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Review Article

# Histogical changes in oral mucosa (gingiva) as a method for estimating post-mortem interval: A literature review

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ABSTRACT

Estimating the post-mortem interval (PMI) is an essential step in forensic investigations, particularly those involving homicides and unwitnessed deaths. However, traditional methods occasionally yield inconsistent estimates. Histological and molecular techniques are considered crucial in forensic pathology and are frequently employed to estimate the time interval of death. The gingiva is an oral mucosal tissue used to estimate PMI. This review aimed to examine the potential of histological methods to determine PMI using oral mucosal tissue, namely the gingiva, and to investigate changes that occur in oral mucosal tissue at different time intervals when compared with those in normal tissues. The oral mucosa comprises layers of stratified squamous epithelium and connective tissue. Similar to other body tissues, changes are known to occur in the gingiva after death, and these cellular and tissue changes should also be considered. Alterations in the gingiva include homogenisation, karyorrhexis, pyknosis, karyolysis, chromatin clumping, eosinophilia, collagen fibre degradation, and the loss of tissue architecture. Reviews collating the results of original trials have consistently reported how the oral mucosa is altered by autolysis and how such changes can be observed in histological tissue morphology after death. Histology is an acceptably accurate technique for estimating PMIs.

## 1. Introduction

The post-mortem interval (PMI) is fundamental in forensic science (Mahalakshmi et al., 2016) and is the time elapsed since the body was found. Determining the time interval of death is an essential step in the investigation of homicides or unwitnessed death.(Donaldson & Lamont, 2014) An accurate estimation of PMI can include and exclude suspects based on their whereabouts at the time of death.(Donaldson & Lamont, 2014; Pasca & Ulasan, 2014).

After death, chemical or physical changes that occur in tissues until decomposition can be used to determine the PMI.(Patro et al., 2021; Yadav et al., 2015) There are various methods for estimating PMI, such as physical (algor mortis and livor mortis), microbiological (decomposition), biochemical (electrolyte concentration and enzyme activity), and entomological and psychochemical (rigor mortis) changes.(Mahalakshmi et al., 2016).

Traditional methods occasionally provide large time estimates and may contradict each other. Several factors have been found to impact the estimation of the time of death. One of these factors is the time since death before the body is detected. The greater the delay in finding the body, the less accurate the estimate of the time of death.(Pasca & Ulasan, 2014) Therefore, it is necessary to identify other methods to determine the time since death.(Mahalakshmi et al., 2016; Mazzotti et al., 2019) Histological and molecular techniques are essential in forensic pathology.(Mazzotti et al., 2019).

Histology is the science of microscopic examination of biological materials and structures. Histological examinations can be used to examine the condition of tissue cells and architecture. Chemical or physical exposure, including death, can alter the tissue structure.(Al-Taee et al., 2019) A combination of histology and immunohistochemistry has been used to identify morphological parameters in human tissues and the appearance of marker proteins to determine the time since death.(Mazzotti et al., 2019).

Oral mucosa, such as the gingiva, is a potential source of tissue samples for PMI estimation. Gingiva is used owing to its location within the oral cavity, which is typically protected from the external environment, resulting in tissue stability from external factors.(Fais et al., 2018) PMI estimation using histological techniques is performed by examining

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changes in epithelial and submucosal tissues collected from the oral mucosa, such as the gingiva. This review aimed to examine the potential of histological methods to determine PMI using oral mucosal tissue, namely the gingiva, and to investigate changes that occur in oral mucosal tissue at different time intervals when compared with those in normal tissues.

## 2. Literature review

### 2.1. Oral mucosa

Histologically, the oral mucosa comprises layers of stratified squamous epithelium and connective tissue. Stratified squamous epithelium, also called the oral epithelium, varies in thickness and degree of keratinisation depending on its location and functional requirements. Beneath the epithelium is a connective tissue called the lamina propria, and irregular connective tissue is found in the deep or submucosa. (Groeger & Meyle, 2019; Wang et al., 2019).

The oral mucosa is classified as the lining mucosa (lips, soft palate, cheeks, ventral part of tongue, floor of mouth), masticatory mucosa (gingiva and epithelial attachment, free and fixed gingiva, interdental papillae, and hard palate), or specialised mucosa (tongue papillae, taste buds, and umami taste modality).(Chiego, 2019).

Each oral mucosa is unique, and the mucosal lining is soft and non-keratinised. The masticatory mucosa is keratinised to overcome abrasion and attrition during mastication.(Chiego, 2019) The presence of connective tissue beneath the epithelial layer renders the oral mucosa unique when compared with other epithelial tissues.(Chiego, 2019) The oral mucosal epithelium functions as a barrier or defence mechanism that separates the underlying tissue from the environment.

The oral mucosal epithelium comprises two layers: a squamous epithelial layer, which is stratified on the surface, and a lamina propria on the inside.(Groeger & Meyle, 2019) The oral mucosal epithelium is replaced every 14–21 days because it constantly performs its functions; therefore, the cells undergo turnover. This layer is organised, has no blood vessels, and is semipermeable.(Groeger & Meyle, 2019) In normal tissues, the epithelial layer continues to form an architecture with clearly arranged cells and a clear boundary between the epithelial layer and lamina propria connective tissue.(Groeger & Meyle, 2019).

The lamina propria, a connective tissue found beneath the epithelium, comprises blood vessels, nerves, fibroblasts, macrophages, and mast cells. The lamina propria supports the epithelium and provides nutrients to cells within the epithelial layer.(Waasdorp et al., 2021) The lamina propria comprises two superficial papillary layers and a deep reticular layer. The papillary layer comprises thin and irregular collagen fibres. The papillary layer is formed by thick collagen fibres parallel to the surface.

The lamina propria contains collagen type I and V at a ratio of 5:1. (Kaur & Kakar, 2014; Waasdorp et al., 2021) The main cells in this layer are fibroblasts, which synthesise fibres for physiological and wound healing processes.(Groeger & Meyle, 2019) The submucosa, a layer of elastic collagen fibres, lies beneath the lamina propria and contains blood vessels and nerves. However, this layer is not found in fixed gingiva and palate durum because the lamina propria is directly attached to the underlying bone.(Groeger & Meyle, 2019).

### 2.2. Post-mortem changes

Rigor mortis, algor mortis, liver mortis, and tissue decomposition are well-known changes that occur in dead bodies. Tissue decomposition is an interesting research topic and can be observed by performing microscopic and macroscopic examinations.(Simmons Gary T., 2013).

#### 2.2.1. Tissue decomposition

After death, all molecules within the body undergo oxidation and degradation. These mechanisms occur at the macro- and micro-molecular levels. This is because the mechanisms of cell repair and renewal stop working. Tissue decomposition can be divided into two stages: autolysis and putrefaction.(Simmons Gary T., 2013).

Autolysis is an aseptic phenomenon that occurs in the body and refers to cellular destruction. This process occurs via the release of intracellular catalytic enzymes, which catalyse the breakdown of cellular organelles. These catabolic enzymes are physiologically present in cells and are released when cells are damaged or die.(Pasca & Ulasan, 2014; Powers, 2005).

Autolysis is a chemical process affected by both heat and cold conditions. Hot temperatures accelerate autolysis, whereas cold conditions retard autolysis, and freezing can stop this process. Enzyme-rich tissues undergo autolysis more rapidly than enzyme-poor tissues. The autolysis process is slower in the muscle than in the liver, owing to the presence of fewer catabolic enzymes in the muscle.(DiMaio & Kimberley Molina, 2022; Powers, 2005).

#### 2.2.2. Post-mortem histological changes in tissues

Typically, macroscopic changes are visible 0–2 days after death. However, the decomposition process in the cadavers begins within 4 min after death at the beginning of the autolysis process. Histological methods can be used to examine autolysis.(Miller & Zachary, 2017; Powers, 2005) In histological examinations, staining is used to observe changes that occur in the tissue structure, both in the cells and connective tissue and the architecture of the tissue.(Parai & Milroy, 2018) Histological examination of post-mortem tissues included several evaluations of the epithelial surface cells and connective tissue.

*2.2.2.1. Cellular changes on the epithelial surface.* Epithelial surface cell evaluation involves the examination of vacuolisation, cell outline, homogeneity, nuclear outline, nuclear vacuolisation, chromatin clumping, karyorrhexis, pyknosis, karyolysis, epithelial cell separation, antibody-antigen interaction, and loss of tissue architecture. (Muthukrishnan et al., 2018; Patro et al., 2021).

Haematoxylin and eosin (H&E) staining was used to examine changes in the cells. H&E staining is a widely used staining technique. H&E staining imparts a bluish colour to the nucleus and a red-pink colour to the cytoplasmic components.(Alturkistani et al., 2015) This staining technique is easy to perform and inexpensive. However, this staining does not provide all the necessary images; therefore, additional special staining is required.(Alturkistani et al., 2015; Feldman & Wolfe D, 2014; Parai & Milroy, 2018).

Changes in the nucleus, such as pyknosis, karyorrhexis, and karyolysis, occur during death. Pyknosis is defined as nuclear shrinkage owing to the loss of water from the nucleus into the cytoplasm. DNA condenses into a mass that is wrinkled and darker in colour. The condition progressed to karyorrhexis.(Miller & Zachary, n.d.-b) In karyorrhexis, the nucleus is fragmented, observed predominantly in the superficial layer at the beginning of death. Karyolysis is attributed to the loss of chromatin from cells in response to a decrease in intracellular bases, causing the active release of DNAase.(Mahalakshmi et al., 2016; Yadav et al., 2015) Karyolysis is visible on the superficial surface of the specimen.

The nucleus disappears within 1–2 days. Vacuolisation is characterised by the degradation of cellular organelles,(Mahalakshmi et al., 2016; Yadav et al., 2015) which leads to the loss of the constituent compartments of the cell. Homogenisation occurs due to a decrease in the content of cellular glycogen components.(Mahalakshmi et al., 2016; Yadav et al., 2015) During eosinophilia, the cytoplasm turns pink. (Mahalakshmi et al., 2016).

As the condition progresses, cell damage in this epithelial layer leads to the loss of desmosomes. Desmosomes form strong adhesive bonds between cells. The autolysis-mediated breakdown of desmosomes in these cells causes each cell to lose its bonds and become disorganised. This condition leads to the disappearance of the epithelial layer and

connective tissue, which is called loss of tissue architecture.(Mazzotti et al., 2019).

In 2015, Mahalakshmi et al. conducted a study on healthy tissues. (Mahalakshmi et al., 2016) The tissue was fixed based on the defined time since death: 15 min, 30 min, 45 min, 1 h, 2 h, and 4 h. Within the first 15 min, chromatin clumping was observed in the cells, which spread to the surface. Vacuolisation was observed in some cells. Intercellular bridges widened in the superficial layers. At 30 min, specimens exhibited characteristics similar to those observed at 15 min, although an increase in the number of cells and pyknosis was detected. The number of cells increased after 45 min. These cells spread to the layer above the epithelium. After 2 h, eosinophilia and loss of junctional bridges were detected in the superficial layer. At 4 h, homogenisation became more evident, the epithelium began to lose its architectural structure, and the basal nucleus was altered.(Mahalakshmi et al., 2016).

In 2020, Patro et al. conducted a study using 150 cadaver, 75 gingival, and 75 buccal samples. The time intervals since death were < 12.5, 12.5–20.5, and > 20.5 h. The authors examined epithelial changes, connective tissue, and degeneration in the buccal area.(Patro et al., 2021) Histological changes such as homogenisation, cytoplasmic vacuolisation, nuclear shrinkage, and chromatin clumping in cells were observed in tissues at < 12.5 h. Similar findings were detected at intervals of 12.5–20.5 and > 20.5 h, although to a greater extent. Degeneration of the acinar mucosa occurred at intervals of 12.5–20.5 and > 20.5 h.(Patro et al., 2021).

In 2018, Fais et al. conducted a study using gingival samples from the maxillary region.(Fais et al., 2018) The patients were divided into three groups: short PMI (SPMI) at 1–3 days, mid PMI (MPMI) at 4–5 days, and late PMI (LPMI) at 8–9 days. The authors examined the presence of antibody proteins, namely HIF-1α, that appear in tissues after death. The SPMI group exhibited a strong signal of HIF-1α protein in the basal layer, whereas the LPMI group had low levels of protein expression in the basal layer.(Fais et al., 2018).

*2.2.2.2. Changes in connective tissues.* Changes occur in the connective tissue as collagen fibres degrade, vacuolate, and change tissue structure. (Patro et al., 2021) Specific staining techniques can be used to detect changes in connective tissue, including Van Gieson and Masson–Goldner trichrome staining. Van Gieson's staining imparts a red colour to collagen and a yellow colour to other tissues.[4,14] Masson Goldner Trichrome imparts a light green to collagen and blue to other tissues. (Mazzotti et al., 2019; Parai & Milroy, 2018).

Degradation of collagen fibres can occur following a prolonged lack of oxygen supply, causing the chemical breakdown of proteins. After death, blood circulation stops; therefore, the oxygen supply to all tissues also stops.(Mazzotti et al., 2019) During autolysis, the degradation of collagen fibres in connective tissue results in the loss of tissue architecture owing to protein denaturation.(Mazzotti et al., 2019) The amount of collagen fibres in the connective tissue was calculated after staining. Five specimen areas were examined using a selected software. Calculations were performed at a magnification of 40 times. Leica Q-Win software (Leica Microsystems Srl, Cambridge, United Kingdom) is one commonly employed software.(Mazzotti et al., 2019).

In 2020, Mazzoti et al. examined changes in the gingiva at intervals of 1–3 (SPMI), 4–6 (MPMI), and 7–9 (LPMI) days after death. The authors detected significant differences in collagen fibre size changes in the SPMI and MPMI groups when compared with the LPMI group. Collagen fibre size differed substantially between the SMPI, MPMI, and LPMI groups when compared with the control group. In the LPMI group, the number of collagen fibres decreased with the appearance of initial epithelial damage.(Mazzotti et al., 2019).

The collagen fibre size decreased in the following order: 2.26 × 104 μm in the control group; 1.34 × 104 μm in the SPMI group; 0.94 × 104 μm in the MPMI group; and 0.61 × 10 4 μm in the LPMI group.(Mazzotti et al., 2019) Collagen reduction was evaluated based on staining results. In the LPMI group, destruction of the oral epithelium and suboral tissue indicated a reduction in collagen fibres. Conversely, collagen fibres in the control group appeared compact and organised, as indicated by the strong green staining.(Mazzotti et al., 2019).

Patro et al. conducted a study using 150 cadaver, 75 gingival, and 75 buccal samples. The time intervals since death were < 12.5, 12.5–20.5, and > 20.5 h. The authors detected a progressive decrease in collagen fibres, as detected by van Gieson's staining.(Patro et al., 2021).

## 3. Discussion

There are various methods to determine the intervals between deaths, such as observing changes in the body (autolysis, algor mortis, and rigor mortis). However, researchers are still looking for other methods that are more reliable in determining the time interval of death. Histological changes in cells and tissues are one of the proven methods of determining the post-mortem interval by examining body tissues at a given time. (Patro et al., 2021).

Post-mortem tissue changes can be examined histologically; however, tissue fixation must be performed first. After collection from the cadavers and healthy human tissues, 10 % formalin is typically used for fixation.(Fais et al., 2018; Mahalakshmi et al., 2016; Mazzotti et al., 2019; Patro et al., 2021; Yadav et al., 2015) Various staining techniques have been applied, including H&E, Van Gieson's, periodic acid-Schiff (PAS), and Masson Goldner's trichrome staining. Staining techniques are used to visualise important tissue components, i.e., to make the tissue visible.(Alturkistani et al., 2015).

Autolysis can alter the oral mucous membranes, as detected in other tissues in the body. These cellular and tissue changes are considered when utilising the oral mucosa for PMI estimation.(Mazzotti et al., 2019; Yadav et al., 2015) For this purpose, the examination of tissues in the oral cavity is beneficial because these tissues are protected from external factors. Furthermore, the gingiva allows easy and quick access, facilitating sample collection.(Mazzotti et al., 2019).

Changes in tissues are known to occur at the beginning of death and have been well-documented in numerous reports. Yadav et al. reported that homogenisation and eosinophilia occurred within 0–8 h of death, were predominant in the superficial layer, and increased with the time of death. Vacuolisation, karyorrhexis, pyknosis, and karyolysis become more extensive and pass through the epithelial layer at intervals of 8–16 and 16–24 h.(Yadav et al., 2015) Mahalakshmi et al. also detected changes in tissues, such as chromatin clumping, 15 min after death, particularly in the superficial layer. The nuclei exhibited vacuolation in some cells, with the severity increasing with the increasing time interval since death.(Mahalakshmi et al., 2016) The findings reported by Patro et al. supported those of Yadav et al. The authors found the presence of homogenisation, cytoplasmic vacuolisation, nuclear degeneration, and chromatin clumping at the beginning of death, i.e., <12.5 h, with increasing degrees observed with increasing time since death, up to > 20.5 h.(Patro et al., 2021).

Collagen fibres are reportedly reduced in the connective tissues. (Mazzotti et al., 2019) A decrease in collagen fibres can be detected using staining and morphometric measurements, as demonstrated in the study by Patro et al. Using the Van Gieson staining method, a progressive decrease in collagen fibres can be observed with increasing time since death.(Patro et al., 2021).

This literature review has emphasised several aspects. For example, the measurement of tissue changes at each time interval is yet to be standardised. Additionally, certain studies employed markedly short time intervals, which hindered the precise assessment of changes, warranting the examination of longer intervals.

## 4. Conclusion

Histological examination is a technique for estimating the time of death that has great potential in forensic medicine. Changes in cells and

connective tissue as the time of death increases become a guide to determining the post-mortem interval. This technique can be used in cases where macroscopic tissue changes have not been seen and can also be used in cases of late post-mortem interval. Future research should measure these changes at specific intervals to develop a formula for estimating an accurate interval of death using this method.

## Ethical approval

This study obtained the data from online database and did not involve human participants and/or animals. The Ethics Committee of the Faculty of Dentistry, University of Indonesia, has confirmed that no ethical approval is required.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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