Original article:

Utility of vitreous potassium concentration to estimate time since death

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Abstract:

Estimation of time since death has always remained an important requirement in medico legal autopsies. In civil cases also the matter concerning transfer of estate or property may depend upon the time since death. 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. Same is investigated in this study. In the present study 207 cases were studied. The statistical analysis showed a highly significant positive linear correlation between time since death and vitreous potassium concentration with regression equation as, $y = 0.204 x + 9.6315$ with coefficient of correlation of $+ 0.6694$. The rise was found up to 46 hours after death. The present study also showed that 95% confidence limit of over ± 16.74 hours limits the usefulness of this method in estimating time since death.

Key word: Vitreous potassium, time since death, linear correlation

Introduction:

'Forensic medicine' has been defined as that branch of medical discipline which deals with the application of knowledge of principles and practice of medical and paramedical sciences for the purpose of administration of law–both civil and criminal.[1] 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.[2] 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.[3]

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.[4]

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 time since death.[5]

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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.[6] Hence the current study is to check the utility of vitreous potassium in determination of time since death.

Aims and objectives:

1] To know the relationship between changes in vitreous humour potassium concentration with time since death.

2] To find up to what time interval, levels of potassium changes after death.

Material and methods:

The study was done in Forensic Medicine Department of B J Govt. Medical College, Pune during October 2012 to October 2014. 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 + 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 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).

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-2). The statistical analysis done showed that there is highly significant change (p < 0.001) in vitreous potassium concentration with time since death. (TABLE-3). 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 (GRAPH II) showed that the coefficient of correlation (r) was + 0.6694. It means there is a positive linear correlation between TSD and vitreous potassium concentration. The regression equation for the GRAPH II was, y = 0.204 x + 9.6315 (where 'y' is vitreous potassium concentration i.e. independent variable and 'x' is time since death i.e. dependant variable, 'a= 0.204' is the slope of regression line and 'b=9.6315' 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 + 16.74 and Regression coefficient i.e. Standard deviation of x / standard deviation y = 2.19 hrs. This means that an increase of 1mEq/L in potassium concentration will indicate an increase of 2.19 hours in time since death and 95% confidence limit for all cases will be + 16.74 hours.

TABLE 1:- TABLE SHOWING NORMAL VALUES OF POSTMORTEM VITREOUS CHEMICAL ANALYSIS.

	Na Mmol/l	Cl Mmol/l	K Mmol/l	Cr Mg/dL	VUN Mg/dL	Glucose Mg/dL	Ketoacids + or -	R-OH Mg/dL
Vitreous Humour	135-150	105-135	<15	0.6-1.3	08-20	<200	---	---

(Na- Sodium, Cl- Chloride, K – Potassium, Cr – Creatinine, R-OH – alcohol, VUN – vitreous urea nitrogen, mmol/l –millimoles per litre, mg/dL – milligrams per deciliter.)

GRAPH 1:- GRAPHICAL REPRESENTATION OF TABLE NO -03 SHOWING AGE AND SEX WISE DISTRIBUTION OF CASES.
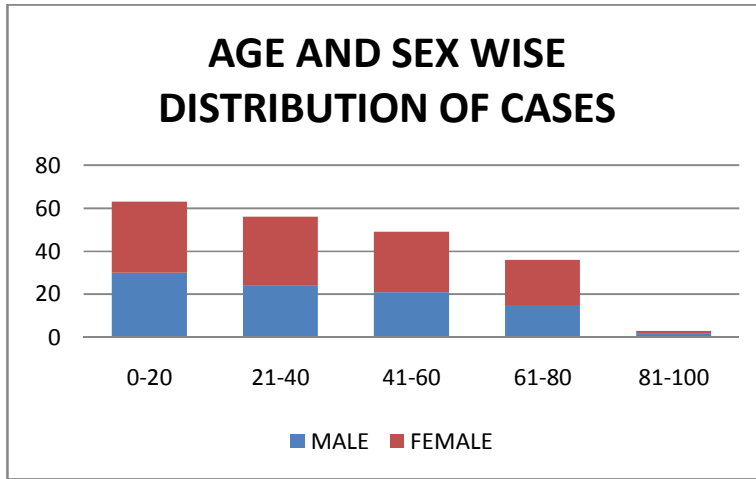
In above graph - On X axis – Age in years. On y axis – Number of cases studied.

Blue colour – Male sex. Red colour - Female sex

TABLE 2:- PERCENTAGE DISTRIBUTION OF CASES DEPENDING UPON TIME SINCE DEATH

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 3:- SHOWING THE LEVELS OF VITREOUS POTASSIUM, DEPENDING UPON THE TIME SINCE DEATH (TSD).

| TSD (hrs)	No. of cases	Vitreous Potassium (mEq/L)		ANOVA F	P value
		Mean	SD		
00 hrs - 12 hrs.	98	10.53	2.826		
12 hrs – 24 hrs.	87	13.51	0.953	52.85	<0.001 HS
> 24 hrs.	22	14.81	1.621		
Total	207	12.24	2.678		

TABLE 4:- SHOWING VARIABLES OF TIME SINCE DEATH AND VITREOUS POTASSIUM CONCENTRATION.

Time since death (hrs.) X variable	Sample size	207
	Mean	13.52
	Standard deviation	8.37
Vitreous Potassium y variable	Sample size	207
	Mean	12.36
	Standard deviation	2.56

TABLE 5:- Rise in vitreous Potassium concentration as studied by various researchers.

Sr. No.	Investigator	Found rise in vitreous potassium up to …hours
01	Sturner (1963)[11]	104
02	Hansson et al (1966)[23]	120
03	Lie (1967)[13]	100
04	Coe J I (1969)[25]	6-24
05	Adjutantis et al (1972)[6]	12
06	Agrawal R L et al (1983)[12]	24
07	Stephens et al (1987) [14]	35
08	Madea et al (1989) [15]	120
09	Gowekar G et al (1996)[16]	60
10	Munoz et al (2001)[17]	41
11	Madea B et al (2001)[18]	120
12	Garg V et al (2004)[3]	104
13	Deokar R et al (2007)[19]	40
14	Present study	46

GRAPH – II :- Scatter diagram showing correlation between time since death and vitreous potassium concentration in sample - 1
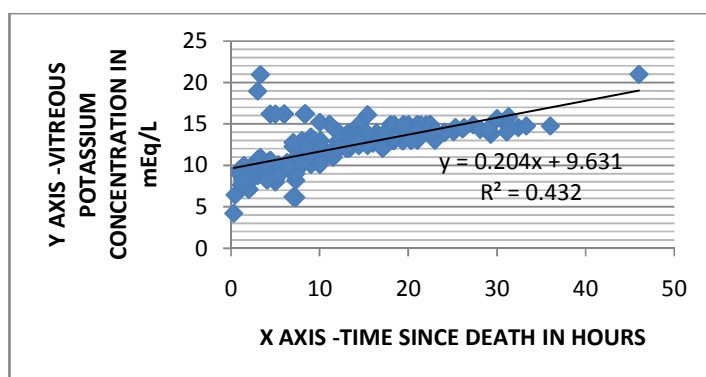
GRAPH II show that there is linear correlation between time since death (on X axis) and vitreous potassium concentration (on Y axis), with positive co-efficient of correlation (r = +0.6694) suggesting that vitreous potassium rises in linear fashion with time since death.

Discussion:

The vitreous humour is present between the lens and retina filling the centre of the eye. The vitreous humour, with volume of about 4 millilitres, constitutes nearly 80% of the globe, making it the largest structure within the eye. [7]

The normal concentration values of different components within vitreous humour is given in TABLE-1.[8] The body maintains a high concentration of potassium in the intracellular fluid. It is reported that the intracellular concentration of potassium is as high as 2 to 40 times the concentration of potassium within the plasma**.** This high intracellular concentration is maintained by a balance between the electrical charges inside and outside the cell membrane and the active metabolic forces that pump the electrolytes selectively across the membrane. A return to equilibrium occurs after death at a steady rate because the pumping mechanism is inactive and the cell wall becomes a semi-permeable membrane that allows the potassium to leak through the membrane to approach equilibrium. The membrane leak occurs at a steady rate because of the mechanical limits of the membrane. The steady rate of potassium leak in the postmortem period provides a form of built in clock that allows a means of projecting back to the time of death and estimate the time since death.[9]

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 larger volume, easy availability and 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.[10] The current study of potassium in the vitreous humour showed that with increasing time since death there was considerable rise in the levels of potassium.

This observation is accordance with studies done by other researcher's previously.[11-18, 20-22] 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 is through ciliary body.[10] 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.[13]

However the findings of some workers [22-24] 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.[22] 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.[23]

A straight line relationship was found between the vitreous potassium levels and the time since death. 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, $y = 0.204 \, x + 9.6315$.

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 (plotted on GRAPH-II) as observed by majority of the investigators [3, 14, 19-21] But few researchers [24, 25] 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.

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. It is summarized in the TABLE-5. It is clear from TABLE-5, 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.[10]

The present study showed that 95% confidence limit of over ± 16.74 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 [26] found the 95% confidence limit of ± 10 hrs, Adjutantis and Coutselinis [6] felt that standard deviation was ± 1.7 hrs. On the other hand Lie [13] in their study found 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.[15]

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.[12] Sample manipulation prior to analysis can also be a reason behind the grater variability in vitreous potassium concentration.[27]

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.[12] 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.[10]

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 [13] and later by Coe [25].

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 Apple demonstrated that potassium concentration obtained by flame photometry was lower than the

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values obtained by direct potentiometry with a potassium ion selective electrode.[28]

Conclusion:

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

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