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Post mortem vitreous magnesium in adult population

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ABSTRACT

Background: The study of post mortem vitreous magnesium (Mg) is less common than sodium (Na), chloride (Cl) and potassium (K) in the forensic literature. There is no accepted normal range for post mortem vitreous Mg and the relationship between post mortem vitreous Mg levels and post mortem interval (PMI), other electrolyte levels, disease conditions, age and sex have not been fully established.
Aim: To investigate the relationship of post mortem vitreous Mg with age, sex, PMI, vitreous electrolyte levels and diabetic status.
Methods: A retrospective study of 20 consecutive cases of diabetics and 20 non-diabetic adult deaths was performed. Spearman correlation and the permutation test were used to explore the relationship between post mortem vitreous Mg and continuous and categorical variables respectively.
Results: The mean post mortem vitreous Mg was 1.03 mmol/L (95%CI: 0.98–1.08 mmol/L). The absolute Spearman correlation coefficients (rho) between post mortem vitreous Mg with PMI, age, and other vitreous electrolytes (Na, Cl, and K) ranged between 0.04–0.21 ($p > 0.19$). Post mortem vitreous Mg was statistically higher in diabetics (mean difference: 0.08 mmol/L; area-under-the-curve = 0.65 on receiver-operator-characteristic curve). No statistical difference was demonstrated between sexes ($p = 0.92$).
Conclusions: In our adult population, post mortem vitreous Mg did not correlate with age, PMI, other vitreous electrolytes (sodium, chloride and potassium) or sex. It was higher in diabetics, however had limited utility as a surrogate marker. Overall, post mortem Mg is steady in the early post mortem period with a mean of 1.03 mmol/L.

1. Introduction

Vitreous humor is a good sample for post mortem biochemical analysis as it is relatively resistant to microbiological contamination and mirrors plasma chemical composition [1]. Amongst the commonly analysed post mortem vitreous electrolytes, magnesium (Mg) is studied less often in comparison with sodium (Na), chloride (Cl) and potassium (K). Post mortem vitreous Mg is known to be higher in the paediatric population, and decreases with age to stabilize at approximately 10 years of age [2]. Clinically, hypomagnesaemia is associated with type 2 diabetics and diabetics have a lower vitreous Mg levels relative to the general population [3,4]. In the literature, post mortem vitreous Mg was previously used in studying immersion time in bodies recovered from water and to estimate the time of death, but with limited success and application [4–7]. A recent study suggested post mortem vitreous Mg may be useful in assisting the diagnosis of salt water drowning deaths in the adult population [8]. It was observed that during salt water drowning, vitreous sodium and chloride initially elevates from drowning and then may subsequently increase secondary to immersion; whilst magnesium does not elevate during drowning, but from immersion [8–10]. This unique property of Mg was suggested to be useful in the interpretation of vitreous Na and Cl when the immersion time is not fully known [8]. However, there is no accepted range for post mortem vitreous magnesium and its relationship with other electrolyte levels, disease conditions, age and sex are not fully established. This study was carried out to examine the relationships between post mortem vitreous Mg with age, sex, diabetic state (type 2), post mortem interval (PMI) and other electrolytes (Na, Cl, and K) in the adult population.

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2. Material and methods

2.1. Case selection

To establish a correlation coefficient of 0.5 (moderately associated, with $\alpha = 0.05$, $\beta = 0.2$) the sample size (n) required was calculated to be 29. For analyzing the difference in vitreous magnesium in diabetics and non-diabetics, clinical data showed a mean difference of approximately 0.15 mmol/L and a standard deviation of 0.15 mmol/L. To achieve $\alpha = 0.05$, $\beta = 0.2$ a sample size of 19 was needed for each group.

This study subsequently examined 20 consecutive diabetics and 20 non-diabetic adult (age >18 years) deaths between May and October 2017 at the Department of Forensic pathology, LabPLUS, Auckland City Hospital. A full autopsy was performed in each case together with vitreous biochemical analysis. All full post mortem examinations performed at the forensic department are consented and any samples taken are fully disclosed to the Coroner. Any tissue or body fluids kept for analysis are to be returned to the family upon family request after the coronial investigation.

All cases were identified where the pathologist had sampled vitreous fluid for biochemical analysis (including Na, Cl, K and Mg). The case files were reviewed and the post mortem interval (PMI) was determined as the time between time of death and time of autopsy (in hours). The age, sex, cause of death, PMI, and electrolyte analysis results were recorded. The diabetic status was determined via accessing electronic medical records. Deaths from acute metabolic diabetic complications (hypoglycemia or hyperglycemia) were excluded from the study due to possible confounding effects on electrolyte concentrations.

2.2. Vitreous electrolyte analysis

Vitreous fluids were collected by aspirating through the sclera using a 21-guage needle and a 10 ml syringe. In all cases the tip of the needle was placed near the center of the globe and all vitreous fluid from both eyes were collected. The collected vitreous fluid from both eyes were mixed together and placed either in total into a plain tube for biochemical analysis or split into half (one for toxicological analysis and the other for biochemical analysis). The vitreous fluids for biochemical analysis were sent to a local accredited laboratory (Department of Biochemistry, LabPLUS, Auckland City Hospital, Auckland, New Zealand) for analysis. Prior to analysis, the vitreous samples were heat treated (100 °C for 5 min) and spun down as per protocol [11].

Na, Cl and K were measured by ion selective electrode on a Roche Cobas ISE module (Roche Diagnostics). Vitreous Mg was measured on a C502 (Roche Diagnostics) using a xylidyl blue method. A validity study using xylidyl blue method was undertaken using bovine eye samples. In the validity study, varying volumes of a weighed-in magnesium chloride solution (ACS Reagents, Mexico) was added to bovine vitreous specimens (<10% v/v) following heat treatment. Comparisons between the measured concentration of the mixture was in agreement with the concentration calculated (n = 20; N = 13 separate eyes; Fig. 1). The calculated concentration was based on initial concentration as measured by Roche xylidyl blue method and the volume of magnesium chloride solution added. The typical imprecision of our method as measured by the coefficient of variation for Mg was 3.7% (0.9 mmol/L, mean concentration).

2.3. Statistical analysis

Continuous variables were described using means and standard deviations. Medians, 95% confidence interval, minima and maxima were also presented. Categorical variables were described using counts.

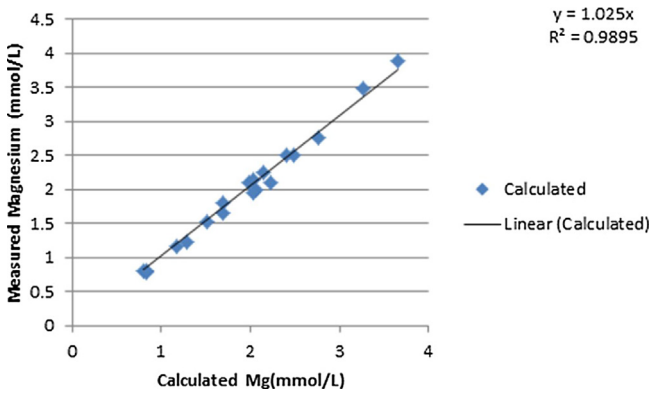

Fig. 1. Validation curve for measuring post mortem vitreous Mg using bovine vitreous.

Assuming monotonic and non-parametric relationships between vitreous electrolytes, age and post mortem interval, Spearman's correlation coefficient was determined between continuous variables and post mortem vitreous Mg. For dichotomous relationships (sex and diabetic state), vitreous magnesium was compared using resampling permutation test which is robust to departures in model assumptions such as normality and constant variance. Depending on the result of the permutation test, the receiver–operator–characteristic (ROC) curve was plotted and the area-under-the-curve (AUC) was determined. The optimal cut-point was determined as by minimizing the Euclidean distance to the perfect predictor (sensitivity of one and false positive rate of zero).

Statistical analysis was performed using R (version 3.4.1, The R Foundation for Statistical Computing). A p-value of <0.05 was considered significant.

3. Results

In the 40 cases identified, there were 29 natural deaths (22 cases of cardiovascular causes, 6 infective causes and 1 from gastrointestinal hemorrhage), 8 drug and alcohol related deaths, 2 traumatic deaths and 1 case of hypothermia. In the diabetic group, all cases were type 2 diabetics. A summary of the demographics, PMI and post mortem vitreous electrolytes is shown in Table 1.

Vitreous Mg did not show any statistical significant correlations with age, PMI, and measured vitreous electrolytes (Fig. 2,Table 2). The absolute Spearman's coefficient was between 0.04–0.21 and none reached statistical significance ($p > 0.05$).

Permutation test (Table 3) showed vitreous Mg levels were higher in diabetics ($p = 0.04$, Fig. 3) but there was no difference in sex ($p = 0.92$). The AUC on the ROC curve constructed from post mortem Mg and diabetic state was 0.65 (Fig. 4). The optimal post mortem vitreous Mg cut-point to discriminate diabetic state was 1.05 mmol/L which translated to a sensitivity of 0.35 and specificity of 0.95.

4. Discussion

In the presented study, post mortem vitreous Mg did not demonstrate statistically significant correlations with age, PMI, and vitreous electrolyte levels (Na, Cl and K), and there was no demonstrable difference between sex. Statistically, diabetics appeared to have higher vitreous Mg with a mean difference of 0.08 mmol/L, but this difference was not useful as a discriminatory test for diabetes with an AUC of 0.65 on the ROC curve.