ORIGINAL ARTICLE

Vitreous cardio-renal biochemical parameters and minerals as biomarkers for forensic discrimination of salt water drowning

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Abstract

Background: Drowning is an episode occasioned by respiratory disturbances resulting from either submersion or immersion in liquid. The World Health Organization ranked drowning globally as the third cause of unintentional death, with an estimated annual mortality of about 236,000. Statistics on drowning in Nigeria are not available, but it is quite prominent amongst causes of unintentional death. Poor discriminatory measures of drowning resulting from suicidal, homicidal, or accidental causes are empirically handicapped due to scientific gaps. In addition, the type of water bodies drowning occurs is very important in drowning investigations. *Aim and Objectives:* This study was designed to use vitreous cardiorenal biomarkers and minerals to substantiate drowning due to saltwater intoxication. *Methodology*: The sample size was determined by Mead's formula, resulting in the use of twenty-four (24) albino rabbits randomized into four groups: the Control Death (CD) group, the Chloroform Death (CFD) group, the Post-mortem Drowned Death (PDD) group, and Saltwater Drowned Death (SDD) group. The saltwater was harvested from the Brass Sea of Bayelsa State, Nigeria, whereas vitreous humour was extracted using the Coe method. The vitreous biochemical parameters employed included creatinine, urea, uric acid, electrolytes, Lactate Dehydrogenase (LDH), troponin I (TnI), and creatine kinase. *Results*: The results show a significant increase ($p < 0.05$) in vitreous zinc, creatinine, and creatine kinase concentrations, whereas vitreous calcium concentration decreased in the SDD group compared to other groups. Similarly, vitreous calcium, sodium, and magnesium strictly and significantly increased ($p < 0.05$) in the PDD group compared to all the different groups. Parameters such as vitreous chloride and LDH concentrations increased significantly ($p < 0.05$) in the PDD and CFD groups compared to the other groups, though more conspicuous in the latter. Furthermore, the vitreous activities of creatinine kinase in the CFD and SDD groups significantly increased ($p < 0.05$) compared to the other groups. Also, a significant increase was noted in the vitreous activities of troponin I in the PDD and SDD groups when compared to the other groups. *Conclusion*: Conclusively, the observed distortions could be harnessed as adjunct investigations in the discrimination of death due to saltwater drowning.

Keywords: vitreous, saltwater, drowning, biomarkers, electrolytes

Introduction

Drowning is an episode occasioned by respiratory disturbances resulting from either submersion or immersion in liquid. The World Health Organization (WHO) ranked drowning globally as the third cause of unintentional death [1]. This accounts for 7% of all injury-related deaths, and an estimated mortality of about 236,000 in 2019, making it a major public health problem. Poor discriminatory measures of drowning resulting from suicidal, homicidal, or accidental causes are empirically

handicapped due to scientific gaps. These gaps in drowning are exploited by criminals, disguising homicidal drowning as either accidental or suicidal drowning, thereby hampering justice or the jurisprudence [2]. Saltwater, also known as seawater, is water from a sea or ocean. On average, salt water in the world's oceans has a salinity of about 3.5% [3]. This means that roughly every liter by volume of seawater has approximately 35 grams of dissolved salts (predominantly sodium (Na^+) and chloride (Cl^-) ions. The water body, where drowning occurs to a large extent, plays a crucial role in its investigation. Saltwater was the choice water body used for this study, as fresh and brackish water bodies have been investigated earlier by the Dr. Eni-Yimini Solomon Agoro-led research team [4-5].

Vitreous Humour (VH) is an emerging choice sample for forensic and toxicological research due to its resistance to early autolysis and putrefaction, unlike blood [4]. It is a colourless gelatinous mass located between the lens and the retina, occupying about four-fifths of the volume of the eyeball [5]. The VH shares similar biochemical compositions with blood, though with varying concentrations of inorganic ions such as sodium, potassium, chloride, and calcium [6]. These vitreous bio-chemical parameters are useful in disease diagnosis and forensic investigations [7-8]. This study investigated the usefulness of vitreous parameters such as electrolytes, uric acid, creatinine, urea, creatine kinase, lactate dehydrogenase, and troponin I in discriminating true saltwater drowning from post-mortem saltwater drowning (disguised). A minority of studies have investigated fresh and brackish water drowning using vitreous cardiorenal markers [9-10]. However, there seems to be a dearth of literature on the use of cardiorenal markers in true saltwater drowning discrimination.

The sole use of autopsy findings in drowning investigation is quite difficult due to discrepancies in histological and cytological patterns [11]. A complete autopsy can only determine the presence of asphyxia symptoms in drowning [12]. In general, it is also found that the physical/external and autopsy examinations are not as specific in drowning cases as complete laboratory examinations [13]. These handicaps associated with drowning diagnosis brought about the application of the diatom test. Diatom testing is the most important test in drowning cases in forensic investigation. Diatoms (Bacillariophyceae) have been classified as a group of algae that are unicellular, photosynthetic, and eukaryotic microorganisms [14]. Applying the diatom test and fingerprinting in drowning investigation has added a bit of credence to the autopsy [11,15-16].

The discrimination of postmortem drowning from true drowning needs more empirical tools and data to create indisputable evidence in a court of law. This study, therefore, explored vitreous cardiorenal biochemical parameters and minerals as a discriminant of saltwater drowning. The findings could serve as a flagship discovery and hallmark in discriminating post-mortem (disguised) saltwater drowning from true saltwater drowning.

Material and Methods

Study location

The animal house located in the Department of Biochemistry Laboratory of the Federal University Otuoke was used for animal breeding, drowning, and sample collection. Similarly, the saltwater used for the artificial drowning was

harvested from the Brass Ocean. The laboratory analysis was conducted at the Eni-yimini Laboratories at Yenezue-gene Epie. All the study sites were located in Bayelsa State, Nigeria. Bayelsa state is situated within Latitude 4^0 151 North and Latitude 5^0 and 231 South of the Federal Republic of Nigeria [4-5]. The study spanned from August 2022 to August 2024, inclusive of the rabbit-rearing period and literature work.

Choice of the animal model

Animal models employed in research are determined based on their similarities to humans. Animals such as mice, rats, and rabbits are the three main research animals of the lower level used for the empirical generation of data to solve human problems. Albino rabbits were used for the study due to the similarities to humans, coupled with the large-sized eyeballs. The blood chemistry of rabbits and humans are quite similar and this forms the basis of the later use as a first-line animal model in place of a human in a lot of pathological studies [17].

Description of the experimental procedure

The research design structure was adopted from the study of Adias *et al*. [18], and the sample size was derived from the formula postulated by Kirkwood & Hubrecht [19]. A total of twenty-four (24) rabbits constituted the sample as validated by Mead's equation [19]. The rabbits were randomly allocated into four groups of six (6) rabbits each. The groups include: the (strangled) Control Death (CD), the Chloroform Death (CFD), the (disguised) Post-mortem Drowned Death (PDD), and the Saltwater Drowned Death (SDD). The albino rabbits were procured from the animal house of the Department of Biochemistry, University of Port Harcourt, Rivers State. The animals were sacrificed based on the group's random allocation. The CD and PDD intoxicated rabbits were strangulated to death, and the latter were drowned, whereas CFD intoxicated rabbits were sedated to death in a desiccator whereas SDD rabbits died from water intoxication. The maximum post-mortem time interval for the sample collection was pegged at twelve (12) hours to reflect the time within which drowned dead bodies are recovered. The vitreous was collected by a scleral puncture on the lateral canthus using a 2 ml syringe and a needle [20]. Vitreous free of tissue contaminants and fragments was transferred into plain containers, centrifuged at 2050 g for 10 min, and the supernatant extracted for laboratory analysis.

Ethical approval/selection criteria

The Committees on Ethics of the Directorate of Research and Quality Assurance (DR&QA) and the Department of Biochemistry of the Federal University Otuoke (FUO) issued the ethical approval letter after thorough peer review and evaluations. The Animal Welfare Act of 1985 of the United States of America for research and the Institutional Animal Care and Use Committee (IACUC) formed the protocol of the study [4-5]. The rabbits used were healthy and active, identified, and authenticated by a registered veterinarian. Rabbits with deformities or exhibiting signs or symptoms of illness were rejected. The age range of the rabbits used was between ten to twelve months, with a weight of 1.8-2.0 kg

Laboratory analysis

Vitreous iron, chloride, sodium, potassium, calcium, magnesium, zinc, inorganic phosphorus, creatinine, urea, uric acid, Lactate Dehydrogenase (LDH), Troponin I (tnI), and creatine kinase were the chosen parameters for the study. These para-

meters cut across all the vital metabolic processes in the body and are suitable for the assessment of systemic dysfunction or compromise. Vitreous creatinine and urea were estimated using modified Jaffes and diacetylmonoxime methods, respectively, whereas vitreous uric acid concentration was measured using the uricase method on the Agappe kit as specified by Agappe Diagnostics (Switzerland). The vitreous electrolytes were estimated using the Ion Selective Electrode (ISE) method. Enzyme Linked Immunosorbent Assay (ELISA) method (Accubind, UK) was used for the assay of LDH, TnI, and CK-MB.

Statistical analysis

Data were analyzed on the Statistical Package for Social Sciences (SPSS) programme (SPSS Inc., Chicago, IL, USA; Version 18–21), Prism Pad, and Microsoft Excel platforms. One-way ANOVA (Post Hoc-LSD) was used to compare the studied parameters of the various groups. The level of significance was considered at a 95% confidence interval.

Results

Table 1 shows a significant increase ($p < 0.05$) in vitreous zinc concentration in the SDD group compared to the other groups. Vitreous calcium significantly increased ($p < 0.05$) in the PDD group compared to all the other groups, whereas that of the SDD group decreased compared to the CD and PDD groups. In a similar vein, vitreous magnesium concentration significantly increased ($p < 0.05$) in the PDD group compared to the other groups. Table 2 shows a significant increase ($p < 0.05$) in the concentration of vitreous sodium in the PDD group compared to other groups. Vitreous chloride concentration increased significantly ($p < 0.05$) in the PDD and CFD groups compared to the other groups, though more conspicuous in the latter. Figure 1 shows a significant increase ($p < 0.05$) in vitreous creatin-ine concentration in the SDD group compared to the other groups. Figure 4 shows a significant increase in the activities of LDH in the PDD and CFD groups when compared to the other groups. Figure 5 shows a significant increase in the activities of vitreous creatinine kinase in the CFD and SDD groups compared to the other groups. Figure 6 shows a significant increase in activities of vitreous troponin in PDD and SDD groups when compared to other groups. All parameters, figures, and tables not listed above were not significantly different ($p > 0.05$).

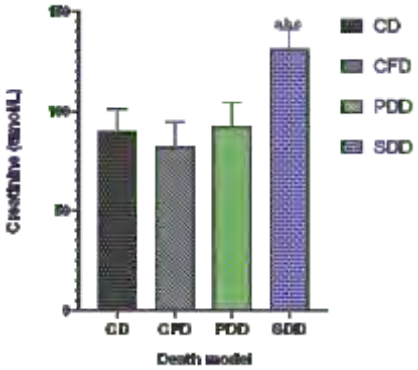

Figure 1: Mean concentrations of vitreous creatinine in various death models

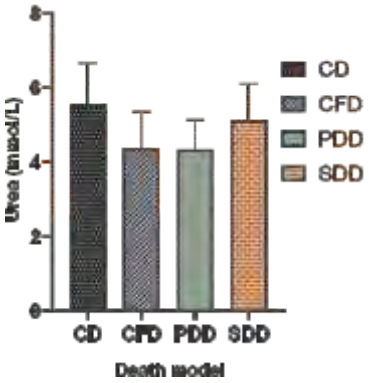

Figure 2: Mean concentrations of vitreous urea in various death models

Table 1: Mean concentrations of vitreous minerals in various death models

Parameters (mmol/L)	CD	CFD	PDD	SDD	F	*p*
Zinc	181.805 ± 36.716	127.705 ± 11.236	152.655 ± 31.577	225.416 ± 57.991[a,b,c]	1.061	0.418
Calcium	5.123 ± 1.842	2.398 ± 1.016[a]	8.206 ± 3.548[a,b]	2.112 ± 0.357[a,c]	3.161	0.086
Potassium	3.642 ± 1.232	8.952 ± 2.440	7.469 ± 2.808	5.374 ± 2.186	1.054	0.420
Iron	36.531 ± 23.074	74.278 ± 15.531	85.719 ± 24.844	71.929 ± 22.926	1.294	0.341
Magnesium	0.884 ± 0.691	0.816 ± 0.567	6.159 ± 2.822[a,b]	1.811 ± 0.148[c]	3.041	0.093

CD-Control Death (a); CFD- Chloroform Death (b); PDD- Postmortem Drowned Death (c); SDD- Saltwater Drowned Death (d) a: $p < 0.05$ vs b, c, & d, b: $p < 0.05$ vs Group C, &d; c: $p < 0.05$ vs Group D
Data are expressed as mean ± SD; Significant at 0.05 Confidence ($p < 0.05$)

Table 2: Mean concentrations of vitreous renal electrolytes in various death models

Parameters (mmol/L)	CD	CFD	PDD	SDD	F	*p*
Sodium	142.333 ± 4.726	145.333 ± 4.933	188.667 ± 4.509[a,b]	158.000 ± 7.000[c]	0.861	0.050
Chloride	143.635 ± 4.343	217.472 ± 42.088[a]	187.714 ± 11.195a[,b]	153.276 ± 27.386[b,c]	3.223	0.082
Potassium	18.416±10.854	16.315±1.917	14.1197 ± 1.948	15.166 ± 2.948	0.304	0.822

CD-Control Death (a); CFD- Chloroform Death (b); PDD- Postmortem Drowned Death (c); SDD- Saltwater Drowned Death (d) a: $p < 0.05$ vs b, c, & d, b: $p < 0.05$ vs Group C, &d; c: $p < 0.05$ vs Group D
Data are expressed as mean ± SD; Significant at 0.05 Confidence ($p < 0.05$)

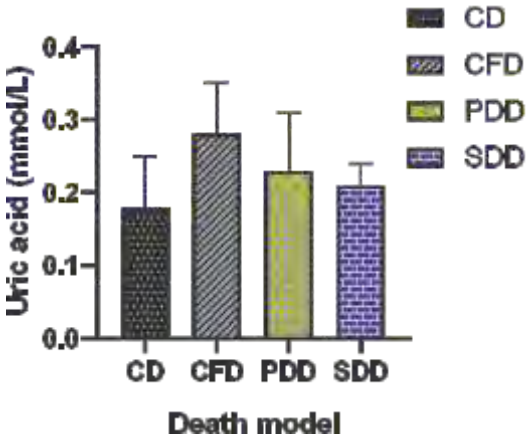

Figure 3: Mean concentrations of vitreous uric acid in various death models

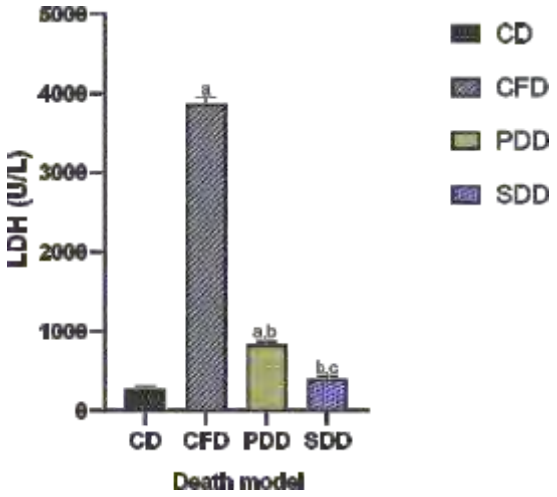

Figure 4: Mean concentrations of vitreous lactate dehydrogenase in various death models

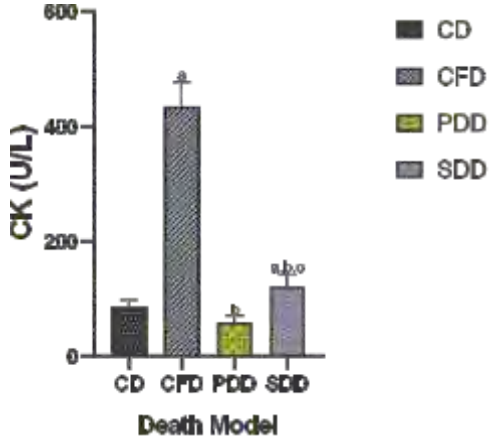

Figure 5: Mean concentrations of vitreous creatinine kinase in various death models

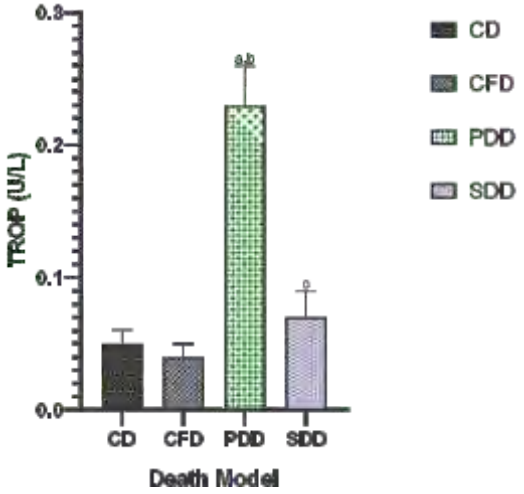

Figure 6: Mean concentrations of vitreous troponin in various death models

Discussion

This study investigated the application of vitreous chemistry in resolving the conundrum of true drowning, employing empirical and randomized tools applied in other studies [21-23].The findings on vitreous minerals revealed a significant increase in the SDD group's vitreous zinc concentration compared to other groups (Table 1). Similarly, vitreous calcium and magnesium concentrations significantly increased in the PDD group compared to all the other groups. Furthermore, vitreous calcium concentration decreased significantly in the SDD group compared to the CD and PDD groups (Table

1). The alterations in mineral concentrations observed could be due to osmotic and diffusional effects. These minerals are known to be present in both the saltwater and the VH. According to Stanford University, in the US, seawater contains 47 minerals and metals. Starting with the most abundant and proceeding to the least abundant, these are chloride, with a concentration of 18,980 Parts Per Million (ppm) in seawater, sodium (10,561 ppm), magnesium (1 272 ppm), calcium (400 ppm), potassium (380 ppm), iron (0.02 ppm), organic phosphorous (0.016 ppm), zinc (0.014 ppm).

The increase in concentrations of vitreous magnesium and calcium in PDD when compared to other groups could be due to the saltwater effect resulting from the diffusion of the Mg and Ca from the water body into the vitreous humor. The vitreous humour is heavily protected by a semi-permeable membrane that restricts the movement of ions. However, in the case of PDD, the membrane could have ruptured due to the earlier postmortem effect. This rupture of the vitreous membrane, coupled with the fact that the concentration of Mg and Ca in salt water is significantly higher than the concentration of Mg and Ca in the vitreous humour, would have led to a higher rate of diffusion of Mg and Ca into the vitreous humor in PDD than in SDD. The finding on vitreous magnesium is in line with that of Farmer *et al.* [24] that observed magnesium in saltwater cases.

Additionally, the study revealed a significant increase in the concentration of vitreous sodium in the PDD group compared to other groups. Similarly, vitreous chloride concentration increased significantly in the PDD and CFD groups compared to the different groups, though more conspicuously in the latter (Table 2). The increased concentrations of vitreous sodium and chloride observed in the PDD group could be ascribed to saltwater and the diffusional effect. Saltwater bodies have high concentrations of sodium and chloride far above the compositions in the VH, hence the favourable gradient movement. However, there was a slight mean increase in vitreous sodium concentration in the SDD compared to others (Table 2). Though insignificant, this observation could be due to hypernatremia as it forms part of the mechanism of drowning. The overt increase in vitreous sodium concentration in the PDD group could serve as a discriminatory tool in mortality resulting from drowning. Farmer *et al.* [24] also advanced similar reasoning in the utilization of sodium in a drowning death investigation. However, Garland *et al.* [25] findings restricted the increase in concentrations of sodium and chloride within an hour for the diagnosis of saltwater drowning; more than an hour was ascribed to immersion. The overt elevation of vitreous chloride concentration in the CFD group could be attributed to cardiac failure resulting from high exposure to chloroform intoxication. A chloride concentration increase has been implicated in heart failure or cardiac arrest. This cascade could be the basis of the elevation in chloride concentration in the CFD.

Moreover, the investigation into the vitreous renal parameters revealed a significant increase in vitreous creatinine concentration in the SDD group compared to the other groups, whereas urea and uric acid were not significant (Figures 1-3). This discriminatory increase could serve as a hallmark in the investigation of saltwater drowning. The increase could be due to muscular contraction and

relaxation resulting from the cascade of events in the drowning process, as the other renal parameters, such as urea and uric acid, were not significant. A handful of vitreous chemistry studies on freshwater and brackish water posited the same scientific basis for increased vitreous creatinine [10-11]. Furthermore, the implication of elevated creatinine concentration may reflect skeletal muscle damage as posited by Zhu *et al.* [26].

Furthermore, the study investigated the suitability of some selected cardiac vitreous markers in elaborating saltwater drowning (Figures 4-6). The findings revealed a significant increase in the activities of vitreous LDH in the PDD and CFD groups compared to the other groups (Figure 4). LDH is widely distributed in the body, with high activities found in the heart, liver, skeletal muscle, kidney, and erythrocytes, whereas lesser amounts are found in the lung, smooth muscle, and brain. An elevated total LDH value is a rather non-specific finding and could be indicative of injuries resulting from organs housing the enzyme [27]. Hence, the increase in LDH activities could have occurred due to organ damage by any of the significant mortalities, but not saltwater drowning. This finding could be of importance in the discrimination of saltwater drowning.

Contrary to vitreous LDH activity, the study showed a significant increase in vitreous creatinine kinase activity in the CFD and SDD groups compared to the other groups (Figure 5). Elevated creatinine kinase activities may indicate skeletal muscle, heart, or brain damage or degeneration [28]. The elevation could be attributed to heart failure, which is associated with drowning death [29]. The cardiac integrity of the mortality models was further investigated using vitreous troponin activities (Figure 6). Vitreous troponin activity was found to be significantly elevated in PDD and SDD groups compared to other groups. Troponin activity is mainly used for the diagnosis of heart attacks and heart-related dysfunctions [30]. Hence, the elevation could be a result of heart failure associated with the mortality model in question. The intensity of the elevation was more conspicuous in the PDD group when compared to the SDD and other groups. This could be due to the severity of the heart attack and the biochemistry of the parameter in discourse. Based on the conspicuous elevation of troponin in the PDD group, it could also be of crucial use in the discrimination of PDD and SDD.

The findings of the study are multifaceted, with arrays of vitreous chemistry distortion. The distortion could be harnessed as an adjunct investigation in the discrimination of death due to saltwater drowning.

Conclusion

A major hindrance in forensic science and medicine has been the ability to use autopsy to make a firm decision on cases of truly drowned death. The use of vitreous parameters in discriminating truly drowned death from cover-up criminal activities is paramount in scientific investigations. The study revealed a distortion in some vitreous parameters that could be used as discriminatory tools in the investigation of saltwater drowning. Vitreous zinc, creatinine, and creatine kinase concentrations strictly increased in the SDD group, whereas vitreous calcium concentration decreased when compared to other groups. Similarly, vitreous concentrations of sodium, chloride, calcium, and magnesium, and LDH activities increased in PDD. Parameters such as vitreous

chloride, LDH, and creatine kinase increased in CFD. Other parameters exhibited no significant difference. The biochemical distortions observed could be employed in the discrimination of death resulting from saltwater drowning.

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