Original Research Article

Medicolegal study of determination of time since death from potassium level in vitreous humour

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ABSTRACT

Background: From the knowledge of science, various workers have established the methods of determining post mortem interval by the use of physical changes after death. Unfortunately the accuracy was low and margin of error high as evident from the literature available. Trends are now shifting towards the use of various chemical methods like estimation of electrolyte concentration of cerebrospinal fluid, synovial fluid and vitreous humour. Vitreous humour is stable, less contaminated by putrefaction, easily accessible in sufficient quantity. Thus, it is suitable to determine time since death.

Methods: The present cross-sectional study was carried out in grant Govt. medical college; Mumbai on 200 cases brought to the mortuary from the period 1st January 2011 to 31st May 2013.

Results: It was found that there was linear correlationship between vitreous humour potassium concentration and increasing post-mortem interval irrespective of age, sex, temperature, humidity and mode of death.

Conclusions: There was linear relationship between vitreous potassium concentration and post-mortem interval. The accuracy of prediction of PMI can be improved by applying present study formula. The rate of rise of potassium level in vitreous humour was 0.23mEq/L/hr No significant difference in vitreous potassium concentration between the two eyes was noted when samples were drawn at the same PMI. There was no significant effect of age, sex, temperature, humidity and mode of death on vitreous potassium level after death.

Keywords: Post-mortem interval, Potassium, Time since death, Vitreous humour

INTRODUCTION

Time since death or post mortem interval (PMI) of a deceased is frequently asked to a medical man while giving evidence in court of law. The autopsy surgeon, hence, during autopsy should keep record of all available data having bearing on this issue. Though it is never possible to fix up exact date and time of death by any findings of autopsy exami- nation, yet some close and reasonable approximation should always be aimed at. Theoretically much reliance may be placed upon progressive changes both physical and chemical that occur in dead body after death but in practice these changes are never constant but variable. Several unpredictable endogenous and exogenous factors such as climatic condition, cause of death, place of death, age, sex, built, nutrition, temperature both inside and outside body etc. apart from individual variation govern the

timings of onset and the rates of change. Therefore doctor should wisely avoid making dogmatic statement regarding duration of post mortem interval. [1,2]

Considering the limitations in estimating time since death by routine methods, the trends are shifting towards the estimation of electrolytes concentration of various body fluids mainly blood, cerebrospinal fluid, synovial fluid and vitreous humour etc.

Like blood, cerebrospinal fluid has been extensively studied but it is frequently difficult to obtain and is contaminated by blood in the process. Further, changes in cerebrospinal fluid occur fairly rapidly and are quite erratic. For this purpose there has been an increasing interest in the use of vitreous humour for biochemical analysis. The eyeball is isolated and well protected anatomically. As a consequence vitreous is usually preserved despite serious trauma to head and is much less subject to contamination or putrefactive change than either blood or cerebrospinal fluid. It is easy to obtained in sufficient quantity for determination of sodium, potassium, urea, chloride and alcohol.[3]

Thus in the present study an effort was made to find out correlation of vitreous potassium level with increasing post-mortem interval, to find out formula for post-mortem interval from changes in level of vitreous potassium, to know whether post-mortem vitreous potassium level has any relationship with age, sex, mode of death, temperature and humidity in Mumbai region where environmental conditions favour early onset of decomposition changes.

METHODS

Two hundred cases brought to the mortuary of Grant Govt. Medical College; Mumbai during the period 1st January 2011 to 31st May 2013 formed the material for collection of vitreous humour in the present cross-sectional study. The institutional ethics committee approval number was IEC/PHARM/701/2010, dated 7/12/2010.

The cases were divided in two groups as:

- Control group with known time of death (100 cases)
- Study group with unknown time of death (100 cases)

Room temperature in degree Celsius (^{0}C) and relative humidity (%) were recorded at the time of collecting samples. Two samples were drawn from each case; sample 1 from right eye and sample 2 from left eye just before the commencement of post-mortem examination at same time and were referred as pair.

All the information about the deceased like name, age, sex, time of death, cause of death were obtained from hospital/ relatives/ eyewitnesses and recorded in the proforma.

Selection criteria

Only clear samples were taken, turbid and blood contaminated samples, cases with known electrolyte imbalance prior to death were discarded. In cases with unknown time of death; samples with normal vitreous urea level were taken for study as normal urea level is a fair indicator of normal electrolyte balance before death.

Methodology of vitreous humour collection

Vitreous humour was collected from the posterior chamber of the eye, slowly and gradually avoiding the tearing of loose fragments of tissues by needle aspiration through a puncture made 5-6 mm away from the limbus using 10 ml syringe and 20 gauze needles and poured in a sterile test tube with rubber stoppered. 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.[4] Liquid paraffin gel was injected in the posterior chamber of eye for cosmetic purposes. The samples were taken immediately to the Central Laboratory, Department of Biochemistry for analysis. The potassium and urea concentration were determined by auto-analyser. Advantages of auto-analyser over conventional flame-photometry are:

- Dilution of serum is not required which avoids the manual error
- Determination of electrolytes is independent of variation in protein and lipid concentration in the sample
- Time required for determination of electrolyte concentration is less as compared to flame photometry.

Statistical analysis

It was carried out using the software "statistical package for social sciences (SPSS15) on control group cases with known time of death and correlation between vitreous potassium concentration and post-mortem interval (PMI), formula for PMI based on potassium level were established. On the basis of formula derived from the statistical analysis on control group cases, the predicted post-mortem interval in study group cases was calculated.

RESULTS

In the present study, out of 100 cases in control group, the minimum and maximum values of actual postmortem interval noted were 2.55 hours and 42.40 hours respectively. The postmortem interval in hours (hrs) was divided in five groups as shown in the BAR diagram (Figure 1).

In the present study the minimum and maximum ages noted in control group were 2 and 76 years respectively. The different age groups in years (years) created and

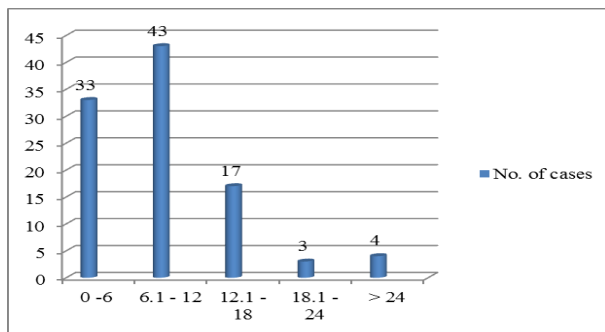number of cases in control group are as shown in the BAR diagram (Figure 2).

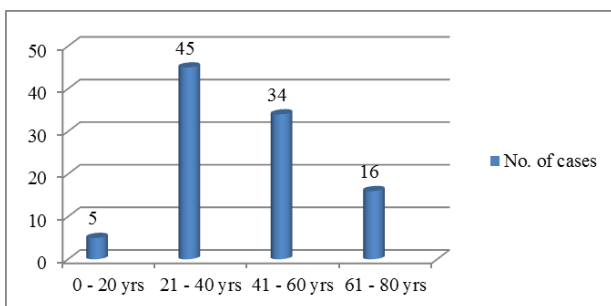**Figure 1: Number of cases in different PMI groups in control group cases.**

Figure 2: Different age groups (in years) in control group cases.

Table 1: Temperature groups in control group cases.

Temperature group (in ^{0}C)	15 - 20	21 - 25	26-30	31 - 35
No. of cases	6	27	37	30

Table 2: Humidity groups in control group cases.

Humidity group (in%)	60 -70	71 -80	81-90	Humidity group (in%)
No. of cases	56	31	13	No. of cases

Table 3: Sexwise number of cases in control group.

Sex	No. of cases (control group)
Male	61
Female	39

The minimum and maximum temperatures noted in control group cases were 17 ^{0}C and 35 ^{0}C respectively. The temperature groups created and number of cases in control group are shown in Table 1.

The minimum and maximum values of humidity noted in control group cases were 60% and 90% respectively. The

different humidity groups created and number of cases in control group are shown in Table 2.

Out of 100 cases in control group the number of cases in different modes of death were 30 cases of asphyxia, 29 cases of syncope and 41 cases of coma.

Table 4: Levels of potassium (K+) in millie equivalent per litre (MEQ/L) in right and left eye in control group.

Eye	No. of cases	Control group	
		Range K$^+$	Mean $\pm$ SD
Right	100	5.9-12.1	8.06 $\pm$1.43
Left	100	5.8-12	8.01 $\pm$ 1.47

There were 61 males and 39 females in control group cases as shown in Table 3.

Table 5: Comparison of potassium level in right and left eye in control group cases, by using unpaired 'T' test.

Comparison	P value	Significance
Right eye with left eye control group	0.800	NO

From the statistical analysis shown in table no. 4 and 5, it was evident that there was no significant difference in potassium level between right and left eye in control group, when withdrawn at the same time.

Table 6: Correlation of vitreous potassium level in right and left eye with pmi in control group cases.

Vitreous constituent	No. of cases	r value	r ^{2}	P value
K+ Right eye	100	0.826	0.681	<0.0001
K+ Left eye	100	0.835	0.698	<0.0001

r - Co-efficient of correlation; r ^{2} - Co-efficient of determination

Table 7: Comparison of actual PMI with estimated PMI by using present study formula (By paired "t" test).

Comparison	P value	significance
Actual PMI with estimated PMI (right eye)	0.995	NO
Actual PMI with estimated PMI (right eye)	0.980	NO
Estimated PMI (right with left eye)	0.921	NO

It was observed that there was highly significant association between vitreous potassium level and PMl. As PMI increases level of vitreous potassium level increases as shown in Table 6.

Table 7: Comparison of increase in the level of vitreous potassium per hour in present study with different past studies.

Study	Present study	Adelson et al[10]	Coe J[7]	Agrawal RL et al[11]	Govekar G et al[15]	Prasad BK et al[16]
Rate of rise of k$^+$ mEq/L	0.23	0.17	0.16- 0.33	0.52	0.15	0.21

Table 8: Comparison of actual PMI and PMI based on autopsy findings.

	Actual PMI in hrs					Autopsy PMI in hrs				
PMI group	0-6	6-12	12-18	18-24	>24	0-6	6-12	12-18	18-24	>24
No. of cases	33	43	17	3	4	34	53	0	13	0

From the two regression graphs as shown in Figure 3 and 4, it was evident that potassium levels were used as dependent variable and PMI as independent variable to calculate the estimated PMI.
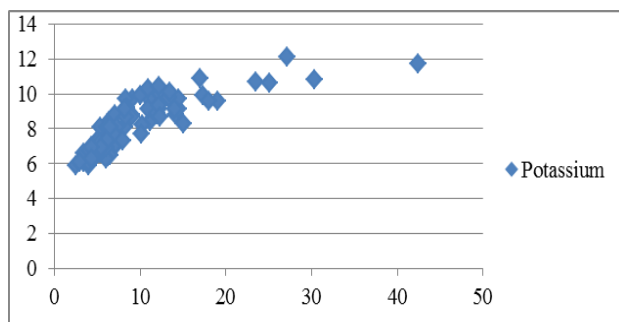
Figure 3: Regression plot of right eye vitreous potassium level against the PMI in control group (X axis - PMI in hours, Y axis- potassium concentration in mEq/L)

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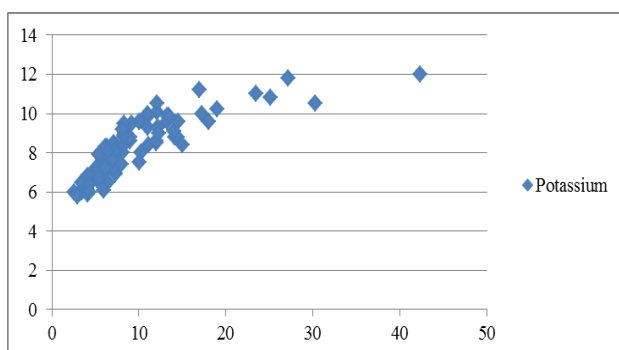
Figure 4: Regression plot of left eye vitreous potassium level against the PMI in control group (X axis - PMI in hours, Y axis - potassium concentration in mEq/L).

The resulting linear regression equation is y=ax+b, where

Y = potassium concentration (mEq/L)

x = actual PMI in hrs

A = slope of regression line, b=intercept

Thus the resulting linear regression formula for PMI is x = y-b/a

For right eye, a is 0.19 and b is 6.34

For left eye, a is 0.2 and b is 6.23

Thus the estimated PMI formulae for right and left eye will be

PMI (right eye) = 5.27y -33.37 ± 2SD, 2SD=15.04 hrs

PMI (left eye) = 5.31y -15 ± 2SD, 2SD=14.86 hrs

The regression coefficient for right and left eyes were 4.34 and 4.22respectively ; this means that increase in vitreous potassium level of 1mEq/L in right and left eye will indicate an increase in PMI of 4.34 Hrs and 4.22Hrs respectively or 0.23mEq/L of potassium level will increase in vitreous humour per hour in both eyes.

From the statistical analyses as shown in table no.7, it was concluded that there was no significance difference between actual PMI and estimated PMI from the present study formula also there was no significance difference between estimated PMI from right and left eye.

It was also found that there was no significant effect of variation of age, sex, mode of death, temperature and humidity on vitreous potassium concentration in control group cases with same PMI.

DISCUSSION

Vitreous humour was preferred in the present study as it was easy to obtain in sufficient quantity, without contamination and its relatively inert nature. Most of the studies in the past admit the utility of vitreous humour potassium analysis as a valuable marker in the determination of PMI.

Correlation of vitreous potassium level with post-mortem interval

The present study shows that there was linear rise in the level of vitreous potassium level with increasing PMI. This observation was also made by most of the past workers namely and Garg V et al[4] Jaffe F[5], Sturner[6], Coe[7], Madea B[8]. In contrast to above study Singh D et al[9] found that the relationship of increasing level of vitreous potassium with increasing PMI was not linear but it was logarithmic.

Comparison of vitreous potassium level between the two eyes

In the present study vitreous humour was aspirated from both the eyes at the same time and it was evident that there was no significance difference between potassium levels in both eyes in control group cases.

This observation was also made by Garg et al (2005)[4], Coe J[7], Adelson et al[10], Agrawal RL et al[11] and Tagliaro et al[12]. But Madea B[8], Balasooriya et al[13] and Pounder DJ et al[14] noted that there was significance difference in potassium level between the two eyes and makes estimation of post-mortem interval from electrolytes levels unreliable.

Comparison of increase in the level of vitreous potassium per hour in present study with different past studies

The increase of 0.23 mEq/L per hour in vitreous potassium level was consistent with the findings of many studies as shown in the table no. 8.

Comparison of estimated PMI with actual PMI and with other studies formulae

After applying appropriate statistical analysis it was evident that there was no significant difference between actual PMI and estimated PMI from the present study formula. The comparison of the present study formula with the established Sturner formula (PMI = 7.14x K^+ mEq/L - 39.1) showed that there was a significant difference in the estimated PMI. But there was no significant difference in the estimated PMI between present study formula and Madea formula (PMI= 5.26xK^+ mEq/L-30.9) as the present study formula and Madea formula are similar.

Comparison of actual PMI and PMI based on autopsy findings

Table number 9 shows that physical changes determining PMI were helpful up to 12 hrs after death with fair degree of accuracy. After 12 hrs, there was a marked fluctuation in actual PMI and PMI estimated by autopsy findings. It can be observed from the table that there were only 3 cases in the actual PMI group 18-24 hrs while as per autopsy findings, the same group contained 13 cases. This suggests that physical changes tend to overestimate the PMI after 12 hrs of death.

Effect of age, sex, temperature, humidity and mode of death on vitreous potassium level

There was no significant effect of these factors on vitreous potassium level. This observation was confirmed by many workers like Agrawal RL et al[11], Myothaik et al[17], Jasnani et al[18], etc. However Singh D et al[9] noted that potassium level in females was more than males with same PMI. Garg V et al (2004)[4] observed that potassium values in burn cases were higher than non-burn cases. Mean sodium/potassium electrolyte ratio was more in winter than in summer in as noted by Singh D et al.[9] As per Madea B and Henssge C[19],the presence of underlying metabolic disorder would result in increase in potassium levels in vitreous level before death, making post-mortem time estimation falsely high.

CONCLUSION

There is linear relationship between vitreous potassium concentration and PMI.

The formula for post-mortem interval on the basis of potassium level in vitreous is PMI (right eye) = 5.27 x (K^+ in mEq/L) - 33.33:

PMI (left eye) = 5 x (K^+ in mEq/L) -31.15

The accuracy of prediction of PMI can be improved by applying present study formula.

The rate of rise of potassium level in vitreous humour is 0.23mEq/L/hr

No significant difference in vitreous potassium concentration between the two eyes was noted when samples were drawn at the same PMI.

There is no significant effect of age, sex, temperature, humidity and mode of death on vitreous potassium level after death.

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