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Vitreous Humor Chemistry: The Use of Potassium Concentration for the Prediction of the Postmortem Interval

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ABSTRACT: Upon review of the literature, extensive disagreement was found as to the usefulness of vitreous humor potassium concentration as a predictor of the postmortem interval (PMI). A pilot study of 1427 cases was performed to address this problem. The requisite statistical analysis for the prediction of PMI is inverse prediction. The 95% inverse prediction interval was found to be approximately ± 20 h. The linear regression equation for the data was $y = 0.238\ x + 6.342$, with a coefficient of determination (r^2) of 0.374. This r^2 value means that 62.6% of the variation of potassium is unaccounted for by the variation in PMI. Further studies are required to attribute this unaccounted variation to quantifiable factors. This would narrow the inverse prediction interval and enable vitreous potassium to be a useful aid in the prediction of PMI.

KEYWORDS: pathology and biology, postmortem interval, vitreous humor

Literature Review

The efficacy of vitreous humor potassium concentration as a predictor of the postmortem interval (PMI) has been in contention for two decades. Confidence in such prediction has ranged from a roseate ± 2.2 h [*1*] to Camps's terse " '. . . the only accurate way of estimating the time of death is to be there when it happened' "[*2*]. Since 1977, the Orange County Sheriff-Coroner's Office has been studying vitreous humor chemistries. Potassium concentration and its use in the prediction of time of death has been part of this project. This pilot study of 1427 cases is presented to elucidate statistics useful in the prediction of time of death. The literature pertinent to PMI prediction starts chronologically with Nauman in 1959.

Nauman analyzed 211 human vitreous humor samples and described the range of postmortem potassium values [*3*]. He discussed his inferences concerning possible antemortem levels and the rise of potassium content postmortem. In 1961, Jaffe [*4*], using 36 determinations from 31 cases, related potassium concentration to the logarithm of time (PMI). He also found ". . . a consistent rise in the level of potassium, commencing shortly after death and continuing for 125 hours."

In 1963, Addleson et al. [*5*] used 349 eyes of 209 individuals to describe, as would all subsequent researchers with the exception of Crowell and Duncan [*6*], a linear relationship

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between potassium concentration and the PMI. In their prediction of PMI, a 95% confidence interval was used. For their data in toto, this 95% confidence interval was ±10 h. In the subgroup of ". . . acute trauma with death within 6 hours . . . ," the interval was ±5.75 h. They concluded that prediction was not accurate and ". . . thus the establishment of time of death using the concentration of the vitreous potassium offers little of practical value . . ."

In 1963, Sturner [7], and in 1964, Sturner and Gantner [8] presented data from 91 cases with a "standard error" of 4.7 h. They further stated that ". . . the standard error does not increase as the death interval lengthens . . . ," and depicted the method as being ". . . remarkably accurate . . ." In 1965, Hughes [9], using 135 cases, took exception to the results of Sturner and Gantner on all points. "There is no question that the variance is more than 5 hours . . . and time of death cannot be put any more accurately than within about 20 hours."

In 1966, Hansson et al. [10] presented data from 203 cases in which

> . . . the dispersion of the potassium values was much greater than in Addleson's series, the standard deviation for a postmortem interval of 0–20 hours being nearly twice as great as that reported by him and increasing further after this interval.

The reference to an increasing ". . . standard deviation for a post mortem interval . . ." is contrary to Sturner and Gantner's report of a nonincreasing "standard error." Though not as sanguine as Sturner and Gantner, the tone of Hansson et al.'s conclusion is much more optimistic than that of Addleson et al., stating that the use of potassium concentration ". . . may be warranted . . ." and ". . . can give some indication of the approximate time of death." So, Hansson et al. state conclusions of better confidence than did Addleson et al. Surprisingly, they did this with data that exhibit greater dispersion than Addleson's.

In April of 1967, Leahy and Farber [11] found the following:

> In 52 patients, values ranging from 4.4–16.6 meq/L were encountered. Any mathematical relationship between vitreous potassium concentration and the postmortem interval in 12 patients who died sudden deaths in this series was not apparent.

In August of 1967, Lie [12], with 88 cases, agreed totally with Sturner and Gantner on all points and was equally optimistic: "It is a remarkably simple and accurate method for estimating the postmortem interval especially after the first 24 hours."

In 1969, Coe [13] reported 145 cases and found himself in agreement with Hansson et al. and their contention that standard error increased with an increase in PMI. Coe further concurred with their conclusions, stating that vitreous potassium concentration ". . . can be of some help in estimating the time of death . . ." Unique to his analysis, Coe described his data in 2 new subgroups: (1) cases with a PMI of less than 6 h and (2) cases with a PMI of 6 h or greater. Each subgroup was described by a separate linear regression equation.

In 1972, Adjutantis and Coutselinis [1], using 120 cases, produced a bifaceted analysis. Following what appears to be the procedures of previous researchers (with the exception of meticulous centrifugation to separate the "vitreous humour" from the "vitreous layer"), they stated ". . . the standard deviation from regression of the X values is $S_{x.y} = 1.7$ hours. Accordingly, in about two-thirds of the cases the real time of death lies between the limits $X \pm 1.7$ hours . . ." This standard deviation of 1.7 h should correspond to a 95% confidence interval of ±3.4 h, better than any previous researcher has claimed. Further, they contend that this PMI prediction can be improved upon by sampling each eye separately at different times after death. Using these two points, a line is extrapolated to the ". . . accepted normal in vivo value of 3.4 mequiv/litre . . ." and the PMI is calculated. For this method they claim ". . . the standard deviation about the regression line is equal to 1.1 hours . . ." and that ". . . in about two-thirds of the cases the actual interval from death will be within the limits ±1.1 hours." This standard deviation of 1.1 h should correspond to a

95% confidence interval of ±2.2 h. They further state ". . . potassium values cease to increase after about 10–12 hours from the time of death . . ." and that this two sample method is proposed only for this postmortem period from 0 to 12 h.

In 1974, Crowell and Duncan [*6*] became the first researchers since Jaffe to relate potassium concentration to the logarithm of time (PMI). In their study of 60 dogs, they presented data with a 95% confidence interval of approximately ±12 h. They also made the interesting observation that the weight of the dog influenced potassium concentration. In 1977, Komura and Oshiro [*14*] performed studies on 90 human cadavers and 30 rabbits to demonstrate that temperature influences vitreous potassium concentration. In their 1980 study of 60 mongrel dogs, Schoning and Strafuss [*15,16*] agreed, stating ". . . potassium increased with temperature when time was held constant." This is directly contrary to the reports of several previous researchers [*4,5,7,8,12*].

In their 1979 study of 127 juvenile cases, Blumenfeld et al. [*17*] obtained a 95% confidence interval of ±26 h for the mean potassium concentration and concluded that ". . . postmortem vitreous humor concentrations . . . cannot be used . . . to establish time of death."

Review of the literature shows a great deal of disagreement on PMI prediction. Data from different researchers appear to be in conflict. Even those researchers with similar data have drawn conclusions that are in conflict. In addition, different statistical methods were used by different researchers making comparisons difficult. The underlying assumption when relating PMI to potassium concentration is that differences in potassium concentration are the result of differences in PMI. But with the possibility of weight of the decedent and ambient temperature being important factors, this assumption may be simplistic. In this study, our hope was that a larger sample size might clarify these areas of disagreement.

Materials and Methods

The samples for this study were obtained from decedents who were coroner's cases of the County of Orange, California. In general, the bodies were unrefrigerated from time of death to the time when the vitreous samples were drawn. Samples were taken at PMIs ranging up to 35 h. The great majority of the decedents were adults, but the ages ranged from newborn to 90 years.

The vitreous humor was aspirated through a puncture at the lateral canthus with an 18-gauge needle and a 5 to 30-mL disposable syringe. To insure an adequate volume of sample for analysis, right and left eye aspirates were usually pooled. All previous researchers have shown no difference between right and left eye samples of the same decedent [*1,5,8,9,12,13*]. Visibly discolored samples were excluded from the study. Samples were routinely filtered to prevent plugging of the small pump tubes during analysis by the Technicon SMAC-20. The method used by the SMAC for the analysis of potassium concentration is the direct potentiometric procedure, by means of a potassium ion selective electrode. The SMAC has a peak selector with a relatively narrow range of 1.9 to 8 meq/L. The vast majority of postmortem potassium values were outside of this narrow range and were subsequently determined by flame photometry.

The wide range of potassium values which we found within groups of samples with the same PMI is in agreement with some of the studies cited earlier [*9,10,17*]. Some authors divided their data into subpopulations as a method of addressing this problem of wide range in the data [*5,8,13*]. We did this as well by editing our data.

Data within each hourly group were ranked according to increasing potassium concentration, and data points that appeared graphically to be outliers were excluded from the study. The mean and standard deviation were then calculated for the remaining points of each hourly group. Data points outside of 2 standard deviations from the mean were then excluded. The data remaining comprise the 1427 samples used in this study. The data are plotted in Fig. 1.

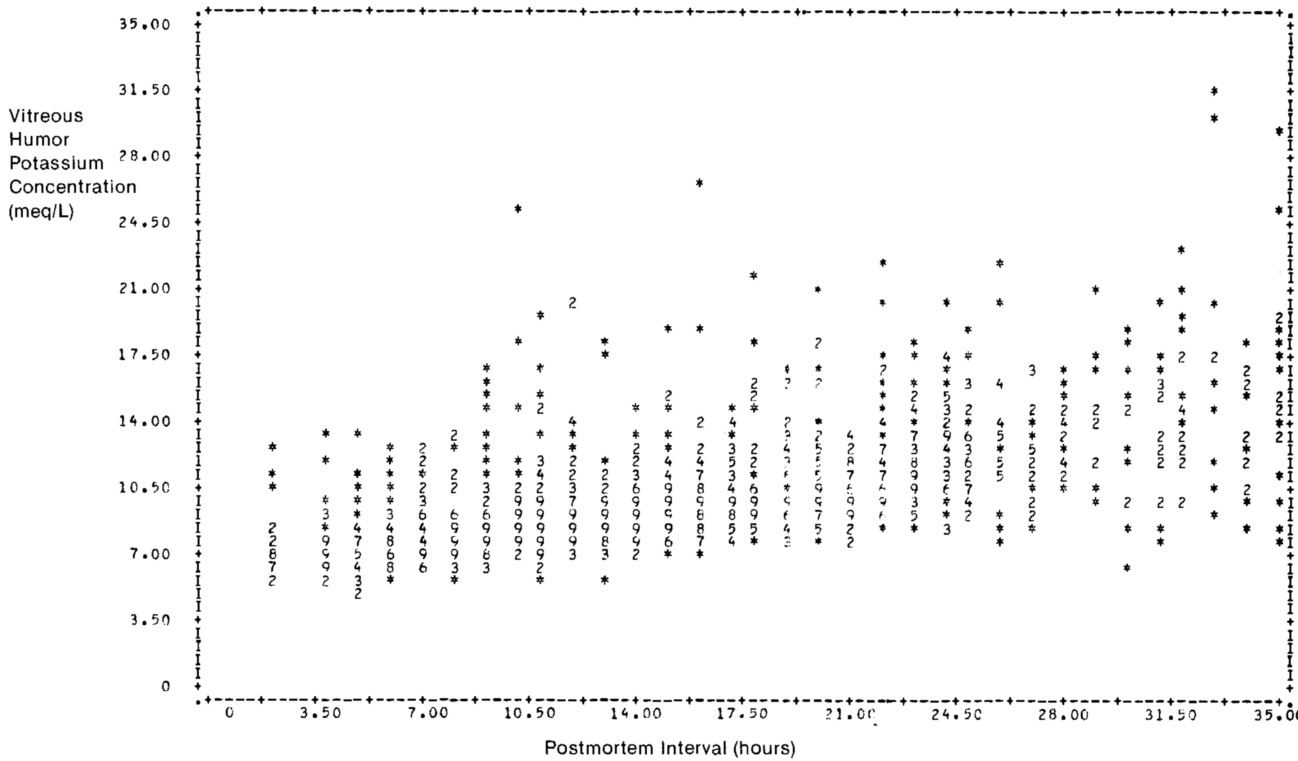

FIG. 1—*Plot of vitreous humor potassium concentration (meq/L) versus postmortem interval (hours). The plot shows the 1427 points used in this study. Numerals indicate the number of points at that position on the graph. The linear regression equation for the data was* $y = 0.238x + 6.342$ *with a coefficient of determination* (r^2) *of 0.374.*

Most of the data points excluded from the study were quite elevated. These high values generally fit into clinically significant subpopulations with death as a result of causes such as Sudden Infant Death Syndrome (SIDS), drowning, electrolyte imbalance, temperature extremes (freezing and burning), and cases in which toxicology studies were required. By dividing the data into subpopulations, we removed these elevated values from our sample and reduced the range of the data. The remaining data should then have a better correlation with time and allow better PMI prediction.

It should be emphasized that the procedure of calculating means and standard deviations from ranked data is not statistically valid. It was used solely as an editing device and gives no measure of central tendency for the data. These statistics were used to search the hourly groups for a "normal" population and thereafter to identify and categorize the "abnormal" clinically significant subpopulations. The final step was the statistical analysis of the data. The *Statistical Package for the Social Sciences* (SPSS) computer statistics package was utilized and the inverse prediction interval was then calculated using the formula

$$\bar{X} + \frac{b(Y_i - \bar{Y})}{K} \pm \frac{t}{K}\sqrt{s^2_{Y \cdot X}\left[\frac{(Y_i - \bar{Y})^2}{\Sigma x^2} + K\left(1 + \frac{1}{n}\right)\right]} \qquad (1)$$

where $t = t_{\alpha(2),(n-2)}$ and $K = b^2 - t^2 s_b^2$ [*18*].

Results

Because of the results of previous research [*5, 7-10, 12, 13*], we had good reason to hypothesize that after death potassium concentration rose in a linear fashion. This hypothesis was verified by least-squares analysis. The linear regression equation obtained for potassium concentration (y) versus time (x) is $y = 0.248\,x + 6.342$ (see Fig. 1).

A pertinent ancillary statistic is the coefficient of determination (r^2). The coefficient of determination, when multiplied by 100, gives the percentage of the potassium concentration variation that is directly attributable to the variation in PMI. In this study, r^2 is 0.374 or 37.4%. This leaves 62.6% of potassium concentration variability that can be attributed to factors other than time [*18-20*]. With the large percentage of potassium concentration variability unaccounted for by PMI, it should come as no surprise that the inverse prediction interval is also large. The 95% confidence limits of inverse prediction for each point of potassium concentration are approximately ± 20 h.

Discussion

In the course of an investigation, a forensic pathologist may wish to use vitreous humor potassium concentration to help establish the PMI. Pragmatically, he will make his prediction from a single sample of vitreous humor. If his prediction is to be accurate, he must use the proper statistics in his analysis. The use of a single potassium concentration (the dependent variable) to predict the PMI (the independent variable) necessitates the use of inverse prediction (more commonly, independent variables are used to predict dependent variables). A confidence interval for a regression line does not include the random error component inherent in the use of a single determination for prediction purposes. Consequently, a confidence interval is too narrow and gives the spurious impression of better predictability than is possible [*21*].

The data of this study, and hence its conclusions, are limited to the range of 35-h postmortem. It would be dangerous (and poor statistical practice) to extrapolate and draw conclusions about the later hours of PMI from this data. For example, the question as to whether the variance (Sturner used the term "standard error," Hughes "variance," and Hansson "standard deviation") increases with an increase in PMI cannot be answered from this study.

As mentioned in the Materials and Methods section, the ultimate effect of the editing process is the reduction in range of the potassium values. This reduction could have narrowed the inverse prediction interval to a degree that would have given one unwarranted confidence in one's ability to predict PMI. In fact, the editing process did narrow the inverse prediction interval, but ± 20 h can hardly make one feel overconfident. More enlightening than the size of the inverse prediction interval is the coefficient of determination, which showed that 62.6% of the variation of potassium was unaccounted for.

If a portion of this 62.6% could be attributed to some known and quantifiable factors, the ability to predict PMI could be greatly enhanced. Future studies may show, however, that some factors such as ambient temperature play a greater role than was previously believed, and may essentially be beyond our control. Other factors, such as sampling technique (that is, force of vacuum upon aspiration, storage temperature of the sample after aspiration), sex, age, race, or cause of death may prove to be quantifiable and improve PMI prediction. The possibility remains that in very early or later hours potassium may not rise linearly. A polynomial curve fit may be in order and improve predictability further. Studies focusing on the use of other chemical determinants in conjunction with potassium may prove promising. We are currently examining phosphorus for this use. These sorts of studies might explain some of the diametrically opposed reports in the literature and help reconcile the research into a practical and useful aid in forensic medicine.

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References

[1] Adjutantis, G. and Coutselinis, A., "Estimation of the Time of Death by Potassium Levels in the Vitreous Humor," *Forensic Science*, Vol. 1, 1972, pp. 55-60.

[2] Knight, B., "Estimation of the Time Since Death: A Survey of Practical Methods," *Journal—Forensic Science Society* (London), Vol. 8, 1968, pp. 91-96.

[3] Naumann, H. N., "Postmortem Chemistry of the Vitreous Body in Man," *American Archives of Ophthalmology*, Vol. 62, 1959, pp. 356-363.

[4] Jaffe, F. A., "Chemical Post-Mortem Changes in the Intra-Ocular Fluid," *Journal of Forensic Sciences*, Vol. 7, No. 2, April 1962, pp. 231-237.

[5] Adelson, L., Sunshine, I., Rushforth, N. B., and Mankoff, M., "Vitreous Potassium Concentration as an Indicator of the Postmortem Interval," *Journal of Forensic Sciences*, Vol. 8, No. 4, Oct. 1963, pp. 503-514.

[6] Crowell, W. A. and Duncan, J. R., "Potassium Concentration in the Vitreous Humor as an Indicator of the Postmortem Interval in Dogs," *American Journal of Veterinary Research*, Vol. 35, No. 2, Feb. 1974, pp. 301-302.

[7] Sturner, W. O., "The Vitreous Humor: Postmortem Potassium Changes," *The Lancet*, Vol. 1, 13 April 1963, pp. 807-808.

[8] Sturner, W. O. and Gantner, G. E., "The Postmortem Interval: A Study of Potassium in the Vitreous Humor," *American Journal of Clinical Pathology*, Vol. 42, No. 2, Aug. 1964, pp. 137-144.

[9] Hughes, W. M. H., "Levels of Potassium in the Vitreous Humor After Death," *Medicine, Science, and the Law*, Vol. 5, No. 3, July 1965, pp. 150-156.

[10] Hansson, L., Uotila, U., Lindfors, R., and Laiho, K., "Potassium Content of the Vitreous Body as an Aid in Determining the Time of Death," *Journal of Forensic Sciences*, Vol. 11, No. 3, July 1966, pp. 390-394.

[11] Leahy, M. S. and Farber, E. R., "Postmortem Chemistry of Human Vitreous Humor," *Journal of Forensic Sciences*, Vol. 12, No. 2, April 1967, pp. 214-222.

[12] Lie, J. T., "Changes of Potassium Concentration in the Vitreous Humor After Death," *American Journal of the Medical Sciences.* Vol. 254, Aug. 1967, pp. 136–143.
[13] Coe, J. I., "Postmortem Chemistries on Human Vitreous Humor," *American Journal of Clinical Pathology,* Vol. 51, No. 6, June 1969, pp. 741–750.
[14] Komura, S. and Oshiro, S., "Potassium Levels in the Aqueous and Vitreous Humor after Death," *Tohoku Journal of Experimental Medicine,* Vol. 122, 1977, pp. 65–68.
[15] Schoning, P. and Strafuss, A. C., "Postmortem Biochemical Changes in Canine Vitreous Humor," *Journal of Forensic Sciences,* Vol. 25, No. 1, Jan. 1980, pp. 53–59.
[16] Schoning, P. and Strafuss, A. C., "Determining Time of Death of a Dog by Analyzing Blood, Cerebrospinal Fluid, and Vitreous Humor Collected at Postmortem," *American Journal of Veterinary Research,* Vol. 41, No. 6, June 1980, pp. 955–957.
[17] Blumenfeld, T. A., Mantell, C. H., Catherman, R. A., and Blanc, W. A., "Postmortem Vitreous Humor Chemistry in Sudden Infant Death Syndrome and in Other Causes of Death in Childhood," *American Journal of Clinical Pathology,* Vol. 71, No. 2, Feb. 1979, pp. 219–223.
[18] Zar, J. H., *Biostatistical Analysis,* Prentice-Hall, Inc., Englewood Cliffs, NJ, 1974, pp. 198–214.
[19] Nie, N. H., Hull, C. H., Jenkins, J. G., Steinbrenner, K., and Bent, D. H., *SPSS: Statistical Package for the Social Sciences,* 2nd ed., McGraw-Hill Book Company, New York, 1975, pp. 293–300.
[20] Klecka, W. R., Nie, N. H., and Hull, C. H., *SPSS Primer: Statistical Package for the Social Sciences Primer,* McGraw-Hill Book Company, New York, 1975, pp. 82–91.
[21] Mendenhall, W., *Introduction to Probability and Statistics,* 5th ed., Duxbury Press, North Scituate, MA, 1979, pp. 343–349.

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