Estimation of Time since Death from Vitreous Glucose Concentration

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ABSTRACT

Introduction: The estimation of time since death (TSD) is undoubtedly one of the most significant research in forensic medicine, and yet it is still considered to be the most controversial and inaccurate one. Various chemical tests to estimate the TSD have been largely developed in last few years. Body fluids which are available for such chemical examination are whole blood, serum, cerebrospinal fluid, aqueous humor, and vitreous humor. Amongst all these available body fluids, the vitreous humor has been largely utilized and correlation of vitreous glucose with TSD is investigated in this study.

Materials and methods: The present study was conducted prospectively on 207 cases brought for postmortem examination during October 2012 to October 2014.

Results: The statistical analysis showed a highly significant linear correlation between TSD and vitreous glucose concentration (VGC) with regression equation as, \( y = -0.5391 (x) +17.81 \) with coefficient of correlation of \(-0.4585\). This correlation was found up to 46 hours after death. The present study also showed that 95% confidence limit of over ±17.14 hours limits the usefulness of this method in estimating TSD.

Conclusion: There is significant decrease in vitreous glucose concentration with increase in TSD and hence, VGC can be effectively used for estimation of TSD.

Keywords: Linear correlation, Time since death, Vitreous glucose.

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INTRODUCTION

Estimation of time since death (TSD) has always remained an important requirement in medicolegal autopsies. Estimation of TSD can directly or indirectly help to find out the time of assault. Time passed between death and postmortem examination and time for which deceased survived after say, sustaining fatal injury considered together give the time of assault.\(^1\) In civil cases also, the matter concerning transfer of estate or property may depend upon the TSD.\(^2\) But even after adapting all possible methods, in many cases only gross estimation is possible with the help of parameters like cooling of body, changes in eye, postmortem staining, rigor mortis, decomposition changes, contents of stomach and bowels, contents of urinary bladder and circumstantial evidence, but besides these changes, many chemical changes also begin to take place in the body after death and progress in a fairly orderly fashion until the body disintegrates which may prove to solve this query.\(^1\)

Various chemical tests to estimate the TSD have been largely developed in last few years. Body fluids which are available for such chemical examination are whole blood, serum, cerebrospinal fluid (CSF), aqueous humor, and vitreous humor. Amongst all these available body fluids, the vitreous humor has been largely utilized.\(^3\)

Till date, different researchers have reported correlation between various vitreous electrolytes and TSD. In the present study, we have investigated changes in vitreous glucose concentration (VGC) with TSD.

MATERIALS AND METHODS

The cases brought for postmortem examination at Department of Forensic Medicine, Byramjee Jeejeebhoy Government Medical College, Pune, Maharashtra, India during October 2012 to October 2014 formed the material for collection of vitreous humor. A total of 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 color 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 humor 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 to 6 mm away from the limbus near outer canthus using 10 mL sterile syringe and 21 gauge needle, directed in such a position that the tip of needle is near retina. Vitreous humor was then slowly aspirated. As much of the vitreous humor as can be aspirated was removed because the vitreous humor 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 humor sample was poured in a fluoride bulb for glucose estimation by glucose oxidase method. Analysis was done immediately after collection of samples without any time delay.

RESULTS

Total number of cases studied during study period were 207. In the study population, there was a female preponderance with maximum number of cases from age group of 0 to 20 years followed by age group of 21 to 40 years. Maximum number of males were from the age group of 0 to 20 years followed by age group of 21 to 40 years (Graph 1).

Distribution of cases with respect to 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 and 24 hours and 22 (10.5%) cases with TSD 24 hours and more than 24 hours (Table 1). The statistical analysis done showed that there is highly significant change (p < 0.001) in VGS with TSD (Table 2). To find the exact correlation between the VGS and the TSD, we did the regression analysis and we charted a graph.

The graphical representation (Graph 2) showed that the coefficient of correlation (r) was −0.4585. It means there...
is a negative linear correlation between TSD and VGC. The regression equation for the (Graph 2) was, $y = -0.5391x + 17.81$, (where 'y' is VGC, i.e., independent variable and 'x' is TSD, i.e., dependent variable, “a = -0.5391” is the slope of regression line and “b = 17.81” is the intercept of regression line). The analysis of Table 3 gave us 95% confidence limit of x, i.e., mean ± 2 (standard deviation of x) = mean mean ± 17.14 and regression coefficient, i.e., Standard deviation of x/standard deviation of y = -0.46 hours. This means that decrease of 1 mEq/L in VGC will indicate an increase of 0.46 hours in TSD and 95% confidence limit for all cases will be ± 17.14 hours.

**DISCUSSION**

“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. In any postmortem examination, determination of TSD, i.e., the interval between death and time of examination of body is an important issue.

The estimation of TSD is undoubtedly one of the most significant research in forensic medicine, and yet it is still considered to be the most controversial and inaccurate one.

Repeated experiences have taught the investigators that they should not rely on any single observation for estimating the time of death and also should wisely avoid to make any confident statements based on such a single observation.

It is known that many of the 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 their own time factor or rate of change. These changes occur especially in body fluids like blood, spinal fluid, and vitreous humor of eye. Thus, it was hypothesized and later confirmed that determination of the chemical quantity could help forensic pathologists to ascertain TSD more precisely.

These chemical changes have been largely studied in the last few decades. Body fluids available for such chemical examination are whole blood, serum, CSF, aqueous humor, and vitreous humor. Amongst all these body fluids, the most widely studied and used method is estimation of vitreous humor electrolytes.

Though no single measurement can give a complete and reliable estimate regarding the TSD, combinations of chemical determinations along with classical methods can be used as a helpful adjunct in cases of unwitnessed deaths.

As compared to other body fluids, vitreous humor 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, CSF, and serum. Hence, it is suitable for many analyses to estimate TSD.

Aqueous humor in the anterior and posterior chambers of the eyes and vitreous humor which is contained within the vitreous body constitutes the intraocular fluid. Because of larger volume, easy availability and lesser or no contamination, vitreous humor was preferred in this study. Also, vitreous humor is relatively inert and slightly influenced by sudden changes in the human blood chemistry.

Researchers have done studies regarding correlation of various vitreous electrolytes and TSD. In present study, we have concentrated on VGCs. The present study shows, there is a significant change in the levels of VGC with change in TSD. The findings of present study are supported by the findings of workers who observed a linear decrease in glucose concentration with increasing time of death. This decrease in vitreous glucose is attributed to continuous postmortem tissue glycolysis and its composition matches a lot to that of aqueous fluid occurring in a matter of few hours in a few individuals. While Mullah et al found no any significant relation between TSD and vitreous glucose.

A straight line relationship (Graph 2) was found between the vitreous glucose levels and the TSD. This observation was verified by the least squares analysis. The resulting linear regression equation in the form of $y = ax + b$ (where ‘y’ is VGC, i.e., independent variable and ‘x’ is TSD, i.e., dependent variable, ‘a’ is the slope of regression line and ‘b’ is the intercept of regression line) was, $-0.5391(x) + 17.81$.

Regression equation for vitreous humor glucose was given by Madea et al as, $y = -0.01x + 1.13$ and by Tumram et al as, $y = -1.358x + 26.25$, where $y =$ glucose concentration (independent variable) and $x =$ TSD (dependent variable) and proposed that vitreous glucose can also be used for estimating TSD.

### Table 3: Correlation between time since death and vitreous glucose concentration

<table>
<thead>
<tr>
<th>Time since death (in hours)</th>
<th>Sample size</th>
<th>207</th>
</tr>
</thead>
<tbody>
<tr>
<td>X variable</td>
<td>Mean</td>
<td>17.28</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>8.57</td>
<td></td>
</tr>
<tr>
<td>Vitreous glucose Y variable</td>
<td>Sample size</td>
<td>207</td>
</tr>
<tr>
<td>Mean</td>
<td>08.81</td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td>08.47</td>
<td></td>
</tr>
</tbody>
</table>
LIMITATIONS

This study cannot be employed if eyes are injured; absent or sample drawn is unclear.

- Procedure of vitreous humor aspiration requires adequate skill, lack of which leads to instrumentation and sampling error.
- Observer’s error can be possible.
- Analysis of vitreous glucose is machine and technique dependent, hence, mechanical and technical errors are possible.
- Analysis of vitreous glucose was done by glucose oxidase method, hence, study cannot be applied if analysis is done by other methods.
- The study cannot be applied in cases where electrolyte imbalance, diabetes, etc., is evident prior to death.

CONCLUSION

The vitreous glucose is a useful indicator for determination of TSD. 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.

REFERENCES