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Correlation between Vitreous Electrolytes and Time since Death: An Autopsy Based Study

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Abstract

Context: Estimation of time since death has always remained an important requirement in medico legal as well as civil cases. In last few years researchers have studied various chemical tests to estimate the time since death. Body fluids which are available for such chemical examination are whole blood, serum, cerebrospinal fluid, aqueous humour and vitreous humour. Amongst all these available body fluids, the vitreous humour has been largely utilized and vitreous potassium concentration has become most widely used method to predict the time since death. *Aims:* To find the correlation between vitreous potassium, vitreous sodium and time since death. *Settings and Design:* The present study is prospective study done at B J Govt. Medical College during October 2012 to October 2014. *Material and Methods:* 207 cases brought for postmortem examination during study period were selected after applying inclusion and exclusion criteria. *Statistical Analysis:* Statistical analysis was done using SPSS software to find coefficient of correlation, regression equation and ANOVA test was applied to study the significance. *Observation and Results:* The statistical analysis showed that there is no any significant correlation between time since death and vitreous sodium. However analysis showed a highly significant positive linear correlation between time since death and vitreous potassium concentration with regression equation as, y = 0.2115 (x) + 8.9122 with coefficient of correlation of + 0.8024. The rise was found up to 46 hours after death. The present study also showed that 95% confidence limit of over ± 17.14 hours limits the usefulness of this method in estimating time since death. *Conclusion:* The vitreous potassium is a very useful indicator for determination of time since death. However one should keep check on sampling errors and method of analysis, as it can make difference in the final results.

Keyword: Vitreous Potassium; Sodium; Time Since Death; Correlation.

Introduction

In any postmortem examination determination of time since death i.e. the interval between death and time of examination of body is an important issue [1]. The estimation of time since death is undoubtedly one of the most significant research in forensic medicine and yet it is still considered as to be most controversial and inaccurate one [2].

It is known that many of chemical changes start in the body immediately or shortly after death. It has also been observed that these changes progress in an orderly fashion till the disintegration of body. Changes in chemical constituents have its own time factor or rate of change. These changes occur especially in body fluids like blood, spinal fluid and vitreous humour of eye. Thus it was hypothesized and later confirmed that determination of the chemical quantity could help forensic pathologists to ascertain time since death more precisely [3].

As compared to other body fluids, vitreous humour of eye is stable and less susceptible to rapid chemical changes and contamination. It is also easily accessible and its composition matches a lot to that of aqueous fluid, cerebrospinal fluid and serum. Hence it is suitable for many analyses to estimate

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time since death [4].

The accurate estimation of time since death carries great value in medico legal investigations of serious crimes. Hence several workers have studied and reported that the accurate prediction of time since death i.e. even within two hours, can be possibly made from vitreous humour potassium [5]. Hence the current study is to find the correlation between vitreous potassium and sodium concentration with time since death.

Material and Methods

The present study was conducted during October 2012 to October 2014 at department of Forensic Medicine, B J Govt. Medical College Pune. The cases brought for postmortem examination formed the material for collection of vitreous humour. Total 207 cases were studied. Cases where exact time of death was known and it correlated with postmortem changes like postmortem lividity, rigor mortis and putrefaction were selected for sample collection. Dead bodies which were kept in cold storage, cases where exact time of death was not known, cases with known ocular disease or trauma, cases whose time of death on enquiry from different sources was found to differ by more than $\pm$ 15 minutes, hospitalized cases where electrolytes or diuretics were given prior to death and cases with known electrolyte disturbances prior to death were excluded from the study.

The information regarding age, sex, cause of death, date and time of death was gathered from police records, hospital records and from eye witnesses, relatives, friends and attendants of the deceased. Examinations of dead bodies were conducted in sufficient light. External examination was carried out to note the appearance and situation of rigor mortis, appearance, site and colour of postmortem lividity, state of eyes with reference to cornea, any external injury to eyes and signs of decomposition. Sample was drawn from right eye of each individual.

After collecting the samples only the clear samples were processed further. Samples having any particulate matter, cloudy, discolored or hemorrhagic were discarded and were not included in the study.

Vitreous humour was collected from the posterior chamber of the eye, slowly and gradually avoiding tearing of loose fragments of tissues by needle aspiration through a puncture made 5-6 mm away from the limbus near outer canthus using 10 ml sterile syringe and 21 gauze needle, directed in such a position that the tip of needle is near retina. Vitreous humour was then slowly aspirated. As much of the vitreous humour as can be aspirated was removed because the vitreous humour next to the retina has a different concentration of solutes than in the central portion of the globe.

Once the sample was aspirated, the syringe was detached from needle. The needle was kept in situ to inject sterile water in the posterior chamber of eye to restore the eyes for cosmetic purposes. The aspirated vitreous humour sample was poured in a rubber stoppered glass vial for sodium and potassium estimation by Flame Photometry Method. Analysis was done immediately after collection of samples without any time delay.

Observations and Results

Total numbers of cases studied during study period were 207. Maximum time since death for which vitreous sample collected was 46.00 hours. In the study population there was a female preponderance with maximum number of cases from age group of 0 to 20 years (33) followed by age group of 21 to 40 years (32). While maximum number of males were from the age group of 0 to 20 years (30) followed by age group of 21 to 40 years (24) (Graph I). Distribution of cases with respect to Time Since Death (TSD) showed that out of 207 cases maximum i.e. 98 (47.4%) were with TSD less than 12 hours followed by 87 (42.1%) cases with TSD between 12 hours and 24 hours and 22 (10.5%) cases with TSD 24 hours and more than 24 hours (Table 1). For vitreous sodium the statistical analysis was not significant (Table 3). However for vitreous potassium the statistical analysis showed that there is highly significant change ($p < 0.001$) in vitreous potassium concentration with time since death (Table 2). To find the exact correlation between the vitreous potassium concentration and time since death we did the regression analysis and we charted a graph. The graphical representation (Diagram 1) showed that the coefficient of correlation (r) was + 0.8024. It means there is a positive linear correlation between TSD and vitreous potassium concentration. The regression equation for the Diagram 1 was, $y = 0.2115 (x) + 8.9122$ (where 'y' is vitreous potassium concentration i.e. independent variable and 'x' is time since death i.e. dependant variable, 'a= 0.2115' is the slope of regression line and 'b=8.9122' is the intercept of regression line). The analysis of Table 4 gave us, 95% confidence limit of x i.e. Mean ± 2 (standard deviation of x) = Mean ±17.14 and Regression coefficient i.e. Standard deviation of x /

standard deviation y = 3.27 hrs. This means that an increase of 1mEq/L in potassium concentration will indicate an increase of 3.27 hours in time since death and 95% confidence limit for all cases will be ±17.14 hours.

Table 1: Percentage distribution of cases depending upon time since death (TSD)

S. No.	TSD (IN hrs.)	No. of Cases	Percentage Distribution.
1.	00 hrs - 12 hrs.	98	47.4
2.	12 hrs – 24 hrs.	87	42.1
3.	≥ 24 hrs.	22	10.5
		207	100.00

Table 2: Levels of vitreous potassium depending upon the time since death (TSD)

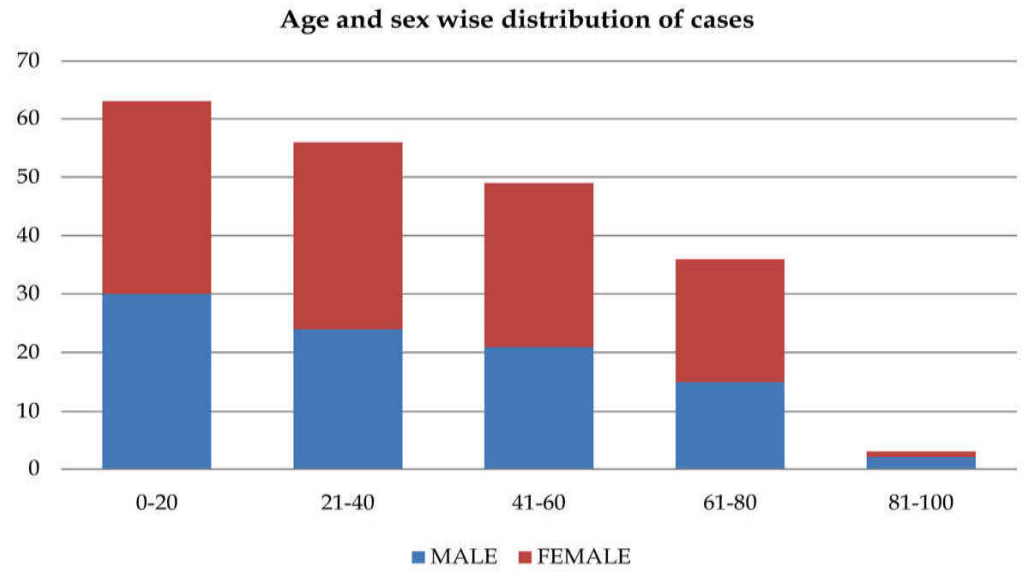
TSD (hrs)	No. of cases	Vitreous Potassium (mEq/L) Mean	SD	ANOVA F	P value
00 hrs - 12 hrs.	81	10.53	2.826		
12 hrs – 24 hrs.	72	13.51	0.953	52.85	<0.001 HS
≥ 24 hrs.	18	14.81	1.621		
Total	171	12.24	2.678		

Table 3: Levels of vitreous sodium depending upon the time since death (TSD)

TSD (hrs)	No. of cases	Vitreous Sodium (mEq/L) Mean	SD	ANOVA F	P value
00 hrs - 12 hrs.	81	176.23	90.905		
12 hrs – 24 hrs.	72	166.47	61.353	1.152	0.319 NS
≥ 24 hrs.	18	147.25	45.479		
Total	171	169.07	75.799		

Table 4: Correlation between time since death and vitreous potassium concentration

Time since death (hrs.) X variable	Sample size	207
	Mean	17.28
	Standard deviation	8.57
Vitreous Potassium y variable	Sample size	207
	Mean	12.69
	Standard deviation	2.10



Graph 1: Age and sex wise distribution of cases.
In above graph - On X axis – Age in years. On y axis – Number of cases studied.
Blue colour – Male sex. Red colour - Female sex

Table 5: Correlation between time since death and vitreous potassium concentration for different durations

Sr. No.	Investigator	Found rise in vitreous potassium up to ...hours
01	Sturner [8]	104
02	Hansson et al [17]	120
03	Lie [15]	100
04	Coe J I [21]	6 - 24
05	Adjutantis et al [5]	12
07	Agrawal R L et al [3]	24
08	Stephens et al[9]	35
09	Madea et al [10]	120
10	Knight B. [22]	100
12	Munoz et al [12]	41
13	Madea B et [13]	120
14	Garg V et al [2]	104
15	Deokar R et al [14]	40
16	Present study	46

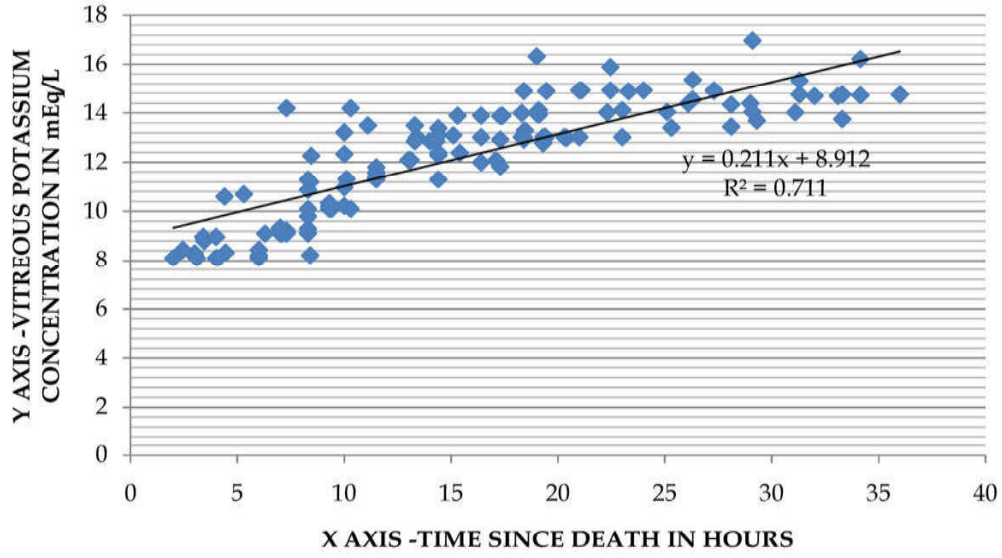


Diagram 1: Correlation between time since death and vitreous potassium concentration

Discussion

Estimation of time since death has always remained an important requirement in medico legal autopsies. Estimation of time since death can directly or indirectly help to find out the time of assault. Time passed between death and postmortem examination and time for which deceased survived after say, sustaining fatal injury considered together give the time of assault. But even after adapting all possible methods, in many cases only gross estimation is possible with the help of parameters like cooling of body, changes in eye, postmortem staining, rigor mortis, decomposition changes, contents of stomach and bowels, contents of urinary bladder and circumstantial evidence, but besides these changes many chemical changes also begin to take place in the body after death and progress in a fairly orderly fashion until the body disintegrates which may prove to solve this query [6].

Various chemical tests to estimate the time since death have been largely developed in last few years. Body fluids which are available for such chemical examination are whole blood, serum, cerebrospinal fluid, aqueous humour and vitreous humour. Amongst all these available body fluids, the vitreous humour has been largely utilized and vitreous potassium concentration has become most widely used method to predict the time since death [1]. Same is investigated in this study. Apart from potassium the postmortem changes of sodium has also been investigated.

Aqueous humour in the anterior and posterior chambers of the eyes and vitreous humour which is contained within the vitreous body constitutes the intraocular fluid. Because of largervolume, easy

availabilityand lesser or no contamination, vitreous humour was preferred in this study. Also vitreous humour is relatively inert and slightly influenced by sudden changes in the human blood chemistry [7]. The current study of potassium in the vitreous humour showed that with increasing time since death there was considerable rise in the levels of potassiumThus vitreous potassium and glucose can be used as indicators to estimate time since death. But the study of vitreous sodium concentration showed no considerable variation and hence was of no use to estimate time since death.

The study shows that with the increase of time since death the levels of potassium in the vitreous humour increased significantly.This observation is supported by workers [2, 3, 8-14].

During lifetime potassium is almost intracellular. Normal vitreous potassium concentration ranges from 2.6 – 4.2 mEql/L. High intracellular concentration of potassium is maintained by sodium potassium pump. After death this pump does not operate and therefore potassium is leaked out of cell, leading to high potassium levels. It is postulated that normal antemortem route of entry of potassium in vitreous humour isthrough ciliary body [7]. After death there is autolysis of the vascular choroids and retinal cells of the eye which release substantial amount of potassium into the vitreous humour causing rise in the potassium levels [15].

However the findings of workers like Hughes W B[16], Hansson L et al [17] and Devgun M S. et al [18]are not in line with present study findings. Hughes W B found that in case of vitreous humour, variance in the internal factors like enzyme supply, surviving oxygen supply etc. make postmortem changes to differ from theoretically predicted one [16]. Hansson et al suggested role of technical difficulties like pipetting and dilution of viscous fluid as the cause of wide spread dispersion of potassium values [17].

A straight line relationship was found between the vitreous potassium levels and the time since death.This is in confirmation with the observation made in most of the previous researches. This observation was verified by the least squares analysis. The resulting linear regression equation in the form of $y = ax + b$ (where 'y' is vitreous potassium concentration i.e. independent variable and 'x' is time since death i.e. dependant variable, 'a' is the slope of regression line and 'b' is the intercept of regression line) was, $y = 0.211 x + 8.9122$.

From this it was found that there appeared a linear relationship between vitreous potassium concentration and time since death and this was a simple straight line (Diagram 1) as observed by majority of the investigators [9, 14, 17, 19, 20].

But Adjutantis and Coutselinis [5], Hughe s[16] and Coe [21] found this line to be biphasic in which the slope of the first few hours after death is steeper than for more prolonged times after death, which is not in agreement with this study.

Regarding vitreous sodium, results of study showed that the levels of sodium in vitreous humour after death wereinsignificantly variable with the increasing time since death. This observation is in agreement with studies made by other workers [7, 15, 19, 21, 22].

In this study it was found that vitreous potassium levels increase up to 46 hours after death. Various researchers found this rise in vitreous potassium for varying hours after death (Table 5).

From above observations it is clear that majority of western workers reported a longer time period as compared to 46 hours in present study. According to some investigators this could be because tropical climate of India, where dead bodies decompose faster leading to faster biochemical changes leading to attainment of diffusion equilibrium of potassium across the cell membrane[7].

The present study showed that 95% confidence limit of over ± 17.14 hours limits the usefulness of this method in estimating time since death. From review of literature it is evident that the reliability of the test showed greater variation with different investigators. While Adelson [23] found the 95% confidence limit of ± 10 hrs, Adjutantis et al [5]felt that standard deviation was ± 1.7 hrs. On the other hand Sturner and Gantner [24] and Lie [15] in their study found a close correlation between vitreous potassium and the postmortem interval and the 95% confidence limit was ± 4.7 hrs. However the greater majority of investigators have found a much greater standard error to be present ranging as high as ± 34 hrs [10]. Further , it is also evident that there is marked variation in the slope of values of potassium derived from various investigators. But majority of the investigators found these slopes to be straight.

Through the researches of last few decades, certain factors have become apparent behind the marked variation in the confidence limits and slope of values of potassium derived from various investigators. These factors can be external such as sampling techniques, different sample size, analytical instruments and environmental temperature during death or internal factors such as age of the individual, the duration of terminal episode, manner of death

like burns, hanging, drowning and electrolyte imbalance especially concentration of blood urea nitrogen or presence of uremia at the time of death [3]. Sample manipulation prior to analysis can also be a reason behind the grater variability in vitreous potassium concentration [25].

Hence during sampling of vitreous humour two precautions must be taken as -

(1) If vitreous aspirate is less than 0.5 ml, it may give unrepresentative results; owing to the uneven distribution of potassium within vitreous body [3]. Hence it is necessary to remove whole of the fluid from the eye that can be aspirated because the vitreous humour next to the retina has a highest concentration of solutes than the central portion of the globe until putrefaction sets in [7].

(2) Secondly, the vitreous must be aspirated slowly to avoid tearing loose fragments of the tissue [12]. Such tissue fragments grossly distort the electrolytes in the vitreous, since it is from those cells from which most of the electrolytes are derived as mentioned by Lie [15] and later by Coe [21].

Recent studies in this field have shown that the values of vitreous potassium can vary with the use of different instruments which are used to measure potassium concentration. Coe and Appledemon-strated that potassium concentration obtained by flame photometry was lower than the values obtained by direct potentiometry with a potassium ion selective electrode [26].

However one should remember that this study cannot be employed if eyes are injured; absent or sample drawn is unclear. The procedure of vitreous humour aspiration requires adequate skill, lack of which leads to instrumentation and sampling error.Analysis of vitreous electrolytes is machine and technique dependent and hence mechanical and technical errors are possible.The study cannot be applied in cases where electrolyte imbalance is evident prior to death.

Conclusion

The vitreous potassium is a very useful indicator for determination of time since death. However one should keep check on sampling errors and method of analysis, as it can make difference in the final results.

Ethical Clearance

Obtained from institutional ethics committee.

Conflict of interest: Nil.

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