A COMPARATIVE BIOCHEMICAL ANALYSIS OF THE POST MORTEM CHANGES IN THE AQUEOUS HUMOR AND BLOOD OF A BOVINE EYE.

BY

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DEPARTMENT OF OPTOMETRY,

FACULTY OF LIFE SCIENCES,

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FEBUARY, 2025

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF OPTOMETRY,

FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY,

IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD

DOCTOR OF OPTOMETRY (O.D) DEGREE IN OPTOMETRY.

FEBUARY, 2025.

CERTIFICATION AND APPROVAL

This is to certify that this research project titled **A COMPARATIVE BIOCHEMICAL ANALYSIS OF THE POST MORTEM CHANGES IN THE AQUEOUS HUMOR AND BLOOD OF A BOVINE EYE** was carried out by **FAVOUR OMORODION** in the Department of Optometry, Faculty of Life Sciences, University of Benin in partial fulfillment of the requirement for the Doctor of Optometry degree in the 2023/2024 Academic Session.

…….. **……**

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PROFESSOR EGBEIBE
(HEAD OF DEPARTMENT)

……..

……
EXTERNAL EXAMINER **DATE**

DEDICATION

I dedicate this project to my Elohim, who has preserved my life and kept me throughout my journey in the study of optometry, at the University of Benin.

I also want to dedicate this project to my lovely parents, Mr. and Mrs. Omorodion, my apostle(Apostle Micheal Omorodion) and siblings for their love, and unwavering moral and financial support.

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Special thanks to God Almighty for his faithfulness and mercy throughout my stay at the University of Benin.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

This study investigates biochemical changes in the aqueous humor and blood of bovine eyes after death to improve methods for estimating the post-mortem interval (PMI) in forensic investigations. The aqueous humor, a clear fluid in the eye's anterior chamber, is relatively isolated from external factors and may serve as a more reliable fluid for PMI estimation compared to blood, which is subject to faster and more variable biochemical changes. Key biochemical markers (e.g., glucose, electrolytes, and proteins) will be measured in both fluids at different times after death. The results obtained will determine if the aqueous humor has more gradual and predictable biochemical changes than the blood or if it changes more rapidly and inconsistently as compared to the blood. This will further suggests that aqueous humor may or may not provide a more stable timeline for estimating PMI, especially when compared to blood samples. This study will be a support to the use of either blood or aqueous humor as a biomarker in forensic settings and calls for further research on post-mortem biochemical dynamics in humans and environmental influences on fluid degradation.

KEY WORDS: Post mortem interval(PMI), Aqueous humor, Biochemical changes, Bovine eye, Blood analysis, Forensic science, Electrolyte levels, Glucose levels, Biochemical markers.

CHAPTER ONE

1.0 INTRODUCTION

Over the past few decades, forensic scientists have increasingly turned to the analysis of biological fluids to improve the accuracy of post-mortem interval (PMI) estimation. Traditional external markers of decomposition, such as livor mortis (the settling of blood), rigor mortis (muscle stiffening), and algor mortis (body cooling), though useful, are limited in their reliability over longer post-mortem intervals and in varying environmental conditions. External factors like temperature fluctuations, the presence of clothing, humidity, and physical location can significantly affect the progression of these signs, introducing variability in PMI estimates. This challenge has led to the exploration of internal biochemical markers, particularly those found in relatively protected body fluids such as the aqueous humor, to complement and enhance the forensic toolkit.

1.1 BACKGROUND INFORMATION

1.1.1 Aqueous Humor

The **aqueous humor** is a clear, watery fluid that occupies the space between the **cornea** and the **lens** in the eye. It is crucial for maintaining ocular health and functionality. The aqueous humor is present in both the **anterior chamber** (between the cornea and iris) and the **posterior chamber** (between the iris and lens). It is secreted by the **non-pigmented epithelium** of the ciliary processes, a part of the **ciliary body** (Forrester et al., 2015). The aqueous humor is composed mainly of water (~99.9%), along with electrolytes (e.g., sodium, potassium), glucose, proteins, and ascorbic acid. The latter protects ocular tissues from oxidative stress (Kiel, 2011).

Mechanism of Production

- The ciliary body utilizes **active secretion**, **ultrafiltration**, and **diffusion** to produce aqueous humor.

Active secretion involves energy-dependent ionic transport mechanisms that pump sodium and other ions into the posterior chamber, drawing water along to maintain osmotic balance

Flow and Drainage: The aqueous humor is produced at a steady rate of approximately 2-3 microliters per minute. It flows from the posterior chamber through the **pupil** into the anterior chamber and exits the eye via two main pathways:

- **Conventional Outflow**: Through the **trabecular meshwork** and into **Schlemm's canal,** draining into the episcleral veins.
- **Uveoscleral Outflow**: secondary route where the fluid percolates through the ciliary muscle and sclera. A less common pathway involving drainage through the uveal tissues (Goel & Picciani, 2019).

This dynamic process is tightly regulated to maintain a balance between production and drainage. Disruption in either can lead to pathological conditions, such as glaucoma (Kiel, 2011).

The aqueous humor resides in the anterior and posterior chambers of the eye:

- **Anterior chamber:** The space between the cornea and the iris.
- **Posterior chamber:** The smaller space behind the iris and in front of the lens.
  The fluid is produced continuously by the **ciliary body**, specifically by the **non-pigmented epithelial cells** of the ciliary processes. This structure is located near the lens and forms part of the uveal tract (Forrester et al., 2015).

KEY FUNCTIONS OF THE AQUEOUS HUMOR

1. **Maintaining Eye Pressure**
The aqueous humor regulates intraocular pressure (IOP), typically ranging between 10-21 mmHg, to preserve the structural integrity of the eye (Kiel, 2011). By maintaining a stable volume, the aqueous humor ensures a constant IOP that supports the globe's structure. Dysregulation of IOP, whether due to overproduction or impaired drainage, can cause conditions such as ocular hypertension or hypotony (Weinreb et al., 2014).
2. **Nutrient Supply and Waste Removal:** The aqueous humor nourishes avascular structures (e.g., the cornea and lens) and facilitates the removal of waste products. For instance, it provides antioxidants like ascorbic acid to combat oxidative stress, especially critical for protecting the

transparent structures of the eye (Goel & Picciani, 2019).The fluid removes metabolic waste products from the eye, maintaining a healthy ocular environment (Kiel, 2011).

3. **Optical Transparency**

Due to its clarity and refractive properties, the aqueous humor contributes significantly to the optical quality of vision (Forrester et al., 2015).

Clinical Importance

1. **Glaucoma**

An imbalance in aqueous humor production or drainage can lead to elevated IOP, a primary risk factor for glaucoma. This condition damages the optic nerve, potentially resulting in vision loss if untreated (Weinreb et al., 2014). The most common condition associated with aqueous humor dysfunction is glaucoma.

Open-angle glaucoma involves reduced outflow through the trabecular meshwork, while closed-angle glaucoma results from physical obstruction of the drainage angle.Treatments target IOP reduction, either by enhancing outflow (e.g., prostaglandin analogs, surgery) or reducing production (e.g., beta-blockers, carbonic anhydrase inhibitors).

2. **Surgical Relevance**

Treatments like **trabeculectomy** or **laser trabeculoplasty** are designed to enhance the outflow of aqueous humor to lower IOP in glaucoma patients (Goel & Picciani, 2019).

3. **Disease and Injury**

Disruptions in the clarity or composition of the aqueous humor, such as in **hyphema** (blood in the aqueous humor) or infections, can impair its functionality (Forrester et al., 2015). Blood accumulation in the anterior chamber, often due to trauma, can mix with the aqueous humor, impairing vision and increasing IOP.

4. **Aging and Aqueous Humor:** As individuals age, the efficiency of aqueous humor outflow often declines, leading to an increased risk of glaucoma (Forrester et al., 2015).

5. **Therapeutic Advances:** Modern research explores minimally invasive surgical options, such as **microstent implantation** and **laser trabeculoplasty**, which improve aqueous humor drainage. Novel drugs

targeting the trabecular meshwork and Schlemm's canal are also in development.

In summary, the aqueous humor is a fluid which fills the anterior chamber of the eye. It is an attractive fluid for forensic analysis for several reasons. Its isolated location within the ocular cavity shields it from the external environment, reducing the likelihood of rapid microbial invasion or exposure to decomposition accelerants. This protection makes the aqueous humor less variable compared to blood or other bodily fluids like cerebrospinal fluid, allowing for more consistent and predictable changes in key biochemical markers over time. Additionally, because the aqueous humor is involved in maintaining intraocular pressure and supplying nutrients to avascular tissues in the eye, its biochemical composition reflects important physiological processes, such as ion exchange and metabolic activity, which cease after death. Monitoring these biochemical changes could, therefore, provide a timeline of post-mortem events.

One of the most studied biochemical markers in the aqueous humor is potassium. After death, cell membranes begin to lose their integrity due to the cessation of active transport mechanisms, leading to the release of intracellular potassium into the extracellular space, including the aqueous humor. The concentration of potassium in the aqueous humor increases in a relatively linear and predictable manner post-mortem, making it one of

the most reliable markers for PMI estimation. In contrast, other markers such as glucose decline steadily due to the depletion of cellular reserves and the cessation of metabolism, while lactate increases as a result of anaerobic processes taking place during early decomposition. These biochemical shifts, when carefully monitored, provide forensic investigators with a means to estimate the PMI with greater accuracy.

1.1.2 **BLOOD**

Blood, a specialized bodily fluid, plays a critical role in maintaining homeostasis, delivering nutrients, removing waste, and supporting immunity. Blood is a vital fluid responsible for numerous physiological functions, encompassing oxygen transport and immune defense.

Composition

Blood comprises two primary components:

1. **Plasma (55%)**: A straw-colored liquid made up of 90% water and dissolved substances, including plasma proteins (e.g., albumin, fibrinogen, globulins), electrolytes, hormones, and nutrients. Plasma supports nutrient transport, waste removal, and immune responses (Hadjesfandiari et al., 2021).
2. **Cellular Elements (45%)**:

- **Red Blood Cells (RBCs)**: Erythrocytes contain hemoglobin for oxygen and carbon dioxide exchange, vital for cellular respiration.
- **White Blood Cells (WBCs)**: Include immune cells like neutrophils, lymphocytes, and monocytes, crucial for fighting infections.
- **Platelets**: Small cell fragments essential for clot formation and vascular repair (Levy et al., 2023).

Biochemical Properties of blood

1. **RBCs** contain hemoglobin, a protein that binds oxygen via iron in heme groups. They exhibit structural resilience due to cytoskeletal proteins, which are critical for navigating microvascular networks.
2. **Plasma proteins** regulate osmotic pressure, transport hormones, and participate in immune responses. Fibrinogen is crucial for clot formation, while globulins include antibodies essential for immune defense(Levy et al., 2023).
3. **Lipids and metabolites**: Emerging metabolomic and lipidomic studies have revealed biomarkers, such as certain lipids and amino acids, linked to cardiovascular and systemic diseases (Hoffman et al., 2023).

Importance of blood in the body

Blood ensures:

1. **Oxygen delivery** to tissues and carbon dioxide removal.
2. **Nutrient transport**, including glucose, amino acids, and lipids.
3. **Waste removal** via kidneys and liver.
4. **Thermoregulation** through heat distribution.
5. **Immune defense** by carrying WBCs and antibodies.
6. **Hemostasis**, preventing excessive blood loss after injury.

Clinical Significance of blood

1. **Transfusion medicine:** Understanding variability in donor blood (e.g., hemoglobin polymorphisms and platelet sensitivity) improves transfusion outcomes. Molecular profiling of donors also aids in matching high-quality blood components for patients (Hadjesfandiari et al., 2021).
2. **Disease biomarkers**: Changes in blood composition, such as elevated troponin levels, are used in diagnosing conditions like myocardial infarction (Hoffman et al., 2023).
3. **Hematological disorders**: Early detection of blood-related abnormalities, such as in amyloidosis or leukemia, significantly enhances patient outcomes emphasizing the need for prompt diagnosis and treatment. (Levy et al., 2023).
4. **Personalized medicine**:Advances in genetic and biochemical profiling enable tailored treatments, especially for diseases requiring blood-based therapies (Hoffman et al., 2023).

In conclusion, blood is a readily available fluid in post-mortem examinations and is often used in forensic investigations due to the abundance of relevant biochemical markers. However, because blood is part of the systemic circulation and directly exposed to various bodily compartments, it undergoes more rapid and erratic post-mortem changes compared to the aqueous humor. After death, blood is quickly invaded by bacteria, especially from the gastrointestinal tract, leading to accelerated decomposition through putrefaction.

This bacterial activity can cause rapid changes in the levels of biochemical markers such as potassium, glucose, and lactate, making blood a less reliable fluid for estimating PMI in later stages of decomposition. Moreover, external environmental factors such as temperature and humidity can hasten or slow the rate of blood decomposition, introducing significant variability in PMI estimates based on blood analysis alone.

Blood remains a vital focus in clinical research due to its accessibility and the wealth of information it provides about systemic health. Ongoing innovations in molecular diagnostics and therapeutic interventions promise to enhance patient outcomes across various medical disciplines.

1.1.3 BIOCHEMICAL ANALYSIS

Biochemical analysis is a cornerstone of forensic science, allowing for the identification of biological evidence and the elucidation of events in criminal investigations. This discipline integrates chemical, biological, and analytical techniques to provide detailed information about samples encountered in forensic contexts.

Applications of Biochemical Analysis in Forensic Science

1. DNA Analysis

Biochemical techniques are pivotal in the extraction, amplification, and analysis of DNA from biological evidence such as blood, hair, saliva, and skin cells. Techniques like Polymerase Chain Reaction (PCR) and Short Tandem Repeat (STR) analysis allow for precise identification of individuals, aiding in criminal cases, paternity disputes, and mass disaster identifications (Gill & Buckleton, 2023).

2. Toxicology

Biochemical assays detect and quantify drugs, alcohol, and poisons in biological fluids, often using techniques such as gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS). These analyses are critical for determining cause of death, substance abuse, or drug-facilitated crimes (Levine, 2022).

3. Protein and Enzyme Markers

Proteomic approaches identify proteins or enzymes that serve as markers for specific body fluids, such as saliva (amylase), semen (prostate-specific antigen), or blood (hemoglobin). These biochemical markers help confirm the presence of specific fluids at crime scenes (Bachmann et al., 2021).

4. Biochemical Fingerprinting

Emerging methods, such as metabolomics and lipidomics, create unique biochemical profiles for individuals. These profiles are applied to determine post-mortem intervals, establish time since deposition of biological stains, and distinguish between human and animal samples (Beavis et al., 2022).

Key Techniques in Forensic Biochemical Analysis

Spectroscopy and Microscopy:

1. UV-Vis and fluorescence spectroscopy identify trace biological substances.
2. Raman microscopy detects molecules directly in situ on forensic materials (Goodpaster et al., 2022).

Chromatography:

1. GC and HPLC analyze complex mixtures, such as explosive residues or degradation products in environmental samples from crime scenes (Levine, 2022).

Immunoassays:

1. Used for rapid detection of specific antigens or antibodies, for instance, in determining exposure to toxins or infectious agents (Bachmann et al., 2021).

Mass Spectrometry:

1. High-resolution MS helps in identifying drugs, metabolites, and unknown compounds in minimal quantities with high accuracy (Beavis et al., 2022).

Forensic Importance

1. **Criminal Investigations**: Linking suspects to crime scenes through biochemical evidence such as blood or DNA.
2. **Post-Mortem Analysis**: Determining causes and circumstances of death, including poisonings or trauma.
3. **Wildlife Forensics**: Identifying protected or endangered species in cases of illegal hunting or trafficking through biochemical markers (Goodpaster et al., 2022).

Challenges

- **Sample Degradation**: Environmental factors can degrade biochemical evidence, complicating analysis.
- **Contamination**: Cross-contamination during sample collection and processing can affect reliability.
- **Interpretation of Results**: Complex mixtures and partial profiles often require advanced statistical tools for interpretation (Gill & Buckleton, 2023).

In this study, the choice of **bovine eyes** as a model for human forensic investigations is driven by the structural and biochemical similarities between bovine and human ocular fluids. Bovine models are frequently used in forensic and biomedical research due to their comparable eye size and fluid dynamics. The use of bovine samples allows for controlled laboratory experiments that mimic post-mortem biochemical changes, providing insights that are potentially translatable to human cases. By analyzing the post-mortem biochemical changes in both the aqueous humor and blood of bovine eyes, this study seeks to

determine which fluid provides a more consistent and reliable marker of PMI, while also evaluating the differences in the rate and pattern of decomposition in each medium.

Relevance to Forensic Science:

Forensic investigations often require precise methods to estimate the time of death, particularly in cases of homicide, accidental death, or unexplained fatalities. In some cases, the body may be discovered days or even weeks after death, during which traditional PMI estimation methods may no longer be applicable or reliable. Forensic pathologists have long sought biochemical markers that can remain stable and provide consistent data over an extended post-mortem interval. The aqueous humor, due to its relatively stable environment, has been identified as one such medium that holds potential for forensic application. Its use, however, is still under investigation, with researchers focusing on understanding the exact timeline of biochemical changes and their correlation with PMI under various conditions.

Scientific Gap and Need for Comparative Analysis:

Despite the established role of aqueous humor in PMI estimation, few studies have directly compared its biochemical changes to those observed in blood over time. A detailed comparative analysis is essential for understanding the relative advantages and limitations of using each fluid

for PMI determination. This study addresses this gap by analyzing post-mortem changes in multiple biochemical markers in both the aqueous humor and blood of bovine eyes, providing valuable data on the reliability of each fluid for forensic purposes. Additionally, the study seeks to investigate how these fluids behave under varying environmental conditions, such as different temperatures and exposure levels, which can impact the rate of decomposition and, consequently, the accuracy of PMI estimates.

Potential Forensic Implications:

If the findings of this study confirm that the aqueous humor is a more stable and predictable fluid for PMI estimation compared to blood, it could revolutionize forensic practices by offering a more reliable alternative, particularly in cases where traditional methods fail or blood is too degraded for accurate analysis. The ability to use aqueous humor as a reliable PMI estimator could be especially valuable in advanced decomposition cases, where external signs of death are less informative. Furthermore, this study may pave the way for future research aimed at refining the use of ocular fluids in human forensic applications, potentially leading to the development of new protocols for PMI estimation in real-world death investigations.

By comparing the post-mortem biochemical changes in the aqueous humor and blood, this research contributes to the growing body of knowledge that seeks to enhance the accuracy and reliability of forensic tools. The insights gained from this study may ultimately improve death investigations, aiding law enforcement and forensic pathologists in establishing clearer timelines in legal cases, thus supporting justice in both criminal and civil contexts.

1.2 **STATEMENT OF PROBLEM**

Traditional methods for estimating Post-Mortem Interval (PMI) are unreliable due to environmental factors and degradation of blood. Aqueous humor may offer a more stable alternative, but research is limited.

Key Issues:

1. Inaccuracy in traditional PMI methods
2. Unreliability of blood for PMI estimation
3. Need for a stable alternative (aqueous humor)
4. Limited research comparing blood and aqueous humor

Research Objective:

Investigate if aqueous humor can provide a more reliable medium for PMI estimation, improving forensic accuracy.

1.3 **AIMS AND OBJECTIVES**

1.3.1 Aim of study

To compare the biochemical post mortem changes that occurs in the aqueous humor and blood of a bovine eye.

1.3.2 **Objective of study**

1. To track the temporal changes in key biochemical markers such as potassium, glucose, chlorine, sodium, and protein levels in both the aqueous humor and blood.

2. To compare the rate and pattern of biochemical changes between the two fluids over a range of post-mortem intervals.

1.4 **RESEARCH HYPOTHESIS**

- **Null** Hypothesis(1): There will be no significant changes in the biochemical composition of the aqueous humor with increasing post mortem intervals.
- Null Hypothesis(2):There will be no significant changes in the biochemical composition of the blood at post mortem intervals.
- Null Hypothesis(3): The correlation between biochemical changes in the aqueous humor and blood will not significantly enhance the accuracy of post mortem intervals estimation.

1.4 SIGNIFICANCE OF STUDY

1. Enhancing PMI Estimation Accuracy: The study aims to refine PMI estimation by identifying stable biochemical markers in the aqueous humor and blood. Improved accuracy in PMI estimation can significantly

benefit forensic investigations, allowing for more precise time-of-death estimates, especially in cases where external factors compromise traditional indicators.

2. **Contributing to Forensic Methodology:** By providing empirical data on post-mortem biochemical changes in bovine models, the study contributes to forensic science by validating the reliability of using the aqueous humor as an alternative to blood. These insights can help establish new standard operating procedures for PMI estimation.

3**. Application in Human Forensic Science**: Since bovine eyes share anatomical and biochemical similarities with human eyes, findings from this study may be transferable to human forensic practices. This could support the adoption of aqueous humor analysis in human autopsies, especially in cases where blood samples are unavailable or heavily degraded.

4. **Support for Comparative Biochemistry in Forensics**: This study supports the value of comparative biochemistry by highlighting the differences in post-mortem behavior between blood and aqueous humor. It reinforces the importance of choosing the right biological fluid for PMI analysis, especially in cases where one fluid may yield more reliable results than another.

5. **Advancements in Veterinary Forensics:** While this research focuses on bovine eyes as a model, its findings may also directly benefit veterinary forensics. Establishing reliable PMI markers in bovine species could support investigations in agricultural or animal-related criminal cases, offering methods for more accurate assessments of time of death.

6. **Foundation for Future Research on Additional Fluids**: This study can encourage further research into other bodily fluids as potential PMI indicators. With advancements in molecular and biochemical technologies, future studies could build on these findings to explore fluids such as cerebrospinal fluid or synovial fluid, expanding the range of reliable PMI markers available to forensic scientists.

7.**Training and Education in Forensic Pathology**: Findings from this study could be integrated into educational materials and training programs for forensic pathology, providing aspiring forensic scientists with a deeper understanding of biochemical changes in different post-mortem fluids and their implications for death investigations.

8. **Potential for Global Forensic Standardization**: By validating PMI markers in an animal model widely used in forensics, the study may contribute to global efforts in forensic standardization, potentially leading to internationally recognized protocols for using aqueous humor and

blood in PMI estimation across different climates and environmental contexts.

CHAPTER TWO

2.0 LITERATURE REVIEW

Comparative Studies of Aqueous Humor and Blood

The pioneering work of Jaffe (1962) first highlighted the potential of the aqueous humor in forensic science, noting the post-mortem changes in electrolyte levels, particularly potassium, as a possible indicator of the time since death.

Adelson and Sunshine (1963) conducted one of the earliest comparative studies, showing that while potassium levels in both blood and aqueous humor increase post-mortem, the rise in aqueous humor was more linear and predictable, providing a more accurate timeline for death estimation. Similarly, a study by Palmiere et al. (2013) demonstrated that the aqueous humor is less affected by environmental factors compared to blood, making it a more stable fluid for forensic pubResearch on bovine models has also been informative, given the similarities between bovine and human eyes in terms of anatomical structure and biochemical composition.

Studies using bovine eyes have confirmed that the post-mortem changes in potassium, glucose, and lactate levels in the aqueous humor follow similar patterns to those observed in human forensic cases,

reinforcing the value of animal models in understanding PMI estimation (Coe & Appleby, 1985). Bovine studies have allowed researchers to conduct controlled experiments and closely monitor the biochemical changes over time, providing insights that are difficult to achieve with human subjects due to legal and ethical constraints responses.

In forensic science, the estimation of post-mortem interval(PMI) is crucial for death investigations, as it aids in establishing the time of death—a key factor in many forensic cases (DiMaio & DiMaio, 2001). Over the years, researchers have explored various methods to accurately estimate PMI, with a growing interest in the biochemical analysis of body fluids as potential indicators (Schleyer et al., 2016; Madea & Musshoff, 2007). Traditionally, blood has been the primary medium used for PMI estimation due to its accessibility and abundance in the body (Garg & Oberoi, 2014). However, alternative fluids like the aqueous humor have gained attention for their stability and resistance to rapid decomposition, particularly in the advanced stages of post-mortem changes (Baldwin et al., 2018). This literature review aims to explore the current understanding of post-mortem biochemical changes in blood and aqueous humor and their implications for PMI estimation (Dix & Graham, 2000; Cordeiro et al., 2015).

Traditional methods of determining PMI, including body temperature (algor mortis), muscle stiffness (rigor mortis), and post-mortem discoloration (livor mortis), have been used for decades. These methods are effective for early post-mortem interval estimations but become less reliable as the body decomposes further, or when environmental factors such as temperature, humidity, and exposure to the elements interfere with natural processes (Madea, 2016). Moreover, these methods are subject to significant variability based on the individual case, making it difficult to provide precise PMI estimates after 24–48 hours post-mortem.

Biochemical Markers in Blood Post-Mortem:

Blood has long been used in forensic science to estimate PMI by measuring the levels of specific biochemical markers. Among these,potassium concentration in the blood is often cited as a reliable indicator of the PMI. Following death, the breakdown of cell membranes leads to the release of intracellular potassium into the bloodstream, resulting in an observable increase in potassium levels (Schleyer et al., 2018). Despite this, blood as a medium for PMI estimation has limitations. It is prone to rapid post-mortem changes due to microbial invasion, exposure to the external environment, and systemic decomposition. Studies have shown that after death, the degradation of red blood cells

and other cellular components accelerates, leading to erratic changes in markers like glucose and lactate (Coe, 1972).

Lactic acid, which accumulates as a byproduct of anaerobic metabolism, also increases significantly in blood post-mortem, reflecting tissue hypoxia and cellular breakdown. However, the rapidity of these changes, coupled with the variability caused by microbial contamination, makes blood a less reliable fluid in cases where decomposition is advanced (Hossain et al., 2016). Other markers, including glucose and urea, have shown potential for PMI estimation, but their levels are subject to quick post-mortem depletion, especially when environmental conditions promote rapid decomposition (Girela et al., 2002).

The aqueous humor, has emerged as a promising alternative to blood for PMI estimation due to its relatively stable and isolated environment within the ocular cavity. The aqueous humor is less susceptible to microbial contamination and is protected from external environmental influences, making it a more stable fluid than blood for forensic analysis. The pioneering work of Jaffe (1962) first highlighted the potential of the aqueous humor in forensic science, noting the post-mortem changes in electrolyte levels, particularly potassium, as a possible indicator of the time since death.

Studies have consistently shown that potassium levels in the aqueous humor rise in a predictable and linear manner after death, making it one of the most reliable markers for PMI estimation (Adelson et al., 1963; Sturner, 1963). This increase is attributed to the breakdown of cellular membranes and the release of intracellular potassium into the extracellular space, a process that occurs at a steady rate due to the relative isolation of the aqueous humor from systemic circulation.

In addition to potassium, other biochemical markers in the aqueous humor, such as glucose, sodium, and urea, have been investigated for their potential use in PMI estimation. Glucose levels in the aqueous humor decrease steadily post-mortem, reflecting the cessation of metabolic processes. Lactate concentrations, similar to those in blood, increase as anaerobic metabolism dominates in the absence of oxygen, though the changes in the aqueous humor occur at a slower rate due to its protected environment (Coe, 1974). Sodium and urea, while less frequently studied, have also shown potential as indicators of PMI, with concentrations fluctuating in response to cellular breakdown and fluid shifts after death (Madea, 2015).

The literature suggests that while both blood and aqueous humor hold promise for post-mortem interval estimation, the aqueous humor presents distinct advantages due to its relative isolation from external

contamination and slower rate of biochemical change. Potassium remains the most reliable marker for PMI estimation in both fluids, but the aqueous humor's more predictable post-mortem behavior makes it a more consistent medium for forensic analysis, particularly in advanced decomposition cases. Comparative studies of bovine models further support the use of the aqueous humor as a viable alternative to blood for PMI estimation, though challenges remain in standardizing methods and identifying additional markers. The continued development of biochemical analysis techniques and further comparative studies are critical for improving the accuracy and reliability of PMI estimation in forensic practice.

Despite the promising potential of the aqueous humor for PMI estimation, several challenges remain. For instance, while potassium levels have shown consistent post-mortem increases, the rate of change can be influenced by factors such as the cause of death, ambient temperature, and the individual’s physiological state at the time of death (Wetterich et al., 2013). Therefore, more research is needed to establish standardized reference ranges for biochemical markers in both the aqueous humor and blood across different populations and environmental conditions.

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.0 METHODOLOGY

3.1. STUDY DESIGN

An experimental study was conducted.

3.2 STUDY LOCATION

This study was conducted at the Biochemistry laboratory, Department of Biochemistry, Faculty of Life Science,University of Benin, Benin City, Edo state, Nigeria.

3.3 STUDY POPULATION

Healthy Bovine eyes was gotten from an abattoir(located at benin ewah slope) in Benin city.

3.4 SAMPLE SIZE

The sample size was n=10

3.5 SAMPLING TECHINIQUE

A simple random sampling was used for this study.

3.5.1 Potassium Concentration Model:

A common model used in forensic pathology is based on the linear increase of potassium concentration in vitreous humor. The rate of

increase is often found to be around 0.12 to 0.16 mmol/L per hour post-mortem. By measuring the potassium concentration and applying this rate, forensic experts can estimate the PMI.

Formula Example:

PMI(hours)=(K+concentration−baseline concentration)/rate of increase

If the potassium concentration in the aqueous humor is 15 mmol/L and the baseline (normal) concentration is 3 mmol/L with a rate of increase of 0.14 mmol/L per hour, the PMI would be:

PMI=(15−3)/0.14≈86 hours.

3.6 STUDY DURATION

The study was carried out for the period of three days(0,12,24,48and

72hours).

3.7 RESEARCH MATERIALS

- Bovine eyes
- Blood Samples
- Alcohol solution
- Ice pack
- Paraffin wax
- Needles
- Centrifuge

- Vacuum system
- GSEP gel serum test tube
- Automatic chemical analyzer
- Plastic/container
- Glucose kits
- Electrolyte kits
- Gloves

Fig: Image of a chemical analyzer.

Source: https://sl.bing.net/eck998xBg16

Fig:image of electrolyte test kits

Source:https://sl.bing.net/friPCu1sGtM

Fig:GSEP gel serum test tube.

Source:https://sl.bing.net/kADEHxGLpVQ

3.7.1 Instruments used for aqueous sampling:

1. Needle for Fixation:

Type: 20G needle

Specifications: 0.9mm × 38mm

Purpose: Permanently inserted through the limbus to facilitate fixation of the eyeballs wrapped in paraffin.

2. Vacuum System:

Purpose: It was used for collecting aqueous humor samples at specified time intervals (0, 12, 24, 48, and 72 hours) post-mortem.

Function: Creates suction to draw and collect 200μL of aqueous humor from each eyeball.

3.7.2 Instruments Used for Blood Sampling:

3. ZSEP GEL SERUM Test Tubes:

Type: Specific type of test tube used for blood collection.

Function: It was used to collect blood samples from the jugular veins at the moment of slaughter.

4. Centrifuge:

Type: High-speed centrifuge

Specifications: Operates at 3000 rpm

Purpose: Used to separate serum from whole blood by spinning samples at high speeds for 10 minutes.

5. Automatic Chemical Analyzer (Architect C4000):

Type: Automated analyzer

Manufacturer: Abbott Park, IL, USA

Function: Analyzes serum samples for electrolyte concentrations (Na+, K+, Cl-) and glucose levels.

Process: Samples were thawed prior to analysis, and the analyzer provides quantitative measurements of the biochemical markers.

These instruments are essential for carrying out precise and standardized sampling procedures for aqueous humor and blood in the research study. They ensure the accuracy and reliability of the biochemical analyses conducted on the collected samples.

- ***3.8 Inclusion Criteria***

1.Bovine eyes collected within 1hour of slaughter.

2.Eyes with no visible signs of disease or trauma.

3.Post-mortem intervals ranging from 0 to 72 hours.

4.Eyes from cattle of various ages and breeds.

- ***3.9 Exclusion Criteria***

1.Eyes with visible signs of disease, trauma, or infection.

2.Post-mortem intervals exceeding 72 hours.

3.Eyes from cattle with known ocular or systemic diseases.

3.10 DESCRIPTION OF PROCEDURE

3.10.1 SAMPLE COLLECTION

- ***.1 Animal Sampling***

Fifteen pieces of healthy eyes from freshly slaughtered animals was obtained from an abattoirs in Benin City. The eyeballs were obtained within an hour of death before roasting of the animal commence. The obtained eyeballs were placed in a bucket of ice so as to slow the rate of degeneration to ensure accurate observations of the changes that occur in the aqueous humour and blood after death.

- ***.2 Blood Sampling***

At the moment of slaughter, when the jugular veins was cut, blood samples was taken in 5 mL Z SEP GEL SERUM test tubes, after which the blood was centrifuged at 3000 rpm ×10 min in order to separate the serum. The samples of serum was stored at −20 °C until processing. Before analysis, the samples were thawed and analyzed in an automatic chemical analyzer. Electrolyte concentrations (Na+, K+, Cl−) and glucose levels (mmol/L)was established.

- ***.4 Sampling the Aqueous Humor***

After sampling, the eyes were wrapped in paraffin and a needle was inserted permanently into the eye and fixed (20G, 0.9 mm × 38 mm) through the limbus . The aqueous humor of the eye was sampled from a total of 5 cattles (N = 10), the eyes for biochemical testing was stored at +4 °C and, using a vacuum system for collecting blood at each time interval (0, 12, 24, 48, and 72h), 200µL of aqueous humor was collected. The centrifuged samples of aqueous humor was stored at −20 °C until analysis. Before analysis, the samples were thawed and analyzed in an automatic chemical analyzer The concentrations of sodium (Na+), potassium (K+), chlorine (Cl−), and glucose (mmol/L) was then measured.

- ***3.10 Data Analysis***

Statistical analyses was conducted to determine the relationship between the biochemical changes in the aqueous humor over the post-mortem intervals. (ANOA)

Simple linear regression analysis, and other relevant statistical tests were employed to assess the predictability and reliability of biochemical markers for estimating the post-mortem interval (PMI).

3.11 DATA ANALYSIS

A One-way analysis of variance (ANOVA) with repeated observations was used to determine comparison between two variables.

- Potassium and postmortem interval at 0 , 12, 24, 48 and 72 hours.
- Sodium with postmortem interval at 0,12, 24, 48 and 72 hours.
- Chloride with postmortem interval at 0, 12, 24, 48 and 72 hours.
- Serum glucose with postmortem interval at 0, 12, 24, 48 and 72 hours.
- A Simple linear regression graph was drawn for comparison of the relationship between two continuous variables.

3.12 Ethical consideration

The study was conducted in compliance with ethical guidelines for the use of animals in research. All procedures were approved by the relevant institutional review boards and adhered to national and international guidelines for animal welfare.

3.12.1 ETHICAL APPROVAL

Ethical approval to conduct this study was obtained from the Research and Ethics Committee of the Department of Optometry, University of Benin.

CHAPTER FOUR

4.0 RESULT

TABLE 4.1: Mean Values of Substances in the Aqueous at Different Post-Mortem Intervals

	0 hour	12 hours	24 hours	48 hours	Total
	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
CL	0.65 ± 0.04	0.80 ± 0.03	0.78 ± 0.01	0.89 ± 0.01	0.78 ± 0.09
K	0.67 ± 0.05	0.57 ± 0.08	1.18 ± 0.01	1.48 ± 0.003	0.97 ± 0.38
NA	0.75 ± 0.03	0.12 ± 0.12	0.48 ± 0.24	0.82 ± 0.01	0.54 ± 0.31
GL	0.03 ± 0.01	0.29 ± 0.01	0.03 ± 0.02	0.04 ± 0.003	0.10 ± 0.11

The table shows the mean values of chlorine, potassium, sodium and glucose in the aqueous at different post-mortem intervals.

TABLE 4.2: Descriptive Statistics of Substances in the Aqueous at Different Post-Mortem Intervals

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
CL	0 hours	10	0.6502	0.04254	0.01345	0.6198	0.6806	0.60	0.69
	12 hours	10	0.7952	0.02984	0.00944	0.7739	0.8165	0.75	0.82
	24 hours	10	0.7804	0.00797	0.00252	0.7747	0.7861	0.77	0.79
	48 hours	10	0.8927	0.01497	0.00474	0.8820	0.9034	0.87	0.90
	Total	40	0.7796	0.09124	0.01443	0.7504	0.8088	0.60	0.90
K	0 hours	10	0.6731	0.04691	0.01483	0.6395	0.7067	0.63	0.74
	12 hours	10	0.5687	0.08005	0.02531	0.5114	0.6260	0.49	0.66
	24 hours	10	1.1751	0.01417	0.00448	1.1650	1.1852	1.16	1.20
	48 hours	10	1.4803	0.00263	0.00083	1.4784	1.4822	1.48	1.48
	Total	40	0.9743	0.37877	0.05989	0.8532	1.0954	0.49	1.48
NA	0 hours	10	0.7493	0.03333	0.01054	0.7255	0.7731	0.72	0.79
	12 hours	10	0.1197	0.11671	0.03691	0.0362	0.2032	0.02	0.26
	24 hours	10	0.4762	0.24346	0.07699	0.3020	0.6504	0.33	0.83
	48 hours	10	0.8153	0.00732	0.00231	0.8101	0.8205	0.81	0.82
	Total	40	0.5401	0.30675	0.04850	0.4420	0.6382	0.02	0.83
GL	0 hours	10	0.0286	0.00830	0.00263	0.0227	0.0345	0.02	0.04
	12	1	0.2881	0.00899	0.0028	0.2817	0.2945	0.28	0.30

	hours	0			4				
	24 hours	10	0.0297	0.01638	0.00518	0.0180	0.0414	0.01	0.05
	48 hours	10	0.0417	0.00287	0.00091	0.0396	0.0438	0.04	0.05
	Total	40	0.0970	0.11228	0.01775	0.0611	0.1329	0.01	0.30

Chlorine ($\mu = 0.89$), potassium ($\mu = 1.48$) and sodium ($\mu = 0.82$) levels were highest at 48 hours post-mortem. Glucose level was highest at 12 hours post-mortem ($\mu = 0.29$).

TABLE 4.3: Comparing the Means of Substances in the Aqueous at Different Post-Mortem Intervals Using Analysis of Variance

ANOVA						
		Sum of Squares	**df**	**Mean Square**	**F**	**P-Value**
CL	Between Groups	0.298	3	0.099	132.888	0.000
	Within Groups	0.027	36	0.001		
	Total	0.325	39			
K	Between Groups	5.516	3	1.839	834.162	0.000
	Within Groups	0.079	36	0.002		
	Total	5.595	39			
NA	Between Groups	3.003	3	1.001	54.071	0.000
	Within Groups	0.666	36	0.019		
	Total	3.670	39			
GL	Between Groups	0.488	3	0.163	1526.325	0.000
	Within Groups	0.004	36	0.000		
	Total	0.492	39			

There was a significant difference between the levels of chlorine, potassium, sodium and glucose in the aqueous at different post-mortem intervals ($p < 0.05$).

TABLE 4.4: Post Hoc Test Comparing Substances in The Aqueous at Different Post-Mortem Intervals

Multiple Comparisons							
Tukey HSD							
Dependent Variable	**(I) Group**	**(J) Group**	**Mean Difference (I-J)**	**Std. Error**	**P-Value**	**95% Confidence Interval**	
						Lower Bound	**Upper Bound**
CL	0 hours	12 hours	-0.14500*	0.01222	0.000	-0.1779	-0.1121
		24 hours	-0.13020*	0.01222	0.000	-0.1631	-0.0973
		48 hours	-0.24250*	0.01222	0.000	-0.2754	-0.2096
	12 hours	0 hours	0.14500*	0.01222	0.000	0.1121	0.1779
		24 hours	0.01480	0.01222	0.624	-0.0181	0.0477
		48 hours	-0.09750*	0.01222	0.000	-0.1304	-0.0646
	24 hours	0 hours	0.13020*	0.01222	0.000	0.0973	0.1631
		12 hours	-0.01480	0.01222	0.624	-0.0477	0.0181
		48 hours	-0.11230*	0.01222	0.000	-0.1452	-0.0794
	48 hours	0 hours	0.24250*	0.01222	0.000	0.2096	0.2754
		12 hours	0.09750*	0.01222	0.000	0.0646	0.1304
		24 hours	0.11230*	0.01222	0.000	0.0794	0.1452
K	0 hours	12 hours	0.10440*	0.02100	0.000	0.0479	0.1609
		24 hours	-0.50200*	0.02100	0.000	-0.5585	-0.4455
		48 hours	-0.80720*	0.02100	0.000	-0.8637	-0.7507

NA	12 hours	0 hours	-0.10440*	0.02100	0.000	-0.1609	-0.0479
		24 hours	-0.60640*	0.02100	0.000	-0.6629	-0.5499
		48 hours	-0.91160*	0.02100	0.000	-0.9681	-0.8551
	24 hours	0 hours	0.50200*	0.02100	0.000	0.4455	0.5585
		12 hours	0.60640*	0.02100	0.000	0.5499	0.6629
		48 hours	-0.30520*	0.02100	0.000	-0.3617	-0.2487
	48 hours	0 hours	0.80720*	0.02100	0.000	0.7507	0.8637
		12 hours	0.91160*	0.02100	0.000	0.8551	0.9681
		24 hours	0.30520*	0.02100	0.000	0.2487	0.3617
	0 hours	12 hours	0.62960*	0.06085	0.000	0.4657	0.7935
		24 hours	0.27310*	0.06085	0.000	0.1092	0.4370
		48 hours	-0.06600	0.06085	0.701	-0.2299	0.0979
	12 hours	0 hours	-0.62960*	0.06085	0.000	-0.7935	-0.4657
		24 hours	-0.35650*	0.06085	0.000	-0.5204	-0.1926
		48 hours	-0.69560*	0.06085	0.000	-0.8595	-0.5317
	24 hours	0 hours	-0.27310*	0.06085	0.000	-0.4370	-0.1092
		12 hours	0.35650*	0.06085	0.000	0.1926	0.5204
		48 hours	-0.33910*	0.06085	0.000	-0.5030	-0.1752
	48 hours	0 hours	0.06600	0.06085	0.701	-0.0979	0.2299
		12 hours	0.69560*	0.06085	0.000	0.5317	0.8595
		24 hours	0.33910*	0.06085	0.000	0.1752	0.5030

GL	0 hours	12 hours	-0.25950*	0.00462	0.000	-0.2719	-0.2471
		24 hours	-0.00110	0.00462	0.995	-0.0135	0.0113
		48 hours	-0.01310*	0.00462	0.036	-0.0255	-0.0007
	12 hours	0 hours	0.25950*	0.00462	0.000	0.2471	0.2719
		24 hours	0.25840*	0.00462	0.000	0.2460	0.2708
		48 hours	0.24640*	0.00462	0.000	0.2340	0.2588
	24 hours	0 hours	0.00110	0.00462	0.995	-0.0113	0.0135
		12 hours	-0.25840*	0.00462	0.000	-0.2708	-0.2460
		48 hours	-0.01200	0.00462	0.062	-0.0244	0.0004
	48 hours	0 hours	0.01310*	0.00462	0.036	0.0007	0.0255
		12 hours	-0.24640*	0.00462	0.000	-0.2588	-0.2340
		24 hours	0.01200	0.00462	0.062	-0.0004	0.0244

*. The mean difference is significant at the 0.05 level.

The post hoc test shows that there were significant differences between chlorine level in the aqueous 0 hours post-mortem and 12, 24 and 48 hours post-mortem ($p < 0.05$).

There were also significant differences between potassium levels at different post-mortem intervals ($p < 0.05$).

Similarly, there were significant differences between sodium levels at 0 hour, 12 hours and 48 hours post-mortem ($p < 0.05$). However, there was no significant difference between sodium levels at 0 hours and 48 hours post-mortem ($p > 0.05$).

There were significant differences between glucose levels at 0 hour, 12 hours and 48 hours post-mortem ($p < 0.05$). There was no significant difference between glucose levels at 0 hours and 24 hours post-mortem ($p > 0.05$).

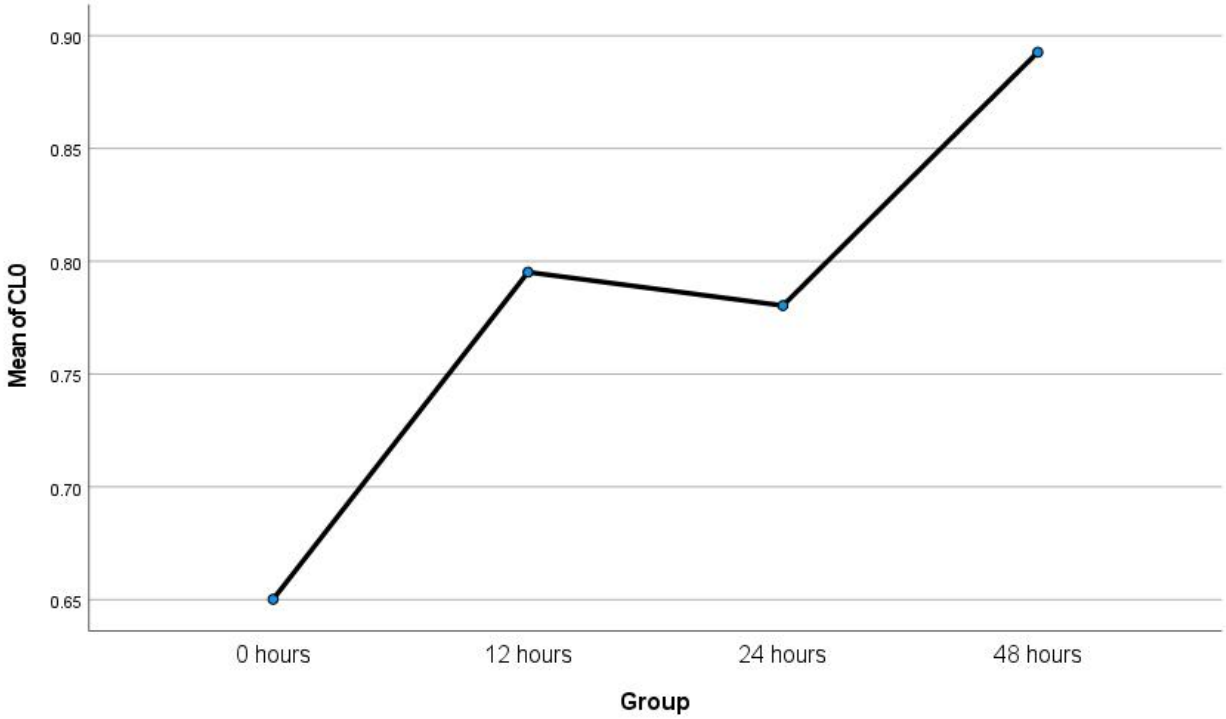

FIGURE 4.1: Means Plot of Chlorine in the Aqueous at Different Intervals

The plot shows that chlorine levels were highest in the aqueous 48 hours post-mortem.

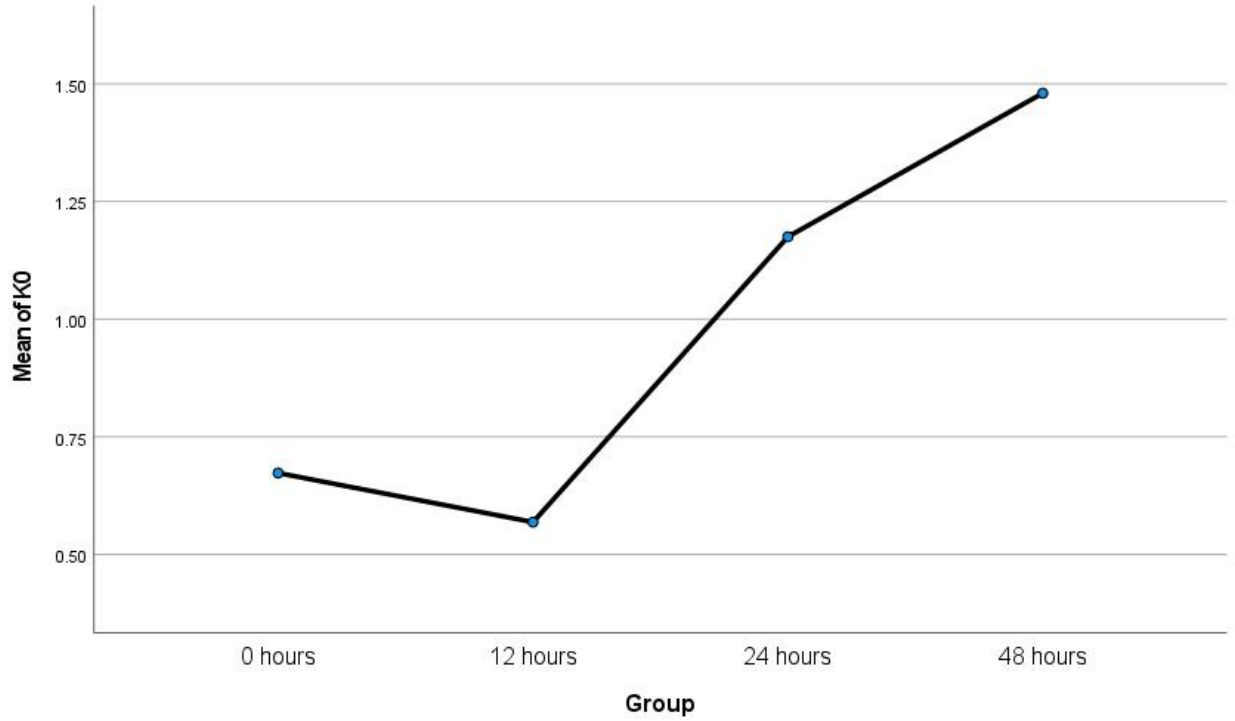

FIGURE 4.2: Means Plot of Potassium in the Aqueous at Different Intervals

The plot shows that potassium levels were highest in the aqueous 48 hours post-mortem.

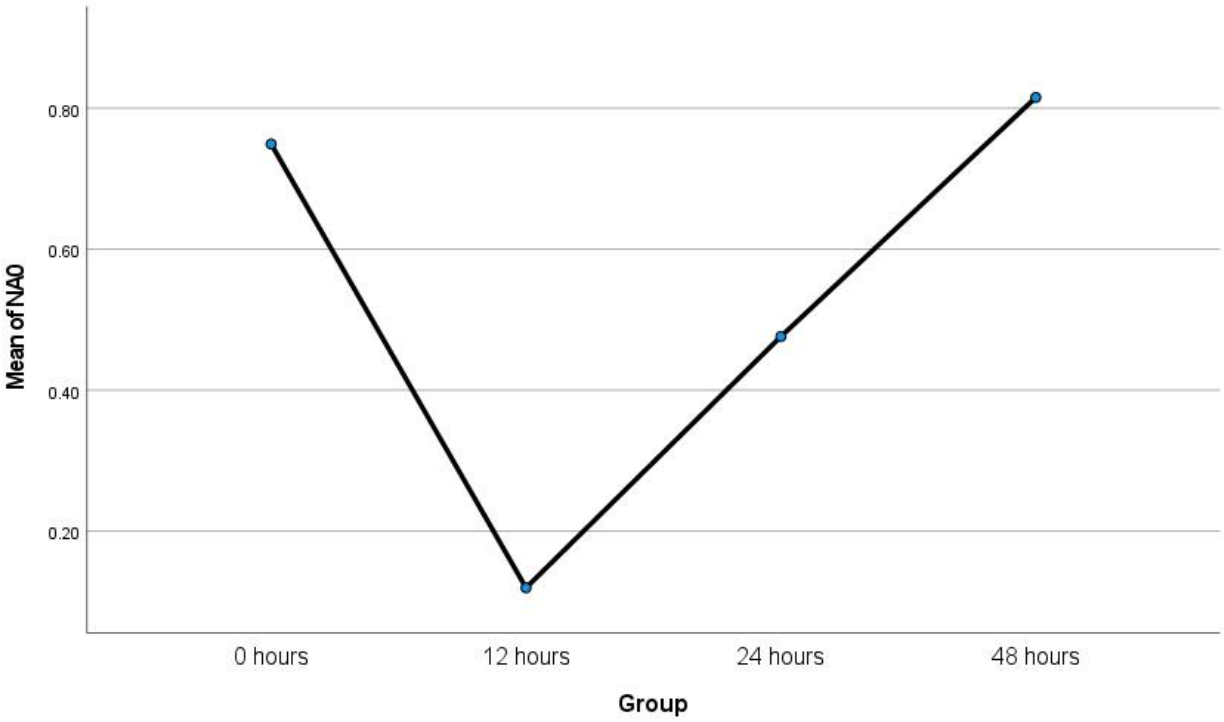

FIGURE 4.3: Means Plot of Sodium in the Aqueous at Different Intervals

The plot shows that sodium levels were highest in the aqueous 48 hours post-mortem.

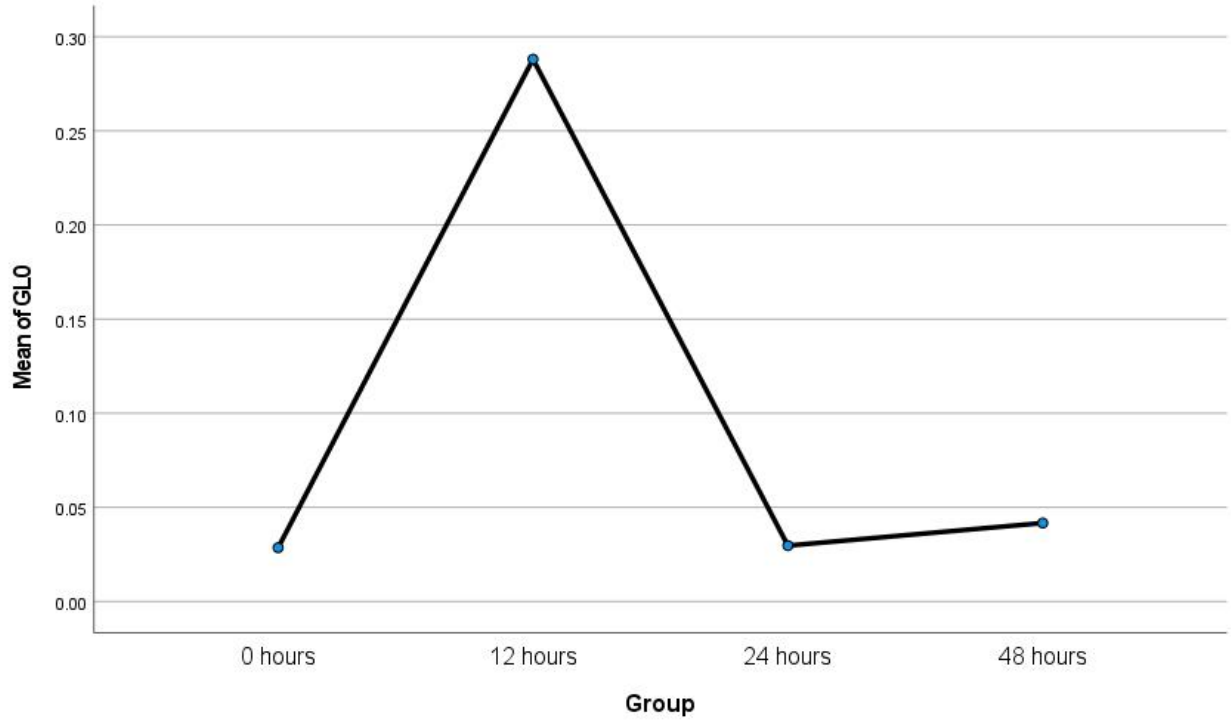

FIGURE 4.4: Means Plot of Glucose in the Aqueous at Different Intervals

The plot shows that glucose levels were highest in the aqueous 12 hours post-mortem.

TABLE 4.5: Mean Values of Substances in the Blood at Different Post-Mortem Intervals

	0 hour	12 hours	24 hours	48 hours	Total
	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
CL	0.63 ± 0.05	0.82 ± 0.03	0.76 ± 0.01	0.78 ± 0.01	0.75 ± 0.08
K	0.76 ± 0.03	0.72 ± 0.07	0.74 ± 0.002	0.75 ± 0.07	0.74 ± 0.05
NA	0.68 ± 0.05	0.08 ± 0.03	1.37 ± 0.01	1.39 ± 0.01	0.88 ± 0.55
GL	0.18 ± 0.04	0.30 ± 0.02	0.20 ± 0.01	0.16 ± 0.01	0.21 ± 0.06

The table shows the mean values of chlorine, potassium, sodium and glucose in the blood at different post-mortem intervals.

TABLE 4.6: Descriptive Statistics of Substances in the Blood at Different Post-Mortem Intervals

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
CL	0 hours	10	0.6314	0.05421	0.01714	0.5926	0.6702	0.57	0.68
	12 hours	10	0.8249	0.03043	0.00962	0.8031	0.8467	0.80	0.86
	24 hours	10	0.7636	0.01005	0.00318	0.7564	0.7708	0.75	0.78
	48 hours	10	0.7823	0.00613	0.00194	0.7779	0.7867	0.78	0.79
	Total	40	0.7506	0.07927	0.01253	0.7252	0.7759	0.57	0.86
K	0 hours	10	0.7591	0.03082	0.00975	0.7371	0.7811	0.72	0.79
	12 hours	10	0.7212	0.07234	0.02288	0.6695	0.7729	0.63	0.80
	24 hours	10	0.7403	0.00206	0.00065	0.7388	0.7418	0.74	0.74
	48 hours	10	0.7456	0.07218	0.02283	0.6940	0.7972	0.64	0.79
	Total	40	0.7415	0.05310	0.00840	0.7246	0.7585	0.63	0.80
NA	0 hours	10	0.6843	0.05492	0.01737	0.6450	0.7236	0.62	0.74
	12 hours	10	0.0818	0.02567	0.00812	0.0634	0.1002	0.05	0.10
	24 hours	10	1.3687	0.01021	0.00323	1.3614	1.3760	1.36	1.38
	48 hours	10	1.3857	0.00873	0.00276	1.3795	1.3919	1.38	1.40
	Total	40	0.8801	0.54853	0.08673	0.7047	1.0556	0.05	1.40
GL	0 hours	10	0.1835	0.04422	0.01398	0.1519	0.2151	0.13	0.23
	12	10	0.2970	0.02279	0.0072	0.2807	0.3133	0.26	0.31

hours				1				
24 hours	10	0.1974	0.00873	0.00276	0.1912	0.2036	0.19	0.21
48 hours	10	0.1569	0.00745	0.00235	0.1516	0.1622	0.15	0.17
Total	40	0.2087	0.05903	0.00933	0.1898	0.2276	0.13	0.31

Chlorine ($\mu = 0.82$) and glucose ($\mu = 0.30$) levels were highest at 12 hours post-mortem. Potassium level ($\mu = 0.76$) was highest at 0 hours post-mortem and sodium level ($\mu = 1.39$) was highest at 48 hours post-mortem.

TABLE 4.7: Comparing the Means of Substances in the Blood at Different Post-Mortem Intervals Using Analysis of Variance

ANOVA						
		Sum of Squares	**df**	**Mean Square**	**F**	**P-Value**
CL	Between Groups	0.209	3	0.070	69.627	0.000
	Within Groups	0.036	36	0.001		
	Total	0.245	39			
K	Between Groups	0.007	3	0.002	0.866	0.468
	Within Groups	0.103	36	0.003		
	Total	0.110	39			
NA	Between Groups	11.700	3	3.900	4045.825	0.000
	Within Groups	0.035	36	0.001		
	Total	11.735	39			
GL	Between Groups	0.112	3	0.037	57.519	0.000
	Within Groups	0.023	36	0.001		
	Total	0.136	39			

There was a significant difference between the levels of chlorine, sodium and glucose in the blood at different post-mortem intervals ($p < 0.05$). There was no significant relationship between the level of potassium in the blood at different post-mortem intervals ($p > 0.05$).

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Interval	(J) Interval	Mean Difference (I-J)	Std. Error	P-Value	95% Confidence Interval	
						Lower Bound	Upper Bound
CL	0 hours	12 hours	-0.19350*	0.01415	0.000	-0.2316	-0.1554
		24 hours	-0.13220*	0.01415	0.000	-0.1703	-0.0941
		48 hours	-0.15090*	0.01415	0.000	-0.1890	-0.1128
	12 hours	0 hours	0.19350*	0.01415	0.000	0.1554	0.2316
		24 hours	0.06130*	0.01415	0.001	0.0232	0.0994
		48 hours	0.04260*	0.01415	0.023	0.0045	0.0807
	24 hours	0 hours	0.13220*	0.01415	0.000	0.0941	0.1703
		12 hours	-0.06130*	0.01415	0.001	-0.0994	-0.0232
		48 hours	-0.01870	0.01415	0.555	-0.0568	0.0194
	48 hours	0 hours	0.15090*	0.01415	0.000	0.1128	0.1890
		12 hours	-0.04260*	0.01415	0.023	-0.0807	-0.0045
		24 hours	0.01870	0.01415	0.555	-0.0194	0.0568
K	0 hours	12 hours	0.03790	0.02387	0.398	-0.0264	0.1022
		24 hours	0.01880	0.02387	0.860	-0.0455	0.0831
		48 hours	0.01350	0.02387	0.942	-0.0508	0.0778
	12 hours	0 hours	-0.03790	0.02387	0.398	-0.1022	0.0264
		24 hours	-0.01910	0.02387	0.854	-0.0834	0.0452

		48 hours	-0.02440	0.02387	0.738	-0.0887	0.0399
		0 hours	-0.01880	0.02387	0.860	-0.0831	0.0455
	24 hours	12 hours	0.01910	0.02387	0.854	-0.0452	0.0834
		48 hours	-0.00530	0.02387	0.996	-0.0696	0.0590
		0 hours	-0.01350	0.02387	0.942	-0.0778	0.0508
	48 hours	12 hours	0.02440	0.02387	0.738	-0.0399	0.0887
		24 hours	0.00530	0.02387	0.996	-0.0590	0.0696
		12 hours	0.60250*	0.01388	0.000	0.5651	0.6399
	0 hours	24 hours	-0.68440*	0.01388	0.000	-0.7218	-0.6470
		48 hours	-0.70140*	0.01388	0.000	-0.7388	-0.6640
		0 hours	-0.60250*	0.01388	0.000	-0.6399	-0.5651
	12 hours	24 hours	-1.28690*	0.01388	0.000	-1.3243	-1.2495
NA		48 hours	-1.30390*	0.01388	0.000	-1.3413	-1.2665
		0 hours	0.68440*	0.01388	0.000	0.6470	0.7218
	24 hours	12 hours	1.28690*	0.01388	0.000	1.2495	1.3243
		48 hours	-0.01700	0.01388	0.616	-0.0544	0.0204
		0 hours	0.70140*	0.01388	0.000	0.6640	0.7388
	48 hours	12 hours	1.30390*	0.01388	0.000	1.2665	1.3413
		24 hours	0.01700	0.01388	0.616	-0.0204	0.0544
		12 hours	-0.11350*	0.01142	0.000	-0.1442	-0.0828
GL	0 hours	24 hours	-0.01390	0.01142	0.620	-0.0446	0.0168

	48 hours	0.02660	0.01142	0.110	-0.0041	0.0573
	0 hours	0.11350*	0.01142	0.000	0.0828	0.1442
12 hours	24 hours	0.09960*	0.01142	0.000	0.0689	0.1303
	48 hours	0.14010*	0.01142	0.000	0.1094	0.1708
	0 hours	0.01390	0.01142	0.620	-0.0168	0.0446
24 hours	12 hours	-0.09960*	0.01142	0.000	-0.1303	-0.0689
	48 hours	0.04050*	0.01142	0.006	0.0098	0.0712
	0 hours	-0.02660	0.01142	0.110	-0.0573	0.0041
48 hours	12 hours	-0.14010*	0.01142	0.000	-0.1708	-0.1094
	24 hours	-0.04050*	0.01142	0.006	-0.0712	-0.0098

*. The mean difference is significant at the 0.05 level.

TABLE 4.8: Post Hoc Test Comparing Substances in The Blood at Different Post-Mortem Intervals

The post hoc test shows that there were significant differences between chlorine level in the blood 0 hours post-mortem and 12 hours, 24 hours and 48 hours post-mortem ($p < 0.05$).

There was no significant difference between potassium levels at any of the different post-mortem intervals ($p > 0.05$).

There were significant differences between sodium levels at 0 hour, 12 hours, 24 hours and 48 hours post-mortem ($p < 0.05$).

There were significant differences between glucose levels at 0 hour and 12 hours post-mortem ($p < 0.05$). There was no significant difference between glucose levels at 0 hours, 24 hours and 48 hours post-mortem ($p > 0.05$).

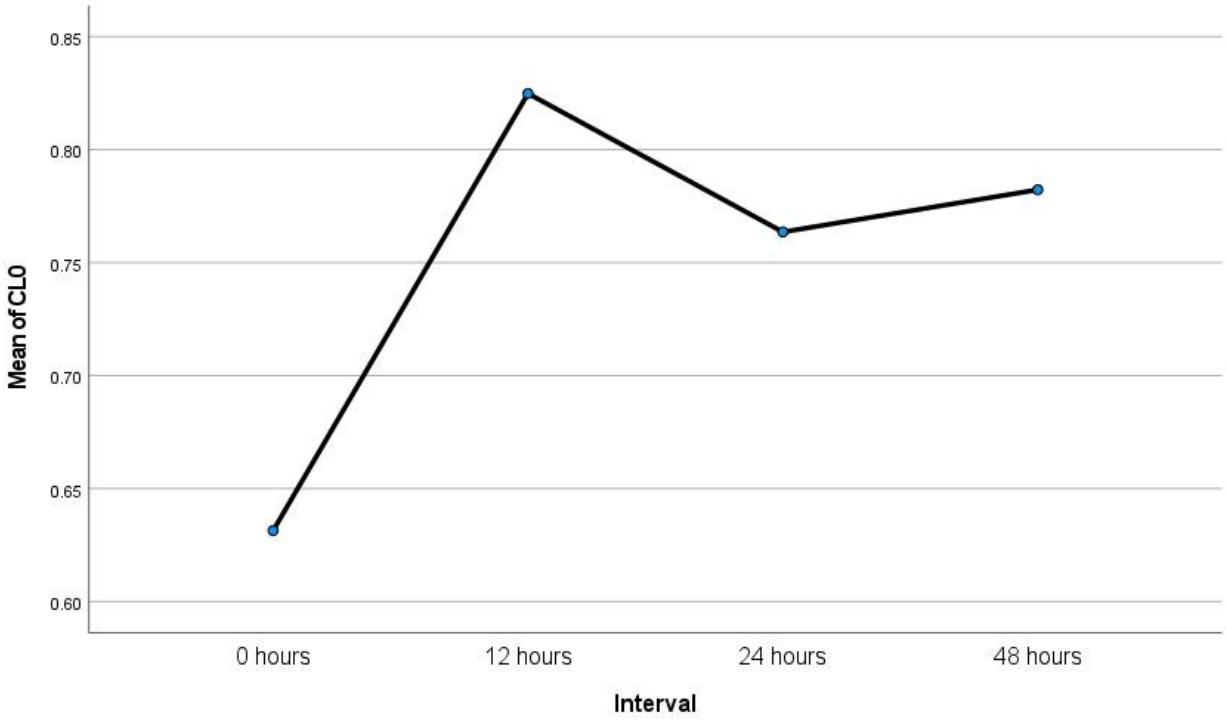

FIGURE 4.5: Means Plot of Chlorine in the Blood at Different Intervals

The plot shows that chlorine levels were highest in the blood 12 hours post-mortem.

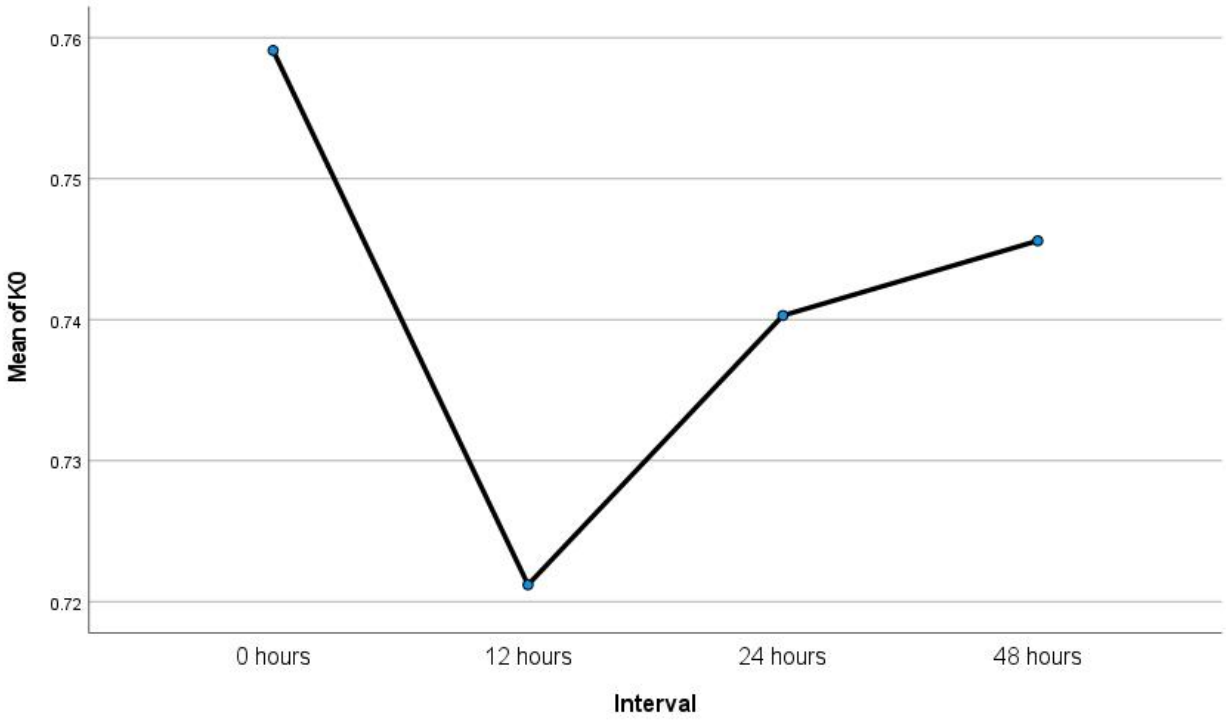

FIGURE 4.6: Means Plot of Potassium in the Blood at Different Intervals

The plot shows that potassium levels were highest in the blood 0 hours post-mortem.

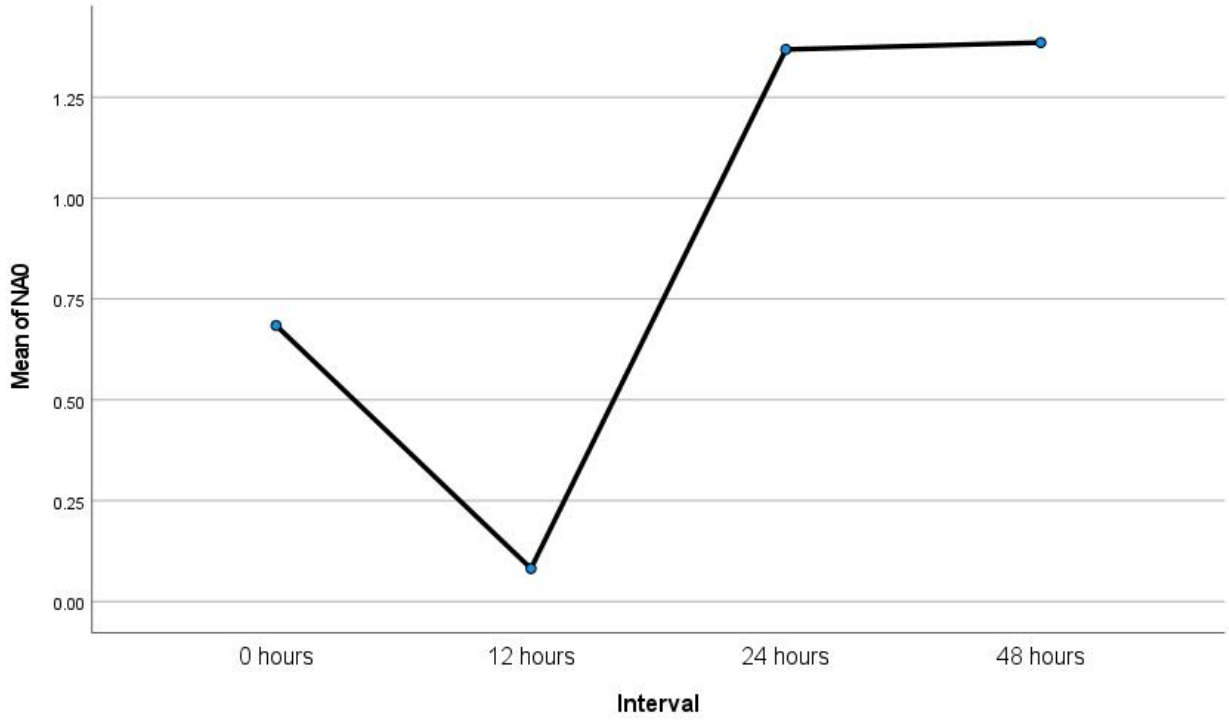

FIGURE 4.7: Means Plot of Sodium in the Blood at Different Intervals

The plot shows that sodium levels were highest in the blood 48 hours post-mortem.

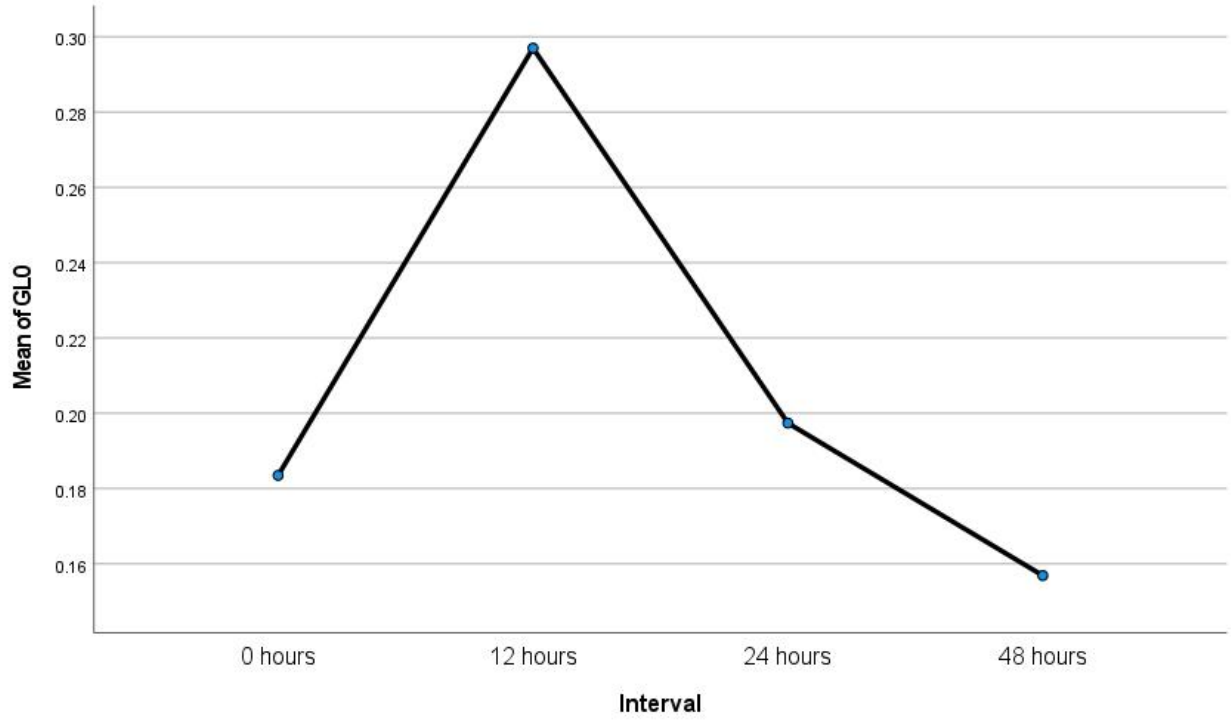

FIGURE 4.8: Means Plot of Glucose in the Blood at Different Intervals

The plot shows that glucose levels were highest in the blood 12 hours post-mortem.

CHAPTER FIVE

5.0 DISCUSSION

This is an experimental study that was conducted to determine the comparative biochemical analysis of the post mortem changes that occurs in the aqueous humour and blood of a bovine eye at 0hrs, 12hrs, 24hrs, 48hrs and 72hrs . The result gotten would add to existing literature on the possible associations between time of death and forensic analysis.

Biochemical changes can tell a lot about cause of death. Feasibility of the study and estimation depends upon the sampling, contamination, temperature, microbial activities etc. of the fluid chosen for the analysis. E.g., aqueous humour and blood. However, ocular fluid provide better results as compared to Blood/serum or other bodily fluids due to easy sampling and lower chances of contamination.

Biochemical changes at later stages of death can also be taken into consideration for estimation of time since death. Bacterial action, biochemistry of decomposition and autolysis etc, can provide better approximation to the degradation that occurs. Overall determination of post mortem activities through the analysis of biochemical changes is a better, emerging and more reliable way as compared to other ways.

The findings of this study in table (4.1) displays the mean values of chlorine, potassium, sodium and glucose in the aqueous at different post-mortem intervals. It comprises of 0hours, 12hours, 24hours and 48hours. Post mortem changes for 72hours could not be determined as the entire organ(bovine eye) has decayed and degraded and as a result, a pure sample of the aqueous humour could not be extracted. Thus the experiment ended at 48hours readings.

Descriptive Statistics of Substances in the Aqueous at Different Post-Mortem Intervals was recorded in table (4.2). In the table, it was noted that Chlorine ($\mu = 0.89$), potassium ($\mu = 1.48$) and sodium ($\mu = 0.82$) levels were highest at 48 hours post-mortem while Glucose level was highest at 12 hours post-mortem ($\mu = 0.29$).

Table (4.3) emphasized on comparing the Means of Substances in the Aqueous at Different Post-Mortem Intervals Using Analysis of Variance. Here, it was observed that there is a significant difference between the

levels of chlorine, potassium, sodium and glucose in the aqueous at different post-mortem intervals ($p < 0.05$).

Table (4.4) showed a Post Hoc Test Comparing Substances in The Aqueous at Different Post-Mortem Intervals. The post hoc test shows that there were significant differences between chlorine level in the aqueous 0 hours post-mortem and 12, 24 and 48 hours post-mortem ($p < 0.05$). There were also significant differences between potassium levels at different post-mortem intervals ($p < 0.05$).
Similarly, there were significant differences between sodium levels at 0 hour, 12 hours and 48 hours post-mortem ($p < 0.05$). However, there was no significant difference between sodium levels at 0 hours and 48 hours post-mortem ($p > 0.05$).
There were significant differences between glucose levels at 0 hour, 12 hours and 48 hours post-mortem ($p < 0.05$). There was no significant difference between glucose levels at 0 hours and 24 hours post-mortem ($p > 0.05$).

In figure 4.1, there is a Means Plot of Chlorine in the Aqueous at Different Intervals which revealed that chlorine levels were highest in the aqueous 48 hours post-mortem.

FIGURE 4.2 shows a Means Plot of Potassium in the Aqueous at Different Intervals which shows that potassium levels were highest in the aqueous 48 hours post-mortem.

FIGURE 4.3, shows a Means Plot of Sodium in the Aqueous at Different Intervals which revealed that sodium levels were highest in the aqueous 48 hours post-mortem.

In FIGURE 4.4, there is a Means Plot of Glucose in the Aqueous at Different Intervals which shows that glucose levels were highest in the aqueous 12 hours post-mortem.

The biochemical comparison between post mortem changes which occurs in the aqueous humour and blood of bovine eyes using certain biochemical markers such as chlorine, potassium, sodium and glucose

revealed that glucose level in the blood maintained a steady rise from 0hours post mortem to 12hours where it hits its highest peak and then slowly declined during 24hours post mortem before observing a sharp break which declined further at 48hours. Sodium levels in the blood started with a steady decline from 0hours post mortem afterwhich it maintained a steady increase from 12hours to 24hours where it attained it highest peak and maintained this range till 48hours. Potassium levels on the other hand began with a sharp decline from an high level within 0hours post mortem and continued it decline to 12hours where it attained its lowest value and then ascended within 24hours to 48hours in an almost linear pattern. However, chlorine attained its highest peak at 12hours post mortem after a steady increase from 0hours. It then began experiencing a sharp decline linearly within the 24 and 48hours interval.

In aqueous humour, the statistics and results obtained revealed a relationship between chlorine, potassium, sodium and glucose during the different post mortem intervals. This shows that aqueous humour level of degradation and decomposition followed a more structured and orderly pattern which a steady degradation rate being tested with the following biochemical markers listed above. Potassium levels which was not stable during PMI for blood showed more reliable values in the aqueous.

The result obtained from this study shows that the null hypothesis was rejected which state that:

- **Null** Hypothesis(1): There will be no significant changes in the biochemical composition of the aqueous humor with increasing post mortem intervals.

- Null Hypothesis(2):There will be no significant changes in the biochemical composition of the blood at post mortem intervals.

- Null Hypothesis(3): The correlation between biochemical changes in the aqueous humor and blood will not significantly enhance the accuracy of post mortem intervals estimation.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION
6.1 CONCLUSION

After a careful analysis of the comparison of biochemical markers changes during post mortem intervals in the aqueous humour and blood component of a bovine eye, it can be concluded that there is a significant difference between the levels of chlorine, sodium, and glucose in the blood at different post mortem intervals ($p<0.05$) and there is no significant relationship between the level of potassium in the blood of a cattle at different post mortem intervals ($p>0.05$).
It can however be deduced from these findings that there is a significant difference between the levels of chlorine, potassium, sodium, and glucose in the aqueous humor of a bovine eye at different post mortem intervals ($p<0.05$).

In conclusion, aqueous humour prove more reliable than blood in the accurate estimation of post mortem changes as their stability in composition, anatomical location and structure gives it an advantage over blood. This findings supports the pioneering work of Jaffe (1962) and the study done by Adelson and Sunshine(1963).

6.2 RECOMMENDATION

Further studies should be conducted with larger sample sizes and in diverse population, regions and animals to clarify the reliability of ocular fluids such as the aqueous humour and body fluids such as blood in the accurate estimation of post mortem interval estimation which is invaluable in forensic analysis and educational purposes.

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APPENDIX

Tests of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
CL0	Based on Mean	29.792	3	36	0.000
	Based on Median	2.603	3	36	0.067
	Based on Median and with adjusted df	2.603	3	20.679	0.079
	Based on trimmed mean	24.971	3	36	0.000
K0	Based on Mean	33.719	3	36	0.000
	Based on Median	7.016	3	36	0.001
	Based on Median and with adjusted df	7.016	3	15.663	0.003
	Based on trimmed mean	32.179	3	36	0.000
NA0	Based on Mean	33.716	3	36	0.000
	Based on Median	2.456	3	36	0.079
	Based on Median and with adjusted df	2.456	3	12.711	0.110
	Based on trimmed mean	26.265	3	36	0.000
GL0	Based on Mean	16.436	3	36	0.000
	Based on Median	4.546	3	36	0.008
	Based on Median and with adjusted df	4.546	3	17.964	0.015
	Based on trimmed mean	16.454	3	36	0.000

Robust Tests of Equality of Means

		Statistic[a]	df1	df2	Sig.
CL0	Welch	175.897	3	17.562	0.000
	Brown-Forsythe	132.888	3	19.492	0.000
K0	Welch	2645.000	3	15.368	0.000
	Brown-Forsythe	834.162	3	15.226	0.000
NA0	Welch	124.917	3	15.496	0.000
	Brown-Forsythe	54.071	3	13.341	0.000
GL0	Welch	2171.216	3	16.954	0.000
	Brown-Forsythe	1526.325	3	19.624	0.000

a. Asymptotically F distributed.

CL0

Tukey HSD[a]

Group	N	Subset for alpha = 0.05		
		1	2	3
0 hours	10	0.6502		
24 hours	10		0.7804	
12 hours	10		0.7952	
48 hours	10			0.8927
Sig.		1.000	0.624	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

K0

Tukey HSD[a]

Group	N	Subset for alpha = 0.05			
		1	2	3	4
12 hours	10	0.5687			
0 hours	10		0.6731		
24 hours	10			1.1751	
48 hours	10				1.4803
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

NA0

Tukey HSD[a]

Group	N	Subset for alpha = 0.05		
		1	2	3
12 hours	10	0.1197		
24 hours	10		0.4762	
0 hours	10			0.7493
48 hours	10			0.8153
Sig.		1.000	1.000	0.701

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

GL0

Tukey HSD[a]

Group	N	Subset for alpha = 0.05		
		1	2	3
0 hours	10	0.0286		
24 hours	10	0.0297	0.0297	
48 hours	10		0.0417	
12 hours	10			0.2881
Sig.		0.995	0.062	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

Tests of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
CL0	Based on Mean	66.666	3	36	0.000
	Based on Median	4.644	3	36	0.008
	Based on Median and with adjusted df	4.644	3	14.866	0.017
	Based on trimmed mean	61.305	3	36	0.000
K0	Based on Mean	17.684	3	36	0.000
	Based on Median	3.328	3	36	0.030
	Based on Median and with adjusted df	3.328	3	16.563	0.045
	Based on trimmed mean	15.831	3	36	0.000
NA0	Based on Mean	44.503	3	36	0.000
	Based on Median	4.785	3	36	0.007
	Based on Median and with adjusted df	4.785	3	12.101	0.020
	Based on trimmed mean	40.833	3	36	0.000
GL0	Based on Mean	29.608	3	36	0.000
	Based on Median	5.568	3	36	0.003
	Based on Median and with adjusted df	5.568	3	18.054	0.007
	Based on trimmed mean	27.704	3	36	0.000

Robust Tests of Equality of Means

		Statistic[a]	df1	df2	Sig.
CL0	Welch	37.849	3	17.570	0.000

	Brown-Forsythe	69.627	3	15.175	0.000
K0	Welch	1.365	3	15.060	0.291
	Brown-Forsythe	0.866	3	21.091	0.474
NA0	Welch	7820.944	3	18.429	0.000
	Brown-Forsythe	4045.825	3	14.013	0.000
GL0	Welch	124.070	3	18.410	0.000
	Brown-Forsythe	57.519	3	14.905	0.000

a. Asymptotically F distributed.

CL0

Tukey HSD[a]

		Subset for alpha = 0.05		
Interval	N	1	2	3
0 hours	10	0.6314		
24 hours	10		0.7636	
48 hours	10		0.7823	
12 hours	10			0.8249
Sig.		1.000	0.555	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

K0

Tukey HSD[a]

		Subset for alpha = 0.05
Interval	N	1
12 hours	10	0.7212
24 hours	10	0.7403
48 hours	10	0.7456
0 hours	10	0.7591
Sig.		0.398

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

NA0

Tukey HSD[a]

		Subset for alpha = 0.05		
Interval	N	1	2	3
12 hours	10	0.0818		
0 hours	10		0.6843	
24 hours	10			1.3687
48 hours	10			1.3857
Sig.		1.000	1.000	0.616

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

GL0

Tukey HSD[a]

		Subset for alpha = 0.05		
Interval	N	1	2	3
48 hours	10	0.1569		
0 hours	10	0.1835	0.1835	
24 hours	10		0.1974	
12 hours	10			0.2970
Sig.		0.110	0.620	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.