ORIGINAL ARTICLE

A STUDY TO EVALUATE OF TIME SINCE DEATH FROM POTASSIUM LEVEL OF VITREOUS HUMOUR

Umang P Patel¹, Jigneshkumar Patel², Pranav Prajapati³, Ganesh Govekar⁴

Author's Affiliations: ¹Resident, ²Assistant Professor, ⁴Professor & Head, Dept. of Forensic Medicine, Government Medical College, Surat; ³Associate Professor, SMIMER, Surat, Gujarat Correspondence: Dr Umang Patel Email: pprajapti79@gmail.com

ABSTRACT

Introduction: Estimation of time since death plays a crucial roles in law enforcement agencies in most of the unnatural deaths. Estimation of post-mortem interval from changes of potassium level in vitreous humour is more diagnostic tool for forensic field.

Material & methods: We prospectively studied 200 cases having known time since death for the present study at the Dept. of Forensic Medicine & Toxicology, Govt. Medical College, Surat in the year of 2015-2016. Level of potassium in vitreous humor in all subject were analysed in Bio-chemistry laboratory on the same day and various statistical tests performed.

Observation: In this study age of the subjects were mostly from 26-35 years and predominantly male gender. Samples collected for this study were from 12-24 hours of post mortem interval with linear rise of potassium level in both eyes.

Conclusion: Time since death was more accurately calculated with the help of potassium concentration of vitreous humour.

Keywords: Post mortem interval, Potassium, vitreous humour, P value

INTRODUCTION

Now a day hardly a day passes without the news of unnatural death likes homicide, suicide or accidental. In all these cases, time since death is the important key point of starting investigation by investigating team. In many cases apparent cause of death is always given by investigation officer which is consistence with the opinion given by doctor. But the question is time since death and time of incidence which give the starting point of investigation.

Reddy KSN and Murty OP¹ mentioned that it might enable to exclude some suspects and search for the likely culprits, to confirm or disprove an alibi and to check on suspects' statement. In court of law the prosecution and defense lawyer both requires the time of incidence and the time passed before examination, because they want to fix the exact duration of incidence to punish the guilty or to save his client. This shows the importance of estimation of time since death for the people of investigations and law enforcement agency.

There are many ways to estimate time since death in natural and unnatural cases like cellular changes like

cooling of the body, post-mortem lividity, rigor mortis, and decomposition changes like putrefaction, adipocere, and mummification. These are the changes of body after death give the time since death but the range of time since death was more and different from subject to subject where reliability is question as many environmental factor affect them.

From all body fluids, vitreous humor is the only fluid which is unique and preferred because it is anatomically separated, resistant to putrefaction for a long time and most sterile. Vitreous humor containing biochemical constituents, especially potassium, have been widely used in the post-mortem interval (PMI) estimations. The time dependant rise of various bio chemical parameters in the post-mortem period has been considered to be helpful in PMI determinations. So this study was carried out from potassium level in both eyes and to formulate the practical method for estimation of time since death.

METHODOLOGY

The present study was carried out in the Department of Forensic Medicine & Toxicology, Government Medical College & New Civil Hospital, Surat. A total of 200 subjects were taken up for this study which has known time of death. The sample of vitreous humour was taken from both eyes. The information regarding exact time of death was gathered from police inquest report, death certificate, death slip, clinical details from hospital records; correlated and checked from relatives, friends and attendants of the deceased, eye witnesses and concerned investigating officers.

Vitreous humour was collected from the posterior chamber of both eyes. The sample was kept in plain sterile vacuttee. Then sample was heated at 100 degree Celsius for 5 min followed by cooling in room temperature to clear the particulate matter. The sample was properly labelled and will be sent to biochemistry laboratory within 15 min of sample taken, and analysed at bio- chemistry laboratory. The sample was centrifuged at 3000 rpm for 10 min by ERBA COULTER XL 640 AUTOMATED ANALYZER; all the bio chemical analysis was carried out immediately post extraction. The sample was analysed for potassium content of vitreous humour. Finally the data were analysed and various statistical tests were performed on it.

RESULTS

In the present study there were 200 subjects, out of these maximum subjects were from 26-35 years age group.

Table 1: Distribution of cases according to age group (N=200)

Age (In years)	No. (%)
05 - 15	13 (6.5)
16 – 25	41 (20.5)
26 – 35	55 (27.5)
36 – 45	43 (21.5)
46 – 55	23 (11.5)
56 - 65	22 (11.0)
66 - 75	01 (0.5)
76 – 85	02 (1.0)

Table: 2 Distribution of cases according to gender (N=200)

Gender	No. (%)
Male	149 (74.5)
Female	51 (25.5)
Total	200

According to the post-mortem time interval, distribution of 200 subjects placed as 59 subjects were of 0-12 hours, 61 subjects were of 12-24 hours, 56 subjects were of 24-36 hours, 18 subjects were of 36-48 hours 4 subjects were of 48-60 hours and 2 subjects were of 60-72 hours.

Table 3: Distribution of cases according to postmortem interval time (N=200)

Post-mortem Interval Time (PMI)	No. (%)
0 - 12 hours	59 (29.5)
12 - 24 hours	61 (30.5)
24 - 36 hours	56 (28.0)
36 - 48 hours	18 (9.0)
48 – 60 hours	04 (2.0)
60 – 72 hours	02 (1.0)

Table 4: Potassium concentration level (mmol/l) of both eyes

Eye	Mean	Me- dian	SD	Min	Max	P value
R	8.7	9	2.19	5	19	0.538
L	8.74	9.00	2.26	5.00	18.00	

Table 5: Concentration of potassium in vitreoushumour with post-mortem internal

Eye	Postmortem interval (hrs)						
		0-12	12-24	24-36	36-48	48-60	60-72
R	Mean	6.28	8.41	10.35	12.23	14.33	19
	SD	0.69	0.55	0.58	1.092	0.57	0
	One w	vay AN	NOVA t	est, P val	lue- 0.00	001	
L	Mean	6.52	8.34	10.25 12	2.32	14.11	18
	SD	1.64	0.59	0.73 1.	170	1	0
	One way ANOVA test, P value- 0.0001						

According to Figure-1 & 2, a correlation between potassium concentrations with post-mortem interval (PMI) was observed. We have reached a conclusion that the level of potassium is increased against the post-mortem time internal.

The concentration level of potassium was not much more different in both eye and the P value between two eyes was not significant.

For determining regression line from 200 subjects for the samples having time since death less than 72 hours, we have calculated the following linear regression with SPSS software. We have applied regression formula on the level of potassium level of right eye.

Model	R	R Square	Adjusted R Square	SE
1	0.988^{a}	$0.97\bar{7}$	0.977	1.973
a Predicto	ors (Cons	stant) Right e	eve potassium level b T)ependent

a. Predictors: (Constant), Right eye potassium level b. Dependent Variable: Actual TSD

In above table the accuracy of linear regression formula is 97%.

M. 1.1	Un standardized Coefficients		Standardized Coefficients		Sia
Woder	В	Std. Error	. Beta or	- L	Jıg.
Con- stant	- 31.041	0.590		- 52.652	.000
Right eye K	5.996	0.066	0.988	91.298	.000

a. Dependent Variable: Actual TSD

In above table the constant value for the linear regression formula is -31.041. So finally regression formula for right eye potassium is, Y = (5.996 x X) - 31.04.

Where, Y =time since death X =potassium concentration



Figure 1: Co-relation of Potassium concentrations with Post mortem interval in Rt. Eye



Figure 2: Co-relation of Potassium concentrations with Post mortem Interval in Lt. Eye

DISCUSSION

In the present study, maximum number of cases was found in the age group of 26 to 35 years followed by 36 to 45 years and 16 to 25 years [Table-1]. These findings are almost consistent with most of the studies. 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.

As males are predominantly more active in doing outside works so more prone to unnatural deaths, that why in the present study male subjects were higher in number than female [Table-2]. These findings were also consistent with studies of other authors. In the present study, in 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 [Table-3].

In the present study, observed value of potassium ion concentration of both eyes had a p value of 0.538. Thus there is no significant difference of potassium ion concentration between both eyes [Table 4]. Various studies also show no any difference of potassium ion concentration between both eyes.

Adelson et al ² 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 al³ hypothesised in his study that the concentration of vitreous biochemicalconstituents 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 15.81meq/, which is comparable to the values reported by Govekar G⁴. 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., 7.04 mEq/L.

In the present study, maximum potassium ion concentration (right eye 18.6 mmol/l & left eye 18.4 moml/l) was observed in the subject with postmortem interval of 60-72 hours and least concentration (right eye 5.2 mmol/l & left eye 5.1 mmol/l) was observed in the subject with postmortem interval 0-12 hours [Table-4]. As observed from table 5 and figure 1 & 2 there is linear increase in potassium ion concentration in vitreous humor with increasing postmortem interval. This suggest that rise in potassium ion concentration is directly proportional to the increasing postmortem interval.

After applying regression formula, we noted the concentration of potassium in vitreous humour was continuously increased in particular pattern against the 12 hrs of postmortem interval. We noted that every 12 hrs of postmortem interval approximate 2 mmol/l of concentration of potassium in vitreous humour was increased in significant pattern. So, it was much more helpful to estimate time since death from changes in potassium level of vitreous humour.

Various studies have been done on concentration level of electrolyte in vitreous humour, in an attempt to know the post-mortem interval. Nauman et al⁵ in the year of 1959 did extensive study on 211 post-mortem cases. He demonstrated rise in the Vitreous K+ values, but did not attempt to correlate this with the Post mortem interval. He also found an average concontration of 7.2 mg/dl with an average Post-mortem interval of 9 hrs. Jaffe⁶ in the year of 1962 analyzed 31 cases (none of them had uraemia or electrolyte imbalance) and related to the K+ concentration in vitreous to the post-mortem interval. He found a consistent rise in the level of K⁺ starting shortly after death, which continues for 125 hours. There was no significant difference between refrigerated bodies and those kept at room temperature.

Sturner^{7, 8} in the year of 1963 alone and later with Gantner in the year of 1964, did a more detailed study (54 coroner and 37 hospital cases). In 15 of these, vitreous humour was drawn from both the eyes simultaneously and the average difference between the two eyes was 0.1 meq/L. In the 54 coroner cases, there was a linear relationship of the K+ values, obtained by flame photometry and the post-mortem interval. Coe⁹ in the year of 1969 found a linear rise in vitreous K⁺ levels with increasing Post-mortem interval up to 100 hrs, but found this to be biphasic. There was more rapid rise in the first few hrs after death and the 95% confidence limit was approximately \pm 12 hrs.

Gregora et al¹⁰ in the year of 1978, employed the method of Atomic absorption Spectrophotometry, to estimate the proportion of K+ in vitreous humour in 47 deceased persons. The amount of both was found to increase in linear proportion, to the time elapsed since death. Blumenfeld TA et al¹¹ in the year of 1979, studied vitreous humour from the eyes of 127 children and found that the vitreous K+ concentration increased with increasing Post-mortem interval in a linear fashion but 95% confidence limit was ±26 hrs. Hence they concluded that the vitreous K+ concentration cannot be used to establish the time of death. Choo-Kang E et al¹² analyzed 105 cases and found a linear relationship between vitreous K+ concentration and the Post-mortem interval. The rise was biphasic having steeper slope in early hours of Postmortem period than the later hours.Prasad et al13 in the year of 2003 studied correlation of K+ level of vitreous and the postmortem interval and found that the rise in K+ level after death has a strong correlation with the Post-mortem interval's.

The potassium ion concentration in the present study for different age groups for both eyes was found to be not significant with p value 0.538 [Table 4]. Similar inferences have been drawn by Rathinam RD et al¹⁴.

CONCLUSION

It is observed that every 12 hrs of postmortem interval approximate 2 mmol/l of concentration of potassium in vitreous humour was increased in significant pattern. It follows the rule of 12. Significantly increasing the level of potassium against the time since death is useful in law agencies.

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