

Estimation of postmortem death interval from autopsied tongue tissue: A cross-sectional study

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Abstract

Context: Estimation of time since death is the preliminary step in any postmortem examination. Although there are various physiological methods to conclude the postmortem, interval histological changes can be applied to obtain precision. However, the utility of oral tissues for such an event is still evolving.

Aims: The present study was conducted to assess the efficacy of postmortem histological changes that occur in tongue to conclude the postmortem interval (PMI).

Materials and Methods: After obtaining institutional human ethical committee, tongue tissue was collected for during routine autopsy procedure. The study comprised twelve autopsied tongue tissues. The tissue specimens were subjected to routine laboratory tissue processing procedure and the histological changes were evaluated.

Conclusion: This is the first study of this kind in the scientific literature to explore the tongue tissue to estimate the PMI. There were definite changes in the epithelium and the connective tissue of the tongue, and these features were highly remarkable at various postmortem time intervals.

Keywords: Death interval, histology, oral autopsy, postmortem, time since death, tongue

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INTRODUCTION

Forensic odontology usually involves personal identification and crime investigation through comparative and reconstructive dental identification mainly by analyzing antemortem and postmortem dental records, dental profiling, bitemark, cheiloscropy, rugoscropy, dental DNA analysis, facial reconstruction and superimposition. Most of these procedures involve hard tissue component of the facial and dental apparatus, while the soft tissue analysis is rarely sought (rugoscropy). Histological analysis

of the oral hard tissues is proved and well established in age estimation;^[1] however, microscopic analysis of oral soft tissues in at postmortem examination is at its primitive stage. The most important medicolegal issue in any postmortem examination is to determine time elapsed since death which is also referred as postmortem interval(PMI),^[2,3] and still there are many researches in this regard to get appropriate time of death.^[4] Although the physical signs such as algor mortis, rigor mortis, livor

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mortis, muscle excitability, gastric content, retinal, iris changes and biochemical changes can elucidate the time since death,^[4,5] microscopic changes can be considered as an appropriate method.^[6-8] Hence, in this study, we made an attempt to observe and correlate the various changes in the histology of the tongue with the PMI.

MATERIALS AND METHODS

The study was conducted after obtaining human ethical clearance from Chengalpattu Medical College and Hospital. The study sample included twelve tongue tissue samples collected during routine autopsy procedure at the mortuary in Chengalpattu Government Hospital, Tamil Nadu. The relatives and the investigating police officer were informed about the study details, and written consent was obtained from them. Demographic data and postmortem number of the corpse were collected from the relatives, and there was no gender discrimination. Inclusion criteria considered for the study are as follows: corpse in which death occurred due to road traffic accident without injury to the face and jaws specifically related to tongue. Exclusion criteria comprised all other cases reported to the department of forensic medicine for autopsy, including road traffic accident with injury to the face and jaws related to the study site, pathological lesion involving the study site and frozen corpse. All the twelve specimens were collected at varied time intervals of death.

The specimens were immediately washed under running water and transferred to a container with 10% formalin for fixation. Routine tissue processing was done and stained

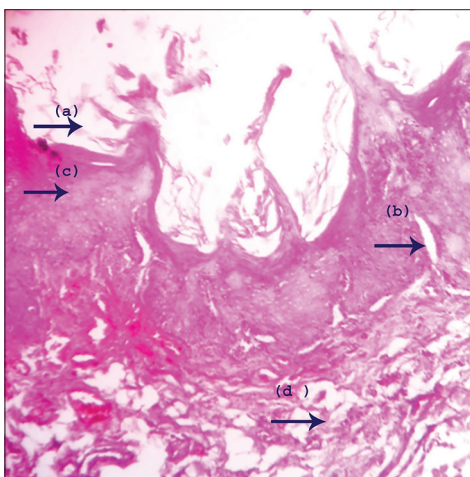


Figure 1: Hematoxylin and eosin-stained tongue tissue section (16–24 hrs) at low-power view (x10) shows (a) epithelial shredding and disruption, (b) split between the epithelium and connective tissue, (c) cytoplasmic eosinophilia, homogenization and karyolysis in the superficial layer and (d) homogenization and clumping of collagen in connective tissue

with hematoxylin and eosin. The microscopic examination of the tissue changes was carried out by two individual observers, and they were blinded to avoid any bias. The histopathological changes at architectural and cellular level were tabulated and correlated with the time interval of death as reported by Yadhav A *et al.*, 2012.^[9]

RESULTS

The observed histological changes in the epithelium and the connective tissue of the tongue are given in Table 1. The histological changes could be categorized as architectural changes, nuclear changes and cytoplasmic changes. The architectural changes include epithelial disruption, shredding and separation of epithelium from the connective tissue and are shown in Figure 1. Nuclear changes and cytoplasmic changes include pyknosis, vacuolation, karyolysis, karyorrhexis, cytoplasmic vacuolation, cytoplasmic eosinophilia and homogenization and are given in Figures 2 and 3. Early stages (0–8 hrs) of PMI show minimal changes at the cellular level; the nuclear changes and the cellular changes are more evident at 8–16 hrs; while cytoplasmic and nuclear alterations, architectural changes are exceedingly conspicuous at 16–24 hrs. The interobserver variability was calculated for various histological changes and is given in Table 2.

DISCUSSION

Death of a person under suspicious condition demands enormous queries about the nature of the incident such as the cause of death and time of death. Although careful physiological examination can unveil the mystery, the forensic experts at time seek specialist opinion to demystify some unknown fact.

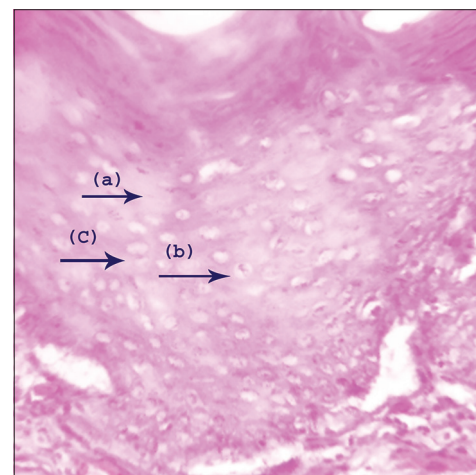


Figure 2: Hematoxylin and eosin-stained tongue tissue section (16–24 hrs) at high-power view (x40) shows (a) karyolysis, (b) pyknosis and (c) nuclear vacuolation

Table 1: Histopathological changes in tongue at different postmortem interval

Features	PMI <8 hrs (n=6)	PMI 8-16 hrs (n=4)	PMI 16-24 hrs (n=2)
Sub-epithelial split	Nil	Nil	Present
Suprabasilar split	Nil	Nil	Nil
Epithelium disruption	Nil	Nil	Present
Epithelium shredding	Nil	Present	Present
Epithelium ballooning	Nil	Spinous and basal layer	Spinous and basal layer
Cytoplasm: Homogenization (%)	100	100	100
Cytoplasm eosinophilia (%)	100	100	100
Cytoplasm vacuolation	Nil	Basal and spinous layer	Basal and spinous layer
Nuclear changes: Karyolysis	Superficial layer	Superficial and spinous layer	Throughout the epithelium
Pyknosis	Superficial layer	Superficial and spinous layer	Superficial and spinous layer
Karyorrhexis	Superficial and spinous layer	Throughout the epithelium	Throughout the epithelium
Nuclear vacuolation	Superficial and spinous layer	Throughout the epithelium	Throughout the epithelium
CT: Distribution of collagen	Homogenized	Homogenized and clumped	Homogenized and clumped
Fibroblast vacuolation	Present	Absent	Absent
Inflammation: Distribution	Focal subepithelial region	Diffuse subepithelial region	Diffuse subepithelial region
Type of inflammation	Absent	Lymphocytes	Lymphocytes
Muscle tissue degradation	Intact	Focal area of degradation	Diffuse area of degradation

CT: Connective tissue, PMI: postmortem interval

Table 2: Kappa test for inter-observer variability

Histopathological changes	κ
Architectural changes	0.824
Cytoplasmic changes	0.556
Nuclear changes	1.000
Connective tissue changes	1.000

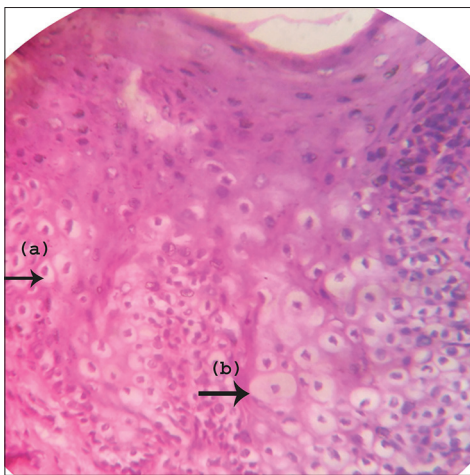


Figure 3: Hematoxylin and eosin-stained tongue tissue section (8–16 hrs) at high-power view (x40) shows (a) cytoplasmic vacuolation and (b) ballooning of epithelial cells at the spinous layer

Once the vital functions are halted, hypoxia is preceded by ischemia, leading to downregulation of oxidative respiration, which at end stage causes irreversible cell injury and cell death. The cell death occurs due to mitochondrial dysfunction, followed by rupture of lysosomal membranes, resulting in enzymatic action.^[4] These changes are sequential and may be correlated with PMI.

The microscopic changes of tongue tissue observed in our study are consistent with the study by Yadhav *et al.*,^[9,10] They have reported cytoplasmic eosinophilia, homogenization and mild nuclear changes in the superficial layers of the

epithelium at early stages of post-mortem (before 8 hrs). Beyond 8hrs nuclear changes such as pyknosis, vacuolation, karyolysis, karyorrhexis and cytoplasmic vacuolation were evident in the spinous layer and mild nuclear changes in the superficial layers of the epithelium at early stages of postmortem, i.e., before 8 h, whereas nuclear changes such as pyknosis, vacuolation, karyolysis, karyorrhexis and cytoplasmic vacuolation were evident in the spinous layer as the PMI prolongs (8–16 h). Epithelial disruption, shredding, separation of epithelium from the connective tissue and clumping of collagen fiber are more evident in the later stages of PMI (16–24 hrs). In addition to these findings, we noticed degradation of muscle tissue in the deeper layers beyond 16 h. In the post-mortem study on gingival tissue by Pradeep *et al.*,^[11] cytoplasmic eosinophilia and homogenization that was noticed within 10 hrs, faded as the time elapsed similar to our study. Whereas cytoplasmic vacuolation present at all stages was more appreciated beyond 20 hrs. They observed nuclear changes at all time intervals in the spinous layer, but intensity varied as the time elapsed. They considered PMI interval of 10 hrs, which contradicted our study (8 hrs time interval), but the findings in both were parallel. Mahalakshmi *et al.*, 2016, in their study on antemortem gingival tissue fixed the tissue at the time range of 15 min, 30 min, 45 min, 1 hrs, 2 hrs and 4 hrs; however, substantial microscopic changes such as homogenization loss of epithelial architecture and nuclear changes were evident at 4th hrs.^[12] Muthukrishnan *et al.* in their study on postmortem gingival specimen considered the time frame of 2 hrs and 4 hrs. In their study, they observed cytoplasmic homogenization and other cytoplasmic and nuclear changes within 2 hrs, but there was no significant difference at 4th hr.^[13]

The interobserver variability test shows that there was 100% ($\kappa = 1.000$) agreement between the observers with

regard to nuclear and connective tissue changes, while 80% ($k = 0.824$) agreement in case of architectural alterations and cytoplasmic changes brought moderate agreement with k value of 0.556, which shows that there is slight confusion over these. The dispute in the interobserver error can be reduced by involving only one researcher for selected variables, restricting the study to the researcher only and repeated review of the parameter.^[14]

The tongue tissue comprising numerous papillae is a specialized mucosa, which shows varied degeneration pattern similar to other types of oral mucosa and can be taken as reliable marker of PMI estimation.

CONCLUSION

Applicability of microscopic alterations in PMI determination is still at its primitive, and this is the first study in the scientific literature to explore the microscopy of tongue tissue for such an event. This study gives a path to future forensic pathologists to consider microscopic changes in tongue as a factor to determine PMI.

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Conflicts of interest

There are no conflicts of interest.

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