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Elevation of *post mortem* vitreous humour sodium and chloride levels can be used as a reliable test in cases of suspected salt water drowning when the immersion times are less than one hour

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ABSTRACT

Background: Previous studies in salt water drowning deaths (SWD) demonstrated an observable elevation of *post mortem* vitreous sodium and chloride (PMVSC) levels. It remains unclear what the underlying mechanism responsible for this change is: whether this is due to rapid electrolyte changes from salt water inhalation/ingestion during drowning or from electrolyte diffusion and/or osmosis across the outer coats of the eyeballs during immersion. A recent animal study using sacrificed bovine eyeballs immersed in salt water demonstrated no significant elevations in PMVSC when immersed for less than one hour. Assuming similar physical properties between human and bovine, we extrapolate that an elevation in PMVSC in SWD with immersion times of less than one hour (SWD-1) would not be from immersion, but from drowning.
Aim: Investigate whether there is an elevation in PMVSC in SWD-1.
Methods: Retrospective study comparing PMVSC in SWD-1 with controls from 2012 to 2015 inclusive.
Results: PMVSC in SWD-1 was significantly elevated compared with controls. A PMVSC of 259 mmol/L has a sensitivity, specificity and likelihood ratio of 0.9, 0.9 and 7.6, respectively.
Conclusion: The elevation in PMVSC in SWD-1 is due to drowning. A PMVSC of 259 mmol/L and above is a reliable ancillary test in diagnosing drowning in bodies immersed in salt water for less than one hour.

1. Introduction

Drowning is the process of experiencing respiratory impairment from submersion/immersion in a liquid [1]. During drowning, large volumes of liquid may be inhaled and/or ingested, and death can occur within minutes. The mechanism of death is thought to be by asphyxiation/hypoxia [2]. Making a diagnosis of drowning can be challenging due to the absence of specific *post mortem* findings and unreliable ancillary tests [2].

The authors have previously reported an elevation in *post mortem* vitreous humour sodium and chloride levels (PMVSC) in salt water drowning deaths (SWD) compared with immersion deaths not related to drowning but recovered from water (DNRD) [3]. It is hypothesized that the PMVSC elevation seen in SWD is related to blood electrolyte changes secondary to large amounts of salt water inhalation and/or ingestion which results in PMVSC elevation [3].

A major confounding factor causing PMVSC elevation is electrolyte diffusion and/or osmosis across the outer coats of the eyeball during salt water immersion. Although prolonged salt water immersion causes PMVSC elevation, previous studies [4–6] did not investigate PMVSC with shorter immersion times. Studying PMVSC changes with shorter immersion times is critical as death due to drowning can occur within minutes. A recent study carried out by the authors using sacrificed bovine eyeballs to investigate

Abbreviations: DNRD, immersion deaths not related to drowning but recovered from salt water; DNRD-1, immersion deaths not related to drowning but recovered from salt water; (DNRD), with immersion time of less than one hour; PMVSC, *post mortem* vitreous sodium and chloride; SWD, salt water drowning death; SWD-1, salt water drowning death (SWD) with immersion time of less than one hour.

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the changes in PMVSC with shorter immersion times in salt water did not show any significant elevation in PMVSC when the eyeballs were immersed for less than one hour [7]. With comparable permeability between human and bovine eyes, the authors concluded that any PMVSC elevation in humans with an immersion time of less than one hour would not be from immersion [7].

It is therefore reasonable to investigate whether there is an elevation in PMVSC in SWD with immersion times of less than one hour (SWD-1) in humans. An elevation in PMVSC in SWD-1 would not be from immersion, but from drowning, and would support the hypothesis that during salt water drowning, the inhaled and/or ingested salt water causes electrolyte changes in blood which are reflected in an elevation in PMVSC.

This study compared PMVSC in 24 cases of witnessed or clinically confirmed SWD-1 with 96 controls (non-immersion deaths) during 2012–2015 (inclusive).

2. Method and material

A retrospective study comparing the PMVSC in SWD-1 and controls during 2012–2015 (inclusive) was performed at the Department of Forensic Medicine, Newcastle, Forensic & Analytical Science Service, New South Wales Health Pathology. The specified study time period was chosen deliberately as routine PMVSC analysis in bodies recovered from water was not performed prior to that time.

All witnessed or clinically confirmed SWD-1 deaths admitted to the department during the study period were selected. Twenty-four SWD-1 cases were identified by selecting all SWD deaths in which there was a clear documentation of immersion time of less than one hour in the referring police report. The age, gender, location of death, circumstances relating to the death, time between death and autopsy, and vitreous humour sodium (Na) and chloride (Cl) levels were recorded.

A previous study by the authors used DNRD as controls [3]. Since then, we have performed an experimental study immersing sacrificed bovine eyeballs in salt water and shown that PMVSC was unchanged within the one hour time window [7]. Therefore, for this study DNRD data were no longer needed as controls. Controls (non-immersion deaths) were selected sequentially in cases for which vitreous humour Na and Cl were analyzed at *post mortem* during the same study period in the same department. Coronial cases such as sudden unexpected infant deaths, suspicious deaths, homicides, and bodies which were decomposed, incinerated or recovered from water were excluded. Conditions which possibly have an impact on the vitreous humour Na and Cl levels were also excluded (such as end stage liver disease, hyponatraemia, and diabetic ketoacidosis or any other with suspected metabolic derangement). The number of controls was chosen to be four times the number of SWD-1 (96 cases) which gives the study 80% power to detect a 6.5 mmol/L difference in PMVSC at the 5% significance threshold (assuming a standard deviation of 10 mmol/L [3]). The age, gender, cause of death, time between death and autopsy, and vitreous humour Na and chloride Cl levels were recorded.

All vitreous humour samples were analyzed for Na and Cl by a local accredited biochemistry lab (Pathology North, John Hunter Hospital, New Lambton Heights, New South Wales, Australia) using an ion selective electrode analyzer (Abbott chemistry analyzer C16000/C8000 or an Olympus 5400 Auto-analyzer). In both SWD-1 and controls, the PMVSC was calculated by adding vitreous humour Na and Cl levels.

2.1. Statistical methods

SAS 9.4 (SAS Institute, Cary, North Carolina, USA) was used for analysis.

Continuous variables were described using means and standard deviations. Medians with minima and maxima were presented. Categorical variables were described using counts and percentages for cases and controls. Differences between cases and controls in the age, time between death and autopsy, vitreous humour Na, vitreous humour Cl, and PMVSC were assessed using Students *t*-test, and between-group differences in gender was assessed using the Pearson Chi-square test.

Logistic regression models were used to assess the predictive ability of the vitreous humour Na, vitreous humour Cl, and PMVSC measures. We first created a baseline logistic regression model including variables that differed between cases and non-cases. We then assessed the incremental improvement in fit (according to the likelihood ratio test) and discriminative ability (according to area under the Receiver Operating Characteristic) by separately adding vitreous humour Na, vitreous humour Cl, and PMVSC to the baseline model. Optimal cut-points are presented for all measures, where the value is chosen to minimize the Euclidean distance to the perfect predictor (sensitivity of one and false positive rate of zero). Sensitivity, specificity, positive and likelihood ratios are also presented for the optimum cut-points.

3. Results

In the 24 cases of SWD-1 identified during the period, the average age was 40 years (range: 7–70) with a male predominance (male to female ratio of 19:5). The average time between death and autopsy was 2.9 days (range: 1–6 days). The locations of the deaths were from local beaches from our catchment area, being along the east coast of Australia facing the Tasman Sea, a part of the Pacific Ocean. The most common reason for entering the sea was recreational swimming (14 cases), followed by accidentally falling or being washed off rocks into the sea when fishing (4 cases), diving (2 cases of SCUBA diving; 1 case of non-SCUBA diving), and surfing (2 cases).

In the 96 sequential controls, we excluded certain known conditions such as diabetic ketoacidosis, end stage liver disease, insulin toxicity, pancreatitis, and hyponatraemia. The average age was 51 years (range: 7–98) with a male predominance (male to female ratio of 58:38). The average time between death and autopsy was 3.3 days (range: 1–6 days).

The cases differed significantly to the controls in PMVSC and vitreous Na and Cl (Table 1). Vitreous Na, vitreous Cl, and PMVSC were very good discriminators of the outcome (cause of death was drowning), with AUCs of 0.895, 0.905 and 0.908 (95% CIs: [0.83, 0.96], [0.84, 0.97], [0.84, 0.97]) for vitreous Na, vitreous Cl and PMVSC, respectively (Table 2). There was substantial improvement in model fit and discrimination from a baseline model including only age, gender and time from drowning to autopsy (LR *p*-value = <0.0001) (Table 3, Fig. 1). Of all the measures, PMVSC had comparable sensitivity (0.8) and the greatest specificity (0.9) and positive likelihood ratio (7.6).

4. Discussion

The definition of drowning has changed over the years [8], and is now defined by the World Health Organization (WHO) as the process of experiencing respiratory impairment from submersion/immersion in liquid [1]. Drowning as a cause of death in Australia has an incidence of approximately 1.5–2 deaths per 100,000 [9] and accounts for approximately 19% of child injury deaths [10].

The pathophysiology of drowning is as a result of alveolar collapse secondary to inhalation of liquid, leading to ventilation-perfusion mismatch and eventually asphyxia/hypoxia [2]. Although commonly encountered, diagnosing drowning in bodies