The use of vitreous renal chemistries in the discrimination of postmortem fresh water drowning

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Abstract

Background: The World Health Organization (WHO) has estimated drowning as the leading cause of unintentional death in the world, with 372,000 deaths reported annually. Not all drowning are unintentional; some could be disguised to cover up an act of heinous criminality. This study was aimed at using some vitreous renal function biochemical parameters as a discriminant of postmortem fresh water drowning. Twelve albino rabbits constituted the sample size as validated by Mead’s formula. The study was divided into three groups; the control, postmortem-drowned and truly drowned as mimicked using an artificial fresh water pond. Vitreous humours were extracted using Coe method. The vitreous renal chemistries were analysed using diacetyl monoxime, Jaffe’s test, uricase and ion-selective electrode (ISE) methods respectively.

Result: The mean of vitreous creatinine, urea, uric acid, Na⁺, K⁺, Cl⁻, Ca²⁺, glucose and CO₂ of the control, postmortem-drowned and truly drowned groups were compared using One-way Anova (post-hoc-LSD) with the aid of SPSS Inc., Chicago, IL, USA; Version 18–21 package. The findings revealed a significant increase in concentrations of vitreous creatinine, glucose, Ca²⁺ and K⁺ of the drowned death group, whereas vitreous concentrations of CO₂ and urea significantly decreased when compared to the controls and/or postmortem-drowned death. Conclusion: The study has shown that some of the studied vitreous biochemical parameters could be used as an ancillary tool in discriminating death due to fresh water drowning from that of disguised or postmortem-drowned death.

Keywords
Forensic Science, drowning, vitreous humour, urea, creatinine, electrolytes

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Introduction

Drowning is defined based on professional angles and perspectives. The most classical definition of drowning was provided by Roll¹ as the hampering of respiration by obstruction of mouth and nose by a fluid medium (usually water). The World Congress on drowning that convened in Amsterdam in 2002 adopted the definition of drowning as the process of experiencing respiratory impairment from submersion or immersion in a liquid.²

Vitreous humour (VH) is a transparent, colourless, gelatinous mass that fill the space in the eye between the lens and the retina. The VH makes up four-fifth of the volume of the eyeball and consist of inorganic ions such as Na⁺, K⁺, Cl⁻, and Ca²⁺.³ The anatomical configuration makes some vitreous contents stable and less susceptible to autolytic changes.⁴,⁶ This attribute is the major reason the VH is heavily patronized in forensic toxicology as against

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blood that is easy affected by postmortem interval (PMI), or autolysis.

Globally, drowning is the third leading cause of death accounting for 7% of all injury related deaths. In 2015, the World Health Organization (WHO) estimated that 360,000 individuals died of drowning. This excludes those caused by large scale natural disasters such as flood, transportation accidents and intentional deaths resulting from suicide or homicide.7

In Africa, drowning has been revealed to be one of the leading causes of injury mortality with rates much higher in United States and other developed countries.8 In Nigeria, even though no verified data exist, it has been of increasing concern in parts of the country predominated with water bodies especially in the Niger Delta region.

Not every dead body found in water could be attributed to accidental death; some are disguised to cover up of the act of criminality. A good number of individuals are murdered and thrown into water bodies to conceal the real cause of death. The important task of forensic identification is to distinguish and ascertain the true cause of death. In the practice of forensic identification, drowned death is based generally on comprehensive considerations such as cadaveric signs, histopathological tests, diatom tests with exclusion of other potential cause of death. This alone has created much of a dilemma to the pathologist in coming up with scientific proof of differential evidence in discriminating death due to drowning from that of disguised or postmortem drowning.

The application of vitreous renal chemistries in crime investigation is gradually on the increase. A similar study by Agoro et al.,9 indicated some vitreous proteins and lipids as emerging tools that could aid in discriminating true fresh water drowning from that of disguised or postmortem drowning. In the same vein, a study on the use of vitreous renal biochemical markers and electrolytes in the discrimination of carbon monoxide death by Agoro et al.10 laid credence to disguised death investigation. Based on the difficulty encountered in differentiating true drowning death from disguised one, the concept laid by Agoro et al.10 for acute carbon monoxide intoxication death was applied to fresh water drowning.

An assault on the circulatory system is known to have palpable alternative impact on renal function markers and electrolytes. The impediments to the circulatory system usually lead to the failure of the renal system; this consequentially yields retention of creatinine and urea in the body. The retention cascades to the migration of creatinine and urea into intracellular and interstitial spaces of the body of which vitreous humour and blood are the most prominent. Similarly, electrolytes are volatile molecules vulnerable to alterations by a lot of pathological and non-pathological conditions. Based on this premise, the use of renal function parameters and electrolytes in discriminatory studies are gradually creating niche in the forensic domain.10

Biochemical presentation that arose from true drowning could be different from postmortem drowning due to cascades of events such as hypoxia, suffocation, muscular contractions, respiratory alterations, and heavy intake of water. These events are known to alter biochemical parameters which could serve as a marker of true fresh water drowning death. This study was therefore aimed at using vitreous renal biochemical markers and electrolytes in discriminating death due truly to drowning from that of postmortem drowning.

Methodology

Study area

The study was conducted at the Biochemistry Laboratory of the Federal University Otuoke, Bayelsa State, Nigeria. Bayelsa state is located within Latitude 4°151 North and Latitude 5° and 231 South of the Federal Republic of Nigeria.6

Study population

This study utilized the Mead’s resource equation for sample size calculation.11 A total of twelve (12) rabbits were used for the study as validated by the Mead’s resource equation. The study comprised of three groups of four rabbits each; the control (CD), postmortem-drowned death (PDD) and truly drowned death (TDD). The control group rabbits were aesthetically euthanized mechanically without exposure to submersion. The PDD group was sacrificed before submersion, whereas the TDD group died of fresh water submersion. It is worth noting that rabbits randomly allocated to the CD and PDD groups were exposed to chloroform before mechanically sacrificed.

Ethical approval

The ethical clearance was obtained from the Bayelsa State Ministry of Health and the Committee on ethics of the Biochemistry Department of the Federal University Otuoke (FUO). The Animal Welfare Act of 1985 of the United States of America for research and Institutional Animal Care and Use Committee (IACUC) protocol were stringently followed.

Selection criteria

The choice animal for the study was New Zealand white albino rabbit. The rabbits (male, 8-month old, approximately 2.0 kg) were obtained from Lagos State of Nigeria. Rabbits used were healthy and active as confirmed suitable for research by the University Veterinarian. Each was housed in an individual metal cage in a specific pathogen-free facility maintained at 25°C with a 60% relative humidity and a 12 hrs light/dark cycle. All rabbits had ad libitum access to standard rabbit chow purchased from
animal feed store in Yenagoa, Bayelsa State Nigeria and filtered water. Rabbit the exhibited signs or symptoms of illness prior to the exposures were excluded from the study.

Sample collection
The vitreous humour samples were collected by the method of Coe.12 Briefly, using a 2 mL syringe and a needle, a scleral puncture was made on the lateral canthus and the total extractable vitreous humour was aspirated from the eye. Adequate care was taken to gently aspirate the fluid to avoid tearing of any loose tissue fragments surrounding the vitreous chamber. On an average 1.0 mL of vitreous humour was collected from each rabbit eye. Only crystal clear liquid free of tissue contaminants and fragments were used in the study. Immediately after sample collection in each case, the vitreous humour was transferred into plain containers for the biochemical analysis. Prior to analysis the vitreous samples were centrifuged at 2050 g for 10 min. The supernatants were separated and used for the analysis.

Determination of vitreous renal chemistries
An ion-selective electrode [ISE] (Analyzer ISE 4000, France) was used for the determination of vitreous electrolytes (sodium, potassium, chloride, carbon dioxide and calcium). Vitreous glucose concentration was determined using the glucose oxidase method. Similarly, vitreous urea and creatinine concentrations were estimated using diacetyl monoxime and Jaffe’s test respectively,13 whereas, vitreous uric acid concentration was determined using the uricase method.

Statistical analysis
Data were analysed using the Statistical Package for Social Sciences (SPSS) programme (SPSS Inc., Chicago, IL, USA; Version 18–21) and Microsoft Excel. One-way ANOVA (Post Hoc-LSD) was used in comparing the means of the studied parameters of the various groups. The level of significance was considered at 95% of interval confidence.

Results
Significant decrease in concentrations of vitreous sodium, chloride, carbon dioxide and glucose were observed when PDD and TDD were compared to CD, on the contrary, vitreous potassium increased respectively in Table 1. A significant increase in vitreous creatinine concentration in the TDD when compared to CD and PDD was observed in Figure 1, whereas Figure 2 indicated a significant decrease in vitreous urea concentration in the TDD when compared to CD and PDD. In the same vein, a significant decrease in vitreous uric acid concentration in the PDD and TDD when compared to CD was exhibited in Figure 3.

Discussion
The study revealed distortions in electrolytes balance occasioned by fresh water drowning (Table 1). A significant decrease in vitreous CO2 was observed in the TDD estimated to about 18 and 13 times to that of CD and PDD respectively. The strict significant decrease in concentration of vitreous CO2 could be ascribed to drowning as the mechanism of drowning posited by Armstrong and Erskine,’ supported the stance of this study. Blood transports

Table 1. Concentrations of some vitreous electrolytes of the studied groups.

| Vitreous Lipid Profile | CD      | PDD     | TDD     | f-value | p-value |
|------------------------|---------|---------|---------|---------|---------|
| Sodium (mmol/l)        | 152.00  | 133.50  | 129.50  | 103.740 | 0.000   |
| Potassium (mmol/l)     | 6.44    | 8.70    | 9.95    | 36.606  | 0.000   |
| Chloride (mmol/l)      | 112.00  | 101.50  | 98.50   | 39.325  | 0.000   |
| Carbon Dioxide (mmol/l)| 14.50   | 10.00   | 0.80    | 42.796  | 0.000   |
| Calcium (mmol/l)       | 6.54    | 7.19    | 11.01   | 8.843   | 0.008   |
| Glucose (mmol/l)       | 4.63    | 0.63    | 3.20    | 34.626  | 0.000   |

Control Death (CD); Postmortem-Drowned Death (PDD); Truly Drowned Death (TDD).
Symbols – a: P < 0.05 vs CD, b: P < 0.05 vs PDD.
Data are expressed as mean ± SD; Significant at 0.01 Confidence (p < 0.01).
Carbon dioxide is exchanged for oxygen in the lungs for systemic sustenance. The absence of oxygen halts the production and transportation of CO2. In the absence or deprivation of oxygen, the exchange is made barren leading to the fall in CO2 concentration or non-production at all. This could be adduced to the significant decrease in CO2 concentration observed strictly in TDD when compared either to CD or PDD.

In the same vein, the non-conspicuous decrease in concentration of vitreous glucose in the TDD compared to the PDD could also be adduced to cardiac arrest mechanism. Increase in potassium concentration is known to be a hallmark of heart failure.21,22 This is buttressed by the higher vitreous potassium concentration in the TDD animals when compared to the PDD that was elevated due to postmortem effect. If postmortem effect was the only reason, the PDD would have had higher vitreous potassium concentration as studies have shown that the longer the time since death (TSD), the higher the vitreous potassium concentration.23,24

Similarly, a significant decrease in concentration of vitreous sodium was observed in the PDD and TDD when compared to CD. The decrease was more conspicuous in the TDD than PDD. The decrease observed could be attributed to haemodilution occasioned by drowning. Drowning involve intake of large volume of water. Increase inappropriate intake of water is known to cause hyponatraemia.25,26 The finding of Roger and Glenda27 agreed with the stance of this study.

A significant increase in vitreous creatinine concentration and stability of vitreous urea concentration were observed in the TDD when compared either to PDD and CD (Figures 1 and 2). These points to fact that the alterations as observed is not a manifestation of renal failure, rather could be due to muscular contractions resulting from struggles in the process of drowning. It is a known fact that the process of drowning involves aggressive melees and high energy expenditure. A study using carbon monoxide as a case study has presented that a strict increase in vitreous creatinine concentration with a contrast pattern of vitreous urea and uric acid concentrations could be attributed to muscular alteration due to struggle or flight.10

Hence, this revelation could be used as a hallmark in discriminating drowned death from that of disguised or.

**Figure 2.** Vitreous urea concentrations of the study groups. Control Death (CD); Postmortem-Drowned Death (PDD); Truly Drowned Death (TDD). F-Value = 7.624. P-value = 0.012. Significant @ P < 0.05 (post hoc: CD vs TDD; CD vs PDD; PDD vs TDD).

**Figure 3.** Vitreous uric acid concentrations of the study groups. Control Death (CD); Postmortem-Drowned Death (PDD); Truly Drowned Death (TDD). F-Value = 4.254. P-value = 0.050. Significant @ P < 0.05 (post hoc: CD vs TDD; CD vs PDD; PDD vs TDD).
postmortem which presented a stable vitreous creatinine and urea concentrations.

Conclusion
The dilemma of resolving truly drowned death using autopsy is a major impediment in forensic science and medicine. This brought about the need for other ancillary investigations that could serve as adjunct parameters in confirming the true cause of death due mainly to drowning. The study revealed a markedly elevated vitreous creatinine, potassium and calcium. On the contrary, vitreous CO₂ decreased significantly. The findings of this study could serve as a hallmark in defining fresh water drowning death. It has also opened up a robust discourse for scientific interactions on the importance of vitreous chemistry in confirming fresh water drowning.

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Author contributions
The first author did the analysis and literature gathering. The second author read the study and did the narratives. The third author did the statistical analysis.

Availability of data and materials
Data are available for any interested researcher as far as the source will acknowledge and credit accorded.

Declaration of conflicting interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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