“Estimation of time since death by means of changes in the eye – Vitreous humour calcium levels”

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ABSTRACT:

Introduction: 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 postmortem interval (PMI).

Methodology: The present study was carried out in Shri. Vasantrao Naik Government Medical College, Yavatmal, Maharashtra. 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 with PMI or time since death.

Conclusion: A significant linear correlation, at various degrees, 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.

Key words: Calcium, vitreous humour, time since death.

INTRODUCTION

Time since death is a question of day to day challenge faced by teams of investigators during their course of investigations. The physical signs require a definite experience and are subject to variations in interpretations from person to person, hence are extremely subjective in nature. It was a quest for us to arrive at an objective method for determination of time since death which would be objective and simple, cheap and easily available, undeterred by external influencing factors, providing a reliable method of estimation of time since death. 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 postmortem interval (PMI).

In the present study, vitreous analytes have been investigated to establish their correlation with PMI or time since death. A linear correlation, at various degrees, was seen to exist between PMI and vitreous calcium. Main objectives of the present study were to investigate the utility of vitreous calcium levels in estimating post mortem interval (PMI) and also to find the accuracy of vitreous calcium levels in estimating time since death.

MATERIALS AND METHODS

The study was carried out in Shri. Vasantrao Naik Government Medical College, Yavatmal, Maharashtra. The dead bodies brought to the mortuary at Shri. Vasantrao Naik Government Medical College, Yavatmal were used as material for collection of vitreous humour sample from the
eyes of deceased. The information regarding exact time of death was gathered from police inquest report, dead body challan, clinical details from hospital records; correlated and checked from relatives, friends and attendants of the deceased. Cases where exact time of death was not known were not included in this study. Vitreous humor samples were collected from the eyes of 152 deceased during January 2006 to May 2007 from the autopsies conducted in mortuary at Shri. Vasantrao Naik Government Medical College, Yavatmal. 96 subjects in all were males and 56 were females in the age group of 07 to 80 years (Mean ± SD, 35.38 years ± 14.37 years) and the known postmortem interval ranges from 1.6 hours to 39.2 hours (Mean ± SD, 13.01 ± 7.22 hours). After sample removal, detail postmortem examination carried out and the general relevant findings were documented in the proforma with details of livor mortis, rigor mortis, external and internal sign of decomposition. The vitreous samples drawn from the eyes of deceased at the beginning of postmortem examination of dead body to the mortuary, (Only the clean and clear samples were taken, samples having any particulate matter discarded). All the information about the deceased i.e. age, sex, address, cause of death, exact time of death, time of each sampling, temperature recorded. Vitreous humour was collected at autopsy 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 (sclero-corneal junction) using 10 ml sterile syringe and 20 gauze needle and poured in a rubber stoppered vial. Pre-sterilized syringe was used and rubber stoppered glass vials washed with deionised double distilled water and dried in hot air oven, were used for sampling. Liquid paraffin gels were injected in the posterior chamber of eye for cosmetic purposes. The samples after collection in a rubber stoppered vial were labeled and transported to the biochemistry laboratory for analysis. The vitreous samples were centrifuged at 3000 rpm for 10 minutes; all the biochemical analysis was carried out immediately post-extraction. The samples were analyzed for calcium. The analysis was carried out on the same day as collection using a BECKMAN COULTER LX20 AUTOMATED ANALYZER.

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 40 hours (Mean ± SD, 13.01 ± 7.24) postmortem period. During the studied postmortem period, vitreous calcium represented a fairly linear rise with increasing PMI. The samples were taken from both eyes at same time and evaluated separately, no significant difference was observed.

Out of the estimated concentration in the vitreous humor samples collected from 152 subjects the minimum value for vitreous calcium concentration was 1.2 mmol/L and the maximum value of 3.3 mmol/L hours with an average of 2.0691 mmol/L (Mean ± SD, 2.0691 ± 0.497; SEM, 0.0403).

The minimum value for vitreous calcium concentration noted in the cases of PMI upto 6.0 hours was 1.2 mmol/L and the maximum value of 2.8 mmol/L with an average of 1.9 mmol/L.

The minimum value for vitreous calcium concentration noted in the cases of PMI between 6.1 hours to 12.0 hours was 1.2 mmol/L and the maximum value of 3.1 mmol/L with an average of 2.0 mmol/L.

The minimum value for vitreous calcium concentration noted in the cases of PMI between 12.1 hours to
The minimum value for vitreous calcium concentration noted in the cases of PMI between 18.1 hours to 24.0 hours was 1.4 mmol/L and the maximum value of 3.3 mmol/L with an average of 2.3 mmol/L.

Table-1:

<table>
<thead>
<tr>
<th>Constituent</th>
<th>n</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>152</td>
<td>1.2</td>
<td>3.3</td>
<td>2.0691</td>
</tr>
</tbody>
</table>

The minimum value for vitreous calcium concentration noted in the cases of PMI between 24.1 hours to 40.0 hours was 1.9 mmol/L and the maximum value of 3.2 mmol/L with an average of 2.4 mmol/L.

Table-2: The observed linear regression analyses correlation of the various vitreous analytes with PMI.

<table>
<thead>
<tr>
<th>Constituent</th>
<th>n</th>
<th>r value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>152</td>
<td>0.2778</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

The linear rise of vitreous calcium against increasing PMI is represented in Table No. 2. The linear regression correlation of vitreous potassium and PMI was found to be highly significant (n, 152; R, 02778; P< 0.005).

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:

\[ y = 0.0191 \times + 1.8235. \]

The corresponding formulae to estimate the PMI in the form of: PMI = b^0 + b^1 [Mean of the individual biochemical constituent concentration] where, b^0 is the estimated regression coefficient when no other variable is included in the model and b^1 is the estimated regression coefficient for vitreous calcium) was:

Estimated PMI = 1.065(calcium) - 10.8064.
DISCUSSION

Several authors are in agreement that there is no relationship between calcium concentration in vitreous humor and time since death \cite{4,5,14,15}. Nowak and Balabanova\cite{4} (1989) however established such a relationship on a random sample of 19 cases suffering from heart disease (coronary occlusion) and quote Coe (1969, 1972) who "described also a correlation between PMI and calcium" \cite{6,7,8,9,10}.

The vitreous calcium concentrations observed in the present study are very similar to the concentrations previously reported \cite{Coe6, Nowak and Balabanova4}. The minimum value for vitreous calcium concentration was 1.2 mmol/L and the maximum value of 3.3 mmol/L hours with an average of 2.0691 mmol/L.

There was a significant linear correlation observed for vitreous calcium with PMI\cite{R = 0.2778; P < 0.005}. The rate of rise of calcium concentration was 0.0191 mmol/L per hour.

The significant correlation of vitreous calcium and PMI observed in the present study is similar to the findings of Nowak and Balabanova\cite{4} (1989). However, in the study by Nowak and Balabanova\cite{4}, the significant correlation between vitreous calcium and PMI was only noted in specific groups of death comprising of heart disease and asphyxia.

The present study does not support the previous finding of a significant correlation between postmortem vitreous calcium and PMI in deaths associated with cardiovascular disease \cite{Nowak and Balabanova4}.
CONCLUSION: There is a statistically significant correlation between calcium concentration in vitreous humor and PMI (r = 0.2778) but the range of scatter of the individual calcium values over the PMI is so great that it becomes clear that vitreous calcium can be adjunct to vitreous potassium in estimating post mortem interval so as to reduce the error in estimating time since death.

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REFERENCES

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