Determination of the Late Postmortem Interval by Observing the Electrical Conductivity in Skeletal Muscles of Rats

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ABSTRACT

Objective: To determine the values of electrical conductivity (EC) of cadaveric skeletal muscles of male rats and their relationship with different postmortem intervals (PMI).

Study Design: Experimental study.

Place and Duration of the Study: Department of Forensic Medicine and Toxicology, King Edward Medical University (KEMU), Lahore, in collaboration with the University of Veterinary and Animal Sciences (UVAS) Lahore, from October 2021 to September 2022.

Methodology: The non-probability consecutive sampling technique was used for the sample collection of 98 Sprague-Dawley rats and all were male. All lower-hind limb skeletal muscles of rats with known death intervals were used. Parameters (EC) were measured by using conductivity meter DDS-11A, through the method described by Ekanem and Achinewhu. Healthy adult rats within a specific weight range (250–300 g) and with a known time of death, euthanised using a standardised method such as CO_2 asphyxiation were included, and the rats with pre-existing diseases or injuries affecting muscle tissue, those showing advanced decomposition or bacterial contamination, or with an unknown or inconsistent PMI were excluded.

Results: The results showed that there was a significant correlation between the duration of PMI and EC, and the data fitted well in the quadratic regression equation as $y = -1203.67 + 2.50\ EC + 0.001x^2$ ($R^2 = 0.924$).

Conclusion: This study suggests that measuring the EC of skeletal muscle extracts offers a reliable method for estimating late PMIs, showing a strong correlation with PMI duration and providing a valuable forensic tool.

Key Words: *Postmortem interval, Electrical conductivity, Skeletal muscles, Postmortem changes, Muscle conductivity.*

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INTRODUCTION

Postmortem interval (PMI), the duration between death and the discovery of a corpse, is a critical aspect of medicolegal investigations.[1] It is assessed through corporeal, environmental, and anamnestic evidences.[2] While eyewitness accounts contribute, reliance on scientific methodologies is imperative for robust conclusions.[3] The determination of the PMI is a critical aspect of forensic investigations, aiding in accurate time-of-death estimations. Among various methods, analysing the electrical conductivity (EC) of skeletal muscles offers a promising approach, particularly in the late stages of decomposition. Changes in muscle tissue, such as ion-migration and cellular-breakdown, directly influence the conductivity. This study explores the potential of EC measurements in rats to refine late PMI assessments.

Physical methods for PMI estimation include algor mortis, livor mortis, and rigor mortis. Algor mortis comprises the postmortem decrease in body temperature.[4] Algor mortis, typically applicable within the initial 24 hours postmortem, relies on temperature alterations in specific anatomical regions, including the nostrils, ears, and axillae.[5] However, its reliability diminishes beyond this timeframe. Livor mortis, the gravitational pooling of blood, results in skin discolouration. Livor mortis manifests within 30 minutes to 4 hours postmortem, reaching full development within 8-12 hours.[6] Rigor mortis, the postmortem stiffening of muscles due to biochemical changes, is initiated within minutes after death, progresses through various stages and is influenced by environmental factors such as temperature.[7]

Postmortem decomposition is the enzymatic and bacterial breakdown of tissues postmortem, influenced by variables

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such as temperature and pathology, typically commencing around three days postmortem.[8] Supravital reactions are the physiological responses occurring post-somatic death, facilitating PMI estimation within hours of death, though compromised in cases of severe trauma.[9] Biochemical methods for PMI estimation are the biochemical approaches resistant to environmental factors, thus enhancing PMI estimation accuracy.[10]

Synovial fluid analysis contained within joint cavities emerges as a promising PMI indicator due to its gradual chemical change postmortem, offering an extended analysis timeframe compared to other bodily fluids.[11] Electrolyte analysis relies on electrolyte concentrations, notably potassium (K^+), which exhibits a significant correlation with PMI due to postmortem cellular membrane changes, leading to K^+ leakage.[12] Other electrolytes, including sodium (Na^+), calcium (Ca^+), and chloride (Cl^-), exhibit less significant associations with PMI.[13] Synovial fluid analysis, particularly electrolyte assessment, holds promise in forensic studies, particularly in Asian contexts.[14] Researchers have also highlighted the utility of electrolyte analysis, particularly potassium, for an accurate PMI estimation owing to its compartmentalisation and resistance to contamination and putrefaction.[15]

This study aimed to determine the value of EC of cadaveric skeletal muscles at different PMIs and to correlate these with late PMIs by using cadaveric male rats.

METHODOLOGY

It was an experimental study. The study was conducted at the Department of Forensic Medicine and Toxicology, King Edward Medical University (KEMU), Lahore, in collaboration with the University of Veterinary and Animal Sciences (UVAS) Lahore, from October 2021 to September 2022, with an IRB approval reference number 149/PEC/RC/KEMU. The non-probability consecutive sampling technique was used for sample collection. The sample size comprised of 98 Sprague-Dawley rats. The muscles of the left-lower hind limbs of the rats were removed at different PMIs and were sent immediately to the laboratory for EC testing. The parameters (EC) were measured by using conductivity meter DDS-11A, through the method described by Ekanem and Achinewhu.[16]

The minimum recorded value of EC with the conductivity meter was determined as 689 µC/cm. Before analysing the samples, the muscles were homogenised with 50 mL of de-ionised water and stirred while the dynamic EC was determined in the liquid.

The study subjects were cadavers that were brought to the outdoor medicine department of UVAS Lahore, for postmortem examination. The muscles of the left lower limb were collected from a total of 98 rats at different PMIs of 0 hour, 4 hour, 8 hour, 12 hour, 16 hour, 20 hour, 24 hour, 48 hour, 72 hour, 96 hour, 120 hour, 144 hour, 168 hour, 192 hour, 216 hour, and 240 hour. All the rats were male. The muscle samples were taken to the laboratory of the Forensic Medicine and Toxicology Department KEMU, for quantitative analysis of EC. All the muscles were separated from the bone of the hind limbs and then crushed with Mili-Q water until the dynamic EC was determined.

The inclusion criteria were: Healthy adult rats within a specific weight range (250–300 g) and with a known time of death, euthanised using a standardised method such as CO_2 asphyxiation, while the rats with pre-existing diseases or injuries affecting muscle tissue, those showing advanced decomposition or bacterial contamination, or with an unknown or inconsistent PMIs were excluded. Statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 23. Quantitative variable such as EC was presented as mean ± standard deviation (SD) while PMI was measured in hours. For comparison between PMI and EC, regression analysis was applied.

RESULTS

The quadratic regression equation is PMI (Y) = -1203.67 + 2.50 EC + $0.001x^2$ (R^2 = 0.924). This above-stated regression model is based on a scatterplot non-linear trend between PMI and EC with R^2 = 0.924.

The mean EC values measured at different PMI durations are depicted in Table I. There was a trend toward increased EC with increasing PMI values.

The relationship between BMI and EC is also shown in Figure 1 and 2. EC showed a significant increase in values with the increase of PMIs.

Table I: Electrical conductivity (EC) values of muscles according to postmortem intervals (PMIs).

PMI (h)	EC (µC/cm) Mean ± SD
0	722.71 ± 12.88
6	700.85 ± 19.27
12	699 ± 15.38
18	722.28 ± 20.62
24	700.57 ± 27.50
48	747.85 ± 21.47
72	748.57 ± 42.92
96	815.42 ± 27.54
120	893.71 ± 60.82
144	819.71 ± 74.55
168	1051.85 ± 147.72
192	957.28 ± 115.07
216	1052.14 ± 147.77
240	1054.57 ± 187.80

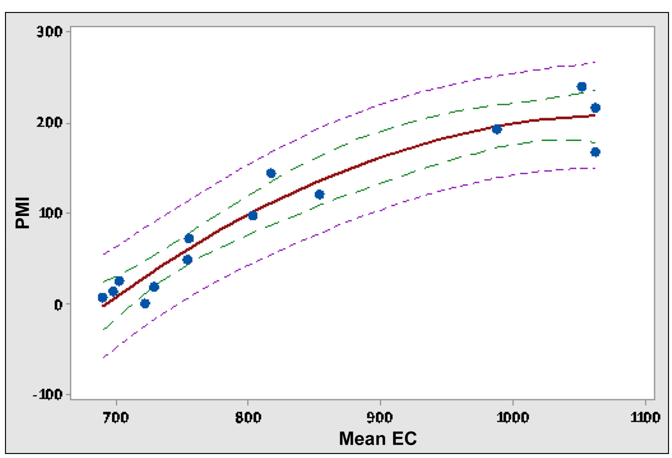

Figure 1: Scatter-plot between postmortem intervals (PMIs) and electrical conductivity (EC).

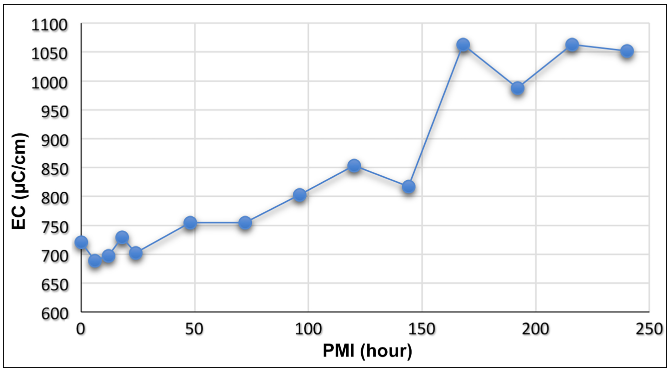

Figure 2: The curves between postmortem intervals (PMIs) and electrical conductivity (EC).

DISCUSSION

Accurate estimation of PMI has always been a challenge to forensic pathologists.[17] In this study, the cadaveric hind limb muscles of rats were analysed to measure the EC of the muscles by using a conductivity meter. EC is used to measure how well any solution or mixture conducts electricity and is applied in many fields such as food science, geology, clinical medicine, and forensic environment science.[18]

EC was used in this study because it dissolved the total conductive substances from the cadaveric muscles of the hind limbs of rats. For simplicity, the EC values were measured at the specific temperature of 25°C. Within the first 24 hours of PMIs, there was no significant change. However, EC value started to increase slowly in the next 48 hours, rapidly from 72 hours to 216 hours, after which it reached peak values. Currently, there is no single reliable method for the determination of the long PMIs. Therefore, it is more challenging for forensic professionals to estimate long PMIs as compared to short PMIs. The authors believe that the measurement of the EC of the cadaveric muscle is likely to become a useful method for measuring long PMIs.

The findings of this study suggest that EC is indeed a promising indicator of PMI. As the PMI progresses, the EC of skeletal muscles appears to increase steadily. This correlation can be attributed to the biochemical changes that occur in the muscle tissue after death, such as the breakdown of cellular structures and the release of ions.[19] The study's findings align with previous research that has explored the relationship between EC and postmortem changes in various tissues. However, this research contributes to the field by focusing specifically on skeletal muscles and by investigating the late stages of PMI.

Several studies have investigated the use of EC for estimating PMI in different tissues, including the liver, spleen, and kidney.[20] These studies have generally found a positive correlation between EC and PMI.

To assess its effectiveness, it is essential to compare it with existing methods, such as those utilising vitreous humour and synovial fluid. Vitreous humour, a clear gel-like substance found in the eye, has been widely used for PMI estimation due to its protected environment, which minimises postmortem changes. Potassium levels in vitreous humour have been particularly studied, as they tend to increase postmortem at a relatively consistent rate. While vitreous humour offers a reliable method for estimating PMI, it may not be suitable for cases where the eyes are damaged or inaccessible. Additionally, factors such as temperature and individual variations can influence potassium levels, potentially affecting the accuracy of PMI estimation.[21]

Synovial fluid, found in joints, has also been explored for PMI estimation. Similar to vitreous humour, it offers a protected environment and can provide insights into postmortem changes. Studies have investigated various analytes in synovial fluid, including glucose, lactate, and potassium, as potential indicators of PMI. Synovial fluid may be limited in availability, especially in cases where joints are damaged or inaccessible.[22] Moreover, factors such as joint type, activity level, and postmortem conditions can influence the composition of synovial fluid, potentially affecting the accuracy of PMI estimation.

The present study on skeletal muscle EC offers several advantages over vitreous humour and synovial fluid. Skeletal muscles are generally accessible in most forensic cases, making them a more practical option for PMI estimation. This study's focus on late-stage PMI estimation is particularly valuable, as existing methods may become less reliable over time. The use of EC represents a novel approach to PMI estimation, potentially offering unique insights and overcoming the limitations of traditional methods.

While vitreous humour and synovial fluid have been established as valuable tools for PMI estimation, the findings of this study suggested that skeletal muscle EC may offer a promising alternative, particularly for late-stage cases. However, it is important to note that further research is needed to fully evaluate the accuracy and reliability of skeletal muscle EC for PMI estimation. Factors such as temperature, humidity, and individual variations in muscle composition may influence the results. Additionally, larger sample sizes and studies on different species are necessary to strengthen the generalisability of the findings.

Regardless of technical methodology advancements for the estimation of PMI, the accuracy of these methods still leaves room for improvement. So, there is a need for the development of more precise and reliable techniques as they will not only prove beneficial in the field of forensic medicine but will also eliminate chances of any error as well.

CONCLUSION

The findings from this study suggest that measuring the EC of skeletal muscle extracts offers a reliable method for estimating late PMIs, showing a strong correlation with PMI duration and providing a valuable forensic tool.

ETHICAL APPROVAL:
The data were collected after the approval of the Institutional Review Board of the King Edward Medical University, Lahore (Approval No: 149/PEC/RC/KEMU).

COMPETING INTEREST:
The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:

SU: Manuscript writing and interpretation of data.
MS: Introduction writing.
NK: Data collection.
MH: Data analysis.
UH, DZ: Revision of the manuscript.
All authors approved the final version of the manuscript to be published.

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