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Postmortem Interval Assessment: Correlating Histological Changes and Antioxidant Enzyme Activity in Human Kidney Tissues

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Abstract: In forensic medicine, estimating the post-mortem interval is crucial for forensic analysis. Thanato-chemistry, also known as the chemistry of death, is used for this purpose. The current study aims to correlate histological and enzymatic changes in kidney tissue samples at 12, 24, 48, 72, and 96 hours postmortem. In human kidney samples, histological changes included alterations in renal tubular cells, obliteration of the tubular lumen, tubular necrosis, cytoplasmic architectural distortion, and distortions in the glomerular tufts. Alongside histological examination, UV spectroscopy was used to estimate enzyme levels, including catalase, glutathione reductase, and glutathione peroxidase. When analysing histopathological changes from 12 to 96 hours, UV spectroscopy showed a significant decrease in antioxidant enzyme levels. The post-mortem morphological alterations were progressive, steady, and slow, indicating that PMI determination is essential. Between 48 and 96 hours postmortem, cellular features and components were observed to undergo necrosis. PMI may be estimated either independently or in combination with changes in the histological structure of tissues, reflecting the time elapsed after death, and from measurements of antioxidant enzyme levels in human kidney tissue samples using UV spectroscopy.

Keywords: *Histology, Enzyme Analysis, Time Since Death, U V Spectroscopy.*

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I. INTRODUCTION

In forensic medicine, estimating the postmortem interval is crucial for reconstructing the events surrounding the crime. Time of death is an essential aspect of the criminal justice system that helps identify the culprits. (Gelderman et al., 2019). The evidence used to estimate time since death can come from 3 possible sources: anamnestic, environmental, and corporal. (Siddhamsetty et al., 2014). One of the problems that forensic pathology has to deal with is the accurate calculation of PMI, since it is determined by the physicochemical and biochemical changes that occur in the decomposing body (Fais et al., 2018) (Rosana Gerometta et al., 2019). Most of the existing protocols are based on visual changes that occur in the corpus(Apurba Nandy, 2015; Viera Valencia & Garcia Giraldo, 2019) such as urine in the bladder, biochemical changes, electrical and mechanical stimuli in skeletal muscles, chemical stimulation in the eye pupils (Corradini et al., 2015; Siddhamsetty et al., 2014; Zilg et al., 2015)(Chandrakanth et al., 2013; Ubelaker et al., 2015). The degenerative changes observed are well designed to help the forensic pathologist achieve reasonable accuracy. Various techniques have been used to accurately and methodically calculate the PMI in the immediate, early, and late stages following death (Henssge, 1988). The study of microscopic tissue architecture—the arrangement of a tissue's cells—is known as histology. Sample tissues are prepared using a technical process called "histological staining" to facilitate microscopic inspection. Histological staining involves five fundamental steps: fixation, processing, embedding, sectioning, and staining (James & Geoffrey Rolls, n.d.)(Alturkistani et al., 2015).

Histology is vital in forensic investigations for determining the cause, time, and manner of death. With advancements, tissues are chemically fixed and stained for

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examination, commonly using hematoxylin-eosin (H&E) stain. This stain highlights different cell components, with red cytoplasm, blue nuclei, and red erythrocytes. Histopathology involves analysing tissues or cells under a microscope to diagnose and explore tissue disorders. (Dr Rachel Brown, n.d.). Sikandar A. et al. provided fresh insights into the origins and pathogenesis of diseases using histological methods.(Alturkistani et al., 2015). Also, estimation of the levels of antioxidant enzymes in the liver and kidney, e.g., glutathione reductase, glutathione peroxidase, and catalase, could help in the analysis of the postmortem interval. (Sakr et al., 2023). Estimating PMI (post-mortem interval) is essential because it provides a time frame that helps identify human remains and aid in investigating and advancing case resolution.(Gelderman et al., 2018). Various pathological conditions lead to histological changes, which may not be observed in histological studies related to PMI, COD, and Examination of Injury. There are a few approaches that are pretty accurate under specific controlled conditions. Still, the unpredictable nature of external factors—such as the environment—has made it impossible to make a trustworthy prediction. The best estimates of the time of death may be established early in the PM investigation, before many environmental factors significantly impact the outcomes (Guerrero-Urbina et al., 2022). Forensic histology is extensively valued for assessing injuries, determining the cause of death, and measuring the post-mortem period. Histological alterations in kidney tissue after the post-mortem interval (PMI) were illustrated in this article.

The kidneys are essential for filtering blood, removing waste and excess fluid, maintaining electrolyte balance, and regulating blood pressure. Millions of nephrons in each kidney filter blood and create urine, which is transported via the ureters to the bladder. Changes in the renal tubular and glomerular structures, along with debris buildup, are often the primary focus of histological investigations (Stephens & Tim Jewell, 2018).

In the present study, we focused on detecting the effects of different postmortem intervals on antioxidant enzyme levels using UV spectroscopy. The enzymes are analysed at various time intervals, and the enzyme concentration and specific activity are observed. In histological analysis, we sectioned and fixed the sample at the respective time interval in formalin, then processed it for block and slide preparation. This histological analysis was conducted to develop a reliable method for determining the time elapsed since death.

II. METHOD

➢ *Study Design:*

This is a descriptive & comparative study conducted from July to October 2023.

➢ *Ethical Considerations:*

- The Institutional Ethics Committee (IEC) of the University School of Sciences, Gujarat University, approved the study with reference ID: GU-IEC(NIV)/02/Ph.D./005.
- The Institutional Ethics Committee (IEC) from B J Medical College & Civil Hospital, Ahmedabad, approved the study with reference No.: EC/Approval/16/2023/31/03/2023.

➢ *Study Population:*

The study was conducted on 14 autopsy cases from the Department of Forensic Medicine and Toxicology, BJ Medical College, Ahmedabad. In these autopsy cases, only male samples were considered, with causes of death including sudden death, hanging, and road traffic accidents [spot death]. Cases excluded from this study were putrefied cadavers and those with a past medical history of diseases to prevent interference with the results, as such factors could affect cellular morphology and biochemical analysis. Further studies were conducted at Gujarat University with assistance and support from the Department of Biochemistry and Forensic Science. In this study, the collected tissues were divided into two sections: one for biochemical analysis and the other for histological analysis.

Table 1 Demographic Features of the Cases Analyzed.

Cases	Age (years)	Sex (M-Male, F- female)	Cause of Death
1	15	M	Hanging
2	22	M	Hanging
3	24	M	Road Traffic Accident
4	26	M	Hanging
5	26	M	Road Traffic Accident
6	29	M	Hanging
7	35	M	Road Traffic Accident
8	38	M	Road Traffic Accident
9	40	M	Road Traffic Accident
10	40	M	Road Traffic Accident
11	45	M	Road Traffic Accident
12	44	M	Road Traffic Accident
13	50	M	Road Traffic Accident
14	50	M	Sudden Death

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➢ *Inclusion Criteria:*

- The case must be registered under the Department of Forensic Medicine and Toxicology, Civil Hospital, Ahmedabad.
- The cases under 50 in age were considered for the research analysis.
- Death on the spot of the accident [road traffic accident, sudden death, hanging]

➢ *Exclusion Criteria:*

- Death due to any medical conditions.
- Age above 60 years.
- Cases with any of the organ disorders.
- Time of death is more than 15 hours.

The sample is stored in an acrylic chamber, sectioned every 24 hrs for 6 days, and fixed in formalin for histological analysis.

III. BIOMARKERS ANALYSIS

➢ *Sample Preparation:*

We have collected about 1-2 g of tissue samples. The extraction from the tissue samples was performed by homogenizing the tissue using liquid nitrogen and a motor pestle. The samples were then added to a microcentrifuge tube along with 1 ml of extraction buffer and centrifuged at 5000 rpm for 10 to 15 minutes. The supernatant was collected, transferred to a fresh tube, and stored at a cool temperature.

➢ *Enzymatic Assay:*

- *Glutathione Reductase:*

NADPH reduces glutathione disulfide (GSSG) via glutathione reductase, yielding reduced glutathione (GSH), which is essential for the survival of most aerobic organisms. Various glutathione transferases also utilize GSH to activate and deactivate a range of toxic and carcinogenic electrophiles.(Mannervik, 1999) Glutathione reductase plays a crucial role in regenerating the reduced form of glutathione from its oxidized state. Most of the total glutathione concentration in normal cells is in its reduced form, and the maintenance of the redox state depends on glutathione reductase and the availability of NADPH.(Mbemba Fundu et al., 2020).

- *Glutathione Peroxidase:*

Glutathione Peroxidase is a vital antioxidant enzyme responsible for regulating the cellular redox state through the GSH/GSSG ratio, in conjunction with other redox couples such as NADP/NADPH and FAD/FADH2.(Mbemba Fundu et al., 2020) It converts glutathione (GSH) to its oxidized form (GSSG), thereby protecting cells from reactive oxygen species and moderate oxidative stress. This enzyme is predominantly found in hepatocytes and erythrocytes and belongs to the selenocysteine family, which is essential for its enzymatic activity. Typically composed of four subunits, each containing a selenium atom in the form of selenocysteine, Glutathione Peroxidase serves as the primary enzyme for detoxifying hydrogen peroxide across a variety of biological organisms.(Mbemba Fundu et al., 2020).

- *Catalase:*

It is a crucial enzyme found in nearly all living organisms exposed to oxygen, including bacteria, plants, and animals. Its primary function is to catalyse the decomposition of hydrogen peroxide (H2O2) into water (H2O) and oxygen (O2), thereby protecting cells from oxidative damage caused by reactive oxygen species (Adam Augustyn., n.d.; *What Is Catalase Enzyme? - Chemical Composition, Function, Reaction*, n.d.). It is a tetrameric enzyme composed of four polypeptide chains, each exceeding 500 amino acids in length. It contains four heme groups, each containing an iron atom, which are essential for its catalytic activity. The enzyme exhibits one of the highest turnover numbers, capable of converting millions of hydrogen peroxide molecules per second. (Adam Augustyn., n.d.). The presence of catalase is critical for cellular health, as it helps mitigate potential damage from oxidative stress, which can lead to various diseases, including cancer, heart disease, and neurodegenerative disorders.(Hadwan, 2016; Nandi et al., 2019).

IV. HISTOLOGICAL ANALYSIS

➢ *Sample Collection.*

The tissue samples (kidney) are collected from the post-mortem room of a civil hospital in Ahmedabad, Gujarat. Sterile gloves and other equipment are used to prevent infections. Approximately 2-3 grams of the tissue were collected and stored in a sample bottle. This sample bottle is carried in a thermos ice box with ice packs, in excellent condition, to avoid any contact with environmental conditions.

➢ *Sample Preparation.*

These samples are brought to the Department of Biochemistry and Forensic Science, Gujarat University, Ahmedabad, for Analysis. The tissue samples are removed from the sample container and stored separately on small (sterile) Petri plates. These are transferred to a closed acrylic chamber. After every 12 to 24 hrs., the transversely sectioned tissue is preserved in 37 to 41 % formalin solution for fixation. Sectioning is performed at 12, 24, 48, 72, and 96 hr. The tubes are sealed using paraffin film and processed further for block and slide preparation.

➢ *Tissue Preparation*

Once all the samples are labelled and ready for analysis, they are taken to a histopathology laboratory. These samples are processed, embedded in paraffin wax, and sectioned to prepare blocks. Using the prepared blocks, slides are prepared.

➢ *Slide Preparation*

For slide preparation, the blocks are sectioned using a microtome. These sections are placed on slides, stained with H&E (Hematoxylin-eosin), and heat-fixed. Finally, the slide

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is entirely fixed by using a DPX mount and a coverslip. Once the slides are mounted using the mounting medium, they are ready for examination.

➢ *Observation*

Once the slides are fixed, mounted, and dried, they are ready for examination. The slides are observed microscopically at 40x magnification. Grading of the histological changes that are observed in the tissue is represented as follows:

Table 2 Grading of the Histological Changes.

Grade	Histological appearance	Changes
Grade 0	Minimal changes	High cellular density with a defined structure
Grade 1	Minimal-mild changes	Intense staining and more dispersed nuclei
Grade 2	Mild changes	Lesser intensity in the staining of the nuclei
Grade 3	Mild to moderate	Complete disappearance of the nuclei
Grade 4	Severe changes	Complete cellular density has been distorted

➢ *Statistical Analysis*

The sample size was calculated based on the data acquired. Interpretation is done using MS Excel. Testing methods include autoregression and exponential decay models for comparing across groups. P-value ≤ 0.005 was considered statistically significant—data expressed as Mean standard deviation.

V. RESULTS

➢ *Biochemical Assay Results:*

The enzymatic activity of Glutathione Reductase, Glutathione Peroxidase, and Catalase in renal tissue (kidney tissue) displayed distinct temporal patterns across post-mortem intervals (PMI: 12, 24, 48, and 72 hours), indicating significant biochemical changes over time. Glutathione Reductase exhibited a gradual decline in activity, with mean (SD) values decreasing from 278 (100) at 12 hours to 137 (39) at 24 hours, 81 (20) at 48 hours, and 53 (30) at 72 hours, suggesting a slow reduction in the reductive capacity of renal tissue. Similarly, Glutathione Peroxidase showed a notable decrease, with mean (SD) values falling from 2169 (287) at 12 hours to 1763 (282) at 24 hours, 1363 (252) at 48 hours, and 1006 (417) at 72 hours, indicating a declining oxidative defence mechanism as the PMI advanced.

In contrast, catalase activity showed an increasing trend, with mean (SD) values rising from 111 (27) at 12 hours to 150 (34) at 24 hours, 199 (38) at 48 hours, and 253 (26) at 72 hours, indicating an enhanced oxidative stress response in the later post-mortem stages. These findings highlight the time-dependent changes in enzymatic activity in kidney tissue, emphasizing the biochemical processes of oxidative stress and tissue degradation relevant to forensic and pathological studies.

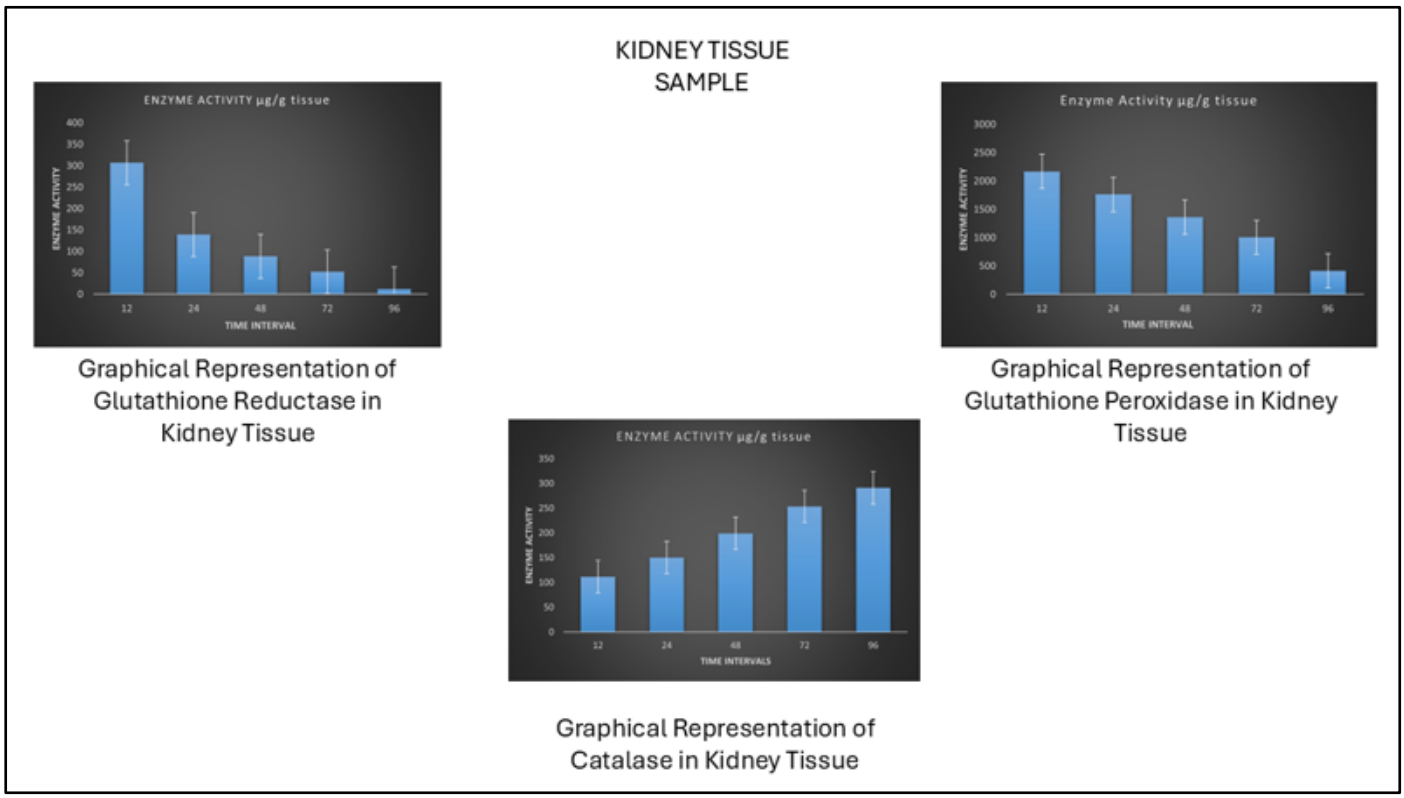

Fig 1 Graphical Representation of Enzyme Activity in Kidney Tissue Sample.

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➢ *Histological Results:*

The samples were collected from the postmortem room of Civil Hospital, Ahmedabad, Gujarat, for analysis. The samples were transported in accordance with proper protocols to prevent tissue contamination. Each sample was washed and then stored in an acrylic chamber for decomposition. Tissue sections were collected at specific time intervals: 12 hr, 24 hr, 48 hr, 72 hr, 96 hr, and 120 hr. The collected tissue sections were fixed in a 37-41% formalin solution. These preserved and fixed samples were then sent for block and slide preparation. In the kidney tissue section, Figure 2 illustrates various stages of autolysis.

In stage A (minimal autolysis), the renal tubules and glomerular tufts are slightly altered, with swollen tubular cells causing lumen obliteration and the accumulation of pinkish debris. Stage B (minimal to mild autolysis) shows degenerative changes, including necrosis of the tubular epithelium and distortion of tissue architecture, characterised by moderate pinkish material. Stage C (mild to moderate autolysis) features tubular cells without nuclei, obliterated lumens filled with pinkish debris, and reduced glomerular cellularity. Stage D (moderate to severe autolysis) exhibits distorted glomeruli and widened inter-tubular spaces. Finally, stage E (severe autolysis) shows a lack of intercellular demarcation in all renal tubules, complete necrosis, cytoplasmic distortion, and increased inter-tubular spaces.

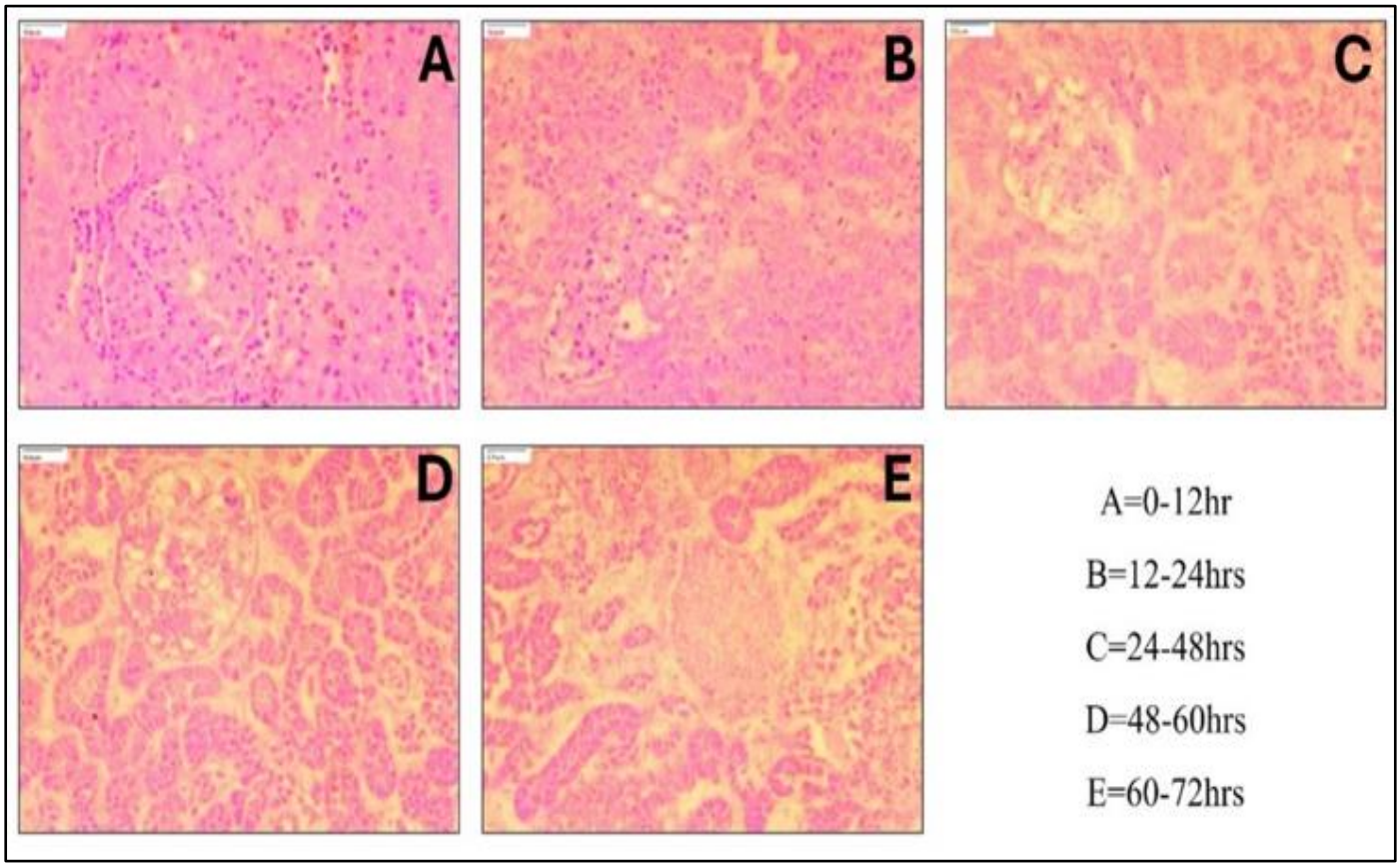

Fig 2 Images of the Kidney Tissue Sectioning

VI. DISCUSSION

Estimating the post-mortem interval (PMI) is crucial for forensic investigations because it helps reconstruct the circumstances of death. To identify potential indicators of PMI, this study analysed histological and enzymatic changes in human kidney tissue over time. The findings provide valuable insights into post-mortem biochemical and morphological processes by assessing tissue structure and antioxidant enzyme activity at multiple time points. As cellular components gradually deteriorate and oxidative balance is lost, both hepatic and renal tissues exhibit a consistent decline in enzyme activity throughout the PMI.

Enzymatic changes are observed in glutathione reductase, which is susceptible to post-mortem cellular alterations, as evidenced by a notable decrease in glutathione reductase activity over time in the sample tissues. Given that glutathione reductase is essential for preserving low glutathione levels, its action may be a precursor to PMI (Sakr et al., 2023). A decline in glutathione peroxidase activity indicates a gradual depletion of antioxidant defence systems. Studies of oxidative stress during decomposition have shown similar results (Mbemba Fundu et al., 2020; Sakr et al., 2023). Catalase enzyme activity initially increased in kidney tissues, before declining. This transient rise might reflect an early response to accumulating reactive oxygen species (ROS) before the enzyme's degradation becomes dominant. (Nandi et al., 2019). In the kidney, Histological alterations ranged from tubular necrosis and lumen obliteration at early intervals to complete cellular collapse and loss of intercellular demarcation by 96 hours. The findings which were similar to

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time dependent autolytic patterns in renal/kidney tissues are corroborated by the findings which were found in the research conducted by Guerrero-Urbina et al.

This study's histological and enzymatic patterns align with previous research on post-mortem changes in human tissues. For instance, Sakr et al.(Sakr et al., 2023) demonstrated that oxidative biomarkers, including glutathione-dependent enzymes, degrade progressively post-mortem, offering reliable PMI indicators. Alturkistani et al. (Alturkistani et al., 2015) He emphasised the utility of histological grading for tracking autolytic changes, particularly in high-metabolism organs such as the liver and kidneys. However, numerous trials have shown differences in enzyme breakdown, like for example an accelerated decomposition of catalase enzyme under certain conditions (Mbemba Fundu et al., 2020; Sakr et al., 2023) The environmental effect must be taken into account, and this necessity of following standardized procedures demands emphasis.

The strength of the present study lies in its dual approach, integrating enzymatic assays and histological analyses to enhance the accuracy of PMI estimation. Furthermore, external variability was reduced under controlled settings. The study has several drawbacks, including environmental variables such as temperature, humidity, and microbial activity, which are known to influence decomposition rates. (Gelderman et al., 2019).

The current study is important for forensics because it combines histological grading and enzyme testing to provide a reliable way to estimate the post-mortem interval (PMI) during the first ninety-six hours after death. Improving time of death calculations can be achieved by including kidney histology and enzyme activities such as catalase, glutathione peroxidase, and glutathione reductase. Future research should explore how environmental factors like temperature and humidity affect these changes. Additionally, advanced techniques such as transcriptomics or proteomics, and expanding the study to include other organs like the heart and skeletal muscles, may lead to more precise temporal indicators.

VII. CONCLUSION

This study advances forensic science by providing new insights into estimating the postmortem interval (PMI) through analyzing histological and biochemical changes in human kidney tissues. The findings show that postmortem changes are systematic and measurable, making them valuable tools in forensic investigations. The biochemical results are supported by these morphological changes, observed at specific intervals, adding an extra trustworthy dimension to PMI assessment. The activities of two important enzymes, glutathione reductase and glutathione peroxidase, decrease steadily over time, indicating a loss of antioxidant defenses. Conversely, catalase activity fluctuates—increasing initially and then decreasing—reflecting an early response to oxidative stress that diminishes through enzymatic degradation. These patterns form the basis for determining the time since death and reveal the molecular mechanisms involved in tissue decay. This research highlights the benefits of an interdisciplinary approach in forensic science by combining histological and biochemical techniques. This integrated method enhances PMI estimation accuracy and broadens our understanding of the molecular processes behind tissue deterioration. Future studies should explore how environmental factors like humidity and temperature affect postmortem changes. PMI assessment could also be improved by extending investigations to different tissue types and employing advanced molecular techniques such as transcriptomics or proteomics. These developments would increase the practical application of these methods in forensic cases.

The results of this study highlight the importance of integrating histology and biochemical testing in estimating PMI. By improving these methods for real forensic situations, investigators can achieve more precise and reliable results, promoting the progress of forensic science.

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- *Author Contribution:*

Sai Soumya Gadepally – Conceptualization and design of the study, sample preparation, visualization, methodology, and writing. Dr. Harishkumar T. Khubchandani – PM sample collection and review. Dr. Rushikesh Joshi– Conceptualization, Supervision, review and editing. All authors approved the submitted version of the manuscript.

- *Conflict of Interest*

The authors declare no conflict of interest.

- *Ethical Approval*

- The Institutional Ethics Committee (IEC) of the University School of Sciences, Gujarat University, approved the study with reference ID: GU-IEC(NIV)/02/Ph.D./005.
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