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# Discriminatory potentials of vitreous electrolytes and renal indices in the autopsy of deaths suspected of disguise by hanging in rabbits.

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**Abstract**

The absence of biomarkers that can reveal the cause of death when the cause of death is disguised constraints the verdicts of the coroner. This study aims at exploring the discriminatory potentials of vitreous electrolytes and renal indices in the autopsy of deaths suspected of disguise by hanging in rabbits. 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 sodium, potassium, carbon iv oxide, chloride, calcium, glucose, urea and creatinine using standard methods. SPSS (version 18-21) and one-way ANOVA were used for data analysis. The results show that postmortem vitreous levels of Na, Ca and creatinine 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 striking differences in the levels of these notable analytes can be utilized either as primary or confirmatory tests to reveal and discriminate between death disguised as hanging and death by actual hanging

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## Introduction

Judicial decisions involving the discrimination of deaths due to an actual hanging incident and deaths caused by other means but disguised as hanging with the intention of misleading law enforcement agents have always been mostly speculative (Rao, 2016). This is because there are no bioindicators that can precisely unmask the cause of death when the cause of death is veiled. Blood, the usual analytical specimen, is unsuitable as a sample when an autopsy is conducted long after death because it decomposes shortly after death. This far-reaching diagnostic gap of making a distinction between an actual cause of death and a purported cause of death is being exploited by murderers to evade justice while homicidal crimes are on the rise. Sadly, studies have shown that hanging is one of the most patronized means of covering up murder crimes and escaping justice (Nath et al., 2020). Hence, the search for bioindicators that can end such error-prone courtroom conjectures, plug this lacuna for getting away with murder crimes and strengthen the legal processes by providing it with correct empirical tools for reaching accurate and valid judgments.

Asphyxia has been identified as the pathophysiological mechanism that mediates death by hanging. Within few minutes of hanging, asphyxia leads to anoxia, which then results in the build-up of carbon iv oxide in the tissues, thereby causing a disturbance in cellular metabolism like the prevention of cellular respiration. This is followed by unconsciousness and the eventual death of the individual (Dillon et al., 2023). Hanging can be

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classified depending on the degree of suspension. Complete hanging involves the suspension of the whole body from the ligature material and no portion of the body is touching the ground. Complete hanging is considered as suicidal in nature. While partial hanging involves the partial suspension of the body where either part of the body is touching the ground. In partial hanging the deceased body may be in kneeling or sitting position (Abouhashem et al., 2020). Another method of classification involves the placement of the material used in hanging, it is divided into two types. The first is known as typical hanging, where the ligature runs from the midline, above the thyroid cartilage, symmetrically upwards on both sides of the neck, to the occipital region and the knot is placed over the central part of the back of neck. The second type, known as atypical hanging, here the knot of the ligature can be found in the front, side of the neck but not the central back of the neck (DeBarma, 2014). Abouhashem et al., (2020) carried out a study on 36 cases of deaths due to hanging, and reported that skin haemorrahage was positive in 38.9% of cases, petechial hemorrhage was positive in 50% of cases, conjunctival hemorrhage in 55.6% of cases and other periligature injuries were positive in 11.2% of cases. Homicidal hanging was greatly associated with skin haemorrahage in 84.6%of cases, petechial hemorrhage in 69.2%of cases, conjunctival hemorrhage in 61.5% of cases (Garetier et al., 2017).

Because of the great similarity in the biochemistry and evolutionary pattern between certain species of animals and human beings, some of them have found significant use in medical research. The findings of such studies provide an empirical basis for an extension of such studies to humans (Ferreira et al., 2005). Animal studies are almost inevitable in cases where the use of human subjects for research is impossible or is fraught with serious ethical barriers. The use of animal models in research has contributed immensely to several and diverse scientific discoveries with multiple Nobel Prizes won for researches involving the use of animals. Experiments involving the use of animals are to conform to conventional ethical and legal standards. Compliance with such approved protocols helps to prevent issues of animal abuse (Manjeet et al., 2012).

Vitreous humour, sometimes referred to as “the vitreous body” or just “vitreous”, is a transparent, jelly-like structure that accounts for four fifths of the eye volume. The vitreous humour is located between the lens and the retina filling the center of the eye. The vitreous humour, with an approximate volume of 4 mL, constitutes nearly 80% of the globe, making it the largest structure within the eye (Agoro et al., 2017a). However, relatively little is known about this important structure than any other part of the eye. The vitreous body is an extracellular matrix that contains fibrillar structural proteins associated with varying amounts of hyaluronic acid and various types of proteins, glycoproteins and proteoglycans. Collagen is the major structural protein of the vitreous. Hyaluronic acid is the major glycosaminoglycan present in the vitreous humour. The vitreous is a conglomerate of various biochemical substances that are divers in structures and functions. The diversities are the attributes that had made the vitreous a peculiar postmortem sample (Agoro et al., 2017b). Justifiable basis why vitreous humour holds promising autopsic relevance are: it has a relatively stable chemical composition when compared to blood and cerebrospinal fluid (Kondo et al., 2009); it is relatively inert and only slightly influenced by sudden fluctuations in the blood chemistry (Garg et al., 2004); it is resistant to microbial contamination associated with the period after death (Chandrakanth et al., 2013).

The aim of this study is to explore the discriminatory potentials of vitreous electrolytes and renal indices in the autopsy of deaths suspected of disguise by hanging in rabbits. The objective of this study is to measure and compare the postmortem vitreous levels of levels sodium, potassium, carbon iv oxide, chloride, calcium, glucose, urea and creatinine of dead experimental subjects in different groups based on differences in the causes of death in order to identify metabolites with significant variations in quantities amongst the studied groups that can be used as bioindicators for making distinctions amongst them.

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

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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.

## Materials and methods

### Study area

This study was conducted in Yenagoa, the capital of Bayelsa State in Southern Nigeria. Its geographical coordinates are $4^0$55'29"N and $6^0$15'51"E. Its vegetation is composed of four ecological zones – coastal barrier island forests, mangrove forests, freshwater swamps and lowland rain forests. Apart from being a government administrative metropolis and a trade centre for agricultural produce, petroleum exploitation activities within its surroundings have introduced some vocational diversifications with associated commercial and technological advances (Agoro et al., 2021).

### Animal specimen and study population

The research utilized 96 male albino rabbits of close age and weight brackets. 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 had 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 was 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

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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 meant for glucose concentration measurements were transferred into fluoride oxalate tubes, while those designated for investigations involving electrolytes and renal indices 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 sodium, potassium, carbon iv oxide, chloride, calcium, glucose, urea and creatinine.

**Analysis of samples**

The selected vitreous electrolytes concentrations were determined using an ion selective electrode (ISE) analyzer method (analyzer ISE 4000) as stated by Bolarin and Azinge (2010). Vitreous glucose concentration was estimated quantitatively using glucose oxidase method as specified by Randox Laboratories (United Kingdom). The glucose kit used was a product of Randox and the standard operating procedure as stated in the manual was strictly followed. Vitreous urea was estimated by diacetyl monoxime method. The diacetyl monoxime salts are stable at room temperature and could adapt to harsh environment (Bolarin and Azinge, 2010). Determination of vitreous creatinine concentration was by Jaffes method. It was chosen because of its stability and consistency (Bolarin and Azinge, 2010).

**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 results show that Na level in DHD (15.600±0.00408mmol/l) is significantly lower than in HD (149.000±0.040825mmol/l) and in the controls (139.000±0.040825mmol/l) (145.000±0.040825mmol l) ($p \leq 0.05$) respectively (Fig. 1). K level in HD (32.800±0.040825mmol/l) is significantly lower than in control 1 (38.300+0.04082mmol/l) (Fig. 2). $CO_2$ level in HD (7.000±0.40825mmol/l) is significantly lower than in control 2 (16.000±0.40825mmol/l) (Fig. 4). Ca level in DHD (3.546±0.00041mmol/l) is significantly higher than in HD (1.734±0.0004mmol/l) and in the controls (1.221±0.00041mmol/l) (1.359±0.0004mmol/l) respectively (Fig. 5). Cl and glucose levels were not statistically different in all groups (Fig. 3). Urea level in HD (4.933±0.00041mcmol/l) is significantly lower than in the controls (10.617±0.00041mcmol/l) (13.275±0.000.41mcmol/l) respectively (Fig. 6). Creatinine levels in DHD (37.083+0.0041mcmol/l) is significantly lower than in HD (65.192±0.0041mcmol/l) and in control 1 (83.022±0.0041mcmol/l) but lower in control 2 (28.663±0.00041mcmol/l) (Fig. 7).

The observed low level of Na in DHD (15.600± 0.00408mmol/l) when compared with HD (149.000±0.040825mmol/l) and the controls (139.000±0.040825mmol/l), (145.000±0.040825mmo l/l) may be attributed to disturbances of the carcass of the animal whilst staging the disguise (Zilg et al., 2022). D'souza et al., (2011) had established that movements of a body after death affect post-mortem findings. While Gill and Landi (2011), reported that certain post-mortem discoveries may not be the direct result of the means of death but may be due to other associated physical, chemical and biological processes. The observed low level of K in HD (32.800±0.040825mmol/l) when compared with control 1 (38.300±0.04082mmol/l) may be due to the different means of death. In HD, the means of death is by ligature strangulation, while in control 1 it is by manual strangulation. The pathophysiological mechanism associated with both means of death varies

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(Thierauf and Pollak, 2013). The differences in the agonal period associated with both means of death impacts on neurotransmitters released and brings about a shift in the cell ion concentrations which then eventually affects the post mortem levels of potassium in the vitreous humour (Apparaj, 2020). The observed high level of $CO_2$ in control 2 (16.000±0.40825mmol/l) when compared with HD (7.000±0.040825mmol/l) is because chloroform is metabolized to yield $CO_2$ (Foxall, 2007). This outcome agrees with the report of EPA. (2001) and further corresponds with the findings of Cui et al., (2002). The observed high level of Ca in DHD (3.546±0.00041mmol/l) is when compared with HD (1.734±0.0004mmol/l) and the respective controls (1.221±0.00041mmol/l) (1.359±0.0004mmol/l) may be the effect of the post-mortem movements of the carcass while carrying out the disguise as physical activity is capable of interfering with the calcium-dependent on-set of rigor mortis (Shedge et al., 2022). The values of this study correspond with the findings of Gonzalez-Montana et al (2019) who reported the differential stability in the values of post-mortem vitreous calcium. The observed higher levels of urea in the respective controls (10.617±0.00041mcmol/l) (13.275 ± 0.00041mcmol) may be due to the effects of epinephrine which is released during the longer agonal periods connected with the controls. Epinephrine decreases glomerular filtration rate, thereby increasing plasma levels of urea, from where it diffuses into the vitreous (Begum et al., 2018; Gurler et al., 2015). The observed lower level of creatinine in DHD (37.083±0.0041mcmol/l) when compared to HD (65.192 ± 0.0041mcmol/l) may be attributed to the post-mortem activities involving the corpse whilst implementing the disguise (D'Souza et al, 2011). The lower level of creatinine in HD (65.192±0.0041mcmol /l) when compared with control (83.022±0.0041mcm ol/l) may be due to the decreased renal clearance associated with adrenaline induced decrease in glomerular filtration rate as a result of a longer agonal period linked to deaths by means of control 1 (Zilg et al., 2022; Begum et al., 2018). The observed low level of creatinine in control 2 (28.663±0.00041mcmol/l) can be explained by the hyper-permeability of creatinine at the glomerulus due to chloroform induced nephrotoxicity (Liu *et al*., 2013).

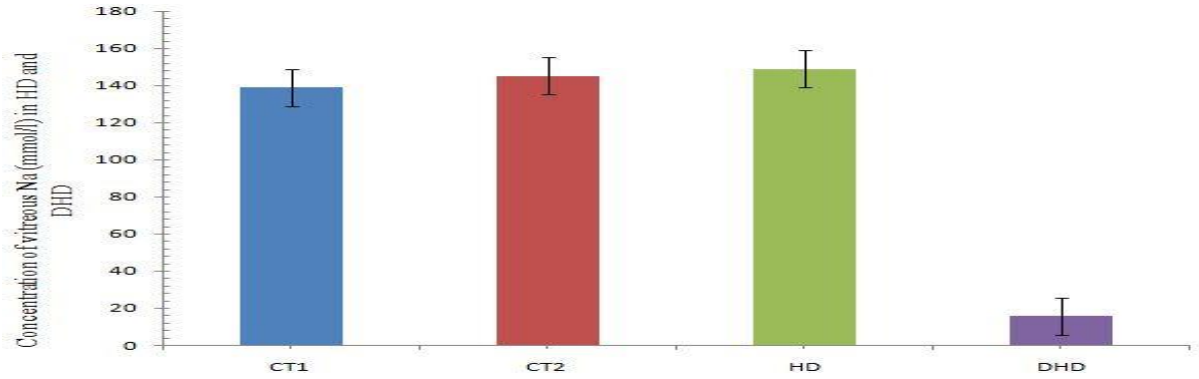


Fig. 1: Chart showing postmortem vitreous levels of Na in HD and DHD

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

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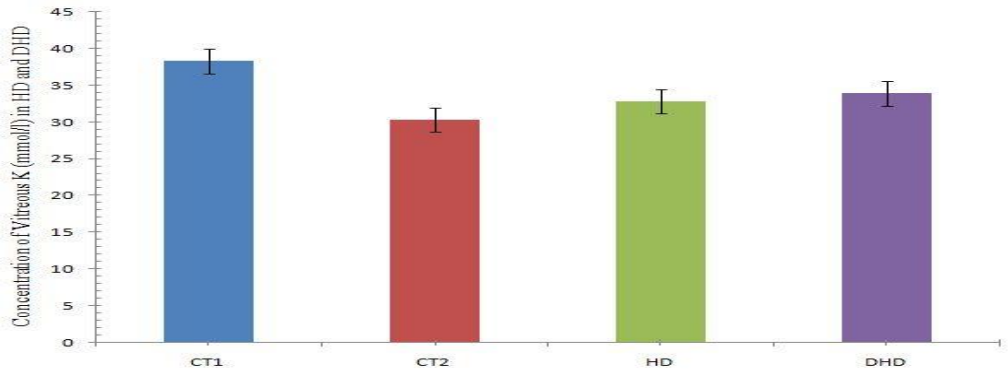


Fig. 2: Chart showing postmortem vitreous levels of K in HD and DHD

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

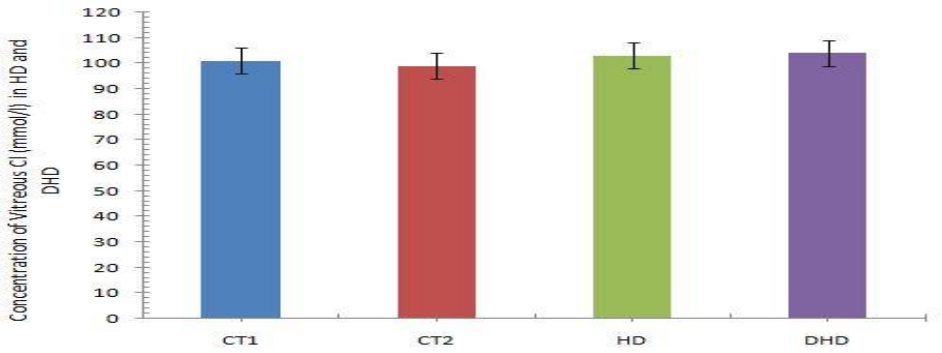


Fig. 3: Chart showing postmortem vitreous levels of Cl in HD and DHD
CT1: Control 1 (Strangulation death); CT2: Control 2 (Chloroform death); HD: Hanging Death
DHD: Disguised hanging death (strangled to death before hanging)

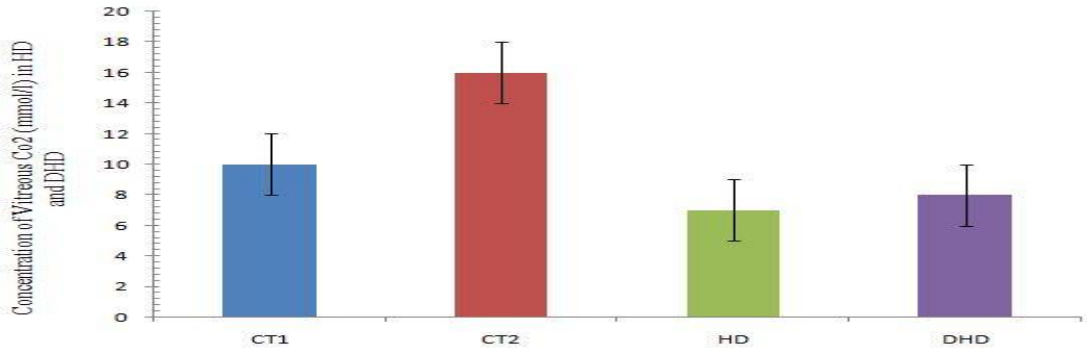


Fig. 4: Chart showing postmortem vitreous levels of $CO_2$ in HD and DHD

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

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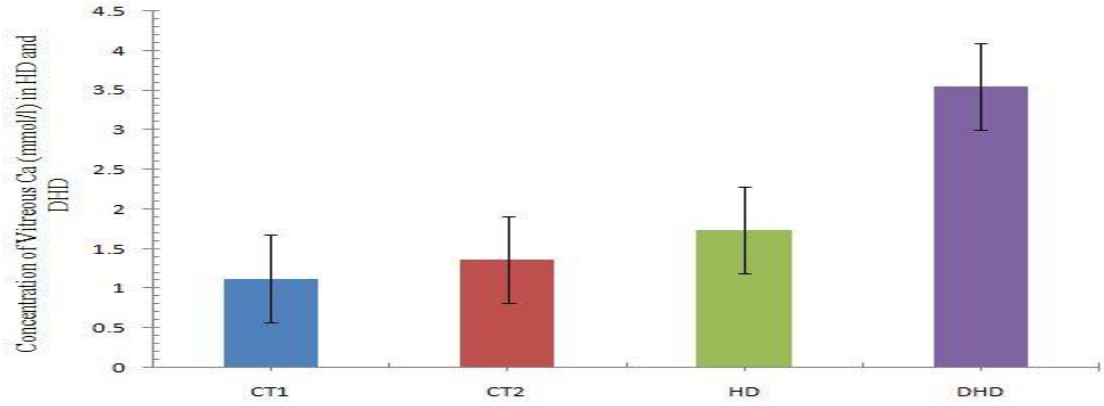


Fig. 5: Chart showing postmortem vitreous levels of Ca in HD and DHD

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

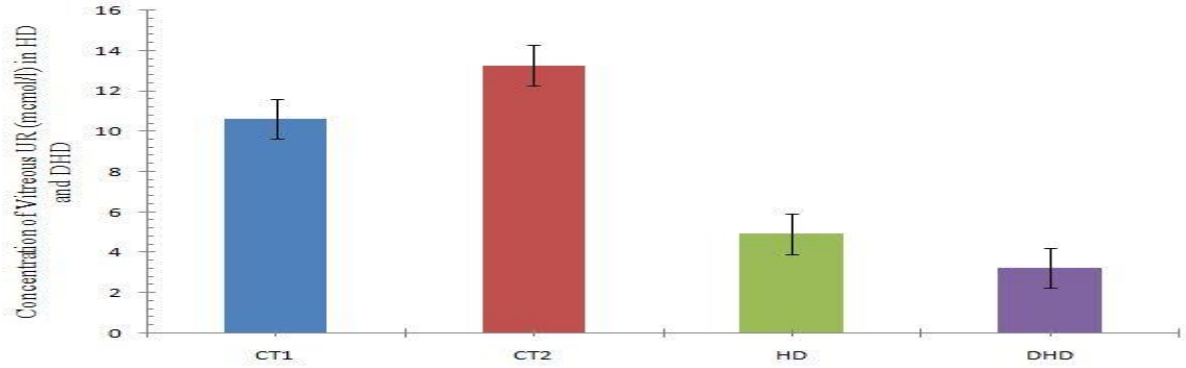


Fig. 6: Chart showing postmortem vitreous levels of urea in HD and DHD

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

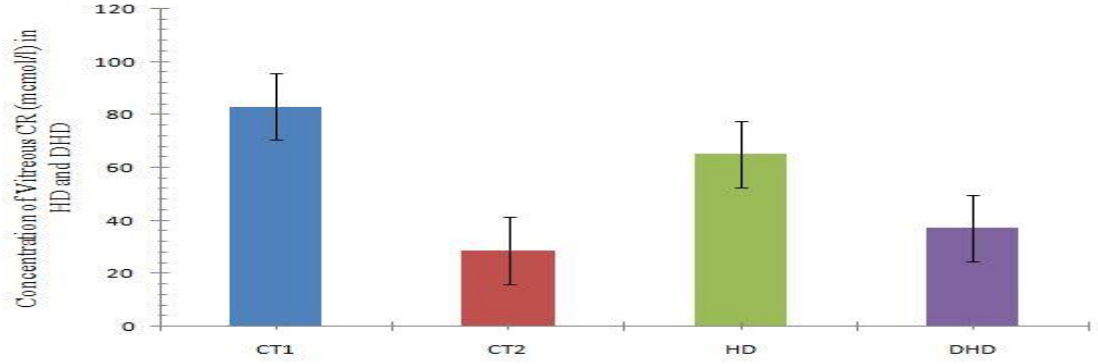


Fig. 7: Chart showing postmortem vitreous levels of creatinine in HD and DHD

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

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**Conclusion and recommendation**

Postmortem vitreous levels of Na, Ca and creatinine 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 K and urea differed significantly (P≤0.05) between samples from deaths by means of hanging and deaths by means of strangulation. Postmortem vitreous levels of $CO_2$ differed significantly (P≤0.05) between samples from deaths by means of hanging and deaths by means of chloroform toxicity. The striking differences in the levels of these notable analytes can be utilized either as primary or confirmatory tests to reveal and discriminate between death disguised as hanging and death by actual hanging. It is recommended that further studies with longer postmortem intervals be carried out.

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