**Research Article**

Vitreous Lipid and Protein Biomarkers as Indicators of Brackish Water Drowning: A Forensic Biochemistry Study

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<https://doi.org/10.4314/njbmb.v39i4.4>**ABSTRACT**

Deaths resulting from submersion or immersion in water account for about 7% of trauma-induced mortalities. Several cases of drowning deaths are not unintentional but criminally disguised to evade justice. Also, the type of water body in which drowning occurred is important in making an empirical closure on the intent of occurrence. Therefore, this study was designed to utilize vitreous proteins and lipids in discriminating postmortem brackish water drowning from true brackish water drowning. Sixteen (16) albino rabbits constituted the sample size which were further divided into four (4) groups; the control (CD), the chloroform group (CFD), the postmortem drowned (PDD), and the brackish drowned death (BDD). Vitreous humours were extracted from the eyes of the animals, and consequently processed, and analysed for proteins and lipids. The results showed a significant decrease ($P > 0.05$) in the concentration of vitreous albumin in PDD (0.190 ± 0.071) and BDD (0.109 ± 0.043), when compared to the CD (0.485 ± 0.307), whereas it increased significantly ($P > 0.05$) in CFD (1.837 ± 0.231). In a similar vein, there was a significant increase ($P > 0.05$) in vitreous triacylglycerol in the experimental groups (CFD- 0.218 ± 0.116 ; PDD- 1.051 ± 0.627 ; BDD- 0.826 ± 0.172) when compared to the controls (0.082 ± 0.032). Additionally, the increase in vitreous triacylglycerol was more prominent in the PDD when compared to the BDD and CFD. The findings have revealed that vitreous albumin and triacylglycerol could be useful in discriminating against deaths resulting from brackish water drowning.

Keywords: Homicide, drowning, brackish, vitreous humour, proteins, lipids**INTRODUCTION**

Brackish is a term derived from the Dutch root word brak and is used to describe water bodies with salinity higher than freshwater, but lower than seawater (Ramasamy et al., 2014). Brackish water could be formed as a result of the mixing of salt and freshwater bodies (Agoro and Kpomah, 2024). It is a common water body where drowning can take place considering the vastness, salt content, and deepness. Vitreous humour is a gel-like fluid in the eyeball between the lens and the retina and is found in the vitreous chambers of humans and other vertebrates. The importan-

ce of vitreous humour to forensic scientists is enormous as it is used in estimating post-mortem interval (PMI), diagnosing diseases, and confirming the causes of death (Agoro et al., 2017; Agoro 2020; Agoro et al. 2020). Vitreous fluid is made up of several biomolecules and substances, inclusive of proteins and lipids. Proteins and lipids are vital cosmopolitan polymers found in both intracellular and extracellular fluids.

Proteins of importance in vitreous chemistry are albumin and globulins which aid in molecular traffic. In a similar vein, lipids such as cholesterol and triacylglycerol are crucial in membrane function, and other metabolic processes in the body. The ubiquitous nature and vulnerabilities to alterations by both internal and external

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factors make proteins and lipids choice parameters for diagnostic and forensic purposes. Drowning remains a leading cause of mortality and morbidity worldwide. Forensic opinion of death resulting from drowning is considered one of the most difficult tasks for both forensic scientists and pathologists (Szpilman *et al.*, 2018). The difficulties associated with drowning investigations have made autopsies most times invalid and inaccurate. Thereby creating loopholes of escape for suspects of drowning crimes. It is worthy of note that there is a dearth of literature on the application of vitreous chemistry in establishing drowning deaths. Related studies in this direction have been conducted using fresh water, but not brackish or salt water (Agoro *et al.* 2020; Agoro, *et al.* 2021).

Additionally, not every dead body found in water could be attributed to accidental or unintentional death; some are disguised to cover up the act of criminality. Several victims of drowning are killed and disposed into water bodies to conceal the real cause of death. It is on this basis that this study was carried out using the concept of vitreous proteins and lipids established in other studies (Agoro *et al.* 2020; Agoro, *et al.* 2021). We, therefore, hypothesized that vitreous lipids and proteins can serve as biomarkers to differentiate between postmortem drowning and actual drowning in brackish water.

MATERIALS AND METHODS

Design of the study

The drowning procedure was mimicked using an artificial brackish water pool. This phase of the study was conducted at the Biochemistry Laboratory of the Federal University Otuoke, the brackish water for the study was harvested from Nembe. Five hundred (500) liters of brackish were collected into sterile drums used for the artificial drowning. The salinity content was measured using a salinity meter (Smart Sensor-AR8012) and the value obtained was 20 parts per thousand (%), with a specific gravity of 1.0126.

The study design and protocol were adopted from that posited by Adias *et al.* (2013).

Rabbit was the chosen animal model for the research work. The suitability of rabbit as a choice animal for this study is attributed to its anatomical and physiological similarities to humans (Gwon, 2008; Los, 2008).

Sample size determination

Mead's resource equation was utilized for the calculation of the sample size (Kirkwood and Robert, 2010). The equation is stated and the components are defined below:

$$E = N - B - T$$

where:

N is the total number of individuals or units in the study (minus 1).

B is the blocking component, representing environmental effects allowed for in the design (minus 1)

T is the treatment component, corresponding to the number of treatment groups (including control group) being used, or the number of questions being asked (minus 1) E is the degrees of freedom of the error component, and should be somewhere between 10 and 20. The study constituted of four treatment groups (T =4), with six (6) animals per group, making sixteen (N=16), without any further stratification (B =0), then E would equal 16, which is within the cutoff of 10-20, indicating that sample size is very suitable for the research.

The rabbits were randomly allocated into four groups of four (4) animals. The groups include the control death (CD), the chloroform death (CFD), the postmortem drowned death group (PDD), and the brackish drowned death (BDD).

The control group rabbits were euthanized mechanically without exposure to submersion, whereas that of the CFD group died of intoxication resulting from chloroform inhalation. Similarly, the PDD group was sacrificed before submersion, whereas the BDD group died of brackish water submersion. The drowning to recovery time of the study was pegged at twelve (12) hours.

Selection criteria/ethical approval

Rabbits used for the study were confirmed healthy, and suitable for the research by the university veterinary doctor; a staff of the Department of Animal and Environmental Biology of the Federal University Otuoke. Only male albino rabbits aged 6-8 months and weight range of 100-120g were recruited for the study. The rabbits used had access to standard rabbit chow and filtered water procured at a market in Yenagoa.

The ethical clearance was obtained from the Bayelsa Ministry of Health and the Committee on Ethics of the Biochemistry Department of the Federal University Otuoke, Nigeria with an approval number of BCH/ECF/076/05/23. Standard procedures for animal care and management of the United States of America for research and Institutional Animal Care and Use Committee (IACUC) protocol were carefully followed (Benjamin and Jean, 2016).

Laboratory methods/procedures

The vitreous humour samples were collected by the methods of Coe (1989) and Tente *et al.* (2004). Briefly, using a 5 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 any loose tissue fragments surrounding the vitreous chamber. On average 1.0 mL was collected from each rabbit's eye. Only crystal-clear liquid free of tissue contaminants and fragments was used in the study.

The samples were collected within 30 minutes postmortem. Immediately after sample collection in each case, the vitreous humour was transferred into plain containers for the vitreous proteins and lipids estimation.

The vitreous samples were centrifuged at 2050 rpm for 10 min. The supernatants were separated and stored at -40C for 24 hours before the laboratory analysis. Only a clear sample void of cell fragments was used for the laboratory analysis. Vitreous total cholesterol and triacylglycerol concentrations were estimated using Agape diagnostic reagents (Switzerland). Similarly, vitreous total protein and albumin concentrations were measured using Randox kits (United Kingdom). However, vitreous globulin concentration was calculated mathematically from vitreous total protein and albumin (Michael et al., 1992).

Statistical analysis

One-way ANOVA (Pos hoc-LSD) was the choice statistical tool computed on the Statistical Package for Social Sciences (SPSS) program (SPSS Inc., Chicago, IL, USA; Version 18-21) software. The confidence interval level was pegged at ≤ 0.05 .

Table 1: Mean concentrations of study parameters in various death model

Parameter	Control death (CD)	Chloroform death (CFD)	Postmortem drowned death (PDD)	Brackish drowned death (BDD)
Total Protein (g/L)	3.448 \pm 1.562	4.807 \pm 1.608	4.149 \pm 1.294	4.416 \pm 1.964
Albumin (g/L)	0.485 \pm 0.087	1.837 \pm 0.231	0.190 \pm 0.071	0.109 \pm 0.043
Globulin(g/L)	2.963 \pm 1.736	2.970 \pm 1.430	3.958 \pm 1.260	4.146 \pm 3.664
Albumin/Globulin ratio	3.448 \pm 1.043	4.807 \pm 1.661	4.148 \pm 1.331	4.255 \pm 1.707
Total Cholesterol (mmol/L)	0.208 \pm 0.087	0.248 \pm 0.056	0.119 \pm 0.095	0.238 \pm 0.076
Triacylglycerol (mmol/L)	0.082 \pm 0.032	0.218 \pm 0.016	1.051 \pm 0.067	0.826 \pm 0.072

Legend Data are expressed as mean \pm SD \pm - Plus-Minus Sign

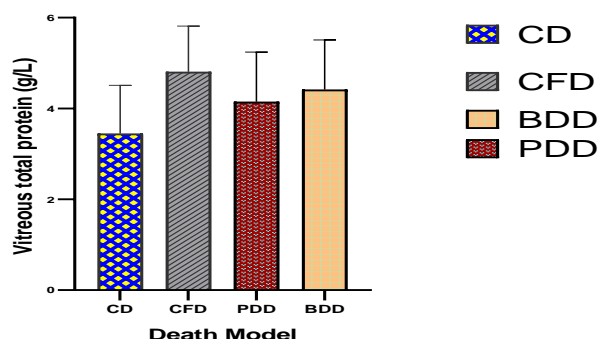


Figure 1. Mean concentrations of vitreous total proteins in the various death model

Legend: CD-Control Death (a); CFD- Chloroform Death (b); PDD- Postmortem Drowned Death (c); BDD- Brackish Drown Death (d). Symbols- 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 \pm SD; Significant at 0.05 Confidence (p < 0.05) \pm - Plus-Minus Sign

RESULTS AND DISCUSSION

Results

Table 1 shows mean and standard presentations of the various death models and the studied vitreous parameter concentrations. Figure 1-5 is the presentations of the vitreous concentrations of the death models. Figure 2 shows a significant decrease ($p < 0.05$) in vitreous albumin concentrations in PDD and BDD, on the contrary, an increase was observed for the CFD when compared to CD. However, the decline was significantly more pronounced in the BDD when compared to PDD. Figure 5 exhibits a significant leap in concentrations ($p < 0.05$) of vitreous triacylglycerol in the various groups of the study when compared to the controls. Other studied parameters were not significant ($p > 0.05$).

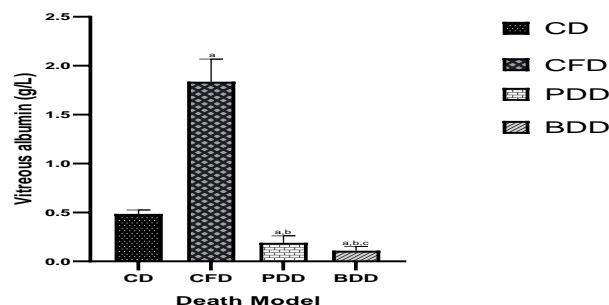


Figure 2. Mean concentrations of vitreous total albumin in the various death model

Legend: CD-Control Death (a); CFD- Chloroform Death (b); PDD- Postmortem Drowned Death (c); BDD- Brackish Drown Death (d). Symbols- 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 \pm SD; Significant at 0.05 Confidence (p < 0.05) \pm - Plus-Minus Sign

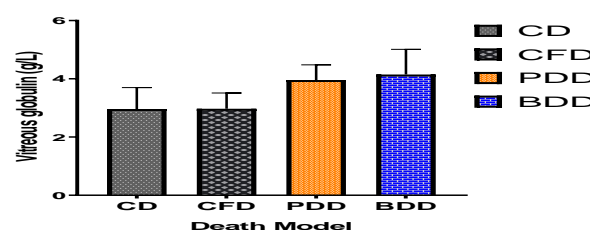


Figure 3. Mean concentrations of vitreous globulin in the various death model

Legend: CD-Control Death (a); CFD- Chloroform Death (b); PDD- Postmortem Drowned Death (c); BDD- Brackish Drown Death (d). Symbols- 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 \pm SD; Significant at 0.05 Confidence (p < 0.05) \pm - Plus-Minus Sign

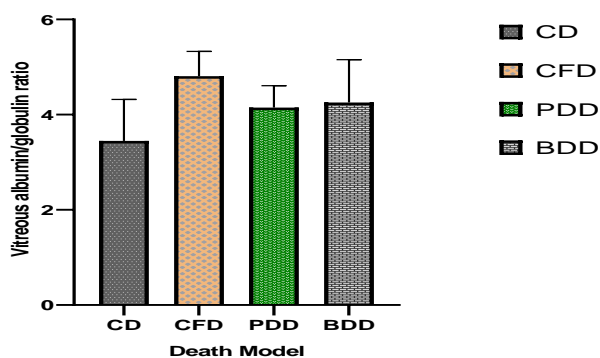


Figure 4. Mean concentrations of vitreous albumin/globulin ratio in the various death model

Legend: CD-Control Death (a); CFD- Chloroform Death (b); PDD- Postmortem Drowned Death (c); BDD- Brackish Drown Death (d). Symbols- *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 \pm SD; Significant at 0.05 Confidence ($p < 0.05$). \pm - Plus-Minus Sign

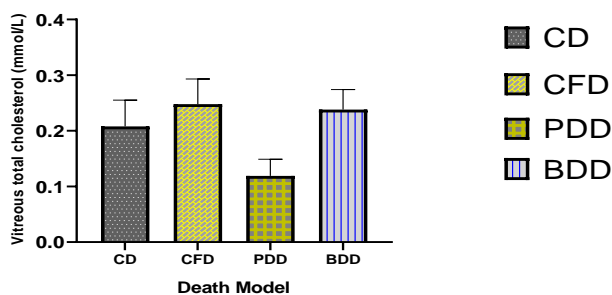


Figure 5. Mean concentrations of vitreous total cholesterol in the various death model

Legend: CD-Control Death (a); CFD- Chloroform Death (b); PDD- Postmortem Drowned Death (c); BDD- Brackish Drown Death (d). Symbols- *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 \pm SD; Significant at 0.05 Confidence ($p < 0.05$) \pm - Plus-Minus Sign

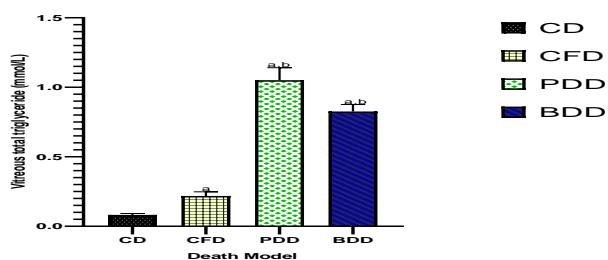


Figure 6. Mean concentrations of vitreous triacylglycerol in the various death model

Legend: CD-Control Death (a); CFD- Chloroform Death (b); PDD- Postmortem Drowned Death (c); BDD- Brackish Drown Death (d). Symbols- *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 \pm SD; Significant at 0.05 Confidence ($p < 0.05$) \pm - Plus-Minus Sign

Discussion

The study revealed a significant decrease in vitreous albumin in the PDD and BDD group when compared to the controls, whereas an increase was observed in the CFD, whereas other parameters were not significant (Table 1, Figure 1-4). The

observed decrease in the concentration of vitreous albumin in the PDD and BDD could be attributed to the diffusional phenomenon resulting from the drowning episode and salinity effect. In this case, the tendency favours brackish water in terms of water migration. The discrepancies in water movement into the vitreous humour could be due to the active nature of the vitreous membrane that regulates molecular traffic. Unlike the BDD, the mechanism of action of water migration was compromised in PDD due to the prior postmortem effect on the vitreous membrane. This phenomenon could be responsible for the observed alteration. The findings of this study concurred with those of Agoro et al., (2020) on freshwater. On the other hand, the significant increase in vitreous albumin concentration in the CFD could be attributed to the chloroform effect. Chloroform is a nonpolar solvent that can interact with the nonpolar regions of proteins and disrupt their structure, leading to denaturation. It can also disrupt the hydrogen bonding and electrostatic interactions that hold the protein's structure together. Based on this premise, it could be argued that the increase in vitreous albumin after exposure to chloroform could be due to the dual interaction leading to chelation, precipitation, or denaturation. This study concurred with a research team that posited the existence of an interaction between chloroform and albumin (Johansson, 2009). In a similar vein, the study revealed a significant elevation in the concentration of vitreous triacylglycerol in PDD and BDD when compared to the control and CFD, whereas cholesterol was not significant (Table 1, Figure 5&6). However, the increase was more significant in the PDD when compared to the BDD and CFD. The increase in vitreous triacylglycerol concentration in PDD and BDD could be due to postmortem changes resulting from lipid degradation, and not necessarily the drowning episode as both groups were exposed to the same water composition and quantity.

Though drowning involves a lot of energy expenditure which could be sourced from lipolysis, the increase in PDD and CFD proved the contrary. This study is in line with the narrative of Agoro et al. (2020) on freshwater drowning. Also, the position of the study agreed with that of Elalfy et al. (2019). However, the findings emanated from this study could serve as an adjunct investigation to autopsy in providing forensic insight into the episode of brackish drowning. The antagonist presentation of vitreous albumin and triacylglycerol could be utilized by pathologists in guiding autopsy report presentation.

Future research direction

The major limitation of this study is the use of animal models in extrapolating the findings on human subjects. There are a lot of physiological and anatomical characteristics that have the preponderance of either compromising or supporting such stances. However, this study has prepared the scientific grounds for advances in this direction by employing human subjects and considering other methods that could further refine advanced techniques, such as genomics and proteomics. In a similar vein, the

comparison of the study with that of salt water was bridged due to the dearth of data on the thematic area of this discourse. This will form another future direction in providing scientific closure to the postmortem chemistry of drowning.

CONCLUSION

The study revealed decreased vitreous albumin concentration, whereas vitreous triacylglycerol concentration increased in brackish drowned death. These findings could serve as a hallmark of brackish drowned death and also ancillary discriminatory investigations to autopsy. The findings have the potential to assist pathologists in scientific decision making as regards autopsy reporting.

AUTHORS' CONTRIBUTIONS

ESA: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources; Software, Supervision, Validation, Visualization. CCT: Roles/Writing – original draft; Writing – review & editing.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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