

Role of CSF Chloride ion in Estimation of Postmortem interval

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ABSTRACT

Aim: To find out the postmortem Chloride level in cerebrospinal fluid and to determine its correlation with the known postmortem interval (PMI).

Study design: Observational study.

Methods: Those Medicolegal autopsy cases conducted at the mortuary of Forensic Medicine Department, King Edward Medical University (KEMU), Lahore were included in which the exact time of death was given. A total number of consecutive 106 samples with known postmortem interval were taken from the cases brought to the mortuary. Unknown and putrefied dead bodies, Poisoning cases and Cases with head injuries were not included. Study was completed in 6 months.

Results: The standard error and the R² value of Chloride indicates that it is not better indicator of time since death as compared to the predicted from the levels of sodium and potassium individually.

Conclusion: The results of this study indicated that the level of postmortem CSF chloride value is not useful in PMI estimation

Keywords: Postmortem interval, Cerebrospinal fluid, Chloride ion

INTRODUCTION

The accurate estimation of the time of death is a critical investigational problem for the authorized medical officer.¹ He has to answer the question regarding time of death which is invariably asked by police officers and courts of law. The answer to this question is extremely difficult because many endogenous and exogenous factors affect the body after death^{2,3}. There are several physical changes that occur after death and are the main parameters for the estimation of time since death such as cooling of the dead body, development of rigor mortis, postmortem lividity etc^{3,4}. Moreover, there are many chemical changes in various body fluids taking place after death. These body fluids are whole blood, serum, CSF, aqueous humor, synovial fluid, and vitreous humor^{5,6}. The literature highlights to put emphasis on the examination of body fluids which are stable chemically and protected from contamination unlike blood after death³.

In this study the cerebrospinal fluid (CSF) has been chosen to estimate time since death. Several studies have been conducted which showed relation between CSF sodium, potassium ions and postmortem interval^{3,7}. The focus of this study is to estimate the postmortem interval from the chloride ion concentration in the cerebrospinal fluid due to its great evidential value in the medicolegal investigation of death. To the best of my knowledge no study has been conducted on this topic in our scenario. We need to conduct a study on this topic to establish the role of CSF in postmortem interval estimation in our setting.

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MATERIALS AND METHODS

Out of the Medicolegal autopsies conducted at the mortuary of Forensic Medicine Department, King Edward Medical University (KEMU), Lahore only those cases were included in which the exact time of death was given by the attending physician who issued Death Certificate, by the law enforcement agencies or near relatives. It was an Observational study. Study duration was 6 months. Unknown and putrefied dead bodies, Poisoning cases and Cases with head injuries were not included. A total number of consecutive 106 samples fulfilling the above mentioned inclusion criteria were taken.

Sampling started from 10th December 2013 and continued up to 20th June 2014. These samples were collected by opening the cranial cavity first, from Rt. or Lt. lateral ventricle of the brain. Liver biopsy needle was attached with 20 mlsyringe and then inserted in the posterior and dependent part of the lateral ventricle of brain up to 1.5 cm depth and as much of the CSF was aspirated as possible.^{9,11} Samples were immediately transferred to the pathology lab, centrifuged and analyzed for chloride ions.

On average, approximately 5-6 ml of CSF was obtained from each subject. Only clear, transparent samples were used. A detailed Proforma was used for each case to fill relevant information. These samples were analyzed for chloride levels were expressed in mEq/l. Before analysis, the samples were centrifuged at speed of 3000 revolutions per minute for 5 minutes. Autoanalyzer, manufactured by Beckman Coulter Centurion Scientific Limited, Model No. CX 5.PRO Synchron/clinical system, made in Ireland was used for the analysis.

RESULTS

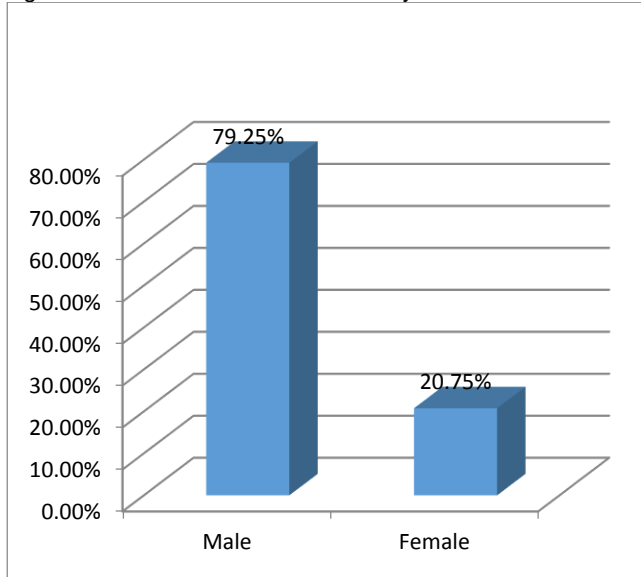
The data analysis was done by using SPSS v-21 (SPSS-Inc, Chicago, USA). Regression and correlation analysis was applied. P-value ≤ 0.05 was considered as statistically significant. Mean, SD, frequencies and percentages were calculated. Tables and graphs were used to present the results. The scatter plots were utilized to show the

relationship between PMI and chloride levels (Mini-tab was used to draw scatter plot). The linear regression equations were obtained from Cl⁻ levels verses time since death. Finally, t-test was used to determine if any two data sets are significantly different or not.

In this study data of 106 cases was obtained. The mean PMI in hours was 12.43±4.44 with range of 5 to 23.45 hours. The mean chloride was 79.11±30.79 with range of 12 to 176mEq/L. The mean age was 32.5±9.69 with range of 12 to 61 years.

Data analysis showed that the majority of cases were males 79.25% (84 cases) and females were only 20.75% (22 cases). Gender distribution of the 106 cases used in this study is shown in the figure 1.

Fig. 1: Gender Distribution of the study cases



The average age of male cases was 33.81 years and female average age was 27.50 years. The average postmortem interval (PMI) for the male cases was 12.74 hours; whereas for the female cases it was 11.23 hours. The average chloride level (Cl⁻) for the male cases was 78.98 mEq/l; whereas for the female was 79.59 mEq/l. The mean level of these variables in male and female cases and standard deviation is given in the table 1.

Table 1: Different variables in male and female cases

| Distribution of Cases based on Gender | | | |
|---------------------------------------|-------------|-------------|--------------|
| Variable | Mean ± SD | | P Value |
| | Male(84) | Female(22) | |
| Age (Years) | 33.81±9.044 | 27.50±10.64 | 0.060 |
| PMI (Hours) | 12.74±4.67 | 11.23±3.25 | 0.156 |
| Cl ⁻ (mEq/l) | 78.98±29.44 | 79.59±36.27 | 0.935 |
| Comparison | t value | p value | Significance |
| Male & Female for Cl ⁻ | 0.07 | p>0.05 | NS |

Table 2 reveals that Firearm Injuries are the predominant (75.47%) cause of death. Then Asphyxia (16.04%), followed by Blunt Weapon Injury (4.72%), and Sharp Edge Weapon Injury (3.75%).

Table 2: Different causes of death in the studied cases

| Cause of Death | n | %age |
|---------------------------------|----|--------|
| Firearm Injury (FI) | 80 | 75.47 |
| Blunt Weapon Injury (BWI) | 5 | 4.72 |
| Asphyxial Death (AD) | 17 | 16.04% |
| Sharp Edge Weapon Injury (SEWI) | 4 | 3.78% |

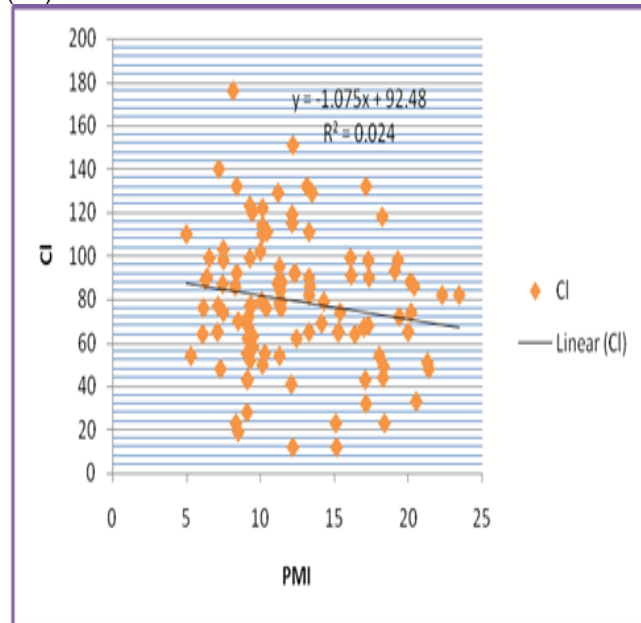
The present study indicates that Cl⁻ level is slightly higher in the sharp edge weapon cases. The t-test was applied again to evaluate the effect on Chloride (Cl⁻) levels due to different causes of death. The chloride level was not dependent on the cause of death. There is only a significant effect on the Chloride levels when making a comparison of death cases due to the sharp edge weapon injuries against the firearm injuries and asphyxia.

Linear Regression Method is again applied for the estimation of PMI (in Hours); whereas, chloride (Cl⁻) levels are used as the known variable. Estimated regression equation showing relationship between PMI and chlorine (Cl⁻) level is given below:

$$PMI(Y) = 92.487 - 1.0755Cl^-(X)$$

Similarly, the scatter plot indicating the relationship between the PMI (in hours) and chloride (Cl⁻) level is shown in the figure 2.

Fig. 2: Regression Model for PMI Estimation using chloride (Cl⁻) level



The equations developed by the Linear Regression Method and R² are shown in Table 3.

Table 3: Regression equations with different electrolytes

| Electrolytes | Cl |
|----------------------|-------------------------|
| Predicted equation | PMI(Y)= -1.0755X+92.487 |
| Correlation with PMI | -0.155 |
| | 0.112 |
| R ² | 0.02 |
| SE of estimate | 30.56 |

DISCUSSION

During criminal investigation of death, the authorized medical officer (working in the ministry of health) has to conduct autopsy. He has to give the postmortem interval and cause of the death. It has always been a challenge for the authorized medical officers to determine the exact time since death. Many complex intrinsic and extrinsic morphological and chemical postmortem changes start in the cadaver immediately after death. At cellular level the earliest phase of decomposition starts which later progresses to gross and obvious changes. These biochemical changes are found to be most useful during this time interval between the cellular death and later decomposition stages. The chemical changes start and progress in the body fluids in an orderly manner^{8,10,12,13}

Firearm Injuries (FAI) were the major cause of death followed by Asphyxia, Blunt Weapon Injury (BWI), and Sharp Edge Weapon Injury (SEWI). Cl⁻ level was slightly higher in the death cases due to the sharp edge weapon injuries and in female cases as compared with male cases. Slope of the regression line for the postmortem CSF chloride was 1.0755mmol/L per hour.

It is essential for the regression slope line to be relatively steeper as the flatter slope lines tend to overestimate the postmortem interval which is based upon the obtained regression line¹⁴.

Regression Equations: The linear regression formulae for PMI estimation are derived by the equation as given below:

$$Y = a + bX$$

Where Y is dependent variable which is PMI in hours and X is electrolyte which is independent variable. "a" is intercept of the regression line also called slope of the regression line and "b" is the regression coefficient. These constants have been estimated from data and substituted within the equation to predict the value of dependent variable. In this method, time is considered as a dependent variable and it is never considered as an independent variable. This is ordinary or direct regression equation. For postmortem CSF chloride concentration the regression equation is;

$$PMI (Y) = 92.487 - 1.0755 Cl^- (X)$$

In this equation, PMI in hours can be calculated by using CSF chloride ion concentration which falls down and rise respectively with the passage of time. In this equation 'a' (intercept of the regression line) is 92.487 and 'b' (the regression coefficient) is -1.0755. Value of R² (coefficient of determination) is 0.0241 and SE (standard error) is 30.56. In general, the higher the R-squared, means the model fits your data better. The standard error and the R² value of

Chloride indicates that it is not better indicator of time since death.

CONCLUSION

The results of this study indicated that the level of postmortem CSF chloride value is not useful in PMI estimation.

REFERENCES

1. Saukko P, Knight B. Knight's Forensic Pathology. Third ed. London, United Kingdom: Edward Arnold (Publishers) Ltd.; 2004. P. 216-20
2. Jashnani KD, Kale SA, Rupani AB. Vitreous humor: biochemical constituents in estimation of postmortem interval. *Journal of Forensic Science*. 2010;55(6):1523-27.
3. Yadav J, Deshpande A, Arora A, Athawal BK, Dubey BP. Estimation of time since death from CSF electrolyte concentration in Bhopal region of central India. *Legal Medicine*. 2007;9(6):309-13.
4. Awan NR. Principles and Practice of Forensic Medicine and Toxicology. 1st ed. Lahore, Pakistan: zubair Pakistan; 2004.
5. Saugstad OD, Olaisen B. Postmortem hypoxanthine levels in the vitreous humour, an introductory report. *Forensic Science International*. 1978;12:33-6.
6. Tumram NK, Bardale RV, Dongre AP. Postmortem analysis of synovial fluid and vitreous humour for determination of death interval: A comparative study. *Forensic Science International*. 2011;204(1):186-90.
7. Singh D, Prashad R, Parkash C, Bansal YS, Sharma SK, Pandey AN. Linearization of the relationship between serum sodium, potassium concentration, their ratio and time since death in Chandigarh zone of north-west India. *Forensic Science International*. 2002;130(1):1-7.
8. Coe JI. Postmortem chemistries on human vitreous humor. *American Journal of Clinical Pathology*. 1969;51:741-50.
9. Lie JT. Changes of potassium concentration in the vitreous humor after death. *Journal of Medical Science*. 1967;254:136-42.
10. Sturner WQ, Gantner GE. The postmortem interval: a study of potassium in the vitreous humor. *American Journal of Clinical Pathology*. 1964;42:137-44.
11. Coe JI, Apple FS. Variations in vitreous humor chemical values as a result of instrumentation. *Journal of Forensic Science*. 1985;30:828-35.
12. Sturner WQ. The vitreous humour: postmortem potassium changes. *The Lancet*. 1963;281(7285):807-8.
13. Madea B, Henssge C, Hanig W, Gerbracht A. References for determining the time of death by potassium in vitreous humor. *Forensic Science International*. 1989;40(3):231-43.
14. Akhtar N. Postmortem Interval Estimation by Sturner's Equation based upon Potassium Level in Vitreous Humor. Lahore: King Edward Medical University, Lahore; 2014;9(1):88-92.