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*Original Article*

# Evaluation of Histologic Changes of the Human Salivary Glands in Post-mortem Period: A Preliminary Study

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**Abstract**

**Background**: Estimation of time of death is one of the important concerns in autopsy practice. Utility of postmortem autolytic changes occurring in the tissues and tissue fluid for estimation of postmortem interval had been evaluated by number of researchers with varied success. **Aim**: The purpose of present preliminary study was to analyze morphological changes in salivary glands and to identify morphological parameters that could help in determining the time of death. **Material and method**: The study consists of 20 human cadaver comprising 14 male and 6 females and their age ranged from 19 years to 42 years. At autopsy, submandibular salivary glands were collected from the submandibular area through the longitudinal midline incision and subjected for light microscopy. **Result**: The results of present study show that the submandibular salivary glands undergo variable morphological changes in the postmortem period and these cellular changes can be observed by light microscopy. **Conclusion:** Currently there is very limited scientific information available regarding the postmortem autolytic process of salivary glands. Further studies involving cold environmental condition may be productive as it was noticed that mucous acini offer greater resistance for autolysis than serous acini and duct cells.

**Keywords**: Autopsy; Death; Post-mortem interval; Time since death; Salivary gland.

## Introduction

Estimation of time of death is one of the important concerns in autopsy practice. Utility of postmortem autolytic changes occurring in the tissues and tissue fluid for estimation of postmortem interval (PMI) had been evaluated by number of researchers. Various tissues such as heart, liver, kidney, uterus, skin, and its appendages, labial mucosa, gingival epithelium and body fluids such as blood cells and cerebrospinal fluid cells were studied with varied results.[1-16]

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Except for few studies on human and animals[17-21], salivary glands in human cadaver have not been thoroughly evaluated for the purpose of estimation of PMI. Furthermore environmental factors such as temperature and humidity, and postmortem period seem to be important factors affecting the salivary glands in postmortem period. Therefore these aspects also need consideration. The purpose of present preliminary study was to analyze morphological changes in salivary glands and to identify morphological parameters that could help in determining PMI.

## Materials and Methods

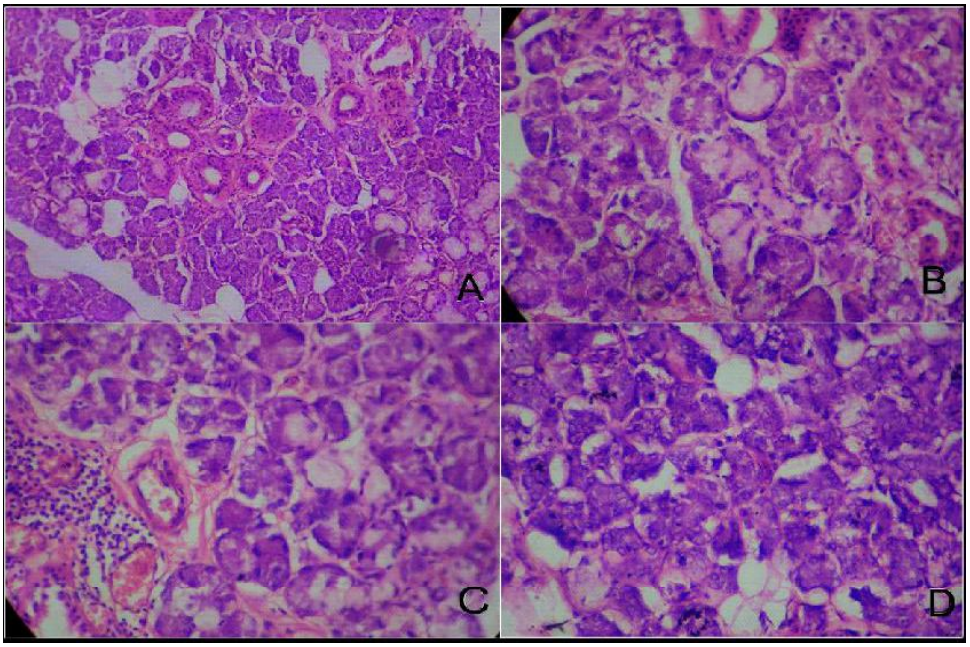
The study was conducted at Department of Forensic Medicine, Govt. Medical College and Hospital, Nagpur from April 2011 to May 2011. The study consists of 20 human cadaver comprising 14 male and 6 females and their age ranged from 19 years to 42 years (mean

age 31.65 years, SD7.60).The death was due to various causes (head injury, n = 12; intracranial hemorrhage, n = 3; consolidation, n = 4; ischemic heart disease, n =1). The bodies were kept at room temperature in the waiting room of mortuary. The average ambient temperature during the period varied from 37° C to 38° C and average humidity ranged from 28% to 37%. At autopsy, submandibular salivary glands were collected from the submandibular area through the longitudinal midline incision.The submandibular gland samples were carefully taken to avoid traumatic artifact. All samples were collected from those bodies where actual time of death was known. All samples were subjected to light microscopy. Tissues were fixed in 10% formalin, embedded in paraffin wax and stained with hematoxylin-eosin (H and E). The salivary glands were observed for mucous acini, serous acini and various ducts. We have excluded cases where there was 1) trauma to face and mandible, 2) acetylcholinesterase inhibitor poisoning and 3) bodies that were kept in cold storage. The cases were divided into 3, 6, 12 and 24 hour interval after death. Only one sample of salivary gland was collected from each cadaver preferably from left side. However, no attempt had been made to note the difference between right and left salivary gland and PMI.

## Results

Before proceeding further, it would be beneficial to revise briefly the normal histology of submandibular gland. Salivary glands are compound tubule-alveolar glands. The secretary elements are called as acini or alveoli. The acini lead into a series of ducts such as intercalated ducts, intralobular ducts and interlobular ducts. On microscopic

**Fig 1: Microphotographs showing Section Through Salivary Gland. A-3 Hours: Mixed Acini showing Serous Cells having Granular and Vacuolated Cytoplasm, Mucous Cells Intact, Nuclei of Both Acini Pyknotic and Duct Cells shows Focal Disruption of Cellular Integrity with Pyknotic Nuclei. (H and E, X 40); B-6 Hours: Mixed Acini showing Intermediary Stage of Autolysis, Acini shows Vacuolated Cytoplasm and Duct Cells shows Focal Disruption (H and E, X 40); C-12 Hours: Serous Acini Cell Ruptured with Nuclei showing Pyknosis and Karyorrhexis, Mucous Cells Begin to Rupture (H and E, X 40); D-24 Hours: shows Stage of Advanced Autolysis with Disorganized Glandular Structure with Ruptured Serous Acini Dispersed amongst Mucous Acini (H and E, X 40)**



examination one can observed closely packed acini with duct scattered between them and supported by connective tissue. The cells lining the acini are either serous or mucous. On H and E staining, serous cells stain dark because of zymogen granules while mucous cells stain light. In submandibular gland both serous and mucous acini are present. The mucous acini are frequently capped by serous crescents or demilunes. [22]

The results of present study show that the submandibular salivary glands undergo variable morphological changes in the postmortem period and these cellular changes can be observed by light microscopy.

By the PMI of 3 hours, morphological cellular elements showed initiation of degenerative process. The section shows mixed population of serous and mucous acini. In most of the cases (n = 16, 80%), cytoplasm appeared granular while in few vacoulation was evident in serous cells. The cytoplasm of mucous cells appeared normal. The nuclei appeared pyknotic in both type of acini. In few cases (n = 5, 25%) the cellular integrity appeared disrupted focally in duct cells with nuclei appeared pyknotic (Fig 1 A).

During PMI of 3-6 hours, nearly all the acini exhibited intermediary stage of autolytic degeneration. In most of the cells of serous acini the cytoplasm was vacuolated while in few cases, the cell membranes were disrupted focally. The nuclei appeared pyknotic. Mucous cells showed changes in cytoplasm that varied from granulations to vacoulation with pyknotic nuclei. The cell membrane appeared intact. The duct cells showed focal disruption of membrane with pyknotic nuclei (Fig 1 B).

During PMI of 6-12 hours, a mixed picture was noted. Most of the cells of serous acini(n = 18, 90%) were ruptured with nuclei showed pyknosis and karyorrhexis. Mucous cells began to rupture and showed pyknotic nuclei. The duct cells showed disruption of membrane with nuclei showed pyknosis and karyorrhexis (Fig 1 C).

With advancing PMI at 12-24 hours, a stage of advanced autolysis was evident with complete disorganization of the glandular structure with ruptured and disrupted serous acini was noted. The nuclear changes consisted of pyknosis and karyorrhexis. Mucous acini could be identified admixed the disintegrated serous acini. The nuclei were pyknotic. The duct cells showed near complete disintegration of cellular integrity. The nuclei showed pyknosis and karyorrhexis (Fig 1 D).

## Discussion

In biological structure, cells are considered as dynamic and complex structures. Cellular death is a state of irreversible injury. After death cellular disintegration occurs in phased manner due to autolysis. The rate of autolysis varies from organ to organ and depends upon number of factors including the hydrolytic enzymes content of the cell. 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.[21]

As far as English literature is concerned, few studies were published related to evaluation of salivary glands in postmortem period.[17-21] Azevedo *et al* (2005) and Moreira *et al* (2006) had studied these glands to assess the age-related differences in the sublingual glands. Tekeda *et al* (1986) studied salivary glands for ascertaining the importance of focal lymphocytic infiltration whereas Dixit *et al* (2001) examined the salivary glands in hanging cases to know whether changes that occurred in these glands may assist to know antemortem nature of the ligature mark. [17-20]However, these studies did not evaluate the salivary glands for the purpose of finding parameters that may assist in determining the time of death. Nery *et al* (2010) had analyzed and quantified morphological acinar postmortem changes in sublingual glands of rats. Aciner autolytic changes were studied at 0, 3, 6, 12 and 24 hour postmortem period. At 0 hour PMI, the glandular structure was found intact. However, with advancing PMI loss of cell limit integrity, granulations in

cytoplasm and nuclei alterations were noted. At PMI of 24 hour, total disorganization of the glandular structure was evident.[21]

Considering the present study, we have sequentially studied the histologic changes in the salivary glands in same environmental conditions with average ambient temperature varied from 37 °C to 38 °C and average humidity ranged from 28% to 37%. With advancing PMI, it was observed that the submandibular salivary gland undergoes variable degree of morphological changes in comparison with progressive morphological changes observed in other body tissues and body fluid.[1-16] By the PMI of 3 hours, the autolytic changes began in serous acini and duct cells. With advancing PMI, the autolytic processes appear accelerated with erratic morphological changes. As the death interval prolonged, the cytoplasm appeared granular and then vacuolated. The nuclear changes consisted of pyknosis and karyorrhexis. The cells began to lose cellular integrity. Eventually there was disintegration and disorganization of morphological features of the gland within 24 hours of death. Moreover, it was noted that mucousacini offer greater resistance to autolysis than serous acini and duct cells.

The findings of the present study are in accordance with findings observed by Nary *et al* (2010) on rat sublingual salivary glands. However, the cellular autolytic changes in present study appear little bit earlier than those of Nary *et al* (2010). The difference could be attributed to environmental difference or biological differences. Environmental temperature is one of the important factors that affect the autolysis. The present study was conducted in hot months of the year and therefore the autolytic changes might have occurred earlier. As far as biological difference is considered, the present study examined human cadaver submandibular salivary glands whereas Nary *et al* (2010) had utilized rat sublingual salivary glands. Apart from environmental temperature, probably this might be one of the reasons for early autolytic change. If biological difference is considered as one of the factor affecting autolytic change then it could be suggested that a different rate of autolysis exists between human cadaver and rat or animal cadaver. The difference in rate of autolysis might be and partly because of difference in metabolic activity or due to difference in enzymatic activity of cells.

In contrast to present observations, where it was observed that autolytic changes occur earlier in serous acini and duct cells,Azevedo *et al* (2005) and Moreira *et al* (2006) had noted delayed autolytic changes in duct cells in comparison with acini cells.[17,18] The variation in autolytic changes in human salivary glands may be attributed to environmental difference.

Currently there are limited scientific information available regarding the postmortem autolytic process of salivary glands. This study is amongst the few studies that had utilized autolytic changes in human salivary glands for determining time of death. [17-21] However, this study is limited by small sample size and evaluation of histologic changes in a common environmental setting. Further studies are required to evaluate these alterations and characterize the postmortem changes in different environmental changes before one could leave such topic because of earlier occurring autolytic changes as observed in the present study.

In view of findings observed in present preliminary study, it can be concluded that the morphological changes in human submandibular gland do not exhibit potential to estimate time of death in early postmortem period. In hot environmental conditions, the autolytic changes occur earlier and the changes are erratic one and hence preclude for proper interpretation and application. However, studies involving cold environmental condition may be productive as it was noticed that mucous acini offer

greater resistance for autolysis than serous acini and duct cells.

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