

Between Eye Difference in Vitreous Electrolytes of Same individual for Identical Time Since Death

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Abstract

Estimation of Time Since Death (TSD) is very important in routine forensic practice. Various techniques and parameters are utilised for determination of this TSD. One of the most recently and vastly studied method of determination of TSD is with the help of vitreous electrolyte concentration analysis. Recently workers have reported a significant between eye differences for same individual at identical TSD. This has doubted the use of vitreous electrolyte analysis in TSD determination. The review of literature highlights various causes behind this fact and need of more research in this topic as discussed in this article.

Keywords: Vitreous Electrolytes; Time Since Death; Between Eye Differences; Sampling Errors; Analytical Errors.

Introduction

In any postmortem examination determination of time since death i.e. the interval between death and time of examination of body is an important issue [1].

However no person knows exact time of his or her departure from this beautiful earth and no medical professional can tell the exact time since death of a person if he or she has not attended the patient at the time of his or her last breath [2].

The estimation of time since death is undoubtedly one of the most significant research in forensic medicine and yet it is still considered as to be most controversial and inaccurate one [3].

Repeated experience 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 single observations [4].

From the second half of 18th century the forensic experts across the globe started using various methods in combinations to estimate time since death. These methods included observations such as cooling of body, changes in eye, post mortem lividity, rigor mortis, signs of decomposition, contents of stomach and bowels, contents of urinary bladder and circumstantial evidences. Though these widely practiced methods give useful information regarding time since death, their range is too wide on most of the times. Hence attention of the researchers has now been drawn towards various biochemical parameters which can be used to narrow down the duration to be opined [5].

It is known that many of 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 its own time factor or rate of change. These changes occur especially in body fluids like blood, spinal fluid and vitreous humour of eye. Thus it was hypothesized and later confirmed that determination of the chemical quantity could help forensic pathologists to ascertain time since death more precisely [6].

These chemical changes have been largely studied in last few decades. Body fluids available for such chemical examination are whole blood, serum, CSF,

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aqueous humour and vitreous humour. Amongst all these body fluids the most widely studied and used method is estimation of vitreous humour potassium concentration [7].

Though no single measurement can give a complete and reliable estimate regarding the time since death, combinations of chemical determinations along with classical methods can be used as a helpful adjunct in cases of unwitnessed deaths[8].

As compared to other body fluids, vitreous humour 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, cerebrospinal fluid and serum. Hence it is suitable for many analyses to estimate time since death [9].

For more than a quarter of a century, determination of time since death by evaluating vitreous biochemistry has been the subject of research in forensic pathology. Various studies performed across the globe to date have hypothesized a variety of linear or piecewise-linear relationships between vitreous humour potassium concentrations and time since death. There is a lack of agreement on different estimated intercepts and slopes of regression lines and also the different reliabilities of these estimates and this is due to the variable number of cases reported from study to study, difference in observed ranges of vitreous potassium and time since death and the unaccommodated effects of factors including age of subject, amount of urea nitrogen, ambient temperature and presence of illness at the time of death on potassium concentration [10].

The accurate estimation of time since death carries great value in medico legal investigations of serious crimes. Hence several workers have studied and reported that the accurate prediction of time since death i.e. even within two hours, can be possibly made from vitreous humour potassium [11].

However few researchers have found a significant difference between the values of vitreous electrolyte concentration obtained from two eyes of same individual for identical postmortem interval. Current article is to highlight the work of those researchers and reasons for the same.

Vitreous Humor Between-Eye Differences or Differences between Vitreous Humour Biochemistry of Two Eyes of Same Individual for identical TSD.

The various factors may have a key role in the various disagreements regarding the utility of vitreous humor in TSD estimations. However, perhaps the most important concern in utilizing vitreous biochemistry for crucial forensic pathology

determinations arises from the observed between eye differences in the same pair of eyes at identical TSD. Many researchers have assumed that the vitreous biochemical concentrations are identical and postmortem changes occur at the same rate in both the eyes. Recent observations have indicated that these presumptions may not be entirely true and between eyes differences at the same TSD have been documented. If these differences were to exist, it would grossly undermine the value of vitreous biochemistry in various forensic pathology applications.

Some early studies had reported that vitreous samples obtained from the same pair of eyes had near-identical biochemical values for the two eyes like Adelson et al [4], Sturmer et al [12], Hughes[13], Lie [14] and Coe[15]. These investigators, however, did not provide the data or their statistical interpretation.

Key Messages

One should not blindly accept that the vitreous electrolytes will be same in two eyes of an individual. There is need to do more and more studies to verify the possibility and causes of between eye differences in vitreous electrolytes of same individual for identical TSD.

Balasoorya et al. [16] reported significant differences in various vitreous biochemical constituents from the same pair of eyes at identical TSD. The authors observed that each of the eyes exhibited independent values and nearly 19% of the results for vitreous potassium varied by more than 10% from the mean of the two values. Out of a total of 59 pairs of eyes, only six pairs had the same potassium concentration. Similar differences were also observed for vitreous sodium where 10% of the results varied by greater than 5% of the mean, vitreous urate where at least 19% of the cases had differences greater than 12% of the mean values of the two eyes.

Madea et al. [17] confirmed these findings and reported deviations up to 10% of the single values of both the eyes in the analysis of potassium, sodium, chloride and calcium. The authors, however, did not observe any such deviations for urea.

Pounder et al. [18] from a later study reported that between eye differences in potassium varied from 0 to 2.34 mmol/L or 0% to 21.8% of the mean. The authors suggested these differences to be significant and erratic, thereby questioning the practical usefulness of vitreous humor in evaluation of TSD. On the contrary, the authors reported that the differences observed for sodium and chloride were

tolerable using their methodology.

Tagliaro et al. [19] explored potassium concentration differences in the vitreous humor of two eyes using micro sampling technique with capillary electrophoresis. The authors reported that no significant differences existed in potassium concentration of the same pair of eyes at identical TSD, thus strengthening the application of vitreous humor as an important tool for TSD estimations.

Garg V. et al [3] studied 200 autopsy cases to find the changes in levels of vitreous humour potassium with time since death. The analyses showed highly significant increase in vitreous potassium with increasing time since death in linear fashion. They also found that when samples from both the eyes were taken at the same time and analyzed separately no significant difference was observed.

Mulla A. et al [20] conducted study to investigate the role of vitreous humour biochemistry in forensic pathology. This study hypothesized that the concentration of vitreous biochemical constituents 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 TSD. The results of this study indicated that there were no significant between-eye differences for all of the vitreous biochemical constituents that were studied. 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 is a useful biochemical marker for TSD estimations.

Discussion

A possible explanation for the ambiguous reports regarding between-eye differences at similar time since death was said to be either the variations in study methods or sample manipulations before analyses. A major variation was found in the aspiration techniques adopted by various investigators. Bito in 1977 reported that there is difference in concentrations of many solutes of the vitreous humor between anterior and posterior vitreous chambers [21].

According to Coe in 1989 the concentration of vitreous solutes next to the retina differed from the concentration in the central portion of the globe, and hence it is very important to aspirate vitreous humor as completely as possible. Such completely aspirated vitreous humour sample can reflect accurately the

concentration levels of all solutes, and serves to eliminate any ambiguity that may occur due to selective vitreous humor aspiration [7].

The aspiration technique used by Balasooriya et al.[16] could highly give distorted values in each eye as they aspirated only the initial 1 mL volume of fluid. It is also interesting to note that, investigators like Coe [15] who removed all the available vitreous humor from both the eyes succeeded in demonstrating near identical concentrations for both eyes.

However in a study by Tagliaro et al. it was found that no statistically significant differences existed for potassium concentrations in the two eyes of the same individual when a micro sampling technique was used for sample collection [19]. The micro sampling technique i.e. aspiration of microliter amounts of fluid, used in their study was different from the technique of complete fluid aspiration employed in the previous studies. Hence it can be proposed that the difference in sampling technique may not be the sole reason that stands for the reported between-eye differences. This is authenticated by two previous studies of Madea et al [17] and Pounder et al[19] who assessed the effect of the sampling technique of the vitreous humor by aspirating the fluid in two installments and found no any significant influence of the sampling technique on the observed between-eye differences.

Hence it can be said that, even though the complete aspiration technique is ideal to give accurate vitreous solute concentration levels, certain other factors may also be responsible for the between-eye differences.

Such other factors may be like the different instrumentation methods used in different studies. It has been suggested that the concentration of vitreous humor constituents will vary with different instruments [22]. It is found that in studies that have proposed significant differences between the same pair of eyes, the method of sample analysis was direct or indirect potentiometry [16,17]. While studies in which near-identical concentration for various vitreous humor solutes were obtained, samples were analyzed by using flame photometry [12,15].

Also it is interesting to find that most of the analytical instruments used in various studies have been used for a clinical range of analysis where compensatory dilution has been an essential procedure in estimating a value for most of the postmortem vitreous humor constituents. Pounder et al. hypothesized that sample dilution prior to analysis can be reason for the between-eye differences in the same pair of eyes, and therefore they suggested measuring the samples undiluted [18]. However,

other studies that have reported no significant between-eye differences for vitreous constituents have performed the required dilutions [12,19].

According to few investigators the long time gap between vitreous-humor sample collection and analysis of the sample may be another responsible factor for the reported between-eye differences in the same pair of eyes. In some studies, the sample was kept frozen at -70°C before biochemical analysis. The improper storage conditions may have impacted the results little bit and it is argued that after indefinite storage at low temperatures, one cannot get accurate results regarding the biochemical concentrations of the vitreous humor [17]. Even though Pounder et al [18] used the technique of immediate analysis post-collection, they reported significant between-eye differences for potassium.

Hence during sampling of vitreous humour two precautions must be taken as

1. If vitreous aspirate is less than 0.5 ml, it may give unrepresentative results; owing to the uneven distribution of potassium within vitreous body [16]. Hence it is necessary to remove whole of the fluid from the eye that can be aspirated because the vitreous humour next to the retina has a highest concentration of solutes than the central portion of the globe until putrefaction sets in [5].
2. Secondly, the vitreous must be aspirated slowly to avoid tearing loose fragments of the tissue [12]. Such tissue fragments grossly distort the electrolytes in the vitreous, since it is from those cells from which most of the electrolytes are derived as mentioned by Coe [15] and Lie[23].

Conclusion

Finally it can be said that vitreous biochemistry, particularly vitreous potassium is useful in time since death estimation; and this utility of vitreous potassium cannot be doubted exclusively on the basis of these reported between eye differences. However more studies are required in future concentrating precisely on this topic.

Acknowledgement

Nil

Conflict of Interest

Nil

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