TOD (hours)} = 101.504 + [-0.384 \times \text{Sodium (mmol/L)}]$$

$$\text{TOD (hours)} = [4.751 \times \text{Potassium (mmol/L)}] - 27.920$$

$$\text{TOD (hours)} = 52.567 + [-1.632 \times \text{Glucose (mmol/L)}]$$

Multivariate linear regression analysis of electrolyte concentration of synovial fluid showed rise in the adjusted r^2 value (0.705–0.710) on addition of sodium to potassium in the equation. However addition of calcium (adjusted $r^2 = 0.705$), chloride (adjusted $r^2 = 0.705$) and glucose (adjusted $r^2 = 0.706$) did not give any significant improvement in the results. The multivariate linear regression equation for estimation of time of death from synovial fluid potassium and sodium concentration is as follows:

$$\text{TOD (hours)} = 4.7 \times \text{Potassium (mmol/L)} - 0.157 \times \text{Sodium (mmol/L)} - 4.356.$$

As adjusted r^2 value is the coefficient of determination which determines the variability explained by the predicted model. In this study r^2 value was 71% using multivariate regression equation, which is usually considered as a value with good predictability (Fig. 2).

However, the accuracy rate was higher when the time of death was predicted using multivariate linear regression equation with observed time of death upto 72 h as shown by scatter graph also.

Table 2
Values of different parameters in synovial fluid.

	Synovial fluid				
	Na^+ (mmol/l)	K^+ (mmol/l)	Ca^+ (mg/dl)	Cl^- (mmol/l)	Glu (mg/dl)
Mean	145.25	15.51	5.22	105.02	4.16
N	210	210	210	210	210
Std. deviation	15.14	5.86	1.04	35.09	8.67
Median	146.00	14.80	5.20	102.60	0.00
Minimum	104	5.2	1.8	39	0
Maximum	186	35.0	12.0	213	69
Variance	229.46	34.38	1.09	1231.81	75.18
Std. error mean	1.04	0.40	0.07	2.42	0.59