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International Journal of Forensic Sciences
ISSN: 2573-1734

Estimation of Time since Death by Vitreous Humor Electrolytes Concentration

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Research Article

Volume 4 Issue 4

Received Date: October 17, 2019

Published Date: November 08, 2019

DOI: 10.23880/ijfsc-16000173

Abstract

Time since death is a question of day to day challenge faced by teams of investigators during their course of investigations. Vitreous humour of eye being relatively stable, less susceptible than other body fluids to rapid chemical changes and contamination, easily accessible; thus it is suitable to estimate post-mortem interval (PMI).The dead bodies brought to the mortuary were used as material for collection of vitreous humour sample from the eyes of deceased. The vitreous analytes have been investigated to establish their correlation ship with PMI or time since death. The results of the present study suggest that the previously reported between eye differences for various vitreous biochemical constituents in the same pair of eyes are insignificant so far as forensic applications are concerned. Vitreous potassium and calcium is a useful biochemical marker for PMI estimations.

Keywords: Forensic Science; Time Since Death; Calcium; Potassium; Vitreous Humor

Introduction

Vitreous humor fluid is relatively well protected from post-mortem degradation and contamination, thus it is suitable to determine 'time since death' [1]. Due to its post-mortem stability, vitreous humour has high utility in forensic pathology. In recent years, most of the work has been concentrated on biochemical changes that occur in different body fluids like vitreous humour, aqueous humour, cerebrospinal fluid and blood. All these fluids show post-mortem time related changes in the electrolyte concentration. Thus a post-mortem examination of body fluids like blood, cerebrospinal fluid and vitreous humour, has come up in a big way in supplementing the knowledge of forensic pathology and has proved to be very useful in medico-legal investigations of death especially to determine 'time since death'. Amongst these the most widely used method is estimation of vitreous humour potassium concentration [2]. Other determinations that may prove valuable include the potassium content of the aqueous humour and lactic acid, ascorbic acid, non protein nitrogen, sodium and chloride [3] and magnesium, phosphate and bicarbonate [4]. Content of the vitreous humor. The normal potassium level in the vitreous humour is about 3.8 mmol/l. There is active transport of potassium from ciliary body into the posterior chamber

and anterior chamber; the lens may also contribute to vitreous levels of potassium [5].

Moreover, studies on the estimation of the post-mortem interval have been performed on vitreous humour [3,4,6-11] and vitreous humour has been used for the clarification of forensic issues [12-15]. The precise prediction of time of death is of immense value in medico legal investigations of severe crimes, thus as a result several authors have reported the possibility of accurate prediction of TSD (within two hours), from measurement of the levels of potassium in the vitreous humour [16]. The single most accurate method to determine PMI is the potassium content of the vitreous humor, which shows a linear rise within the time interval of 12-100 hours after death. The rate of vitreous potassium rise is fairly independent of environmental influence [17].

The most investigated post-mortem analytes in vitreous humour are potassium, sodium, chloride, calcium, magnesium, phosphate, urea, creatinine and lactate [18-21]. In previous studies, in vitreous humour some of the parameters, in particular sodium, chloride, creatinine and lactate, proved to be rather stable in their concentrations post-mortem [19,22,23]. While other analytes showed considerable changes of their concentrations [19,24]. The objective of the present study was to assess the level of potassium in vitreous fluid with increasing TSD.

Materials and Methods

Present study was done on 100 bodies brought for autopsy during August 2015 to March 2017 in Lady Hardinge Medical College, New Delhi. Time of death was recorded from doctor, relatives or police personnel. 2-3ml. of vitreous was aspirated from the eye with a 21 gauze needle in a 10ml syringe from the outer canthus. After obtaining vitreous, the empty vitreous cavity was refilled with normal saline to maintain the shape of eyeball. Estimation of electrolytes in the vitreous was done in the department of biochemistry, Lady Hardinge Medical College, New Delhi by the AVL ion selective electrolyte analyser. Details of the dead body, particularly time of death was kept confidential to avoid any bias in results. The data was statistically analyzed by paired t-test, linear regression analysis using Statistical Package for Social Sciences (SPSS) for Windows™ version 13.0.

Observations

The samples were taken from both eyes at same time and evaluated separately, no significant difference was observed. In the present study, observations were made up to 176 hours (Mean ± SD, 39.18 ± 37.16) postmortem period. During the studied postmortem period, vitreous calcium represented a fairly linear rise with increasing PMI. Out of the estimated concentration in the vitreous humor samples collected from 100 subjects the minimum value for vitreous sodium concentration was 98 mmol/L and the maximum value of 284 mmol/L hours with an average of 143.10 mmol/L (Mean ± SD, 143.10 ± 21.87), vitreous potassium concentration was 4.8 mmol/L and the maximum value of 32 mmol/L hours with an average of 13.86 mmol/L (Mean ± SD, 13.86 ± 6.63) and vitreous calcium concentration was 3.6 mmol/L and the maximum value of 11.4 mmol/L hours with an average of 6.74 mmol/L (Mean ± SD, 6.75 ± 1.57) (Tables 1 & 2).

Constituent	N	Mean	Min	Max	Std. dev
Na+ Rt eye	100	141.83	98	231	21.05
Na+ Lt eye	100	144.38	101	284	22.68
K+ Rt eye	100	13.71	4.8	32	6.59
K+ Lt eye	100	14.01	4.9	31.2	6.70
Ca+ Rt eye	100	6.65	3.6	11.3	1.45
Ca+ Lt eye	100	6.86	3.8	11.4	1.68

Table 1: Descriptive Statistic of constitute in individual eye**.**

Constituent (both eye)	N	Mean	Min	Max	Std. dev
Na+	200	143.10	98	284	21.87
K+	200	13.86	4.8	32	6.63
Ca+	200	6.75	3.6	11.4	1.57

Table2: Descriptive Statistic constitute in both eye.

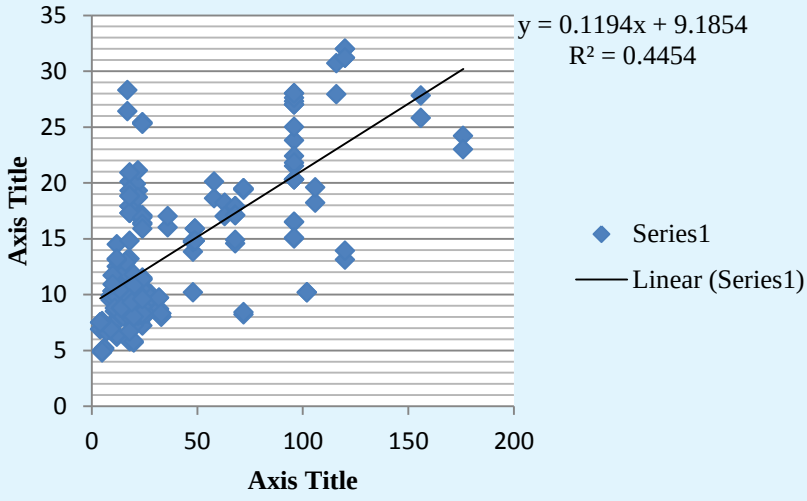
Constituent	N	R	SEE	P value
Na+ Rt eye	100	.013	37.35	.895
Na+ Lt eye	100	.071	37.26	.482
K+ Rt eye	100	.675	27.54	<.001
K+ Lt eye	100	.660	28.06	<.001
Ca+ Rt eye	100	.290	37.75	.003
Ca+ Lt eye	100	.298	37.88	.002

Table 3: The observed linear regression analyses correlation of the various vitreous analytes with PMI in individual eye.

Constituent (both eye)	N	R	SEE	P value
Na+	200	**.030**	**37.14**	**.669**
K+	200	**.667**	**27.67**	**<.001**
Ca+	200	**.261**	**35.88**	**<.001**

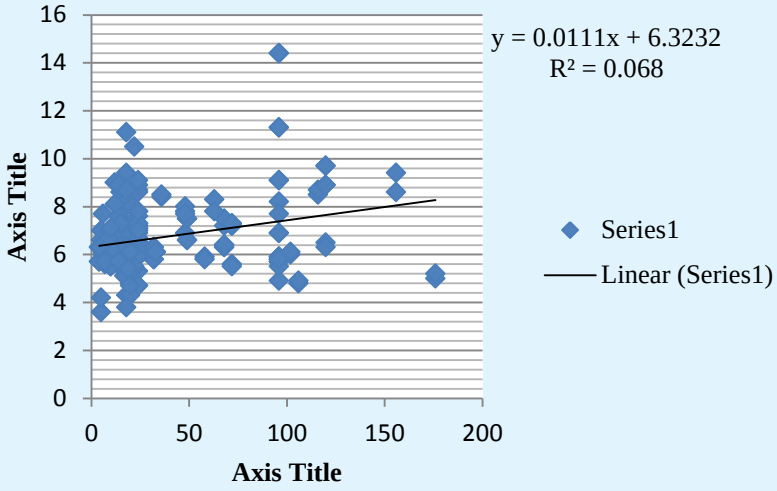
Table 4: The observed linear regression analyses correlation of the various vitreous analytes with PMI in both eye.

The linear rise of vitreous calcium and potassium against increasing PMI is represented in Table 3 & 4. The linear regression correlation of vitreous potassium (R,.667; P< 0.001) and PMI was found to be highly significant. PMI and Vitreous calcium (R, .261; P< 0.001) also shows significant linear regression correlation while Vitreous sodium foundno significant correlation with PMI.



Graph 1: PMI in hours on X- axis and K++ concentration value in mmol on Y-axis.

The vitreous potassium concentrations were used as the dependent variable to calculate the estimated PMI.The resulting linear regression equation in the form of y = ax + b (where, 'y' is vitreous calcium concentration; 'x' is actual PMI in hours; 'a' is the slope of regression line and 'b' is the intercept of the regression line) were:
y = 0.119 x + 9.185.



Graph 2: PMI in hours on X- axis and Ca++ concentration value in mmol on Y-axis.

The vitreous calcium concentrations were used as the dependent variable to calculate the estimated PMI.The resulting linear regression equation in the form of y = ax + b (where, 'y' is vitreous calcium concentration; 'x' is actual PMI in hours; 'a' is the slope of regression line and 'b' is the intercept of the regression line) were:

y = 0.011 x + 6.323.

Discussion

Vitreous humor is a fluid that is relatively well protected from postmortem degradation and contamination. Due to its post-mortem stability, vitreous humor has high utility in forensic pathology. Accurate estimation of PMI (Postmortem interval) has great values to criminal investigation and trial. The levels of chemical components in human vitreous humor are changed with time after death, which can help estimate the PMI [25].

The results of the present study suggest that the between-eye concentration differences evident in the same pair of eyes at identical PMI are not significant, and vitreous potassium levels for individual eyes, as well as mean paired concentrations, were significantly correlated with PMI. Some early studies had reported that vitreous samples obtained from the same pair of eyes had near-identical biochemical values for the two eyes [4,19].

Pounder, et al. found significant differences in vitreous potassium between the two eyes of the same individual [26]. Present study results do not support their conclusion about vitreous potassium but are in agreement with their findings of no significant differences in the same pair of eyes for sodium and chloride. A principal reason for the conflicting reports about the between-eye differences at identical PMI may be the variations in study methods and possible sample manipulations before analyses.

The conflicting views in literature on the subject appear to be a result of non uniform study methodologies and sample manipulations. The present study has attempted to eliminate most of the methodological limitations of sampling techniques and biochemical analysis evident in some previous studies. The insignificant vitreous potassium between-eye differences and a highly significant paired correlation supported by a similar linear correlation at identical levels of significance for right and left eyes with PMI suggest that vitreous potassium is a valuable biochemical marker in PMI estimation. The present study resolves the issue of between-eye differences at identical PMI for vitreous electrolytes and various other vitreous biochemical constituents. The study clearly suggests these differences to be insignificant and therefore the validity of post mortem vitreous humor analysis in forensic pathology applications cannot be solely questioned on the basis of these differences.

The present study shows that there is considerable rise in the levels of potassium in the vitreous humor with increasing PMI, which is supported by other studies

[3,8,19,20,27]. The rise in potassium level is due to the autolysis of the vascular choroids and retinal cells of the eye [27]. There was a linear increase in vitreous potassium level with rise of PMI. This indicates that rise in potassium levels after death has a strong correlation with the PMI [28].

Factors like age, sex, cause of death, season of death, and refrigeration of sample did not influence the vitreous humor potassium values [29]. Two important environmental factors that did not have any effect on the levels of potassium were humidity and temperature, which were in agreement with other studies [2-4,11,30].

In the present study, observations were made up to 176 hours (Mean ± SD, 39.18 ± 37.16) postmortem period. During the studied postmortem period, vitreous potassium represented a fairly linear rise with increasing PMI and significant linear correlation observed for vitreous potassium with PMI(R, .667; P< 0.001).This linear rise of vitreous potassium was consistent in the early PMI with the range of scatter increasing in the later postmortem hours especially after 50 hours into the postmortem period.. These results are in accordance with previous reports in literature on the behaviour of vitreous potassium in the post mortem period [8,11,19].

There was a significant linear correlation observed for vitreous calcium with PMI(R, .261; P< 0.001). Present study found significant correlation of vitreous calcium and PMI. The vitreous calcium concentrations observed in the present study are in non agreement that there is no relationship between calcium concentration in vitreous humor and time since death [31,32]. Nowak and Balabanova [30] however established such a relationship on a random sample of 19 cases suffering from heart disease and quote [14,19]. Who "described also a correlation between PMI and calcium" [2,14,19,33,34].

Conclusion

In present study, linear increase of vitreous humor potassium level with rise in time since death is observed. The results of this study indicated that there were no significant between-eye differences for all of the vitreous biochemical constituents that were studied. It was observed that there was significant correlation between PMI & vitreous potassium (R, 0.731; *P*, <0.0001), PMI & calcium (R = 0.2778; P < 0.005). On a comparison of the actual PMI and the estimated PMI calculated using the formulae derived from the linear regression correlationship, it was found that the lowest standard deviation and the highest correlation ship was obtained for vitreous potassium. The results of the present study suggest that the previously reported between eye differences for various vitreous biochemical constituents in the same pair of eyes are insignificant so far as forensic applications are concerned. A significant linear correlation was seen to exist between PMI and vitreous calcium can be adjunct to vitreous potassium in estimating post mortem interval so as to reduce the error in estimating time since death.Vitreous potassium and calcium is a useful biochemical marker for PMI estimations.

Acknowledgment

We thank our colleagues from Lady Hardinge Medical College who provided insight and expertise that greatly assisted the research, although they may not agree with all of the interpretations/conclusions of this paper.

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