

Histopathological Assessment of Autopsied Salivary Gland Tissue to Estimate the Post Mortem Interval – A Cross Sectional Observational Study

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Abstract

Background: In biological system, cells are considered as dynamic and complex structures likewise cellular disintegration is also a complex process which occurs in a sequence. Therefore, these autolytic cellular changes have been investigated by forensic pathologist in an attempt to find markers that may assist in determining the time of death. In this study we attempt to analyse the efficacy of oral soft tissues specifically salivary gland in concluding the time since death.

Methodology: After obtaining approval from the human ethical committee, informed consent from the relative of the corpse and the investigating police officer, demographic information, as well as post mortem number was recorded. Submandibular Salivary Gland was collected for histopathological analysis during routine autopsy procedure. The specimens were fixed immediately in 10% formalin, processed, sectioned and stained with haematoxylin and eosin. The stained sections were evaluated under light microscope for histopathological changes.

Conclusion. The histopathological changes in the autopsied salivary gland tissue can be used as an adjuvant to estimate the post-mortem interval.

Keywords: autopsy, histopathology, oral tissue, post-mortem interval, salivary gland,

Introduction

Forensic investigation is deemed in case of person identification, to conclude the cause of death and to determine the time of death. Accomplishing the post-mortem interval (PMI) time is most challenging task in forensic investigation. There are variety of methods available to conclude the PMI such as measurement

of body temperature (12-24hr), gross changes that occur after death (unreliable), (Loss of corneal reflex and changes in the eye, algor mortis, livor mortis, rigor mortis, decomposition and putrefactive changes), entomology, muscle action potential, biochemical analysis of food in stomach and intestine, electrolytes in blood, CSF, intraocular and synovial fluids, histological and histochemical study of degenerative changes in various organs and tissues, DNA quantification, analysis of organic compounds from the buried soil, radionucleotide assays.¹ Histopathological aspect of the autopsied tissue can predict the vitality at the time of an event, the time interval since a finding was caused. In some instances histopathology can help in reconstructing an event thereby leading the investigating team, hence

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histopathology had become an integral part in forensics medicine.² Of all the merits it is highly reliable and economic.

Pradeep GL et al., quotes that microscopically evident cellular changes in the post mortem tissues can be appreciated only after 10 hours and so they recommend tissue examination at 10hrs interval while Gururaj N and Sivapathasundharam B noticed significant cellular changes at 24 hrs interval.³ Bardale R⁴ observed significant changes between 6-12hrs, Yadhav AB et al., acclaim 8 hrs interval,⁵ However in our study we have considered the minimal time limit to record the histopathological changes ie., 8hrs interval. There are many studies conducted on the histological analysis of labial mucosa and gingiva but studies on salivary gland is least explored specifically related to post-mortem interval and in this study we analysed the histopathological changes of salivary gland to conclude the PMI.

Materials and Methods

The study sample comprised of autopsied *submandibular salivary gland* tissue (n=12) obtained from the Department of Forensic Medicine, Chengalpattu Medical College and Hospital as per Helsinki Declaration (Institutional Ethics Committee Ref No. SBDCH/IEC/10/2018/20). Only those bodies that were not subjected to refrigeration during the period between death and arrival at the mortuary and the instances of death that occurred due to road traffic accident (RTA)

without any injury to face and jaws involving the study site were included in our study while all other cases including RTA with injury and presence of pathological lesion to the facial region and the study site, freezed corpse were excluded from the study.

Demographic information and post mortem number was recorded for all the corpse included in the study. Autopsied *submandibular salivary gland* tissues were further investigated for histopathological alterations. The acquired specimens were immediately fixed in 10% formalin, followed by routine tissue processing and sectioning, later they were stained with haematoxylin and eosin. The stained sections marked with appropriate reference number were examined under a light microscope by two independent observers, the study details were blinded. The observed histopathological changes in the acini (cytoplasm and nucleus) as well the connective tissue were categorized according to the time interval.^{4,5}

Results

The submandibular salivary gland comprising of both serous and mucous acini undergo degeneration at various intervals. Initially the serous acini undergo cellular alterations followed by mucous acini. The acinar architecture is distorted in mucous acini at prolonged time interval. The various histopathological changes are shown in figure 1 and 2. The observed histopathological changes in the mucous, serous acini and the connective tissue are given in table 1.

Table 1. Histopathological changes in post-mortem salivary gland specimen

S.no	FEATURES		PMI <8 hrs (n=10)	PMI 16-24 hrs (n=2)
1	Acinar Architecture	Serous acini	Well Maintained	Not maintained
		Mucous Acini	Well Maintained	Not maintained
2	Loss of Cell Adhesion		Present	Present
3	Cytoplasm Vacuolation		Present	Present
4	Nuclear Changes: Karyolysis		Present	Present

Cont... Table 1. Histopathological changes in post-mortem salivary gland specimen

5	Pyknosis	Present	Present
6	Karyorrhexis	25%	100%
7	Nuclear Vacuolation	100%	100%
8	CT: Distribution Of Collagen	Less Homogenized	More Homogenized
9	Inflammation : Distribution	Diffuse	Diffuse
10	Type Of Inflammation	Lymphocytes	Lymphocytes

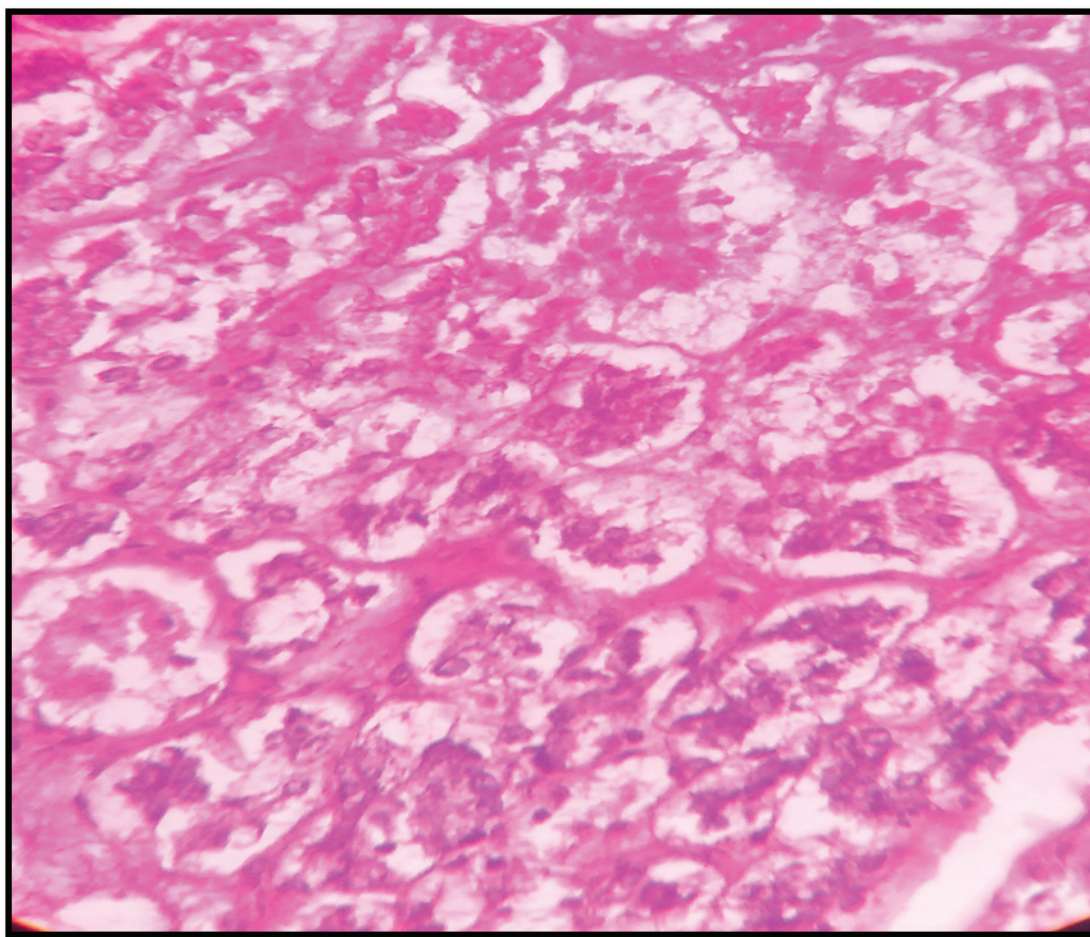


Figure 1. The acinar architecture is maintained in both serous as well as mucous acini at the initial stages but the acini exhibit cellular and nuclear changes.

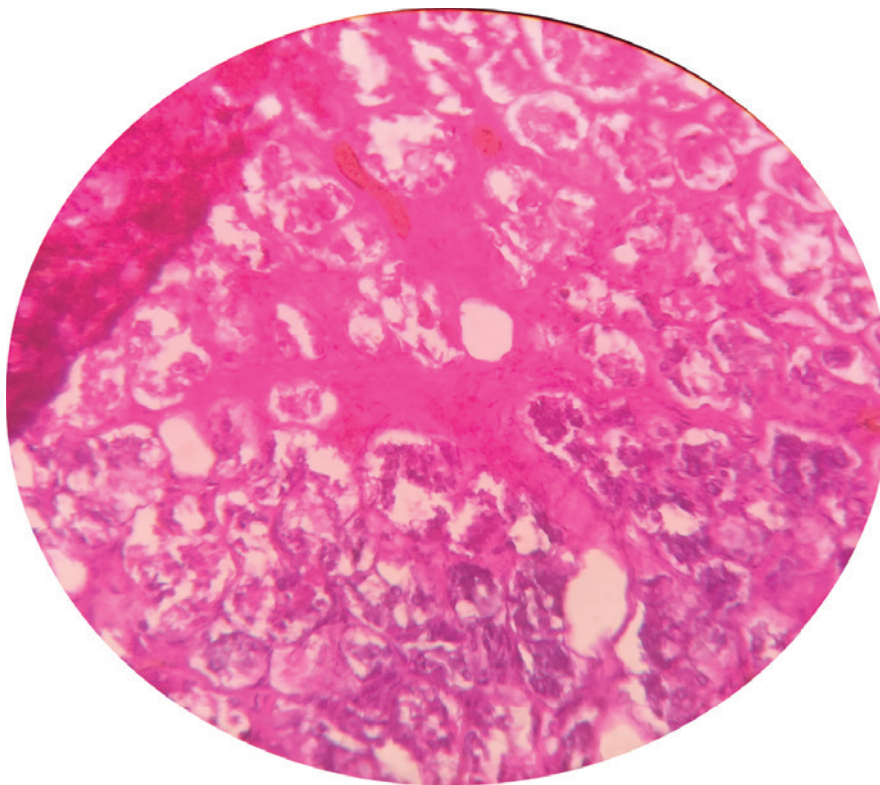


Figure 2. The acinar architecture is distorted in serous and mucous acini at the later stages, also there is homogenization of collagen fibres.

Discussion

Histological assessment of autopsied tissue otherwise known as forensic histopathology clues about the vitality and the time interval of an event such as infliction of wound or aspiration of foreign material. Histopathology also concludes the cause of death,⁶ however the post-mortem interval assessment from the microscopic changes of oral tissues is at primitive stage.

Cellular changes in a naturally degenerating cell involves a complex sequence of events which is usually brought about by variety of enzymes. Tracking these cellular changes at post mortem may clue about many incidents that happened when the individual was alive.³

There are few studies on post-mortem assessment of salivary gland of which one study was related to lymphocytic infiltration in labial salivary gland⁷ while the other was on histological assessment of the salivary gland to study the impact of hanging ligature.⁸ two were

related to age assessment,^{9,10} Nery et al in 2010 studied rat sublingual salivary gland at various post mortem death intervals (0, 3, 6, 12, 24 hrs),¹¹ Bardale R in 2013 studied human submandibular salivary gland to estimate the time since death.

In our study sample of mixed salivary gland (submandibular salivary gland), though there were remarkable cytoplasmic and nuclear changes in both serous and mucous acini they were not so critical to consider as a predictor for post-mortem interval assessment. However, the architectural changes are highly promising, where only the serous acini undergoes degenerative changes at the earlier stage itself. Loss of cell adhesion and cell wall disruption leads to clumping of nucleus to the centre of the acini both serous and mucous forming a filigree pattern in the later stage that is between 16-24hrs. Our study results are consistent with that of Bardale R and Nery et al.,^{4,11} where they have also reported that serous acini are faster to undergo

autolysis while mucous acini undergo degenerative changes close to 24 hrs.

Conclusion

Through this study, it is evident that histopathological changes in mixed salivary gland can aid in predicting post-mortem interval. Though there is environmental influence on the autolysis, different oral tissues from the same person have to be evaluated for exactness of the timing. Also there are very little scientific evidence regarding the utility of microscopic aspect of oral tissues in the prediction of post-mortem interval. The gap must be over-ruled by including forensic dentist and oral pathologist in routine autopsy procedure.

Ethical Clearance – Obtained from Chengalpattu Medical College and from Sree Balaji Dental College.

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Conflict of interest - NIL

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