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# Estimation of Postmorterm Interval by Using Myoglobin Concentration in Blood, Liver And Parotid Glands of Albino Rats

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## ABSTRACT

***Objective:*** To determine the postmortem interval (PMI) by estimating myoglobin concentration in blood, liver and parotid gland of albino rats and to compare the postmortem level of myoglobin among blood, liver and parotid gland.
***Study Design:*** Quasi-Experimental study.
***Place and Duration of Study:*** Department of Forensic Medicine and Toxicology King Edward Medical University, Lahore in collaboration with the University of Veterinary and Animal Sciences, Lahore from 20 June 2021 to 19 June 2022.
***Methodology:*** A total of 72 rats were killed by cervical dislocation and postmortem myoglobin levels were checked in blood, liver and parotid gland at 8 different postmortem intervals (9 rats in each interval) to check its significance in relation to postmortem time.
***Results:*** There was a significant correlation between myoglobin concentration in blood, liver and parotid gland with Postmortem Interval with $p$ value of $< 0.001$. The analysis indicated that the combination of blood, liver, and parotid myoglobin concentrations yielded the highest predictive accuracy ($r = 0.982$). The next best model included liver and parotid myoglobin concentrations ($r = 0.980$), while the model based solely on liver myoglobin concentration had the lowest predictive accuracy ($r = 0.946$).
***Conclusion:*** The myoglobin concentration in glandular tissues is a helpful parameter to determine PMI. As the postmortem interval increases the myoglobin concentration in the blood, liver and parotid gland also increases.

**Keywords:** Blood, Liver, Myoglobin, Parotid Gland, Postmortem Interval (PMI).

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## INTRODUCTION

The postmortem interval (PMI) stands as a crucial endeavor in forensic science investigations, delineating the duration between the cessation of life and the discovery of a deceased individual. Evidentiary foundations for determining PMI encompass corporeal, environmental, and anamnestic evidence, necessitating a comprehensive consideration and assessment before formulating opinions regarding PMI.[1,2] Precise determination of the time of death not only corroborates witness testimonies but also aids in delineating the number of suspects and evaluating their alibis.[3]

Major methodologies employed in determining PMI encompass eyewitness statements and scientific techniques, with the latter category including both physical and biochemical methods. While eyewitness accounts hold weight in criminal justice proceedings, their reliability is often subject to scrutiny, necessitating corroboration with additional evidentiary sources.[4] Physical methods for PMI estimation entail a thorough examination of postmortem physical changes within the body, encompassing algor mortis, livor mortis, rigor mortis, and postmortem decomposition.[5,6] Chemical changes occurring postmortem are influenced by environmental conditions, temperature differentials, and individual factors.[7] Postmortem decomposition signifies the breakdown of soft tissues through bacterial and enzymatic processes, with the onset typically observed after [3] days postmortem. Supravital reactions following somatic death represent the body's response to stimuli in the absence of heart and circulatory function, offering insights into PMI estimation several hours after death. However, these reactions are not applicable in cases involving severely burned or damaged bodies, highlighting their contextual relevance.[8]

Biochemical methods for PMI estimation have garnered attention in recent decades, focusing on chemical changes occurring in bodily fluids postmortem. Synovial fluid emerges as a promising

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substrate for PMI assessment, owing to its well-preserved nature postmortem and reduced susceptibility to contamination and putrefaction.[9] Myoglobin concentration in different organs, particularly skeletal muscle, presents a viable avenue for studying decomposition and detecting breakdown products, albeit necessitating further validation for routine application in PMI estimation. It is well-documented that the concentration of myoglobin in the blood significantly increases after death, with previous research indicating that this protein is released into the bloodstream from the skeletal muscle. While previous studies have shown that organs obtained during autopsies contain relatively low levels of myoglobin compared to skeletal muscle, based on qualitative or semi-quantitative analysis, the specific postmortem alterations in myoglobin levels within organs have not been thoroughly examined.[10] Keeping in view this, we conducted this study that aims at determining the PMI by studying myoglobin levels in different organs and compares myoglobin levels among different organs.

## METHODOLOGY

This Quasi-experimental study was conducted at the Department of Forensic Medicine and Toxicology King Edward Medical University, Lahore in collaboration with the University of Veterinary and Animal Sciences, Lahore over a duration of one year from 20 June 2021 to 19 June 2022 after obtaining necessary ethical approval from Institutional Review Board vide letter number 698/RC/KEMU dated 6 October 2020.

**Inclusion Criteria:** Adult Albino rats were included.

**Exclusion Criteria:** Apparently-sick animals were excluded.

A sample size of 72 albino rats was determined using 95% confidence level (5% level of significance), 80% power of test with expected correlation coefficient of parotid group as 0.987.11 Sample was collected utilizing non-probability consecutive sampling. The division and disposition of rats involved acquiring albino rats within the specified weight range, sacrificed by cervical vertebra dislocation, and divided into 8 groups (Group 1-8), each comprising 9 rats. These groups were allocated time intervals ranging from 30 minutes to 9 days postmortem. Blood, liver and parotid gland samples were collected at designated intervals post-sacrifice.

Group 1: 30 minutes

Group 2: 1 hour

Group 3: 12 hours

Group 4: 1 day

Group 5: 3rd day

Group 6: 5th day

Group 7: 7th day

Group 8: 9th day

**Inclusion criteria:** Adult albino rats of either sex, weighing between 350-400 grams were included in the study.

**Exclusion criteria:** Rats displaying any form of injury or deformity were excluded from the study.

Sample preparation and detection procedures involved the collection and storage of blood, liver, and parotid gland samples, followed by analysis of myoglobin concentrations using ELISA techniques. Liver and parotid gland were separated and stored as entire organ while the blood from left heart was taken at approximately 30 minutes, 1 hour, 12 hours and 1, 3, 5, 7, 9th postmortem day (9 rats consist of each time interval group).

For quantitative analysis of myoglobin in blood, 0.5ml of blood was obtained from left heart. Blood myoglobin concentrations (ug/ml) were determined using the obtained supernatant after centrifugation at 20.000G for 10 minutes (24C) by rat myoglobin ELISA kit. For quantitative analysis of myoglobin in liver (ug/g), 0.1g of liver was removed from the obtained sample and was homogenized with 0.9ml phosphate buffer saline containing 1% bovine serum albumin using an ultrasonic homogenizer. After the centrifugation under the same conditions for the blood, myoglobin concentrations in the supernatant were determined and its content in the liver was calculated by Rat myoglobin ELISA kit. The myoglobin concentration of parotid gland (ug/g) was measured by the quantitative analysis, 0.1g from was removed and homogenized by ultrasonic homogenizer in 0.5ml PBS (Phosphate Buffer Saline) consist of 1% triton X-100, 0.1% SDS (Sodium Dodecyl Sulphate), 0.5% SDC (Sodium Deoxy Cholate), 0.2% SA (Sodium Azide) and the mixture of protease inhibitors. Homogenization was carried out for 1 minute at 40C. Homogenate mixture was centrifugated at 40C for three minutes at 10,000G. The myoglobin concentration was determined through the supernatant after centrifugation by the rat tissue myoglobin ELISA kit at the absorbance wavelength of

45nm by the ELISA reader. Tissue myoglobin concentration was measured in homogenates using ELISA technique based on principle of target antigen or antibody capture and subsequent detection/quantification using an enzyme reaction.

Data analysis involved the entry and analysis of collected data using Social Package of Statistical Science (SPSS version 23), with quantitative variables expressed as Mean±SD. Correlation analysis was done and regression models were employed to estimate PMI, while ANOVA was utilized for comparing the 8 groups (Group 1-8), with statistical significance set at $p<0.05$.

## RESULTS

Myoglobin levels in blood, liver and parotid were measured at different time intervals postmortem. Myoglobin concentration in blood increases steadily from 3.10±0.12 ng/ml at 0.5 hours to 12.20±0.3 ng/ml at 216 hours. Similarly, myoglobin concentration in the liver increased from 2.10±0.11 ng/ml at 0.5 hours to 8.20±0.13 ng/ml at 216 hours. The parotid gland shows the most significant increase, from 1.80±0.09 ng/ml at 0.5 hours to 26.20±0.9 ng/ml at 216 hours. The *p*-value <0.001 indicates that the differences in myoglobin concentrations across the various PMIs are statistically significant for all three tissues as shown in table I.

**Table - I: Myoglobin Concentration Mean Values At Different Postmortem Interval (n=72)**

| Parameters | Post Mortem Interval (Hrs) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0.5 | 1 | 12 | 24 | 72 | 120 | 168 | 216 | *p*-value |
| Myoglobin Conc. in Blood Mean±SD (ng/ml) | 3.10±0.12 | 3.70±0.17 | 4.60±0.34 | 5.80±0.50 | 7.80±0.23 | 9.30±0.14 | 10.30±3.0 | 12.20±0.3 | <0.001 |
| Liver Myoglobin Conc. in Liver Mean±SD (ng/ml) | 2.10±0.11 | 2.60±0.14 | 3.20±0.29 | 4.0±0.29 | 5.40±0.25 | 6.40±0.48 | 7.70±0.15 | 8.20±0.13 | <0.001 |
| Myoglobin Conc. in Parotid Gland Mean±SD (ng/ml) | 1.80±0.09 | 2.30±0.11 | 4.20±0.27 | 12.20±1.21 | 16.10±1.42 | 21.10±1.0 | 25.40±1.4 | 26.20±0.9 | <0.001 |

The concentration of myoglobin increases over time in all three tissues, with the parotid gland showing the most substantial increase, particularly after 24 hours. The significant *p*-values (<0.001) across all comparisons suggest that the differences in myoglobin concentration between the three tissues are statistically significant, making these tissues potentially useful for postmortem interval estimation.

There is strong correlation between myoglobin concentration and postmortem interval, correlation value of blood, liver and parotid gland is r=0.980, r=0.978 and r=0.950 respectively with $p$ value < 0.001 as shown in (Table-III). The correlation analysis shows that there exists a strong correlation between myoglobin concentration in blood, liver and parotid with postmortem interval.

## DISCUSSION

After the irreversible cessation of cardiac function and subsequent death, a series of postmortem changes occur within the human body. These changes encompass the cessation of cellular metabolic processes, leading to biochemical alterations and cellular breakdown as metabolic pathways shift towards autolytic processes. Eventually, structural changes become evident, marking the onset of decomposition.[12]

Death manifests in two stages: immediate (somatic) death and cellular death. Immediate death is characterized by the cessation of movement, breathing, and blood circulation.[13] During cellular death, notable changes occur in the eyes, such as corneal clouding, eye shrinkage, and discoloration of the retina. Early postmortem changes, including rigor mortis, livor mortis, and body cooling, are also observed during this phase.[14]

While it is well-established that blood myoglobin concentrations increase significantly after death, little is known about changes in the very early postmortem period.[15] However, our animal experiment demonstrated a rapid increase in postmortem blood

myoglobin concentrations. Postmortem myoglobin concentration increases in various organs, including the blood, liver, and parotid gland, correlating with the postmortem interval.

In this study, we investigated postmortem variations in myoglobin content in the blood, parotid gland, and liver at different time points postmortem. A total of 72 albino rats were divided into 8 groups and each group contain 9 rats and myoglobin concentration of each group measured at different PMI such as 30 minutes, 1h, 12h, 24h, 72h, 120h, 168h, 216h and it was found to be significantly high postmortem. Moreover, there was a significant correlation between myoglobin concentration (in blood, liver and parotid gland) and PMI. By multiple linear regression analysis, the most expected model was the combination between blood, liver and parotid myoglobin concentrations (R2 = 0.982). The second predictable model was the combination between liver and parotid myoglobin concentrations (R2 = 0.980). The last predictable model was liver myoglobin concentration (R2 = 0.946).

**Table- II: Comparison of myoglobin concentration at different postmortem interval (n=72)**

| PMI (Hours) | *p*-value | | |
|---|---|---|---|
| | Blood vs Liver | Liver vs Parotid Gland | Blood vs Parotid Gland |
| 0.5 | <0.001 | <0.001 | <0.001 |
| 1 | <0.001 | <0.001 | <0.001 |
| 12 | <0.001 | <0.001 | <0.001 |
| 24 | <0.001 | <0.001 | <0.001 |
| 72 | <0.001 | <0.001 | <0.001 |
| 120 | <0.001 | <0.001 | <0.001 |
| 168 | <0.001 | <0.001 | <0.001 |
| 216 | <0.001 | <0.001 | <0.001 |

**Table III: Correlation between Postmortem Interval and Myoglobin Concentration**

| Myoglobin Level | Postmortem Interval (Hours) | |
|---|---|---|
| | r | *p*-value |
| Blood | 0.980 | < 0.001 |
| Liver | 0.978 | < 0.001 |
| Parotid Gland | 0.950 | < 0.001 |

Previous studies have shown significant increases in myoglobin concentration in glandular tissues and organs with longer postmortem intervals. Miura et al. conducted a study on postmortem changes in myoglobin content within organs such as the liver, kidney, heart, and thyroid gland.[16] They investigated these changes at various time intervals, including 30 minutes, and on days 1, 3, 5, 7, and 14 postmortem. Their findings differed from those of our study. They observed that myoglobin concentration in the thyroid gland and lung showed potential for estimating the postmortem interval. Specifically, they noted a significant increase in myoglobin concentration in the thyroid gland by day 1 postmortem, which remained elevated until day 7 postmortem. However, no further increase was observed from day 7 to day [14].

Our study on estimating the postmortem interval using myoglobin concentration in albino rats provides valuable insights into forensic science. While promising, the direct application of these findings to human forensic investigations requires careful consideration and validation. Collaborative efforts among forensic scientists, biochemists, and pathologists will be essential for developing and refining human-specific methodologies, ensuring their reliability and applicability in real-world forensic scenarios.

Chemical methods, such as analyzing potassium levels, rigor mortis pH, and hypoxanthine and xanthine accumulation, provide additional insights into estimating the postmortem interval.[17] Physical methods, including algor mortis, livor mortis, and rigor mortis, also aid in estimating the postmortem interval. However, these methods have limitations, including variability influenced by environmental factors and individual differences.[18]

The study on estimating postmortem interval (PMI) using myoglobin concentration in albino rats presents intriguing possibilities for forensic research. While the findings offer valuable insights, their direct application to human forensic investigations requires careful consideration. Given the inherent biological differences between rats and humans, validation studies correlating myoglobin levels with PMIs in human cadavers are necessary for robust translational potential. Furthermore, comparative analyses between animal models and human samples, alongside interdisciplinary collaboration among forensic scientists, biochemists, and pathologists, will be pivotal for developing and refining human-specific methodologies. Ultimately, these endeavors will ensure the reliability and applicability of myoglobin-based PMI estimation techniques in real-world forensic scenarios.

Limitations: Several limitations exist in this study: Firstly, findings may not directly apply to humans or other animals due to differences in myoglobin metabolism and physiology. Secondly, individual variation among albino rats may affect

myoglobin levels, leading to result variability. Thirdly, environmental factors like temperature and humidity could influence myoglobin concentrations, potentially complicating PMI accuracy. Fourthly, postmortem changes such as autolysis and microbial activity may impact myoglobin levels, affecting PMI reliability. Additionally, myoglobin levels may fluctuate unpredictably over time, reducing PMI estimation accuracy. Lastly, ethical concerns arise from using animals in research, requiring careful justification and adherence to ethical guidelines.

## CONCLUSION

The myoglobin concentration in glandular tissues is a helpful parameter to determine PMI. As the postmortem interval increases the myoglobin concentration in the blood, liver and parotid gland also increases.

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**Conflict of Interest:** None

**Authors' Contribution**

Following authors have made substantial contributions to the manuscript as under:

RB & SI: Data acquisition, data analysis, critical review, approval of the final version to be published.

AA & ARM: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

NK & MM: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

## REFERENCES

1. Maiese A, Scatena A, Costantino A, Di Paolo M, La Russa R, Turillazzi E, et al. Micrornas as useful tools to estimate time since death. A systematic review of current literature. Diagnostics. 2021; 11(1): 1–13.
2. Zissler A, Stoiber W, Steinbacher P, Geissenberger J, Monticelli FC, Pittner S. Postmortem protein degradation as a tool to estimate the PMI: A systematic review. Diagnostics. 2020; 10(12).
3. Pittner S, Bugelli V, Weitgasser K, Zissler A, Sanit S, Lutz L, et al. A field study to evaluate PMI estimation methods for advanced decomposition stages. Int J Legal Med. 2020; 134(4): 1361–73.
4. Głomb K. How to improve eyewitness testimony research: theoretical and methodological concerns about experiments on the impact of emotions on memory performance. Psychol Res [Internet]. 2022; 86(1): 1–11. Available from: https://doi.org/10.1007/s00426-021-01488-4.
5. Hachem M, Said HB, Zakkir S, John A, Salim A, Alkash AS, et al. Emerging approaches for estimation of post-mortem interval in medico-legal practice. 2020 Adv Sci Eng Technol Int Conf ASET 2020. 2020;(June).
6. Chintalwar RS, Gajbhiye SKM, Dhawane SG. Estimation of Time Since Death From Rigor Mortis. Indian Journal of Forensic Medicine & Toxicology. 2016; 10(1): 197.
7. Mostafa HES, El-Shafei DA, Abouhashem NS, Alaa El-Din EA. Could skeletal muscle changes provide a reliable method for estimating the time since death: A histological, biochemical, and DNA study. Aust J Forensic Sci [Internet]. 2023; 55(1): 46–58. Available from: https://doi.org/10.1080/00450618.2021.1921272
8. Pittner S, Bugelli V, Eric Benbow M, Ehrenfellner B, Zissler A, Campobasso CP, et al. The applicability of forensic time since death estimation methods for buried bodies in advanced decomposition stages. PLoS One [Internet]. 2020; 15(12 December): 1–26. Available from: http://dx.doi.org/10.1371/journal.pone.0243395
9. Wenzlow N, Mills DE, Byrd J, Warren M, Long MT. Review of the current and potential use of biological and molecular methods for the estimation of the postmortem interval in animals and humans. J Vet Diagnostic Investig. 2023; 35(2): 97–108.
10. Bonicelli A, Mickleburgh HL, Chighine A, Locci E, Wescott DJ, Procopio N. The 'ForensOMICS' approach for postmortem interval estimation from human bone by integrating metabolomics, lipidomics, and proteomics. Elife. 2022; 11: 1–22.
11. Aprilasari F, Hariani N, Trimurti S. Preliminary study of forensic investigation: Insect Arrival Pattern on Wistar Rat (Rattus norvegicus) Carrion in Samarinda, East Kalimantan. AIP Conf Proc. 2022; 2668.
12. Ibrahim SF, Ali MM, Basyouni H, Rashed LA, Amer EAE, Abd El-Kareem D. Histological and miRNAs postmortem changes in incisional wound. Egypt J Forensic Sci. 2019; 9(1).
13. Kugathasan P, Stubbs B, Aagaard J, Jensen SE, Munk Laursen T, Nielsen RE. Increased mortality from somatic multimorbidity in patients with schizophrenia: a Danish nationwide cohort study. Acta Psychiatr Scand. 2019; 140(4): 340–8.
14. Tang D, Kang R, Berghe T Vanden, Vandenabeele P, Kroemer G. The molecular machinery of regulated cell death. Cell Res [Internet]. 2019; 29(5): 347–64. Available from: http://dx.doi.org/10.1038/s41422-019-0164-5
15. Zelentsova EA, Yanshole L V., Melnikov AD, Kudryavtsev IS, Novoselov VP, Tsentalovich YP. Post-mortem changes in metabolomic profiles of human serum, aqueous humor and vitreous humor. Metabolomics. 2020; 16(7).
16. M. M, T. N, S. M. Postmortem changes in myoglobin content in organs. Acta Med Okayama [Internet]. 2011;65(4):225–30. Available from: http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L362537853%0Ahttp://www.lib.okayama-u.ac.jp/www/acta/pdf/65_4_225.pdf LK - http://resolver.ebscohost.com/openurl?sid=EMBASE&issn=0386300X&id=doi:&atitle=Postmortem+changes+in+myoglo
17. Paula MC, Michelutti KB, Eulalio ADMM, Piva RC, Cardoso CAL, Antonialli-Junior WF. New method for estimating the post-mortem interval using the chemical composition of different generations of empty puparia: Indoor cases. PLoS One. 2018; 13(12): 1–13.
18. Kanz F, Reiter C, Risser DU. Citrate content of bone for time since death estimation: Results from burials with different physical characteristics and known PMI. J Forensic Sci. 2014; 59(3): 613–20.