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Original communication

Estimation of post-mortem interval: A comparison between cerebrospinal fluid and vitreous humour chemistry

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ABSTRACT

Accurate estimation of post-mortem interval is of great importance in medico-legal autopsy cases. The present study is intended to compare the accuracy of estimating post-mortem interval from biochemical parameters of vitreous humour and cerebrospinal fluid (CSF). Both the fluids were collected from 100 medico-legal autopsies with known time of death and analysed for potassium, glucose, sodium, calcium, urea and creatinine. The current study found that vitreous humour is a better fluid in comparison to CSF for estimation of post-mortem interval. It is also observed that among the statistically significant parameters in both the fluids, level of potassium and sodium in vitreous humour are giving more accurate results in comparison to their corresponding parameters in CSF while accuracy of glucose is more or less same in both the fluid. Nevertheless potassium concentration in vitreous humour is a single best time honoured parameter to estimate post-mortem interval.

1. Introduction

A precise estimation of post-mortem interval (PMI) is a useful statistics while examining a missing persons file in search of a likely match, establishing the time frame of crime, narrowing the field of suspects during police inquiry in an unwitnessed crime, solving the civil cases like dispute in property inheritance and insurance policies etc. Estimating accurate PMI has always posed a challenge for the autopsy surgeon. Post-mortem interval is defined as "the amount of time that has elapsed since death of the decedent".[1] After death many physical, metabolic, autolytic, physiochemical and biochemical processes take place in the body. These changes are of paramount importance in estimating PMI as these progress in an orderly manner until the body disintegrates.[2,3] Chances of error are more when physical changes are taken into consideration for quantifying PMI, as these parameters are quickly affected by external environment. Now the focus has been shifted to biochemical estimation of different parameters in closed compartment body fluids like CSF, vitreous humour, pericardial fluid, synovial fluid etc for accurate estimation of PMI as these closed compartments are well-protected and thus, are not contaminated quickly after death.[4,5] Previous studies have shown that proper selection of body fluid, sampling technique and analytical methods are essential for accurate estimation of PMI.[5] Selection of proper body fluid and its comparison with other fluids will help us in taking a conclusive opinion in accurate estimation of PMI. Although numerous studies have been conducted in different parts of the world considering different parameters in various body fluids for estimation of PMI but only few evaluated synovial fluid in comparison with vitreous humour to estimate PMI.[6,7] The present study was undertaken to investigate whether post-mortem analysis of CSF is comparable with vitreous humour for estimation of PMI and if yes; then what parameters are better correlated with PMI.

2. Materials and method

The study was carried out in Department of Forensic Medicine and Toxicology, All India Institute of Medical Sciences, New Delhi,

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India from May 2011 to November 2012. During the study period all medico-legal autopsy cases were evaluated for time of death, cause of death, and demographic profile through death certificate issued from hospitals, police inquest papers and history from eyewitnesses. Those cases with known time of death and PMI of less than 96 h were enrolled in the study. Death due to meningitis, encephalitis, septic shock, vitreous haemorrhage, trauma to eye, metabolic syndrome, and intracranial haemorrhage were excluded from the study. Finally a total of 100 cases were included in the study during the stipulated study period.

Before the autopsy procedure during post-mortem examination, the samples of CSF and vitreous humour were collected after taking informed consent from relatives. In case of delay in post-mortem process the body of the deceased were stored at 4–8 °C in cold storage. With the help of a sterilized disposable spinal needle, 5 ml of clear CSF was aspirated from central cistern puncture by piercing atlanto-occipital membrane and meninges from a point where no resistance was felt. By using a sterilized 16 gauge needle, 2 ml crystal clear vitreous humour was aspirated without exerting much pressure. The needle was introduced 4 mm lateral to limbus and aspirated when tip of the needle reached the centre of the eye ball. After aspiration of vitreous humour same amount of normal saline was injected back into the eye ball to maintain the contour. Every effort was made to collect clear and adequate samples but those cases in which samples were inadequate or turbid were excluded from the study. The collected samples were immediately centrifuged at a rate of 3000 rpm for a period of 10 min. The supernatant solution was collected in a sterile tube and analyzed by auto-analyzer for levels of creatinine (Jaffe method), Urea (enzymatic kinetic method), Glucose(liquid reagent hexokinase method), calcium (Arsenazo method), sodium and potassium (ion-selective electrode method on Beckman auto-analyser). For remote analysis the samples were preserved in −18 °C and −70 °C in deep freezer, to be analyzed within 24 h of short-term storage and long-term storage respectively. Undiagnosed metabolic syndromes in study samples were detected by estimating urea, & creatinine levels in both the fluids and from history by relatives since urea is a suitable indicator to access the ante-mortem electrolyte imbalance as stated by Madea et al.[5] Samples with high urea and creatinine values were discarded.

Data was collected and entered concurrently in a Microsoft Excel 2007 and was analyzed by STATA version 11.2. For the purpose of analysis total 100 cases were randomly divided into two groups i.e. study group containing 70 cases and validated group containing 30 cases each. Correlation between PMI and concentration of different parameters in both fluids were analysed in the study group (n = 70) and regression equation($Y = a+bX$) was formulated for statistically significant parameters. The regression equations obtained from study group were applied in the validated group (n = 30) to calculate estimated PMIs. Then residual PMIs (Actual PMI−estimated PMI) were calculated for different parameters in validated group. Comparison was made between two fluids for mean residual PMI of different statistically significant parameters. For descriptive purpose, only the data of study group is being presented while data of validated group was used for comparison purposes. Data was presented in Mean (SD)/Median (minimum, maximum) or frequency (%). In case of continuous variable within group, comparison was done by paired t-test/Wilcoxson sign rank test and between the group independent t-test/Wilcoxson rank sum test as per condition of normality of data.

3. Results

The known PMI range in the study group was 4.75–78.5 h and 4.5–42 h in validated group. Mean PMI in the study group and validated group was observed as 21.2 h and 19.3 h respectively. Descriptive statistics and comparative values of constituent of vitreous humour and CSF among two groups i.e. study group and validated group are shown in Table 1. It was observed that none of the parameters in the two groups were significant. Hence it can be concluded that both groups are comparable and regression equation, for estimation of PMI, derived from one group can be applied to other group.

For descriptive purpose, only the data of study group is presented. The male: female ratio in the study group was 3.1:1 and mean age among males and females was 35.77 years and 23.29 years respectively. The mean PMI among the males and females was 21.67 h and 20.09 h respectively. The male-female differences in the vitreous humour and CSF parameters were not significant statistically (Table 2). This suggests that there is no difference between male and female while considering these parameters for estimation of PMI in vitreous humour and CSF.

Correlation between PMI and different parameters of both fluids as analysed is presented in Table 3. Potassium, glucose and sodium levels of both fluids showed statistically significant correlations with PMI; but only potassium and glucose in vitreous humour and CSF showed significant difference in their correlation coefficient with increasing PMI but there is no significant difference between correlation coefficient of sodium ion in both fluids. Potassium increases in vitreous humour and CSF after death, and established a statistical significant correlation with PMI; but strength of correlation of potassium in vitreous humour ($r = 0.62$) was stronger than CSF ($r = 0.37$). Other statistically significant correlations were established between Glucose and PMI, sodium and PMI. Glucose in vitreous humour ($r = -0.63$) is strongly correlated with PMI than in CSF ($r = -0.35$) but sodium in CSF ($r = -0.34$) is better correlated with PMI than in vitreous humour ($r = -0.27$). Though sodium in both fluids showed a statistically significant correlation but its 95% confidence interval values were overlapping with each other. The regression equation was established in the study group and applied in the validated group to calculate estimated PMI.

The regression equation for potassium in vitreous humour was found to be

Table 1
Comparison between study and validated group.

Variable	Study group (n = 70)	Validated group (n = 30)	P-value
Age	32.7 ± 14.1	30.7 ± 12.0	0.49
Sex[a]			
▪ Male	53 (67.95)	25 (32.05)	0.39
▪ Female	17 (77.27)	5 (22.73)	
PMI	21.2 ± 10.5	19.3 ± 8.2	0.38
Vitreous			
▪ Potassium	11.5 ± 2.3	10.9 ± 2.2	0.26
▪ Glucose	12.5 ± 5.2	12.8 ± 4.8	0.74
▪ Urea	22.3 ± 8.9	19.2 ± 5.2	0.07
▪ Creatinine[b]	0.5 (0.2–2.9)	0.5 (0.2–1.6)	0.39
▪ Calcium	5.9 ± 1.4	5.8 ± 1.6	0.75
▪ Sodium	137.5 ± 10.5	140.9 ± 16.6	0.22
CSF			
▪ Potassium	15.4 ± 4.7	14.9 ± 5.1	0.64
▪ Glucose[b]	13.5(2–98)	16 (0.7–98)	0.15
▪ Urea	27.8 ± 10.3	24.2 ± 8.3	0.09
▪ Creatinine	1.1 ± 0.4	1.0 ± 0.4	0.36
▪ Calcium	6.2 ± 1.0	6.0 ± 1.8	0.60
▪ Sodium	131.4 ± 11.8	132.0 ± 14.0	0.81

All other values are expressed as Mean ± Standard Deviations.
[a] Values in this category were expressed in numbers and values in parenthesis shows the percentages.
[b] Values were expressed in terms of median and values in parenthesis show range (min. − max.).