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Estimation of postmortem interval using vitreous potassium levels in dogs[#]

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Abstract

Post-mortem interval (PMI) is the time elapsed between death and the discovery of the body and its estimation is crucial in forensic pathology investigations. Numerous methods are available for PMI estimation. In humans, the vitreous potassium concentration (K^+) has shown promise. The usefulness of the method in the estimation of PMI in dogs is explored in the current study. 25 dog carcasses were studied at the Department of Veterinary Pathology, CVAS, Pookode, Kerala and vitreous humour samples were collected and potassium concentration was quantified using the ion-selective electrode method. Concurrently, ambient temperatures were recorded. Our findings unveiled a significant positive correlation between vitreous potassium concentration and PMI. We derived an equation that incorporates ambient temperature. This equation serves as a valuable tool for field veterinarians, enabling them to estimate PMI in dog carcasses up to 96 hours. Consequently, vitreous humour emerges as a distinctive biosignature for accurate PMI estimation in dogs.

Keywords: *Post-mortem interval, vitreous humour, potassium, dog, ambient temperature*

Veterinary forensic medicine is one of the least explored areas in veterinary practice. The determination of time elapsed between death and discovery of carcass, known as post-mortem

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interval (PMI) or time since death (TSD) is a critical component of forensic and vetero-legal procedures. Although many techniques were used for estimating PMI, such as rectal temperature and rigour mortis, the precision of estimation was limited. Thanatochemistry which describes the biochemical changes that occur in body fluids after death, has evolved recently and has become one of the most promising methods for estimating PMI. In this method, the assessment of the concentration of potassium (K^+) in the vitreous humour of the eye was found to contribute significantly to PMI estimation studies in humans (Ahi and Garg, 2011; Akhtar *et al.*, 2015; Zilg *et al.*, 2015). Even with the advantages of optimistic research results and ease of collecting the samples and quantifying potassium, this method is not well-established for PMI estimation in animals. The present study aimed to assess the concentration of potassium in the vitreous humour of dogs with known PMI and to develop a regression formula incorporating ambient temperature to determine PMI in dog carcasses.

Twenty-five dog carcasses brought for post-mortem examination at the Department of Veterinary Pathology, CVAS, Pookode with known PMI were chosen for the present study. Vitreous humour was collected from both eyes in a 2 ml microcentrifuge tube by scleral puncture using a 20-gauge needle. The samples were centrifuged and stored at -25° till the quantification. Only transparent, clear liquid, free from tissue fragments was used for the quantification. The average ambient temperature during the day of collection was recorded. The potassium concentration of the samples was quantified using the ion-selective electrode (ISE) method with the Epsilyte electrolyte analyser (Epsilon Diagnostics, India) and readings were recorded. The statistical analysis was done using Statistical Package for Social Sciences (SPSS) v.20.0. Potassium ion concentration of vitreous humour and corresponding PMI and ambient temperature recorded for 25 dog carcasses are given in Table 1.

The Pearson correlation between the PMI and potassium concentration was 0.942, which was significant at 0.01 level. From analyses of the data, the PMI and potassium concentration of vitreous humour were found to be strongly positively correlated.

Table 1. Vitreous potassium concentration and corresponding ambient temperature of dog carcasses with known PMI

Sl No	PMI (minutes)	Potassium concentration (mEq/L)	Ambient temperature (°)
1	105	6.8	22.58
2	105	6.9	22.32
3	125	8.9	30.5
4	135	11.4	21.36
5	157	9.7	21.08
6	780	15.3	23
7	825	15.8	22
8	960	17.4	23.32
9	1260	17.6	21.95
10	1320	17.8	24.42
11	1455	17.9	23.85
12	1500	18	20.51
13	1800	18.7	21.93
14	1680	18.9	23.8
15	2100	19.2	22.07
16	3120	20.3	24.89
17	3120	21	30.5
18	3285	20.7	26.8
19	4200	22.1	30.5
20	4320	22.5	24.93
21	4500	27.1	30.5
22	4800	28	21.08
23	4860	28.8	30.5
24	5520	28.3	23.93
25	5760	30.5	30.5

The regression model was created for PMI and potassium concentration with an R^2 value of 0.89 with PMI as the dependent and vitreous potassium as the independent variable. A linear regression equation (**A**) was developed which is given below:

PMI (minutes) = 267.486 (K^+) – 2733.107

Pearson correlation was performed between the potassium concentration and ambient temperature and the correlation coefficient was 0.28 at 0.01 level.

To explore the possibility of the existence of a non-linear relationship between the ambient temperature and the vitreous potassium concentration, a multi-linear regression model was created using PMI, vitreous potassium concentration (K^+) and ambient temperature (T). The equation (**B**) is as follows,

PMI (min) = (252.6 K^+(mEq/L)) + (101.7 T (□)) - 4937.4

The validation of both the equations was also performed with the available vitreous potassium data and the residual errors were calculated.

In our study, we found an increase in the potassium concentration of vitreous humour with an increase in PMI. This is also evident in the earlier studies by Nilesh *et al*. (2014), Angayarkanni (2021) in humans, Proctor *et al*. (2009) in dogs and George and Ajayi (2016) in bovine. The impact of ambient temperature was considered in a few earlier studies. Recent studies by Zilg *et al*. (2016) and Rognum *et al*. (2016) indicated an increase in the vitreous potassium concentration in response to an increase in environmental temperature. In our study, it was evident that there was a rise in potassium concentration at high ambient temperature when compared to carcasses of similar PMI maintained at lower ambient temperature. In almost all the carcasses with ambient temperature, 30.5° have a higher vitreous potassium concentration than carcasses kept at lower ambient temperature even though its statistical significance was less. This could be due to the narrow range of ambient temperature (21° to 30.5°) during our study period. The regression model achieved by Munõz *et al*. (2001) in their study yielded an error of 7h without considering ambient temperature. We predicted the PMI by inserting both potassium and ambient temperature data into the regression model. The average difference between actual and estimated error was calculated as 7h at an interval from 24 to 96h of PMI, providing a better estimation of PMI up to a period of 96h compared to the previous studies.

Summary

The present study demonstrates a linear correlation between vitreous potassium concentration and PMI, highlighting the potential of this biomarker in PMI estimation for dogs. The developed mathematical model, incorporating ambient temperature, can serve as a valuable tool for field veterinarians, providing PMI estimates up to 96 hours postmortem. However, further research is warranted to refine the model and enhance its accuracy. Repetitive sampling from eyes maintained under a wide range of temperatures with varying geographical and climatic conditions for assessing PMI could further improve the robustness of the model.

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Conflict of interest

The authors declare that they have no conflict of interest.

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