International Journal of Basic Science and Technology
December 2023, Vol. 9, Number 4, Pages 204 - 211 http://www.ijbst.fuotuoke.edu.ng/ 204
https://doi.org/10.5555/GQEI9081 ISSN 2488-8648

Potentials of selected vitreous biochemical parameters as biomarkers in postmortem determination and discrimination of deaths by hanging using animal models.

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Article Information

Article # 01008
Received: 14th Oct. 2023
1st Revision:19h Oct. 2023
2nd Revision:19th Nov. 2023
Acceptance:23rd Dec. 2023
Available online:
28h December 2023.

Key Words
Postmortem, Vitreous, Biomarker, Strangulation, Crime.

Abstract

This study is an exploration of selected postmortem vitreous parameters for the identification of analytes that can serve as biomarkers for distinguishing between death disguised as hanging and death by actual hanging. Completely randomized block design (CRBD) was used for this study. 96 male rabbits were used for this research and were structured into four groups of twenty-four rabbits each: two treatment (test) groups and two control groups. In one test group, the death of the experimental subjects was caused by hanging. In the second test group, the death of the experimental subjects was caused by strangulation, thereafter, the dead subjects were hanged as a cover up of the actual cause of death. The remaining two groups are the baseline controls. After a postmortem interval of twenty-four hours, vitreous samples were obtained and analyzed for the levels of total protein, albumin, globulin, total cholesterol, triacylglycerol, uric acid, creatine kinase and lactate dehydrogenase using standard methods. Results obtained were then analyzed with SPSS (version 18-21) and one-way ANOVA. The results show that postmortem vitreous levels of uric acid, creatine kinase and lactate dehydrogenase differed significantly ($P \leq 0.05$) between samples from deaths by means of hanging and deaths by means of strangulation but disguised as hanging. The identified significant variations in the quantities of these discovered vitreous metabolites amongst the study groups makes them potential biomarkers for effectively determining and clarifying hanging associated cause of deaths when utilized either separately or as a combination of biomarkers, which is advocated.

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Introduction

There is an alarming increase in crime rates, especially that of murder and genocide (Roberts, 2022). Many of the perpetrators of these crimes escape justice by exploiting the difficulties in the judicial system in the determination of a true culprit. This has allowed mischievous individuals to continuously end the life of others and go scot-free in spite of the fact that human life is sacred and every individual has a constitutional right to life (Epstein, 2020; Lopez, 2021). A survey of literature has revealed two major means by which the perpetrators of murder crimes survive legal scrutiny. One is by disguising the cause of death, which ultimately mislead the jury during courtroom trials. And the other is the fact that blood, the principal specimen for most medical investigations putrefies shortly after death. This limits its capacity for postmortem analysis at long intervals after death (Brandt-Casadevall *et al.*, 2003; Butzbach, 2010). Reports by the WHO reveals that hanging is the second most common method of suicide in the world, with the first being poisoning, as such, hanging is now being widely exploited by murderers to disguise homicide as suicide (Nath *et al.*, 2020).

Postmortem chemistry is the biochemical analyses of body fluids or tissues obtained from dead bodies for the purpose of discerning the cause of death. Postmortem chemistry is becoming increasingly essential in pathology and science (Cristian and Patrice, 2011). Postmortem chemistry may essentially contribute in the determination of the cause of death when the pathophysiological changes involved in the death process cannot be detected by morphological

methods (Maeda *et al*., 2009). A biomarker is a measurable attribute associated with the clinical status of a person, disease and treatment. Biomarkers are in serious demand to aid diagnosis, monitor disease progression and detect patient's response to treatments as well as uncover the underlying causes of death during autopsy (Ward and Schofield, 2010). Some reasons why vitreous humour would make a good sample for post mortem forensic analyses are: unlike blood, it is not degraded for a long period after death (Adam and Gail, 2013); it undergoes very slow post mortem changes (Thierauf *et al.*, 2011); it contains several molecules and metabolites that can be assayed for or monitored (Amith, 2005); it is present in sufficient quantities that can serve as samples for multiple investigations (Garg *et al.*, 2004); it is easy to obtain (Zilg *et al*., 2009).

Rabbits are animals of the phylum *chordata*, class *mammalia*, order *lagamorpha* and family *leporidae* with about eight genera and several species. The applications of rabbits in health research abound in literature. Studies involving the use of rabbits as experimental subjects have resulted in very profound findings that have found widespread utility in medical and related disciplines. Much of the leads for the diagnosis and treatments of human and animal diseases had arisen from rabbits and rats models. Today most scientific hypothesis are first tested on rabbits before extrapolation to humans (Bosze and Houdebine, 2006; Gwon, 2008; Kang and Grossniklaus, 2011; Kondo, *et al.* 2009; Konya, *et al*. 2008; Zahn, 2010).

Hanging is used to describe a special form of compression on the neck. Death by hanging is caused by compression of the cervical structures by rope or other ligature; it depends on the force exerted by the ligature on the neck, the weight of the deceased and the acceleration during the fall (Törö *et al.*, 2008). Hanging injuries are associated with vascular pathology ranging from carotid intimal tears to complete rupture (Kaki *et al.*, 1997). With moderate force the ligature compresses the jugular veins resulting in edema of the face and brain, and subsequent loss of consciousness. An increasing force compresses the carotid and vertebral arteries, causing cerebral ischemia. Pressures on the carotid artery nerve ganglion leads to cardiac arrest (Dipoce *et al.*, 2012). The ligature material's constricting force causes compressive narrowing of the larynx and the trachea, and thereby forces up the root of the tongue against the posterior wall of the pharynx, and folds the epiglottis over the entrance of the larynx to block the airway. This obstruction of the airway causes air hunger and if entry of air in the lungs is completely prevented, then death occurs rapidly with marked signs of asphyxia. A minimum tension of 15kg on ligature blocks the trachea (Nath *et al.*, 2020).

The findings of this study may: reveal unexplored facts that may be useful in the development of biomarkers for distinguishing between death disguised as hanging and death by actual hanging; uncover the potentials of vitreous biochemical parameters in the post mortem discrimination of death disguised as hanging and death by actual hanging; provide useful information to aid the search for better alternative to blood samples in the post mortem investigations of the causes of death; lead to the development of novel technique/methodology for corroborating existing techniques/methodologies in the post mortem investigations of the causes of death; open up opportunities for improving the justice delivery system as it holds enormous promise of providing information that can clarify controversial cases connected with hanging; cascade into a reduction in murder crimes in the society as it may provide novel clues for plugging one of the means by which criminals escape punishment associated with killings disguised as suicidal hanging; provide data that can guide future research in the area of finding scientific solutions to postmortem legal issues.

The aim of this study is to explore the potentials of selected vitreous biochemical parameters as biomarkers in postmortem determination and discrimination of deaths by hanging using animal models**.** The objective of this is to measure and compare the postmortem vitreous levels of total protein, albumin, globulin, total cholesterol, triacylglycerol, uric acid, creatine kinase and lactate dehydrogenase of dead experimental subjects in different groups based on differences in the causes of death in order to identify analytes with significant variations in quantities amongst the studied groups that can be used as biomarkers for making distinctions amongst them.

Materials and methods

Study area

This study was conducted in Yenagoa, Bayelsa State. Yenagoa is an urban town that serves as the capital of Yenagoa local government area and also the capital of Bayelsa State. It is located in the oil-rich Niger Delta region of southern Nigeria and its geographical coordinates are $4^0$55'29"N and $6^0$15'51"E. It has a tropical monsoon climate with temperature fluctuations between 71^0 F and 87^0F. Its weather vacillates between a wet rainy season and a cloudy dry season. Yenagoa has a mixed population of natives and non-natives and majority of residents either work

for the government as civil servants or are engaged in private businesses (Agoro *et al.,* 2021).

Animal specimen and study population

The research utilized 96 male albino rabbits. The age range was between six to eight months. The weight bracket/range was between 1.5kg to 2kg. The animals were obtained from the animal house of the University of Jos, Plateau State. The animals were kept in cages at the animal house in the Biochemistry laboratory of the Federal University Otuoke, Bayelsa State, for 7 days prior to the experiment to allow for acclimatization to the environmental condition at room temperature. Commercial rat pellets and water were provided *ad libitum.*

Mead's resource equation was utilized for the calculation of the sample size (Kirkwood and Robert, 2010). The equation is stated and the components defined. 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 groups (T = 4), with 24 animals per group, making 96 animals in total (N = 95), without any further stratification (B =0), then E would equal 91, which is above the cutoff of 20, indicating that the sample size is very suitable for the research.

Experimental design

The research was structured into four groups of twenty-four rabbits each: two treatment (test) groups and two control groups based on specific means of deaths. Each group was administered a unique treatment in form of cause of death. In one test group, the death of the experimental subjects was caused by hanging. In a second test group, the death of the experimental subjects was caused by strangulation, thereafter, the dead subjects were hanged as a way of disguise or cover up of the actual cause of death. The remaining two groups are the baseline controls, which were deaths by strangulation and deaths by chloroform intoxication. The mechanism of hanging of the experimental subjects was conceptualized from the studies posited by Sabyasachi *et al.,* (2020) and Nath *et al.,* (2020). The observed average agonal period was 6mins for subjects in hanging studies, 10mins in strangulation and 27mins in chloroform. Ascertaining the death status of the experimental animals relied on the prescriptions of the uniform determination of death act (Omelianchuk *et al.* 2022). Subjects in both treatment and control groups were left for 24 hours after death before their vitreous humour samples were collected for analysis, mimicking a scenario of death cover-up and subsequent discovery. The choice of vitreous is based on its long postmortem interval before deterioration, fermentation and putrefaction. In addition to its similarity in biochemical concentrations to blood, coupled with insignificant age and sex influences (Agoro *et al.*, 2018, 2019, 2020). Rabbit was the choice animal model for this research work. The suitability of rabbit as a choice animal for this study is attributed to its anatomical and physiological similarities to human (Gwon, 2008). Treatments and sample collections were conducted at the Biochemistry laboratory of the Federal University Otuoke, Bayelsa State. However, the samples analysis were conducted at the Eni-yimini Laboratories (eL) Ltd, located in Igbogene, Yenagoa, Bayelsa State.

Ethical clearance

Ethical clearance was obtained from the animal research ethics committee of the Nnamdi Azikiwe University, Awka. The Animal Welfare Act of 1985 of the United States of America for research and Institutional Animal Care and Use Committee (IACUC) protocols were stringently adhered to (Benjamin and Jean, 2016).

Selection criteria

Rabbits used were apparently healthy and active as confirmed and approved by a veterinary doctor. Rabbits showing signs and symptoms of illness were excluded from the research. Also excluded were rabbits with any form of derangements. Turbid vitreous humours were rejected.

Collection of samples

The vitreous humour samples were collected by the method of Coe (1993) and Tente (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 of any loose tissue fragments surrounding the vitreous chamber. On an average 1.0 mL was collected from each rabbit's eye. Only crystal-clear liquid free of tissue contaminants and fragments were used in the study. The samples were collected twenty-four (24) hours postmortem.

Preparation of samples

Collected vitreous humour samples were transferred into plain containers. The biochemical analyses were

carried out immediately after extraction. Prior to analysis the vitreous samples were centrifuged at 2050 rpm for 10 min. The supernatants were separated and used for the analyses. The samples were employed for determination of the concentrations of total protein, albumin, globulin, total cholesterol, triacylglycerol, uric acid, creatine kinase and lactate dehydrogenase.

Analysis of samples

Vitreous total protein concentration was estimated quantitatively using Biuret Method as modified by Randox Laboratories (United Kingdom) (Randox kit leaflet). Biuret method is the most widely used method for protein analysis in body fluids (Peters, 1968) and one recommended by the International Federation of Clinical Chemistry (IFCC) expert panel for the determination of total protein (Randox kit leaflet). Vitreous albumin was estimated quantitatively using Bromocresol Green Method as modified by Randox Laboratories (United Kingdom) (Randox kit leaflet). Vitreous globulin concentration was derived by subtracting vitreous albumin from vitreous total protein. The value is an estimate of vitreous globulin. Vitreous total cholesterol concentration was estimated quantitatively using Agappe kit as specified by Agappe Diagnostics (Switzerland) (Agappe Kit Leaflet). Viterous triacylglycerol concentration was estimated quantitatively using Agappe kit as specified by Agappe Diagnostics (Switzerland) (Agappe Kit Leaflet). Viterous uric acid concentration was estimated quantitatively by Uricase Method using Agappe kit as specified by Agappe Diagnostics (Switzerland) (Agappe Kit Leaflet). Vitreous creatine kinase activity was estimated quantitatively using Agappe method as modified by Agappe Diagnostics (Switzerland) (Agappe Kit Leaflet). Vitreous lactate dehydrogenase (LDH) activity was estimated quantitatively using Agappe method as modified by Agappe diagnostics (Switzerland) (Agappe kit leaflet).

Statistical analyses

Each parameter quantity in the samples was determined in triplicates and the data generated were analyzed with Statistical Package for Social Sciences (SPSS) program (SPSS Inc., Chicago, IL, USA; Version 18-21) and Microsoft excel. One-way ANOVA (Post Hoc-LSD) and student T-test were used in comparing the mean levels of the various vitreous parameters amongst the studied groups. The level of significance was considered at $P<0.05$. Pearson correlation was used to determine relationships amongst the data generated.

Results and Discussion

The total protein level in HD (8.393+0.004mmol/l) is significantly higher than in control 2 (4.613±0.00041mmol/l) (Fig. 1). Albumin, globulin and total cholesterol levels were not statistically different in all groups (Fig. 2 and 3). Triacylglycerol level in HD (0.313±0.000mmol/l) is significantly higher than in control 1 (0.010±0.000mmol/l). Uric acid level in DHD (0.021±0.00041mmol/l) is significantly lower than in HD (0.114±0.00041mmol/l) but higher in control 1 (0.632±0.00041mmol/l). Creatine kinase activity is significantly higher in HD (6670.510±0.00408U/L) than in DHD (4966.948±0.00041U/L) and in control 1 (3456.511±0.00041U/L) (Fig. 4). Lactate dehydrogenase activity is significantly lower in HD (6074.835±0.00041U/L) than in DHD (13659.420±0.00409U/L) and in control 1 (17491.880±0.00408U/L) (Fig. 5).

The observed high level of total protein in HD (8.393±0.0004mmol/l) when compared with control 2 (4.613±00041mmol/l) may be due to the chloroform stabilizing effect of tissues, which prevents both protein expression and tissue lysis (Ruchieka *et al.*, 2014; Sacco *et al.*, 2022). The observed higher level of triacylglycerol in HD (0.313±0.000mmol/l) when compared with control 1 (0.010±0.000mmol/l) may be ascribed to the difference in the agonal period between the two causes of death. The longer agonal period associated with death of means of control 1 and the lipolysis associated with sustained trauma may have resulted in the depletion of triacyclglycerol (Thierauf and Pollak, 2013; Zilg *et al.*, 2022). The observed low level of uric acid in DHD (0.0021±0.00041mmol/l) when compared to HD (0.114 ± 0.00041mmol/l) may be due to post-mortem thanatochemical activities unrelated to the causa mortis as reported by Gill and Landi (2011). While the high level of uric acid in control 1 (0.632±0.00041mmol/l) is due to the release of uric acid in larger quantities in hypoxic conditions which is characteristic of a longer agonal period as reported in the findings of Baillie *et al*, (2007). The findings of this study with respect to urea, creatinine and uric acid align with that of Palmiere and Mangin, (2015). The observed high level of creatine kinase activity in HD (6670.510±0.00041U/L) when compared to DHD (4966.948±0.00041U/L) and control 1 (3456.511±0.00041U/L) may be attributed to the greater response of the body to the hypoxic trauma which is more intense in deaths by means of HD than by means of control 1 due to differences in the agonal period before death (Apparal, 2020; Woydt *et al.*,

2018). The observed low level of lactate dehydrogenise activity in HD (6074.835 ± 0.00041U/L) when compared to DHD (13659.420 ± 0.00409U/L) and control 1 (17491.880 ± 0.00408U/L) may be due to the absence of substrate for continual activity as the incidence of death by means of HD occurs faster than by means of control 1 (Spriet *et al.*, 2000; Passarella and Schurr, 2018).

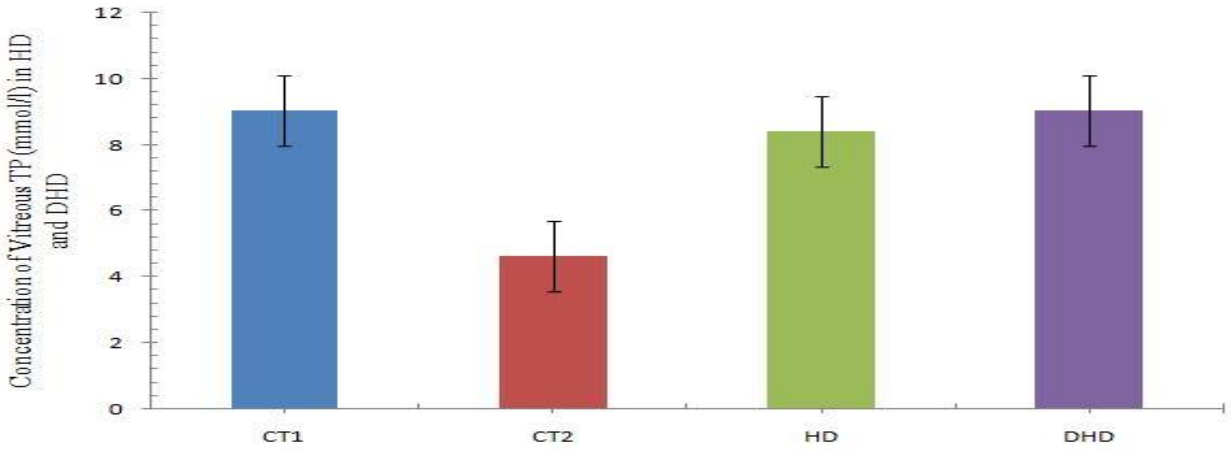

Fig. 1: Chart showing postmortem vitreous levels of total protein in HD and DHD

CT1: Control 1 (Strangulation death); CT2: Control 2 (Chloroform death; HD: Hanging Death; DHD: Disguised hanging death (strangled to death before hanging); TP: Total protein

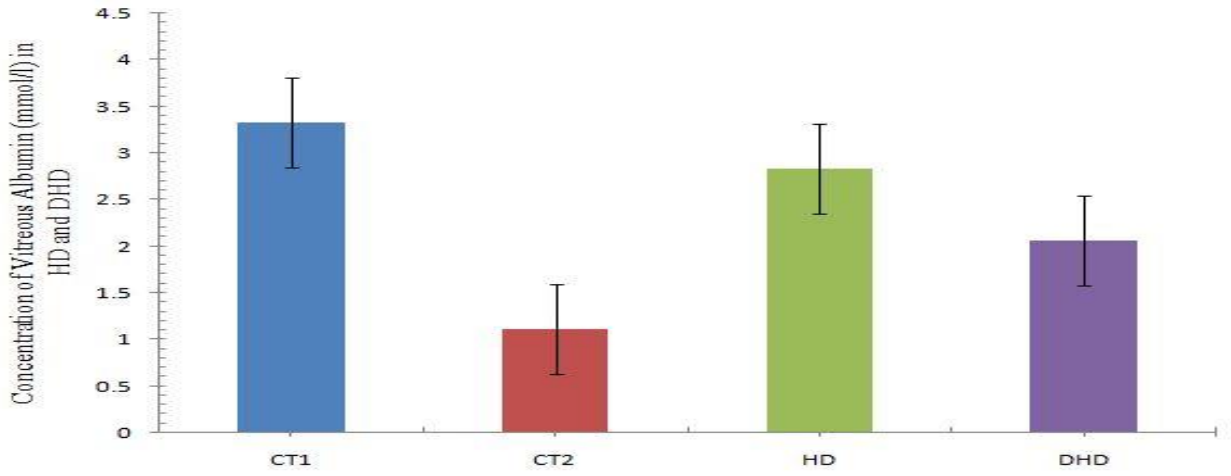

Fig. 2: Chart showing postmortem vitreous levels of albumin in HD and DHD

CT1: Control 1 (Strangulation death);CT2: Control 2 (Chloroform death); HD: Hanging Death; DHD: Disguised hanging death (strangled to death before hanging)

https://doi.org/10.5555/GQEI9081 ISSN 2488-8648

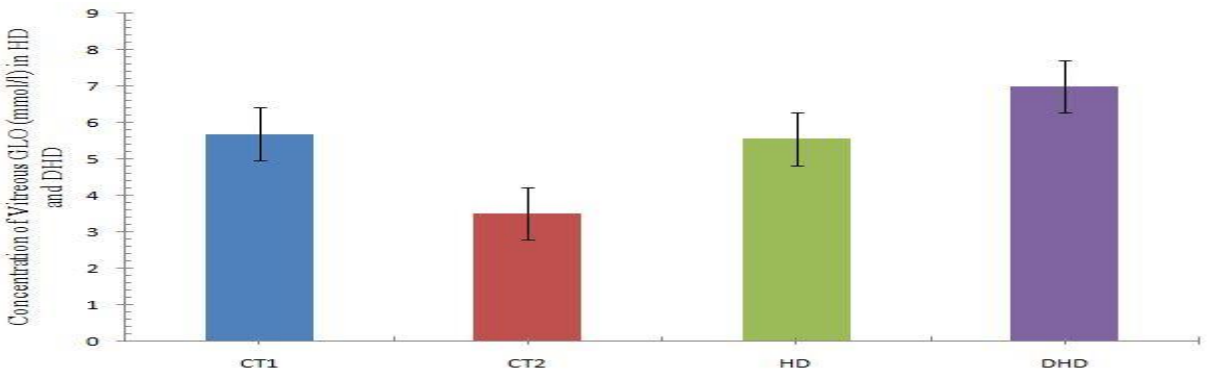

Fig. 3: Chart showing postmortem vitreous levels of globulin in HD and DHD

CT1: Control 1 (Strangulation death);CT2: Control 2 (Chloroform death);HD: Hanging Death; DHD: Disguised hanging death (strangled to death before hanging); GLO: Globulin

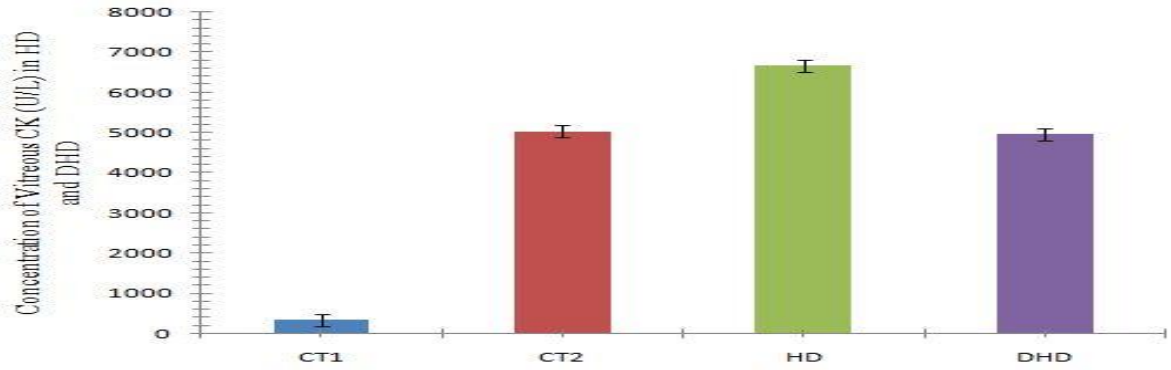

Fig. 4: Chart showing postmortem vitreous levels of creatine kinase in HD and DHD

CT1: Control 1 (Strangulation death); CT2: Control 2 (Chloroform death); HD: Hanging Death; DHD: Disguised hanging death (strangled to death before hanging); CK: Creatine kinase

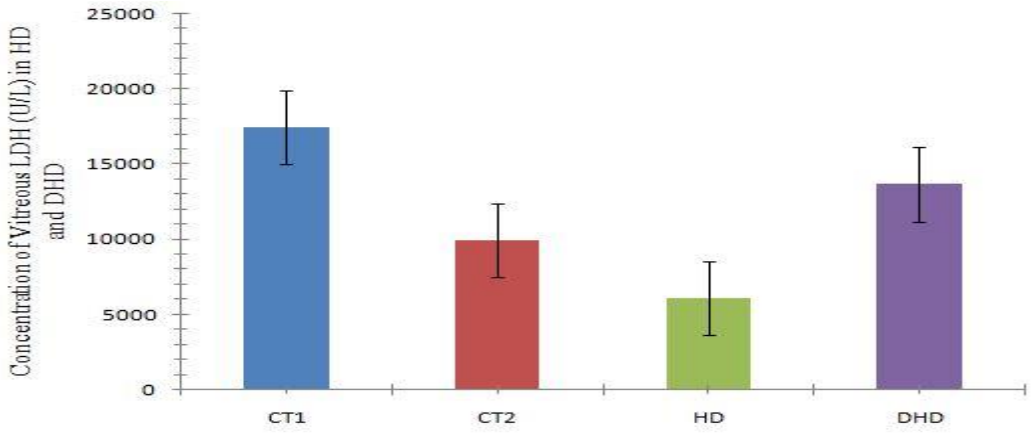

Fig. 5: Chart showing postmortem vitreous levels of LDH in HD and DHD
CT1: Control 1 (Strangulation death); CT2: Control 2 (Chloroform death); HD: Hanging Death; DHD: Disguised hanging death (strangled to death before hanging);LDH: Lactate dehydrogenase

Conclusion and recommendation

Postmortem vitreous levels of uric acid, creatine kinase and lactate dehydrogenase differed significantly ((P≤0.05) between samples from deaths by means of hanging and deaths by means of strangulation but disguised as hanging. Postmortem vitreous levels of triacylglycerol differed significantly (P≤0.05) between samples from deaths by means of hanging and deaths by means of strangulation. Postmortem vitreous levels of total protein differed significantly (P≤0.05) between samples from deaths by means of hanging and deaths by means of chloroform toxicity. The identified significant variations in the quantities of these discovered vitreous metabolites amongst the study groups makes them potential biomarkers for effectively determining and clarifying hanging associated cause of deaths when utilized either separately or as a combination of biomarkers, which is advocated. It is recommended that similar investigations involving the analysis of pH and other physical parameters of the vitreous should be conducted.

References

Adam, N. and Gail, C. (2013). Postmortem Toxicology, "In Clarke's Analytical Forensic Toxicology". 2nd edition, PhP Pharmaceutical press, 189-213.

Agoro, E.S. (2018). Urine composition. Clinical importance of urinalysis. *Semed productions Port Harcourt,* 26 - 27.

Agoro, E.S., Akubugwo, E.I., Chinyere, G.C. and Samuel, R. (2017d). Comparison of Vitreous Protein Profiles of Rabbits subjected to Acute Carbon Monoxide poisoning and normal animal after death. *J Forensic Sci Res., Vol.* 1: 040-045.

Agoro, E.S., Chinyere, G.C. and Akubugwo, E. I. (2020). Emerging concept of vitreous concentrations of proteins and lipids as discriminant of fresh water drowning death. *Journal of forensic and legal medicine, Vol.* 73: 1019 - 1904.

Agoro, E.S., Ikimi, C.G. and Edidiong, T. (2021). The use of vitreous renal chemistries in the discrimination of postmortem fresh water drowning. *Toxicology research and application, Vol.* 5: 1-6.

Agoro, E.S., Okoye, F.B.C., Azuonwu, O. and Ebiere, N.E. (2017a). The Effect of Age and Sex on Vitreous Humour Chemistry and Postmortem Interval (PMI). *Indian Journal of Forensic Medicine and Toxicology, Vol.* 2(2): 173-177.

Agoro, E.S., Wankasi, M.M. and Azuonwu, O. (2017b). The Forensic Application of Vitreous Humour Biochemistry in Postmortem Disease Diagnosis. *Indian Journal of Forensic Medicine and Toxicology, Vol.* 1: 195-199.

Agoro, E.S., Wankasi, M.M. and Ombor, J.O. (2019). Biochemical patterns of cardio-renal biomarkers in serum and vitreous humour of rabbits after chronic CO exposure. *Annals of environmental science and toxicology. Vol*. 3(1): 001-006.

Agoro,E.S., Okoye, F.B.C., Onyenekwe, C.C., Azuonwu, O. and Ebiere, N.E. (2017c). Extrapolation of Three Hourly Post-Mortem Interval using some Vitreous Chemistry Parameters. *Journal of Forensic Research, Vol.* 8(1): 1-5.

Amith, M. (2005). Role of vitreous humour biochemistry in forensic pathology. *A thesis submitted to the University of Saskatchewan. (https://ecommons.usask.ca/handle). Retrieved 17/02/2021.*

Apparaj, D. (2020). Electrolyte composition of vitreous humour and cerebrospinal fluid as a tool to determine the post-mortem interval. *Department of forensic medicine, coimbatore medical college, coimbatore-18.*

Baillie, J.K., Bates, M.G., Thompson, A.A., Waring, W.S., Dartridge, R.W., Schnopp, M.F., Simpson, A., Gulliver-Sloan, F., Maxwell, S.R. and Webb, D.J. (2007). Endogenous urate production augments plasma antioxidant capacity in healthy lowland subjects exposed to high altitude. *Chest, Vol.* 131 (5): 1473-1478.

Benjamin, A. and Jean, L. (2016). Legislative History of the Animal Welfare Act.

Bolarin, D.M. and Azinge, E.C. (2010). Chemical pathology laboratory tests in pregnancy. *Journal of medical laboratory science; Vol.* 19(1): 3-13.

Bosze, Z. and Houdebine, L.M. (2006) Application of rabbits in biomedical research: a review. *World Rabbit Science, Vol.* 14:1-14.

Brandt-Casadevall, C., Krompecher, T., Giroud, C. and Mangin, C. (2003). A case of suicide disguised as natural death. *Science & Justice, The forensic science society, Vol. 43 (1) 41 – 43.*

Butzbach, D.M. (2010). The influence of putrefaction and sample storage on post-mortem toxicology results.

Forensic science medicine and pathology, Vol. 6: 35 – 45.

Coe, J.I. (1993). Postmortem chemistry update. Emphasis on forensic application. *American Journal of Forensic Medicine and Pathology*, 14(2): 91–117.

Cristian, P. and Patrice, M. (2011). Postmortem chemistry update (part 1). *International Journal of Legal Medcine, Vol.*10: 1007-1065.

DiPoce, J., Guelfguat, M., and DiPoce, J. (2012). Radiologic findings in cases of attempted suicide and other self-injurious behavior. *Radiographics, Vol. 32: 2005—2024.*

Epstein, E.J. (2020). Suicides and disguised murders. *Edward Jay Epstein investigates. Fast track press/EJE publications Ltd, New York.* Vol. 4.

Garg, V., Oberoi, S.S., Gorea, R.K. and Kiranjeet, K. (2004). Changes in the concentrationsof vitreous potassium with increasing time since death. *Journal of Indian Association of Forensic Medicine, Vol.* 26: 136–139.

Gill, J.R. and Landi, K. (2011). Putrefactive rigor: apparent rigor mortis due to gas distension. *American journal of forensic medical pathology Vol.* 32: 242-244.

Gwon, A. (2008). The rabbit in cataract surgery. *Tsonis, P.A. (ed.) Animal models in eye research. Elsevier*, pp. 184-204.
Kaki, A., Crosby, E., and Lui, A. (1997). Airway and respiratory management following non-lethal hanging. *Can J Anaesth, Vol. 44(4): 445-50.*

Kang, S.J. and Grossniklaus, H.E. (2011). Rabbit model of retinoblastoma. *Journal of Biomedicine and Biotechnology*, *Vol.* 394730.
Kondo, M., Sakai, T., Komeima, K., Kurimoto, Y., Ueno, S., Nishizawa, Y., Usukura, J., Fujikado, T., Tano, Y. and Terasaki, H. (2009). Generation of a transgenic rabbit model of retinal degeneration. *Investigative Ophthalmology and Visual Science*, *Vol.*50(3): 1371-1377.

Kónya, A. *et al.*, (2008). Animal models for atherosclerosis, restenosis, and endovascular aneurysm repair. *In Conn, P.M. (ed.) Sourcebook of models for biomedical research. Humana Press*, pp. 369-384.
Lopez, G. (2021). 2020's historic surge in murders, explained. *The weeds. Vox media Inc., Washington DC.*

Maeda, H., Zhu, B.L., Ishikawa, T., Quan, L. and Michiue, T. (2009). Significance of postmortem biochemistry in determining the cause of death. *Legal Medicine Tokyo, Vol.* 11(1): 46–49.

Nath, S., Majumder, R., and Pratihar, H. (2020). Homicide hanging - a rare case report. *Forensic Res Criminol Int J. 8(1): 8–9.*

Omelianchuk, A., Bernat, J., Caplan, A., Greer, D., Lazaridis, C., Lewis, A., Pope, T., Ross, L.F. and Magnus, D. (2022). Revise the uniform determination of death act to align the law with practice through neurorespiratory criteria. *Neurology*, Vol. 98 (13): 532 – 536.

Palmiere, C. and Mangin, P. (2015). Urea nitrogen, creatinine and uric acid levels in postmotem serum, vitreous and paricardial fluid. *Int. J. Legal Med. Vol.* 139:201-305.

Passarella, S. and Schurr, A. (2018). I-lactate transport and metabolism in mitochondria of HepG2 cells - The Cori cycle revisited. *Front. Oncol. Vol.* 8(120).

Peters, T. (1996). All About Albumin: Biochemistry, Genetics and Medical Applications. *San Diego, CA: Academic Press Limited.*

Roberts, Y. (2022). All strangulations of women is serious, and it's time for the law to step up. *The Guardian*, Sunday 29th May, 2022.

Ruchieka V., Hitesh, V. and Nirmala, N. (2014). Modified technique for soft tissue processing and staining, *journal of histotechnology*, *Vol.*37(1): 14-20.

Sabyasachi, N., Rupali, M., Pratihari, H. (2020). Homicide hanging - a rare case report. *Forensic Res Criminol Int J. Vol. 8(1): 8–9.*

Sacco, M.A., Cordasco, F., Scalise, C., Ricci, P. and Aquila, I. (2022). Systematic review on post-mortem protein alterations: analysis of experimental models and evaluation of potential biomarkers of time of death. *Diagnostics, Vol.* 12(6): 1490.

Spriet, L.L., Howlett, R.A. and Heigenhauser, G.J. (2000). An enzymatic approach to lactate production in human skeletal muscle during exercise. *Med sci. sports exerc. Vol. 32(4)*: 756-763.

Tente, W., O'Rourke, P., Sherman, S., Kauper, K., McGovern, C., Matteus, S., Dean, B., Toa, W. and Thanos, C. (2004). Sustained Delivery of hCNTF to Rabbit Vitreous Humour by Two Polymer Encapsulated Cell Lines in the NT-502 Device.

Investigative Ophthamology and Visual Science Journal, Vol. 45 (13): 303 -329.

Thierauf, A. and Pollak, S. (2013). Forensic medicine/causes of death. *Encyclopedia of forensic sciences (2nd ed).*

Thierauf, A., Kempf, J., Perdekamp, M.G., Auwärter, V, Gnann, H. (2011). Ethyl sulphate and ethyl glucuronide in vitreous humour as postmortem evidence marker for ethanol consumption prior to death. *Forensic Science International, Vol.* 210: 63-68.

Töro, K., Kristof, I., and Keller, E. (2008). Incomplete decapitation in suicidal hanging - report of a case and review of the literature. *J Forensic Leg Med Vol. 15: 180 - 184.*

Ward, M. and Schofield, E.L. (2010). Biomarkers for brain disorders. *Therapy, future medicine limited*. Vol. 7 (4): 321 -336.

Woydt, L., Bernhard, M., Kirsten, H., Burkhardt, R., Hammer, N., Gries, A., Drebler, J. and Ondruschka, B. (2018). Intra-individual alternations of serum markers routinely used in forensic pathology depending on increasing post-mortem interval. *Scientific reports, Vol. 8* (12811).

Zahn, G. (2010). Assessment of the integrin α5β1 antagonist JSM6427 in proliferative vitreoretinopathy using in vitro assays and rabbit model of retinal detachment. *Investigative Ophthalmology and Visual Science, Vol.* 51(2):1028 - 1035.

Zilg, B., Alkass, K., Berg, S. and Druid, H. (2009), Postmortem identification of hyperglycemia. *Forensic Science International*, *Vol.* 185: 89-95.

Zilg, B., Alkass, K., Kronstrand, R., Berg, S. and Druid, H. (2022). A rapid method for postmortem vitreous chemistry analysis-deadside analysis. *Biomolecules* 12(32): biom12010032.