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Estimation of Time since Death by Potassium Ion (K^+) Level in Cerebrospinal Fluid – A Postmortem Study

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Introduction: Estimation of time since death has always been a point of research with obvious and important medico legal implications. The physical changes which occur after death e.g. cooling of the body, rigor mortis, development of lividity etc., have been known since earliest days of human observation and still continues to be the main basis of estimation of time since death. However, most physical changes in cadaver are not subject to precise measurement and are modified by many factors which tend to introduce considerable errors. Post-mortem chemical measurements have been introduced in Forensic medicine to assist in more precise estimation of the time since death and to demonstrate biochemical abnormalities responsible for death. Emphasis has been placed on the examination of body fluids that are neither altered nor contaminated as rapidly as blood after death and in which the changes taking place in the serum are also well reflected.[1] Many chemical changes begin to take place in the body immediately or shortly after death and progress in a fairly orderly fashion until the body disintegrates. Each change has its own time factor or rate. These changes occur in various body fluids i.e. blood, spinal fluid and vitreous humor of eye. Thus determination of the chemical abnormalities could help forensic pathologists to ascertain time since death more precisely.[2] For investigations of crime, it is very important to determine 'time since death' that is the interval between death and the time of postmortem examination also called as postmortem interval. It provides a clue to the investigating officer to institute suitable enquiries to apprehend the persons likely to be responsible for the crime and to eliminate the innocent ones.[3] In civil cases also the matter concerning transfer of estate or property may depend upon the time of death.[4] The accurate determination of the time of an unwitnessed death is one of the most vital and yet one of the most difficult problems in medico-legal autopsy casework, sometimes a more precise moment of death becomes necessary. Estimation of time since death by (K^+) is interpreted in legal context. The time of death is sometimes extremely important. It is a question almost invariably asked by police officers, sometimes with a touching faith in the accuracy of the estimate. But

determination of accurate time of death is extremely difficult as timings of onset and the rates of postmortem changes are usually governed by unpredictable endogenous and exogenous factors.[4] various body fluids which are available for the chemical examination are whole blood, serum, cerebrospinal fluid (CSF), aqueous humor and vitreous humor, pericardial fluid etc. The biochemical variation of potassium ion (K^+) among various body fluids is somewhat reliable in estimating time since death. It has been shown that linear relationship of increase in potassium ion (K^+) concentration of CSF to the lengthening postmortem interval is both arithmetic and logarithmic.

Aims & Objectives

1. To find out the Post mortem CSF potassium levels until 7 days of death.

2. To find out the correlation between post mortem CSF level times since death.

Key words: Post-mortem interval, cerebrospinal fluid, potassium ion, lumber puncture.

Review of Literature

There is about 125-150 mL of CSF at any one time.[8] This CSF circulates within the ventricular system of the brain. The ventricles are a series of cavities filled with CSF. The majority of CSF is produced from within the two lateral ventricles.

Substance	Presence in CSF
Water Content (%)	99
Protein (mg/dL)	35
Glucose (mg/dL)	60
Osmolarity (mOsm/L)	295
Sodium (mEq/L)	138
Potassium (mEq/L)	2.8
Calcium (mEq/L)	2.1
Magnesium (mEq/L)	2.0–2.5
Chloride (mEq/L)	119
pH	7.33

CSF usually obtained by a procedure called lumbar puncture.[15] Lumbar puncture is carried out under sterile conditions by inserting a needle into the subarachnoid space, usually between the third and fourth lumbar vertebrae. CSF is extracted through the needle, and tested. In the year 2007 Yadav J et al[18] was conducted a study on estimation of time since death from sodium and potassium ion concentration levels in CSF (cerebrospinal fluid). The study was carried out in 100 medico legal autopsies with known time of death in the department of Forensic medicine, Gandhi Medical College in Bhopal region of Central India. CSF was aspirated from lateral ventricles after opening the skull and dura, and concentration of these ions were estimated by flame photometry. Results revealed a significant correlation of sodium and potassium ions in CSF up to 25 h of time since death, with average per hour rise of 1.21 meq/h for potassium and fall of 1.115 meq/h for sodium ions. A useful relationship between sodium potassium ion ratio and PMI (postmortem interval) was also elicited. The study concludes that changes in CSF electrolyte is a significant parameter to estimate time since death. Nidhi Sachdeva et al (2011)[11] was observed that various authors have found the co-relation of vitreous humour Na^+, K^+ & $Ca2^+$ concentration, with increasing post-mortem interval. Some authors have used flame photometry method, while others have used Beckman coulter analyser method. Some authors have found changes in Vitreous Na^+, K^+ & $Ca2^+$ concentration, between the two eyes of the study subjects and gender variation was also noticed. But still various lacunae exist in the present knowledge because of which estimation of post-mortem interval from vitreous biochemistry is not being

practically used till date. So this topic needs further research to bring it in to routine use. Rajanikanta Swain et al (2013)[13] was conducted a study in Department of Forensic Medicine and Toxicology, All India Institute of Medical Sciences, New Delhi, 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. They found that.

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 (r¼ 0.62) was stronger than CSF (r¼ 0.37). The regression equation was established in the study group and applied in the validated group to calculate estimated PMI. They conclude 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. Priyanka Vaitla et al (2017)[12] conducted a study on 50 cases brought to the mortuary at Osmania general hospital, Hyderabad formed the material for collection of vitreous humor. Information regarding time of death was gathered. Cases where exact time of death was not known were not included in the study. Analysis was done immediately after the vitreous humor was aspirated. Samples for vitreous humour potassium were analysed by Ion selective method. Cases were divided into five groups according to time since death and analysed statistically. The postmortem interval varied from 5.15 to 72.45hrs.Vitreous humor potassium levels were significantly increased with increased time since death. There was a linear rise in potassium concentration with increasing time since death and this increase in the level was independent of the factors like age and sex. They conclude that linear relationship between vitreous potassium and time since death. After death, the Na^+-K^+ pump does not operate, therefore K^+ is leaked out of the retinal cells, leading to high postmortem levels of potassium. Therefore, Vitreous humor potassium levels can be a good method of determining time since death along with other traditional methods. Sharma M et al (2017)[14] was conducted a study in Jhalawar Medical College, Jhalawar. 110 samples were collected in the mortuary of the Forensic Medicine Department & Biochemical analysis was done in the Department of Biochemistry, using Beckman Coulter automated analyser while vitreous ascorbic acid was estimated manually. They observed that vitreous potassium increases with time since death, while vitreous sodium, ascorbic acid and alkaline phosphatase do not have significant correlation with time since death. J. O. Idemudia et al (2017)[7] was observed that statistically significant difference between the vitreous fluid potassium and bicarbonate levels of the cadavers that died within 48 hours and those that died after 48 hours

(P-value 0.028 and 0.001 respectively) but there was no significant difference between the vitreous fluid sodium and chloride of cadavers that died within 48 hours and those that died after 48 hours (P-value 0.369 and 0.100 respectively). There was a positive correlation coefficient between potassium and PMI (r=-0.404) and negative correlation coefficient between bicarbonate and PMI (r= -0.339) within 48 hours after death. Same study on vitreous fluid.

Material and Methods

With this expectation and limitation of the available analytical instruments the quantitative study of potassium (K^+) concentration in the C.S.F from dead bodies received for post-mortem examination in the Department of Forensic Medicine in MG and MDM Hospital at Dr. S.N. Medical College, Jodhpur was undertaken. A total of 100 samples have been collected at various known and unknown time interval after death for quantitative K^+ ions concentration to find out the simple and accurate method for estimation of time since death within reasonable limits, during the period of one year. In Mortuary Room, when dead body comes after death, we checked time of death and duration either admitted or brought dead body. Maximum time taken is7 days old death time duration. Then we excluded cases of– spinal Injury, meningitis, decomposed body, serum become haemorrhagic, cases in which the extracted sample is turbid/cloudy/yellow. Now we checked admission ticket , gone through it and find out serum K^+ Level. We also checked if patient got any I/V fluid containing K^+ . If patient took any drugs which are containing K^+. Then we examined the brought dead body through getting in lateral position and giving as much flexion as possible, the lumber puncture needle is inserted in L3-L4 lumber space and collect 2-3ml CSF fluid & take a plastic container & sealed it, write down over plastic container deceased name, cause of death duration of death and date of collecting CSF. We sent it to Biochemistry Lab in SNMC Jodhpur for K^+ Level estimation.

Sample size was calculated in 50 subjects of IPD dead body To assess and expected correlation coefficient of 0.225 between potassium level increased and potassium level decrease (As per reference article). Sample size was calculated at alpha error 0.05 and study power 80%. Sample size was calculated using the below formula for correlation coefficient:-

$$N = \frac{(Z\alpha_2 = Z1-\beta)^2}{\frac{1}{4}\log_e\left(\frac{1+r}{1-r}\right)} + 3$$

Where,

N= Sample size,

($Z\alpha_2$) = Standard normal deviate for Type 1 error (taken as 1.96 for 95% confidence interval)

Z1-β = Standard normal deviate for Type 2 error (taken as 0.84 for 80% study power) r = correlation coefficient (taken as 0.225 as per reference article) This sample size is also adequate to assess correlation coefficient of 0.228 between Increase K^+ level and brought decreased K^+ level. (as per reference article) in brought dead body. Hence for this study, a total of 100 subjects (50 IPD dead body and 50 brought dead body) were taken. After taking the valid consent from legal heirs, 100 medico- legal cases with known time of death were studied between ages of 15-70 years. The serum Potassium ions levels were assumed to be normal at the time of death based on hospital records in the hospitalised cases and the history in the non-hospitalised cases.

Instrument Required

Lumber puncture needle no. 16 and 18 gauge, plastic sterile vial for specimen collection, centrifuge machine, apron, facemask, gloves etc.

Inclusion Criteria

1. All the cases which are admit in MDM & MG Hospital, where the time of death was known, death within 7 days.
2. All brought dead cases come in MG & MDM Hospital Mortuary Room, in which time is given by relatives.

Exclusion Criteria

1. All the case where the time of death was unknown (in some brought dead cases) or body was in advanced state of decomposition.
2. Cases of head injuries , vertebral column and spinal cord injuries.
3. Cases in which due to any reason the extracted sample is contaminated with blood or became haemorrhagic.
4. Cases in which the extracted sample is turbid/cloudy/yellow.

Observations and Results

Table 1: Age wise distribution

Age Group (Yrs)	No. of Cases	Percentage (%)
< 20	8	8
21–30	30	30
31–40	24	24
41–50	16	16
51–60	13	13
61–100	9	9
Total	100	100

Table 2: Sex wise distribution

Sex	No. of Cases	Percentage (%)
Male	75	75
Female	25	25
Total	100	100

Table 3: Cause of death

Cause of Death	No. of Cases	Percentage
RTA	30	30
Poisoning	19	19
Hanging	13	13
Drowning	4	4
Flame Burns	7	7
Electric Burn	3	3
Assault With Head Injury	7	7
Cardiac Arrest	9	9
Tuberculosis	4	4
Snake Bite	2	2
CVA	1	1
ALD	1	1
Total	100	100

Table 4: Times since death

Time (Hours)	No. of Cases	Percentage
0-12	52	52%
13-24	35	35%
25-36	3	3%
37-48	6	6%
49-72	4	4%
Total	100	100%

Table 5: K^+ Level

	K^+ Level
Mean	6.79
SD	1.53

Mean K^+ level was 6.79±1.53 mEq/L.

Table 6: Association K^+ level and times since death

Time	No. of Cases	K^+ Level	
		Mean	SD
0-12	52	5.97	0.51
13-24	35	8.13	0.27
25-36	3	11.34	0.42
37-48	6	11.36	0
49-72	4	12.58	0
p-value	0.001		

Table 7: Correlation between the time since death and K^+ level

	Mean	Std. Deviation	R	R Square	Sig. F Change
Time since death (hours)	11.08	2.53	0.81	0.64	0.000
K^+ level (meq/l)	6.79	1.53			

A significant inverse fair correlation existed between the time since death and K^+ level ($r = 0.81$, $p < 0.001$) by using Pearson's correlation coefficient.

Discussion

In our study out of 100 cases, 30% cases were 21–30 yrs, cases less than 20 yrs were 8%, 13% cases were 51-60 yrs age group followed by 16% cases were 41-50 yrs age group, 24% cases were 31-40 yrs age group and 9% cases were more than 60 yrs age group. These findings are almost consistent with most of the studies[13].These age groups are more productive and hence more vulnerable to road traffic accidents, occupational hazards, suicidal tendency and homicidal activity in country like India Umang P Patel et al[16] was found that there were 200 subjects, out of these maximum subjects were from 26-35 years age group.In our study out of 100 cases, 75% cases were male and 25% cases were female. As males are predominantly more active in doing outside works so more prone to unnatural deaths, that's why in the present study male subjects were higher in number than female. These findings were also consistent with studies of other Authors. Mohit kumar[9] was also found that male cases were more than female. Umang P Patel et al[16] was found that predominantly male gender. Out of 100 cases, 30% death were due to RTA followed by 19% death were due to poisoning, 4 death were due to drowning, 7% death were due to flame burns and 9% death were due to cardiac death, 3% for electric burn, 7% Assault with head injury, 4% Tuberculosis, hanging 13%, Snake bite 2%, CVA 2%, ALD 1% in our study. Due to the fast pace of modernization, basic needs including the requirement of a vehicle for transportation are expanding rapidly and resulting in an epidemic situation of injury everywhere including developing countries. According to the World Health Report 2016, of the global burden of injury, 30.3% morbidity and 28.7% mortality occurred in the South-East Asia Region. Road traffic injuries are among the three leading causes of death for people between 5 and 44 years of age and most common cause of death for people between 5 to 25 years of age.[17] In 52.00 % cases sample was collected within 12 hours after death, in 35 % cases within 13-24 hours, in 3% cases within 25-36 hours in our study. Umang P Patel et al[16] was found that most cases samples were collected within 12-24 hours of death followed by 0-12 hours and 24-36 hours of death. And few samples were collected with more than 48 hours post mortem interval. In our study mean

K^+ level 5.97±0.51 mEq/L was found in within 12 hours duration cases followed by 8.13±0.27 mEq/L was found in within 13-24 hours duration cases, 11.34±0.42 mEq/L was found in within 25-36 hours duration cases, 11.36±0.0 mEq/L was found in within 37-48 hours duration cases and 12.58±0.0 mEq/L was found in within 49-72 hours duration cases. Correlation coefficient (r) between time since death and k Level was found 0.81.It's positive significant correlation. Adelson et al[5] used 349 samples from 269 cases. No significant difference was noted in the K^+ levels of two eyes as determined by flame photometry. Mulla A et al[10] hypothesised in his study that the concentration of vitreous biochemical constituents in the same pair of eyes change at the same rate and this change that occurs in a time dependent fashion may be utilized in accurately estimating the post mortem interval. There was a linear rise of potassium values ranging from 7.04 mEq/L to 15.81 meq/, which is comparable to the values reported by Govekar G[6]. In his study, case of the lower value of potassium was reported to be 3.56 mEq/L, wherein in our study we have found higher levels of potassium i.e., 6.79±1.53 mEq/L.

Summary And Conclusions

Out of 100 cases, 30% cases were 21-30 yrs age group, 13% cases were 51-60 yrs age group followed by 16% cases were 41-50 yrs age group, 24% cases were 31-40 yrs age group and 09% cases were more than 60 yrs age group. Out of 100 cases, 75% cases were male and 25% cases were female. Out of 100 cases, 30 death were due to RTA followed by 19% death were due to poisoning, 13% were due to hanging 4 death were due to drowning, 7% death were due to flame burns, 3% were due to electric burn, 7% death were due to Assault with head injury, 9% were death due to cardiac arrest, 4% were death due to Tuberculosis, 2% were death due to snake bite, 2% were death due to CVA and 1% was death due to ALD. In 52% cases sample was collected within 12 hours after death, in 35% cases within 13-24 hours, in 3% cases within 25-36 hours. Mean K^+ level was 6.79±1.53 mEq/L. Mean K^+ level 5.97±0.51 was found in within 12 hours duration cases followed by 8.13±0.27 was found in within 1324 hours duration cases, 11.34±0.42 was found in within 25-36 hours duration cases, 11.36±0.0 was found in within 37-48 hours duration cases and 12.58±0.0was found in within 49-72 hours duration cases. Correlation coefficient (r) between time since death and K^+ Level was found 0.81. It's positive significant correlation. This is dissertation "Estimation of time since death by potassium ION (K^+) level in cerebrospinal fluid–a postmortem study" is showing K^+ level increase in CSF of dead bodies as time since death increasing.

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