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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), or time since death, remains one of the most challenging components of forensic investigation. Conventional methods based on algor mortis, rigor mortis, livor mortis, and decomposition are influenced by environmental and biological factors. Histopathological examination provides an inexpensive and potentially useful adjunct by documenting sequential autolytic changes in tissues.

Aim:

To assess histopathological changes in autopsied salivary gland tissue and evaluate their association with the post-mortem interval.

Methods:

This cross-sectional observational study was conducted in the Department of Forensic Medicine and the associated Histopathology Department of a tertiary care centre from May 2025 to August 2026. We included 100 medico-legal autopsy cases. We collected representative submandibular salivary gland tissue during routine autopsy, fixed it in 10% neutral buffered formalin, processed it routinely, and stained it with haematoxylin and eosin. Histological parameters including cellular integrity, nuclear changes, cytoplasmic eosinophilia, acinar degeneration, ductal changes, vacuolization, stromal alteration, and autolysis were graded and correlated with the known/estimated PMI. Statistical analysis was performed using SPSS version 26.0.

Results:

The majority of cases were males (72%), and the most frequent PMI category was 13–24 hours (32%). Progressive loss of cellular and nuclear detail, cytoplasmic eosinophilia, acinar degeneration, and ductal epithelial changes increased significantly with increasing PMI. Mild/absent autolysis predominated in cases with PMI ≤12 hours, whereas moderate-to-severe autolysis was predominant after 24 hours ($p<0.001$). A strong positive association was observed between the composite histopathological degeneration score and PMI category (Spearman's $\rho=0.86$, $p<0.001$).

Conclusion:

Histopathological examination of salivary gland tissue demonstrates progressive and statistically significant post-mortem changes and may serve as a useful adjunct for PMI estimation. It should, however, be interpreted alongside conventional forensic findings and environmental and circumstantial factors rather than being used as an independent determinant of time since death.

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

Materials and Methods

Study design and setting

A cross-sectional observational study was conducted in the Department of Forensic Medicine and Toxicology in collaboration with the Department of Pathology at a tertiary care centre.

Study period

The study was conducted from May 2025 to August 2026.

Sample size

We included 100 medico-legal autopsy cases.

Study population

Cadavers undergoing medico-legal autopsy during the study period were screened for eligibility. The PMI was determined from available circumstantial information, police records, last-known-alive time, discovery time, and other standard forensic parameters.

Inclusion criteria

1. Cadavers subjected to medico-legal autopsy during the study period.
2. Cases with reasonably documented information regarding the time of death/discovery sufficient for PMI categorization.
3. Cadavers in whom adequate submandibular salivary gland tissue could be obtained.
4. Cases in which tissue was collected and fixed promptly after autopsy.

Exclusion criteria

1. Severely decomposed or extensively mutilated bodies in which salivary gland architecture could not be assessed.
2. Known salivary gland neoplasms or major inflammatory salivary gland disease.
3. Severe trauma directly involving the sampled salivary gland.
4. Cases with inadequate or poorly preserved tissue.
5. Cadavers subjected to prolonged embalming before tissue collection.

Tissue collection

Representative tissue measuring approximately 1–1.5 cm was collected from the submandibular salivary gland during routine autopsy. Samples were immediately placed in 10% neutral buffered formalin. Tissue processing was performed

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using routine dehydration, clearing, and paraffin embedding procedures. Sections of approximately 4–5 µm thickness were prepared and stained with haematoxylin and eosin.

Histopathological assessment

The following features were evaluated under light microscopy:

- Preservation of acinar architecture
- Cytoplasmic eosinophilia
- Nuclear pyknosis
- Karyorrhexis
- Karyolysis
- Cytoplasmic vacuolization
- Acinar degeneration
- Ductal epithelial degeneration
- Stromal separation
- Loss of cellular outlines
- Overall tissue autolysis

Each parameter was graded semi-quantitatively:
0 = absent, 1 = mild, 2 = moderate, and 3 = severe.
A composite histopathological degeneration score was calculated by summing the individual parameters.

PMI categorization

For analysis, PMI was categorized into:

- ≤6 hours
- 7–12 hours
- 13–24 hours
- 25–48 hours
- 48 hours

Statistical analysis

Data were entered and analysed using IBM SPSS Statistics version 26.0. Continuous variables were expressed as mean ± standard deviation, while categorical variables were expressed as frequencies and percentages. The chi-square test/Fisher's exact test was used for categorical comparisons. One-way ANOVA or Kruskal–Wallis test was used for comparison of continuous histopathological scores across PMI categories, as appropriate. Spearman's correlation was used to assess the relationship between histopathological degeneration score and PMI. A p-value <0.05 was considered statistically significant.

Results

We included 100 autopsy cases.

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%).

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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.

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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 (ρ=0.86, p<0.001).

Discussion

The present study evaluated histopathological changes in autopsied submandibular salivary gland tissue as a potential adjunctive method for estimating the 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 obtained from cases with longer PMI (6).

The underlying mechanism for these observations is post-mortem autolysis. When circulatory function and oxygen supply terminate, cellular metabolic processes gradually deteriorate, ATP-dependent membrane transport systems become inoperative, and lysosomal proteases facilitate intracellular decomposition. Characteristic nuclear alterations, including pyknosis, karyorrhexis, and karyolysis, constitute identifiable phases of cellular disintegration. Comparable sequential histological modifications have been documented in additional tissues examined for forensic postmortem interval assessment. Microscopic evaluation of human tongue striated musculature has revealed systematic structural changes corresponding to different postmortem interval categories, indicating the prospective utility of temporal histopathological analysis. Within this investigation, the integrated histopathological scoring system exhibited a robust positive association with postmortem interval (ρ=0.86; p<0.001). This finding indicates that a methodical assessment framework incorporating diverse morphological characteristics may yield superior diagnostic information relative to examination of isolated microscopic features (7, 8).

Comparable findings have been documented in investigations of bone marrow tissue, wherein advancing cellular deterioration correlated with extended postmortem intervals. These results align with established literature examining salivary gland tissue. Preceding investigations documented postmortem modifications in salivary gland morphology and histochemical properties, whereas a prior Indian epidemiological investigation determined that submandibular salivary gland histological examination could function as a supplementary instrument for postmortem interval determination. The current study advances this methodology through application of an expanded cohort and implementation of a systematic semi-quantitative assessment protocol (9, 10).

However, histopathology should not be interpreted as an independent "clock." Age-related changes in salivary glands, underlying disease, dehydration, temperature, environmental exposure, and storage conditions can influence tissue morphology. Recent studies of minor salivary glands have demonstrated age-associated acinar atrophy, fibrosis, and ductal ectasia, emphasizing the importance of considering demographic and pathological confounders (11).

Contemporary forensic research increasingly investigates biochemical, molecular, and proteomic markers. Recent studies have explored hypoxanthine, protein degradation, cell-free DNA, RNA degradation, and multi-omics approaches for PMI estimation. These techniques may eventually improve precision, but histopathology remains relatively inexpensive and feasible in routine forensic laboratories (12).

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

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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.

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.

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Conflict of Interest

The authors declare that they have no conflicts of interest related to the publication of this case series.

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