CORRELATION OF ACTUAL TIME SINCE DEATH WITH ESTIMATED TIME OF DEATH BY MEASURING PROTEINS CONCENTRATION IN CADAVERIC CEREBROSPINAL FLUID

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Abstract

Background & Objectives: Postmortem Interval (PMI) is the time interval from death upto conducting autopsy of deceased. PMI is an overbearing perspective of medical jurisprudence used to support beholders claim, corroborate the potential in provided proof and serve as evidence for further action. The objective of this study is to estimate the time since death by measuring proteins concentration in cadaveric cerebrospinal fluid.

Methods: This cross-sectional study conducted at Forensic Medicine and Toxicology Department of Allama Iqbal Medical College, Lahore for 1 year from January 2022 to January 2023 in 50 dead bodies included through non probability consecutive sampling. After informed consent, CSF sample was taken by using lumbar puncture technique and quantitative values of proteins were obtained automatically through auto-biochemical analyzer. Data were analyzed through SPSS version 25. The relation between time of death and time estimated on CSF proteins was measured by calculating Pearson's correlation coefficient. P-value ≤0.05 was taken as statistically significant.

Results: Out of 50 bodies, 25 (50%) were male bodies and 25 (50%) were female bodies. The female to male ratio was 1: 1. Mean actual time of death was 73.46 ± 33.82 hours, while the estimated time of death on CSF protein assessment was 71.54 ± 31.92 hours. The mean CSF fluid level was observed was 170.26 ± 88.61 mm^3. A significantly strong positive correlation was observed between time estimated by using protein level in CSF fluid and actual time of death i.e. r =0.952 (p-value <0.0001).

Conclusion: CSF proteins have good correlation value for postmortem interval. It can be beneficial in estimating time of death in unknown bodies.

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To decide the time of death, the expertise of a forensic pathologist is needed to assist in investigating the death of a deceased individual. This support from a forensic expert not only helps to establish a time frame for the investigation but also helps to narrow down the pool of potential suspects in cases of homicide. Time since death also provides valuable information to establish timeline of proceedings leading to death.[1] It is difficult to assess time of death due to multitude of variables affecting accurate calculation. Various authors have conducted research on qualitative and quantitative changes in the expression of protein in the biological samples of humans and animals over specific time periods, in post-mortem study. Nevertheless, literature on this topic is extensive and often lacks consistency, with variations in proteins, tissues, and models being

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appraised. Consequently, in practical, the application of these methods is restricted to-date.[2]

Forensic medicine faces a challenging task of accurately estimating time of death due to the imprecise nature of commonly used methods for determining the postmortem interval. In recent decades, biochemical methods have been introduced to enhance the precision of postmortem interval estimation using postmortem samples. The focus of studies has been on the bio-chemical profiles of body fluids in close compartments as they undergo limited postmortem chemical changes compared to blood. CSF has been identified as a suitable fluid for investigating these changes due to its abundance and ease of sampling.[3]

Accurately estimating the postmortem interval is essential for successful forensic investigation. Various methods have been employed to determine the postmortem interval, including assessment of physical changes occurring after death such as livor mortis, rigor mortis and others.[4] It is still a significant challenge to estimate the postmortem interval, and the current approaches used to determine it often result in broad postmortem intervals.[5] Postmortem changes can ominously affect and modify bio-chemical constituents of body fluids particularly blood leading to controversial results. As a result, studies have shifted their focus towards other body fluids that are present in confined compartments and are less prone to contamination immediately following death. Vitreous humor, CSF, pericardial fluid and synovial fluid are being studied now a days.[6]

CSF is an ultra-filtrate of plasma hence it has small percentage of proteins and small quantity of blood cells which are mainly WBC. In electrolyte concentrations, sodium ions are equal in proportion as in plasma and chloride and magnesium ions are in a bit larger concentrations.[7] Quantity of CSF is almost 150 ml in which 0.3 or 15-45 mg/dl are proteins or even lower in case of children. Protein concentration in CSF also depends on site from where the sample has been taken. Normally protein concertation is lower in cisternal and ventricular area but a bit higher in lumbar region.[8] In a study, the coefficient between actual time of death and estimated time of death by using CSF proteins was noted as 0.1935 (correlation coefficient (r) = 0.44, p = 0.001 9.

Determining correlation among postmortem interval and estimated time of death by using CSF proteins estimation was the rationale of this study. Literature showed that CSF proteins have good correlation value for postmortem interval. It can be beneficial in estimating time of death in unknown bodies. But limited work has been done in this regard and no local study done before. Therefore, we planned this study to get evidence in local population correlating CSF protein levels with PMI in local setting. Time since death assessment has been requisite in investigation of forensic sciences and it needs multiple techniques and methods so that suitable methodology can be applied in any situation accordingly. Thus this study was conducted with an objective to determine the correlation between cerebrospinal protein levels in cadaveric CSF and postmortem interval.

METHODS

It was cross sectional study conducted at Forensic Medicine and Toxicology Department of, Allama Iqbal Medical College, Lahore for a period of one year i.e. 1-1-2022 to 1-1-2023. The sample size was 50 cases with 5% type I error, 10% type II error and correlation coefficient value i.e. r= 0.44 between actual time and estimated time of death on CSF protein.[9] The non-probability, consecutive sampling technique was used. Dead bodies of 5-80 years of either sex received within 1-5 days of death for autopsy with known time since death were included in the study while putrefied dead bodies, bodies with head trauma and known brain diseases were excluded.

After obtaining approval from ERB of institution, fifty dead bodies from morgue section of Forensic Medicine department were obtained after informed consent. Demographic information like age, gender, duration / time of death, cause of death were noted. CSF sample were taken by using lumbar puncture technique from space between L3-L4 and L4-L5 by using 20 gauge lumber puncture needle. Disodium molybdate, pyrogallol and succinic acid were used. The dye bin-

ding method using pyrogallol red was employed for calculation of minimum detectable concentration (0.022 g/l) of total proteins in CSF. Semi-auto biochemical analyzer was used which worked on the principle of spectrophotometry. Quantitative values of proteins were obtained automatically through auto-biochemical analyzer and then were assessed for CSF proteins. Findings were recorded and correlated with postmortem interval according to police record. Data were analyzed by using SPSS version 25. Pearson's correlation coefficient was calculated to measure relation between time of death and time estimated on basis of CSF proteins. P-value ≤0.05 was taken as statistically significant.

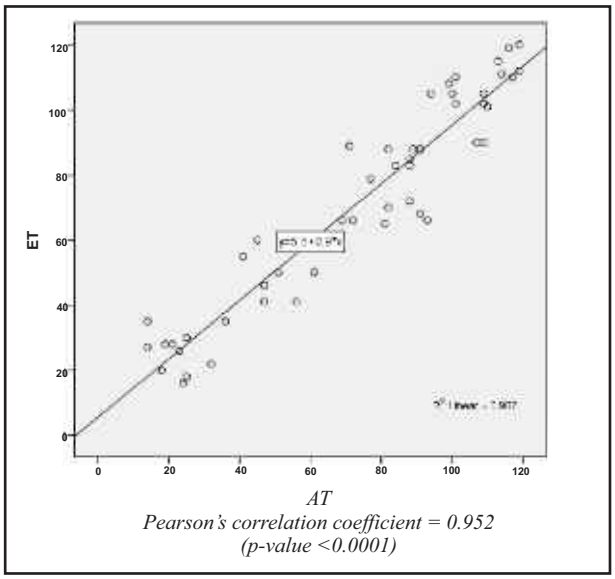
RESULTS

The mean age of dead bodies at time of death was 45.14 ± 13.25 years. There were 25 (50%) male bodies and 25 (50%) female bodies. The female to male ratio was 1: 1. Diabetes was positive among 31 (62%) cases, hypertension was reported in 39 (78%) cases, and smoking in 24(48%) cases and cardiovascular diseases were present in 32 (64%) cases. The major cause of death was natural that was observed in 31 (62%) cases, followed by accident in 12(24%), suicide in 4 (8%) and maternal mortality in 3 (6%) cases. The mean actual time of death was 73.46 ± 33.82 hours, while the estimated time of death on CSF fluid was 71.54 ± 31.92 hours. The mean CSF fluid level observed was 170.26 ± 88.61 mm3. Out of 50 autopsies, 7 (14%) bodes received within 24 hours, 8 (16%) received on 2nd day, 6 (12%) received on 3rd day, 14 (28%) on 4th day and 15 (30%) on 5th day of death. (Table I)

Table 1: Profile of Bodies Examined (n=50)

Feature	Mean ± SD, F (%)
Age (in years)	**45.14 ± 13.25**
Gender	
Male	25 (50%)
Female	25 (50%)
Co-Morbid conditions	
Diabetes	31 (62%)
Hypertension	39 (78%)
Smoking	24 (48%)
Cardiovascular disease	32 (64%)
Cause of death	
Suicide	4 (8%)
Natural death	31 (62%)
Accident	12 (24%)
Maternal mortality	3 (6%)
Actual time of death (hours)	**73.46 ± 33.82**
CSF protein level	**170.26 ± 88.61**
Estimated time of death (hours)	**71.54 ± 31.92**
Time interval between death and receiving of body for autopsies	
Within 24 hours	7 (14%)
After 2 days	8 (16%)
After 3 days	6 (12%)
After 4 days	14 (28%)
After 5 days	15 (30%)

A significantly strong positive correlation observed between time estimated by using protein level in CSF fluid and actual time of death i.e. r = 0.952 (p-value < 0.0001). Figure 1



Pearson's correlation coefficient = 0.952
(p-value <0.0001)

Figure 1: *Correlation between actual and estimated time of death*

DISCUSSION

Estimating the postmortem interval (PMI) in forensic pathology poses a significant challenge. Typically, the calculation is carried out by comparing various parameters, including cadaveric rigidity, body temperature, and hypostasis.[10] Furthermore, the diagnosis is supported by analyzing metamorphic phenomena of the cadaver, postmortem ocular changes, and circumstantial information.[11] The exactitude of estimated postmortem interval (PMI) based on these parameters is

dependent on the time that has passed since death. This means that as time goes on, the calculated time range becomes less precise and more approximate.[2] Vitreous humor is the most commonly used body fluid for this purpose. The renewed literature exists on the study of inquest biochemical analysis of blood and other body fluids.

For many years, postmortem examination of cerebrospinal fluid (CSF) has been conducted. Specific markers in CSF have been studied to diagnose neurodegenerative diseases like Alzheimer's disease and such studies have proved useful. One such marker, Tau protein, is found in patients of Alzheimer's disease. It is studied in CSF along with other components involved in metabolic processes of bio-chemical interest.[12-14] PMI is accessed by various available methods but precise and reliable estimation is still fly-by-night.[15] Studies with larger human sample size and standardized protocols are still required.[16] A valuable and significant parameter for estimation of PMI is CSF albumin.[17]

In this study, we observed that there is a strong positive correlation between time estimated since death by using protein level in CSF fluid and actual time of death i.e.($r = 0.952$) (p-value < 0.0001). In a study, similar findings were reported and regression coefficient of 0.991 was noted.[9] In a study, the regression coefficient between actual time of death and estimated time of death by using CSF proteins was noted as $r^2 = 0.1935$ (correlation coefficient (r) = 0.44, p=0.001[9].

CSF, owing to protected anatomical location suffers little change in early postmortem phase, and is a relatively stable fluid. Although numerous immunehistochemical markers used for clinical diagnostic purposes in tissue biopsy samples can now also be applied to postmortem tissues. There has been no systematic immune-cyto-chemical investigation of postmortem body fluids and for CSF in particular. Such investigations are not established at all. CSF should be examined for a more detailed categorization of the processes in CNS as it directly surrounds the brain. Comparison of traumatized tissue and CSF can provide valuable information for forensic assessment and supplement neuropathological evaluation. It can provide additional evidence for diagnosis and understanding of traumatic brain injury. This information can be crucial in forensic investigation and can aid in determining manner and cause of death.[18]

The concentration of protein in CSF changes not only with advancing age but also to smaller extent depending on the site of sampling. The protein concentration is lowest in CSF of ventricles, intermediary in cisterna magna, and highest in CSF of lumbar region. The difference in protein concentration among cisterna magna and lumbar region is approximately 0.1 g/L.[19] Tau proteins, present in CSF, are well established biomarkers of neuro-degeneration and neuronal damage. It is suggested that Tau proteins can increase in postmortem period due to death of neurons and can serve as potential biomarker of time after death.[20] The dispassion of leptomeningeal lining cells causes rise of mononuclear cells in CSF during first 24 hours of death.[19]

CONCLUSION

CSF protein levels have been found to have a strong correlation with the postmortem interval, and thus have the potential to be employed as biomarker for estimation of time since death with greater precision and accuracy than other methods. It requires fewer techniques, the process is relatively simple with no complications, and the reagents required are readily available and cost-effective. Therefore, if validated by further studies, the use of CSF protein levels as a biomarker for estimating the postmortem interval could be a significant advancement in forensic science.

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