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Estimation of time since death by vitreous humor hypoxanthine, potassium, and ambient temperature

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ABSTRACT

Measurement of vitreous humor potassium (K^+) has since the 1960s been recognized as an adjunct for estimation of time since death. In 1991 we introduced hypoxanthine (Hx) as a new marker. Furthermore we demonstrated that time since death estimation was more accurate when ambient temperature was included in the calculations, both for K^+ and for Hx. In this paper we present a refined method. The subjects consist of 132 cases with known time of death and ambient temperature. One sample from each subject was used in the calculations. Vitreous humor Hx levels were available in all subjects, while K^+ was measured in 106 of the subjects, due to insufficient volume of vitreous humor. Linear regression analysis was applied to model the correlation between vitreous humor Hx and K^+, taking the interactions with temperature into consideration. The diagrams published in 1991, which also included ambient temperature, estimated median time since death with range between the 10th and 90th percentile, whereas the linear regression analysis presented in this paper estimates mean time since death with a corresponding 95% interval of confidence. We conclude that time since death may be estimated with relatively high precision applying vitreous humor Hx and K^+ concentrations combined with ambient temperature.

1. Introduction

In 1991 we published a new method for estimation of time since death based on measurement of hypoxanthine (Hx), potassium (K^+) and ambient temperature [1]. Measurement of K^+ had already been reported by [2–6], however without adjusting the results for differences in ambient temperature. A relationship between vitreous humor Hx concentration and time since death has since been confirmed by several authors [7–13]. However none of these authors did adjust the results for different ambient temperatures.

In our first publication we reported that the slope for both K^+ and Hx increase became steeper with increasing ambient temperature [1]. Based on our data we presented a diagram by which time since death may be estimated from ambient temperature and K^+ and Hx levels in vitreous humor. The diagram was based on 87 cases, with known time of death, kept at different ambient temperatures. Two to four samples obtained at different times from each subject were used for the construction of the diagram [1,14]. In later presentations the diagram was based on one single sample from each of the 132 cases with known time of death [15].

The purpose of this paper is to present a more accurate way of calculating time since death based on multiple regression analyses of vitreous humor K^+ and Hx levels as well as ambient temperature.

2. Materials and methods

2.1. Live controls

Vitreous humor was obtained from 17 patients undergoing vitrectomy, and the samples were analyzed for Hx and K^+. The patients gave written informed consent.

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2.2. Subjects

The subjects included in the study were 132 cases of sudden death (66 male, 66 female). The median age was 77 years (range 15–100 years). The cases are from the years 1988–1998, and were either subjects of autopsy at the former Institute of Forensic Medicine, University of Oslo ($n = 80$) or were sampled after natural deaths at a health institution ($n = 52$). In the latter no autopsy was performed. Time of death was known for all cases. The median post mortem interval (PMI) was 21.9 h (17 min–118 h). Eight deaths were due to accidents, two were homicides, four were suicides and 118 suffered natural deaths (Table 1).

Vitreous humor was sampled from either the right or the left eye by a vacutainer. One sample from each case was included in the dataset. The bodies were kept at different ambient temperatures; 5 °C ($n = 34$), 10 °C ($n = 18$), 15 °C ($n = 18$) and 23 °C ($n = 55$). The temperature range within each group is given in Table 2. Eighty-seven of the cases have been published previously [1]. The study was approved by The National Committees for Research Ethics in Norway.

2.3. Methods

Determination of Hx in vitreous humor was done by high performance liquid chromatography (HPLC) for the 87 cases published previously, and the details regarding this method were given in a previous study [1]. For all new cases included in the present study, Hx was determined by capillary electrophoresis. Results obtained by HPLC have been compared with results obtained by capillary electrophoresis in a previous study, and the coefficient of correlation between the two methods was $r = 0.96$, $p < 0.001$ [14].

2.4. Capillary electrophoresis measurements of Hx

All chemicals used were of analytical or research grade. All buffer solutions, standard solutions and other experimental material were prepared in deionized water and filtered through a filter unit with a 0.45 μm pore size (Gelman Sciences) by means of a syringe. Every sample of vitreous humor was vortexed and pipetted into an Ultrafree-MC 5000 NMWL filter unit (Millipore Corporation). The samples were then centrifuged at 9000 rpm for 90 min at 4 °C and stored at −20 °C/−75 °C until Hx was measured.

Measurement of Hx was performed with a BioFocus 3000 capillary electrophoresis system (Bio Rad Laboratories, Hercules, CA, USA). The columns were made of coated fused-silica capillary tubing (50 μm inner diameter × 30 cm total length for the separation of Hx), which was mounted in a user assembled cartridge (Bio-Rad). Injection of sample was effected by applying a pressure of 15 psi × s. A constant voltage of 10–13 kV (current < 100 μA) was applied; the temperatures of the capillary cartridge and carousel were maintained at 20 °C and 12 °C, respectively. Detection was effected at 254 nm polarity of − to + for Hx. The identity and purity of the peaks were established by high speed scanning (range 200–280 nm) of the sample and of pure standard, in which the eluting peaks were characterized by both migration time and absorption behavior.

Capillary equilibrium between runs was obtained by rinsing the capillary with distilled water for 15 s, 1 mM NaOH for 60 s, distilled water for 15 s, and with the working buffer for 60 s. Biofocus integration software (version 5.0 Bio Rad Laboratories, Hercules, CA, USA) was employed for data conversion and evaluation. A 25 mM sodium tetraborate buffer, pH 9.1, was used for the separation of Hx. Quantitation of Hx was based upon internal four-level calibration using peak area/migration time and having 5–40 μmol/L of Hx containing 15 μmol/L theophylline (internal standard).

Table 1
Manner and cause of death.

Manner of death	Number of cases	Cause of death	Number of cases
Accidental death	8	Aortic rupture	1
		Head and thorax injuries	1
		Thorax injuries	2
		Suffocation	1
		Traumatic brain hemorrhage	1
		Acute intoxication morphine/heroine	2
Homicide	2	Suffocation	1
		Stab wound in thorax	1
Suicide	4	Shot wound in the head	1
		Drowning	1
		Traumatic heart rupture, fall	1
		CO intoxication	1
Natural deaths	118	Sudden cardiac death	60
		Death due to lung disease	32
		Lung artery thrombosis	1
		Intestinal volvulus	1
		Cancer	4
		Renal disease	2
		Acute hemorrhage/cancer	5
		Wernicke's encephalopathy	1
		Sudden death, unknown cause	12

Table 2
Range of ambient temperatures.

Temperature group	Range	Number of subjects
5 °C	3.5–7 °C	34
10 °C	9–12 °C	18
15 °C	13–19 °C	25
23 °C	20–27 °C	55

2.5. Determination of potassium

Vitreous humor K^+ was measured by flame photometry (Department of Medical Biochemistry, Oslo University Hospital).

2.6. Statistical analyses

Linear regression analysis was applied to model the correlation between vitreous humor Hx and K^+, taking the interactions with temperature into consideration. For both Hx and K^+, time since death was used as the dependent variable. For the model describing Hx, vitreous humor Hx, temperature and the product of temperature and vitreous humor Hx (interaction) were used as the independent variables. For the model describing K^+, vitreous humor K^+, temperature and the product of temperature and vitreous humor K^+ (interaction) were used as independent variables. The standard error curves for the predicted values were fitted using a quadratic function.

Goodness of fit for linear correlations was evaluated using r^2 values, with a value of 1 corresponding to a perfect correlation.

The difference in post mortem increases of Hx and K^+ between male and female subjects was evaluated by comparing the estimated slopes of the corresponding linear regression coefficients.

Statistical analyses were performed using IBM SPSS Statistics Version 19 (IBM Company, Chicago, USA) and figures were produced using GraphPad Prism (GraphPad Softare Inc, California, USA). A significance level of 5% was used for all analyses.