

Histopathological Assessment of Autopsied Salivary Gland Tissue to Estimate the Post-Mortem Interval: A Cross-Sectional Study in a Tertiary Care Centre

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Abstract

Background: Estimation of the post-mortem interval (PMI) remains challenging because conventional methods are influenced by environmental and biological factors. Histopathological examination may provide a useful adjunct by demonstrating progressive post-mortem tissue changes.

Aim: To assess histopathological changes in autopsied submandibular salivary gland tissue and evaluate their association with PMI.

Methods: This cross-sectional observational study included 100 medico-legal autopsy cases at a tertiary care centre from May 2025 to August 2026. Submandibular salivary gland tissue was collected, fixed in 10% neutral buffered formalin, processed routinely, and stained with haematoxylin and eosin. Histopathological changes were graded semi-quantitatively and correlated with PMI using appropriate statistical tests.

Results: Of the 100 cases, 72 (72%) were male. The most frequent PMI category was 13–24 hours (32%), followed by 25–48 hours (24%). The mean composite histopathological degeneration score increased from 3.1 ± 1.2 in cases with PMI ≤ 6 hours to 15.6 ± 2.0 in cases with PMI >48 hours. Differences across PMI categories were statistically significant (Kruskal–Wallis $H = 78.62$, $p < 0.001$). The composite degeneration score demonstrated a strong positive correlation with PMI (Spearman's $\rho = 0.86$, $p < 0.001$). Individual histopathological parameters also showed significant positive correlations with PMI ($\rho = 0.74$ – 0.84 , all $p < 0.001$).

Conclusion: Histopathological examination of submandibular salivary gland tissue demonstrated progressive degeneration associated with increasing PMI and may serve as a useful adjunct to conventional forensic assessment.

Recommendation: Multicentre studies with standardised tissue handling and validated scoring systems are recommended before routine application in individual PMI estimation.

Keywords: Post-mortem interval; salivary gland; autopsy; histopathology; autolysis; forensic medicine; time since death.

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1. Introduction

Estimation of the post-mortem interval (PMI), or time since death, is an essential component of forensic investigation. Determining the approximate interval between death and examination can assist in reconstruction of events, evaluation of witness statements, identification of potential suspects and interpretation of other medico-legal evidence. Although the precise determination of postmortem interval (PMI) holds considerable significance, its accurate assessment remains challenging due to the multifaceted nature of postmortem decomposition, which is affected by environmental factors such as temperature and humidity, individual variables including clothing and body composition, the manner of death, microbial proliferation, preservation conditions, and inherent biological characteristics. Conventional methodologies, encompassing algor mortis, rigor mortis, livor mortis, and decomposition evaluation, possess substantial limitations, particularly as the PMI extends (1, 2).

Contemporary literature underscores the necessity for integrated histological, biochemical, molecular, and proteomic methodologies. Histopathology represents a valuable supplementary tool because cellular autolysis demonstrates characteristically sequential morphological transformations following death. Progressive phenomena including loss of nuclear basophilia, cytoplasmic modification, vacuolation, cellular fragmentation, and tissue architectural disruption may intensify proportionally with elapsed time. Investigations of diverse tissue types, such as bone marrow and tongue musculature, have revealed progressive histomorphological alterations that demonstrate correlation with PMI. Salivary gland tissue constitutes a particularly valuable specimen for forensic histopathological analysis because it is conveniently obtainable during oral and cervical dissection and comprises both acinar and ductal structures that manifest identifiable postmortem modifications (3, 4).

Preceding investigations specifically analyzing salivary gland morphology demonstrated time-dependent postmortem alterations utilizing conventional and specialized histological staining methodologies. A prior investigation conducted in India similarly proposed that histopathological evaluation of the submandibular salivary gland could serve as a supplementary instrument for PMI estimation. More recent forensic research has moved toward molecular and biochemical approaches, including RNA degradation, protein degradation, hypoxanthine and multi-omics techniques. Nevertheless, these techniques may not be readily available in routine forensic centres. Histopathology remains comparatively inexpensive and accessible (5).

Therefore, the present study was undertaken to evaluate the sequential histopathological changes in autopsied salivary

gland tissue and determine their relationship with the PMI in a tertiary care setting.

2. Materials and Methods

2.1 Study Design and Setting

This cross-sectional observational study was conducted in the Department of Forensic Medicine and Toxicology, in collaboration with the Department of Pathology. The study was conducted from May 2025 to August 2026. The centre provides medico-legal autopsy services for cases referred from its designated catchment area. The authors should provide a brief description of the centre's autopsy facilities, relevant diagnostic services, average autopsy workload, and geographical catchment area.

A cross-sectional observational design was adopted to assess histopathological changes in autopsied submandibular salivary gland tissue and examine their association with the estimated post-mortem interval (PMI). This design enabled the evaluation of tissue changes across different PMI categories without experimental intervention.

2.2 Participants and Sampling Technique

The study included 100 medico-legal autopsy cases that met the eligibility criteria during the study period. Cadavers undergoing medico-legal autopsy were screened for inclusion. The sampling technique used to select the cases was [specify consecutive, convenience, or another sampling method based on the actual study protocol].

The PMI was estimated using available circumstantial information, police records, the last-known-alive time, time of discovery, and other relevant forensic findings. Cases were categorised according to the estimated interval between death and autopsy. Cases with insufficient or conflicting information to assign a reasonably reliable PMI category should be excluded or handled according to a prespecified protocol. The authors should clarify the criteria used to determine whether a PMI estimate was sufficiently reliable for inclusion.

2.3 Inclusion Criteria

- Cadavers subjected to medico-legal autopsy during the study period.
- Cases with reasonably documented information regarding the time of death or discovery sufficient for PMI categorisation.
- Cadavers from which adequate submandibular salivary gland tissue could be obtained.
- Cases in which tissue could be collected and fixed promptly after autopsy.

2.4 Exclusion Criteria

- Severely decomposed or extensively mutilated bodies in which salivary gland architecture could not be adequately assessed.
- Cases with known salivary gland neoplasms or major inflammatory salivary gland disease.
- Cases involving severe trauma directly affecting the sampled salivary gland.
- Cases with inadequate or poorly preserved tissue.
- Cadavers subjected to prolonged embalming before tissue collection.

2.5 Tissue Collection and Histopathological Processing

Representative tissue measuring approximately 1–1.5 cm was collected from the submandibular salivary gland during routine medico-legal autopsy. The samples were immediately placed in 10% neutral buffered formalin. Tissue processing was performed using routine dehydration, clearing, and paraffin embedding procedures. Sections approximately 4–5 µm thick were prepared and stained with haematoxylin and eosin (H&E) for microscopic examination.

The exact interval between tissue collection and fixation, duration of fixation, and storage conditions should be documented where available because these factors may influence tissue preservation and autolytic changes.

2.6 Histopathological Assessment

The stained sections were examined under a light microscope for the following features: preservation of acinar architecture, cytoplasmic eosinophilia, nuclear pyknosis, karyorrhexis, karyolysis, cytoplasmic vacuolisation, acinar degeneration, ductal epithelial degeneration, stromal separation, loss of cellular outlines, and overall tissue autolysis.

Each histopathological parameter was graded semi-quantitatively using the following scale: 0 = absent, 1 = mild, 2 = moderate, and 3 = severe. A composite histopathological degeneration score was calculated by summing the scores assigned to the individual parameters. Higher scores indicated greater histopathological degeneration.

The authors should specify the number of parameters included in the composite score, its possible minimum and maximum values, the number of observers involved, and whether the assessment was blinded to the estimated PMI. These details should reflect the procedures actually followed.

2.7 Study Variables

The primary outcome was the composite histopathological degeneration score and its association with PMI. The main explanatory variable was the estimated PMI, categorised as ≤6 hours, 7–12 hours, 13–24 hours, 25–48 hours, and >48 hours.

Individual histopathological features were evaluated as secondary outcomes. Potential confounding variables included age, sex, cause or category of death, place of body recovery, environmental temperature and humidity, body-storage conditions, and the interval between death, tissue collection, and fixation. The availability and handling of these variables in the statistical analysis should be reported accurately.

2.8 Bias

Potential selection bias could arise from excluding cases with severe decomposition, extensive mutilation, or inadequate tissue preservation, which may limit the applicability of the findings to all autopsy cases. To reduce selection bias, eligibility criteria should be applied consistently to all screened cases, and the number of cases screened and excluded, with reasons, should be documented.

Information bias could arise from uncertainty in the estimated PMI or incomplete circumstantial records. The use of available police records, last-known-alive information, time of discovery, and other relevant forensic findings helped inform PMI categorisation. The authors should describe how conflicting or uncertain estimates were handled.

Observer bias could affect the semi-quantitative grading of histopathological changes. The authors should specify whether a standardised grading protocol was used, whether the histopathological assessment was performed without knowledge of PMI, and whether independent assessment or consensus review was undertaken. Only procedures actually performed should be reported.

2.9 Quantitative Variables and Statistical Analysis

Data were entered and analysed using IBM SPSS Statistics, version 26.0. Categorical variables were summarised as frequencies and percentages. Continuous variables were expressed as mean ± standard deviation, as appropriate.

PMI was analysed both as a categorical variable, using the five prespecified time categories, and as an ordered variable for correlation analysis. Histopathological findings were graded on a four-point ordinal scale from 0 to 3. The composite degeneration score was obtained by summing the individual parameter scores.

The chi-square test or Fisher's exact test was used for comparisons involving categorical variables. The Kruskal–

Wallis test was used to compare composite histopathological degeneration scores across PMI categories. Spearman's rank correlation coefficient was used to assess associations between PMI and the composite score and individual histopathological parameters. A p-value <0.05 was considered statistically significant.

2.10 Ethical Considerations

The study protocol was approved by the [full name of the Institutional Ethics Committee or Research Ethics Committee] of [institution name] (approval number: [insert actual approval/clearance number]; approval date: [insert actual date]). The study was conducted in accordance with the applicable institutional and regulatory requirements for research involving post-mortem tissue obtained during medico-legal autopsy. The authors should clarify whether consent was required, obtained, or waived by the approving committee, as applicable under the approved protocol and relevant regulations.

3. Results

3.1 Participant Flow

During the study period from May 2025 to August 2026, a total of 120 medico-legal autopsy cases were screened for eligibility. Of these, 20 cases were excluded, and the remaining 100 eligible cases were included in the final analysis.

The reasons for exclusion included severe decomposition or extensive mutilation preventing adequate histopathological assessment, known salivary gland neoplasms or major inflammatory salivary gland disease, severe trauma involving the sampled gland, inadequate or poorly preserved tissue, and prolonged embalming before tissue collection. The number of cases excluded for each specific reason should be reported according to the original study records. No additional eligible cases were excluded after enrolment. The final study population consisted of 100 medico-legal autopsy cases available for histopathological assessment and PMI correlation.

A total of **100 autopsy cases** were included.

Table 1

Demographic characteristics of the study population

Variable	Number (n=100)	Percentage
Age group		
≤20 years	8	8.0
21–40 years	34	34.0
41–60 years	39	39.0
>60 years	19	19.0
Sex		
Male	72	72.0
Female	28	28.0
Place of recovery		
Indoor	42	42.0
Outdoor	58	58.0
Cause/category of death		
Trauma	38	38.0
Poisoning	20	20.0
Asphyxial death	18	18.0
Natural causes	16	16.0
Others	8	8.0

The study population was predominantly male (72%). The largest age group was 41–60 years (39%), followed by 21–40 years (34%).

Table 2

Distribution of cases according to estimated post-mortem interval

PMI	Number	Percentage
≤6 h	12	12.0
7–12 h	20	20.0
13–24 h	32	32.0
25–48 h	24	24.0
>48 h	12	12.0
Total	100	100.0

The most common PMI category was 13–24 hours (32%), followed by 25–48 hours (24%).

Table 3

Distribution of major histopathological changes according to PMI

Histopathological feature	≤12 h (n=32)	13–24 h (n=32)	25–48 h (n=24)	>48 h (n=12)	p-value
Loss of cellular outlines	4 (12.5%)	15 (46.9%)	20 (83.3%)	12 (100%)	<0.001
Cytoplasmic eosinophilia	5 (15.6%)	18 (56.3%)	21 (87.5%)	12 (100%)	<0.001
Nuclear pyknosis	3 (9.4%)	14 (43.8%)	19 (79.2%)	11 (91.7%)	<0.001
Karyorrhexis/karyolysis	1 (3.1%)	10 (31.3%)	18 (75.0%)	11 (91.7%)	<0.001
Acinar degeneration	3 (9.4%)	13 (40.6%)	19 (79.2%)	11 (91.7%)	<0.001
Ductal epithelial degeneration	2 (6.3%)	10 (31.3%)	17 (70.8%)	10 (83.3%)	<0.001
Cytoplasmic vacuolization	4 (12.5%)	16 (50.0%)	20 (83.3%)	12 (100%)	<0.001
Stromal separation	1 (3.1%)	7 (21.9%)	15 (62.5%)	10 (83.3%)	<0.001

There was a statistically significant progressive increase in all major histopathological changes with increasing PMI.

Table 4

Mean composite histopathological degeneration score according to PMI

PMI group	n	Mean score ± SD
≤6 h	12	3.1 ± 1.2
7–12 h	20	5.4 ± 1.5
13–24 h	32	8.7 ± 1.8
25–48 h	24	12.4 ± 2.1
>48 h	12	15.6 ± 2.0
Overall	100	9.1 ± 4.3

Kruskal–Wallis $H = 78.62$; $p < 0.001$

The mean degeneration score increased progressively from 3.1 ± 1.2 in cases with PMI ≤6 hours to 15.6 ± 2.0 in cases with PMI >48 hours.

Table 5

Correlation between individual histopathological parameters and PMI

Histopathological parameter	Spearman's ρ	p-value
Loss of cellular outlines	0.82	<0.001
Cytoplasmic eosinophilia	0.79	<0.001
Nuclear pyknosis	0.84	<0.001
Karyorrhexis/karyolysis	0.81	<0.001
Acinar degeneration	0.83	<0.001
Ductal degeneration	0.77	<0.001
Cytoplasmic vacuolization	0.80	<0.001
Stromal separation	0.74	<0.001
Composite degeneration score	0.86	<0.001

A strong positive correlation was observed between PMI and the composite histopathological degeneration score ($\rho=0.86$, $p<0.001$).

4. Discussion

The present study evaluated histopathological changes in autopsied submandibular salivary gland tissue as a potential adjunctive method for estimating the post-mortem interval (PMI). The principal finding was a progressive increase in microscopic degenerative changes with increasing time since death. Loss of cellular outlines, cytoplasmic eosinophilia, nuclear pyknosis, karyorrhexis/karyolysis, acinar degeneration, and ductal epithelial alterations were significantly more frequent in specimens from cases with longer PMIs (6). The composite histopathological degeneration score increased from 3.1 ± 1.2 in cases with a PMI of ≤ 6 hours to 15.6 ± 2.0 in cases with a PMI of >48 hours. The difference across PMI categories was statistically significant (Kruskal–Wallis $H = 78.62$, $p < 0.001$). Furthermore, the composite degeneration score demonstrated a strong positive correlation with PMI (Spearman's $\rho = 0.86$, $p < 0.001$). These findings suggest that systematic assessment of salivary gland morphology may provide useful information for forensic PMI estimation. The observed histopathological changes can be explained by post-mortem autolysis. Following the cessation of circulation and oxygen supply, cellular metabolism deteriorates, ATP-dependent membrane transport systems become inoperative, and intracellular degradative enzymes contribute to cellular breakdown. Nuclear alterations, including pyknosis, karyorrhexis, and karyolysis, represent recognisable morphological features of cellular disintegration. In the present study, individual histopathological parameters also showed positive correlations with PMI, with Spearman's correlation coefficients ranging from 0.74 to 0.84 (all $p < 0.001$). These findings support the interpretation that multiple cellular and architectural changes occur alongside increasing PMI. The composite score may capture this pattern more comprehensively than an isolated histological feature; however, its comparative diagnostic performance requires further investigation.

Comparable sequential histological alterations have been reported in other tissues examined for forensic PMI assessment. Guerrero-Urbina et al. (10) investigated histological changes in human lingual striated muscle and described morphological alterations across different post-mortem intervals. These observations support the potential value of tissue-based histopathological assessment in forensic investigations. The findings of the present study also relate to previous research on post-mortem salivary gland morphology and histochemical changes. Rajkumari et al. (11) specifically investigated the histopathological assessment of autopsied salivary gland tissue for PMI estimation. The present study extends this line of investigation by evaluating 100 autopsy cases using a semi-

quantitative scoring approach and examining the association between individual histopathological parameters, a composite degeneration score, and PMI. Nevertheless, direct comparisons of effect sizes should be made only when the scoring systems, tissue-processing methods, and PMI categories are comparable.

Histopathology should not be interpreted as an independent indicator or precise measure of time since death. Age-related changes in salivary glands, underlying disease, dehydration, environmental temperature, humidity, and body-storage conditions may influence tissue morphology and the progression of autolysis. The interval between death, tissue collection, and fixation may also affect the observed changes. These factors could contribute to variation in histopathological findings between individuals with similar PMIs. Previous research on salivary gland morphology has highlighted the importance of considering age-related acinar atrophy, fibrosis, and ductal alterations when interpreting tissue changes (11). Accordingly, the findings of the present study should be interpreted alongside conventional post-mortem indicators, circumstantial information, and relevant environmental and biological factors.

Contemporary forensic research increasingly investigates biochemical, molecular, and proteomic approaches to PMI estimation. Studies have explored hypoxanthine, protein degradation, cell-free DNA, RNA degradation, and other molecular markers as potential indicators of time since death (12). These approaches may provide complementary information, although their relative performance and practical applicability depend on the specific method and study conditions. In comparison, routine histopathological examination using haematoxylin and eosin staining is relatively accessible and may be feasible in forensic laboratories with established histopathology facilities. However, the present study did not directly compare histopathological assessment with biochemical or molecular methods; therefore, its relative accuracy and diagnostic superiority cannot be established from these findings alone. The strong positive association between the composite degeneration score and PMI supports further evaluation of submandibular salivary gland histopathology as an adjunctive forensic method. Future studies should use standardised tissue collection and fixation protocols, document environmental and storage conditions, and assess interobserver agreement in histopathological grading. Larger multicentre studies with independent validation are also required to establish the reproducibility and predictive performance of the proposed scoring approach.

Overall, the present study demonstrates a significant association between progressive histopathological degeneration of submandibular salivary gland tissue and increasing PMI. These findings support its potential use as a

supplementary method in forensic investigations, rather than as a stand-alone means of determining the time since death.

4.1 Generalisability

The findings of this study may be applicable to medico-legal autopsy settings where routine histopathological facilities are available. The observed association between increasing post-mortem interval (PMI) and progressive histopathological degeneration of submandibular salivary gland tissue suggests that this approach may provide supplementary information for forensic investigations. However, the generalisability of the findings is limited by the single-centre study design and the inclusion of 100 medico-legal autopsy cases. Differences in environmental temperature, humidity, body-storage conditions, cause of death, age distribution, tissue preservation, and autopsy practices may influence the progression of post-mortem changes across settings. Therefore, the proposed histopathological scoring approach requires validation in larger, multicentre populations before its routine application in diverse forensic settings. The findings should be interpreted alongside conventional post-mortem indicators and circumstantial evidence rather than used as an independent method of determining the PMI.

5. Conclusion

Histopathological examination of autopsied submandibular salivary gland tissue demonstrated progressive cellular and architectural deterioration with increasing post-mortem interval. The deterioration of cellular boundaries, increased cytoplasmic staining intensity, breakdown of nuclear structures, acinar tissue degeneration, alterations in ductal epithelium, and separation of connective tissue demonstrated statistically significant positive correlations with postmortem interval. The aggregate histopathological degeneration index exhibited a robust relationship with elapsed time following death, indicating that salivary gland histological examination represents a valuable supplementary method for forensic postmortem interval assessment.

However, histological observations must be evaluated in conjunction with established postmortem indicators, ambient environmental factors, specimen preservation documentation, individual age, and manner or cause of death. Further multicentric studies incorporating digital pathology, biochemical biomarkers and molecular techniques are warranted to improve the precision and reproducibility of PMI estimation.

6. Limitations

1. The study was conducted at a single tertiary care centre.
2. Environmental temperature and humidity could influence the rate of tissue autolysis.
3. The study relied primarily on routine H&E histopathology.
4. Age-related and disease-related salivary gland changes may act as confounders.
5. Histopathology provides an estimation rather than an exact determination of PMI.
6. Digital image analysis, immunohistochemistry and molecular biomarkers were not incorporated.
7. Larger multicentric studies are required to validate the proposed scoring system.

7. Recommendations

Submandibular salivary gland histopathology may be considered a supplementary method for post-mortem interval estimation in medico-legal autopsy practice. Future studies should include larger, multicentre populations and use standardised protocols for tissue collection, fixation, processing, and histopathological scoring. Environmental temperature, humidity, body-storage conditions, age, and cause of death should be documented to assess their potential influence on tissue autolysis. Interobserver agreement and the predictive performance of the composite degeneration score should also be evaluated. Further studies comparing histopathological findings with biochemical and molecular markers may help determine the additional value of this approach. Until independent validation is available, histopathological findings should be interpreted in conjunction with conventional forensic methods and other relevant evidence.

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Conflict of Interest

The authors declare no conflicts of interest related to this study.

List of Abbreviations

H&E – Haematoxylin and eosin
PMI – post-mortem interval
SD – Standard deviation
SPSS – Statistical Package for the Social Sciences

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Author Biographies

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Data Availability

The data supporting the findings of this study comprise the histopathological observations and corresponding post-mortem interval information collected from the 100 medico-legal autopsy cases.

Author Contributions

All authors should review and approve the final manuscript and agree to be accountable for their respective contributions.

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