ORIGINAL ARTICLE

Histological assessment of cellular changes in gingival epithelium in ante-mortem and post-mortem specimens

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Abstract

Background: Death is an irreversible cessation of circulation, respiration and cerebral function. A number of continuous changes occur in the period after death, and these vary according to the duration of time interval and circumstances of death. Since the rate of post-mortem changes varies between different individuals and there exists a range of normal values during life, extrapolation of the time since death will always reveal an interval, not an exact time point at which death occurred. **Objectives:** This study was conducted to demonstrate the features of decomposition at the cellular level in post-mortem gingival tissues at different time intervals after death and observe the cellular changes in the unfixed ante-mortem gingival tissue at regular time intervals. **Materials and Methods:** Thirty post-mortem and 30 ante-mortem gingival samples were seen microscopically to observe the cytoplasmic and nuclear degenerative changes. The proportion of cells exhibiting nuclear and cytoplasmic alterations in post-mortem gingival samples, during different time intervals, were compared using Chi square test. **Results and Conclusion:** There was a significant association between the time intervals and degenerative changes. The initiation of the decomposition process at the cellular level begins within 10 hours after death and the other clinical features of decomposition occur subsequently.

Key words: Apoptosis, cell death, necrosis, postmortem changes, gingiva, ante-mortem

Introduction

Living cells are extremely complex units, organized for the synthesis of substances essential for their own survival and for producing specialized products specific to the type of cell.[1]

Cell death is a state of irreversible injury.[2] It occurs in a variety of physiological and pathological settings in multi-cellular organisms.[3] It occurs by two modes - *apoptosis*, a programmed, ordered form of cell death, and *necrosis*, an unordered and accidental form of cell death.[4] *Autolysis* is disintegration of the cell by its own hydrolytic enzymes liberated from lysosomes. It can occur in the living body when the cell is surrounded by an inflammatory reaction, or may occur as a post-mortem change with complete absence of surrounding inflammatory response.[2]

Decomposition is a mixed process ranging from cell autolysis due to internal chemical breakdown to tissue autolysis due to liberated digestive fluids. Initial decomposition rate depends on the body conditions and environment. It usually begins after two/three days in an unrefrigerated corpse.[5]

Post-mortem is an examination and dissection of a dead body to determine cause of death or the changes produced by disease, also called *necropsy* or *autopsy*. The body continues to change after death and understanding these changes is of major importance in medico-legal practice.[6] The changes that occur in the body are called post-mortem changes. These changes include autolysis, algor mortis, rigor mortis, livor mortis, postmortem clotting, putrefaction and adipocere.[7]

Materials and Methods

This study was conducted on gingival tissues obtained from deceased individuals collected from the Department of Forensic Medicine, Kempegowda Institute of Medical Sciences, Bangalore.

The study group consisted of:
Post-mortem samples:

a. Ten gingival samples obtained from unrefrigerated deceased individuals, within 0-10 hours of death.
b. Ten gingival samples obtained from unrefrigerated deceased individuals, within 10-20 hours of death.
c. Ten gingival samples obtained from unrefrigerated deceased individuals, within 20-30 hours of death.

Ante-mortem samples:

a. Ten gingival samples from clinically healthy individuals following crown-lengthening procedure, fixed at 10th hour.
b. Ten gingival samples from clinically healthy individuals following crown-lengthening procedure, fixed at 20th hour.
c. Ten gingival samples from clinically healthy individuals following crown- lengthening procedure, fixed between 20-30 hours.

Control group consisted of:

Five gingival samples from clinically healthy individuals following crown-lengthening procedure, fixed immediately.

Thirty gingival samples from the labial gingiva were obtained from deceased individuals. The samples were fixed in 10% formalin immediately. The specimens were processed, sectioned and stained with hematoxylin and eosin and examined under the microscope to determine the post-mortem changes.

Ten gingival samples were obtained from healthy individuals following crown-lengthening procedures. Out of the ten samples, five were divided into four bits and five into three parts (A, B and C). 'A' was fixed in 10% formalin at the 10th hour; 'B' and 'C' were fixed at the 20th hour and between 20-30 hours respectively. The fourth bit of the first five samples were used as controls and were fixed immediately. The specimens were processed, sectioned and stained with hematoxylin and eosin and examined under the microscope to assess the gingival architecture.

The hematoxylin and eosin stained paraffin embedded sections of ante-mortem and post-mortem gingival specimens were examined to assess the cytoplasmic and nuclear changes in the epithelial cells. Ten cells were examined in three high power fields, pertaining to the different epithelial cell layers, i.e. the basal layer, prickle cell layer and superficial layer.

The cytoplasmic features considered were:

- Eosinophilia: The earliest change in the dead cell which stains as bright pink [Figure 1].
- Homogenization: Following eosinophilia, at the tissue level, there is masking of cellular outlines leading to an appearance resembling a glossy, homogenous mass which is also eosinophilic [Figure 2].
- Vacuolation: Small clear vacuoles seen within the cytoplasm representing vacuolar degeneration [Figure 3].

The nuclear features considered were:

- Chromatin clumps: The chromatin is fragmented and spread out within the nucleus and nucleolus is not clearly visible [Figure 4].
- Pyknosis: It is characterized by nuclear shrinkage and increased basophilia where the nucleus resembles an 'ink – spot' [Figure 5].
- Vacuolation: This is characterized by formation of vacuoles within the nucleus [Figure 6].

Based on the degree of cellular changes, the initiation of decomposition was assessed in the post mortem samples. In ante-mortem samples, the cellular changes were noted with respect to the time of delay in fixation.

Results

On comparing cytoplasmic and nuclear changes in ante-mortem and post-mortem samples, at different time intervals, it was found that there was a significant association between time intervals and changes in ante-mortem [Table 1] and post-mortem samples [Table 2].

The following cytoplasmic changes were observed in both ante-and post-mortem samples:

- Cytoplasmic vacuolation was the predominant feature, present throughout the thickness of the epithelium. It was most prominent in the prickle cell layer. Cytoplasmic vacuolation was present in samples collected at all time intervals, but it was very significant in samples collected beyond 20 hours of death.
- Homogenization and eosinophilia were seen predominantly in the superficial layers of epithelium *only in the post mortem samples.* These changes were prominent in samples collected within 0-10 hours of death. A few cells in samples collected between 10-20 hours of death exhibited eosinophilia in the superficial layer. These findings lead one to conclude that eosinophilia and homogenization are the initial

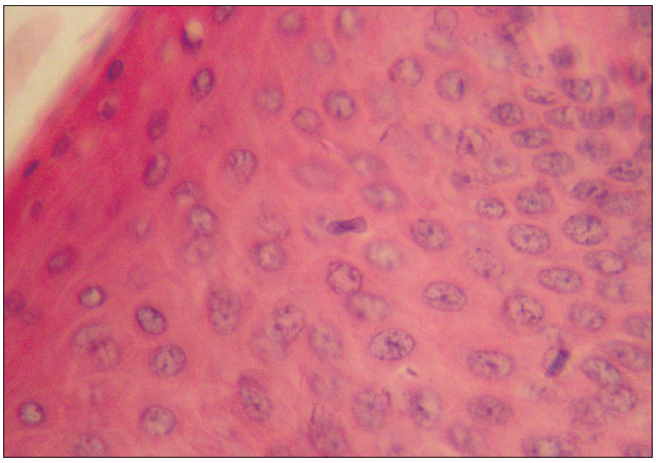

Figure 1: Photomicrograph showing eosinophilia (400×)

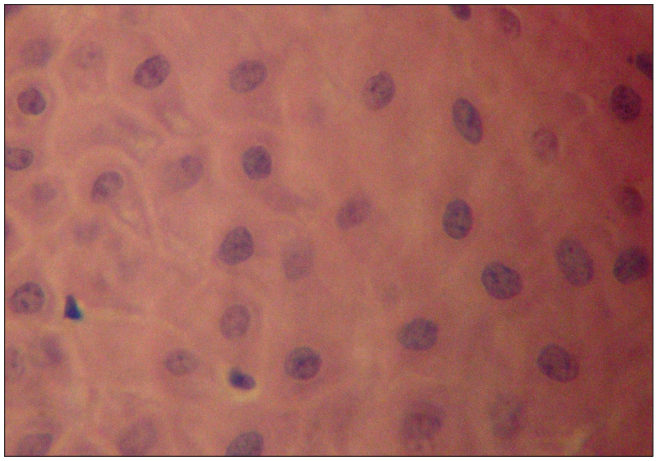

Figure 2: Photomicrograph showing homogenization (400×)

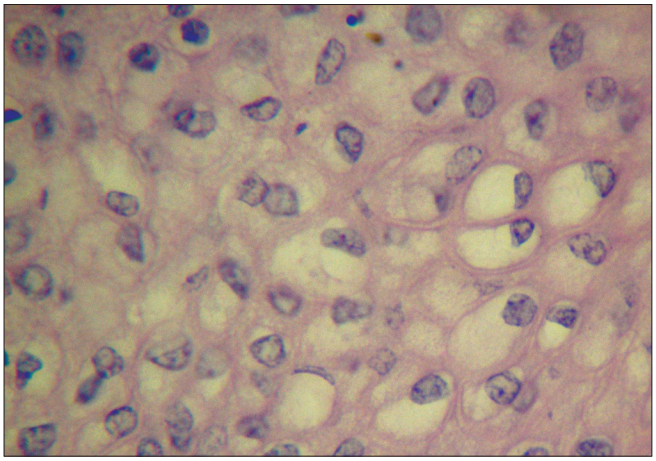

Figure 3: Photomicrograph showing cytoplasmic vacuolation (400×)

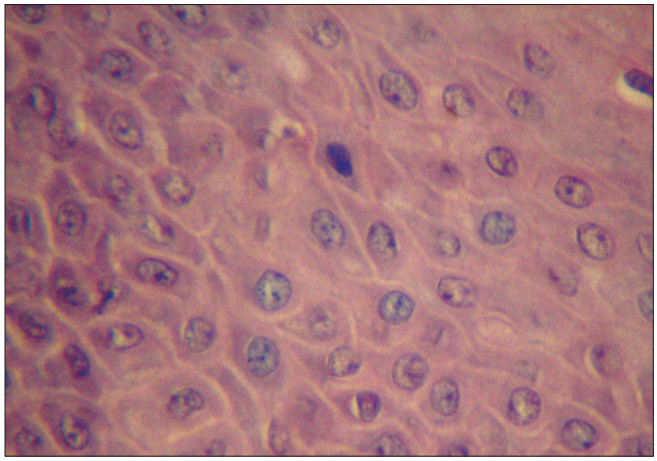

Figure 4: Photomicrograph showing chromatin clumps (400×)

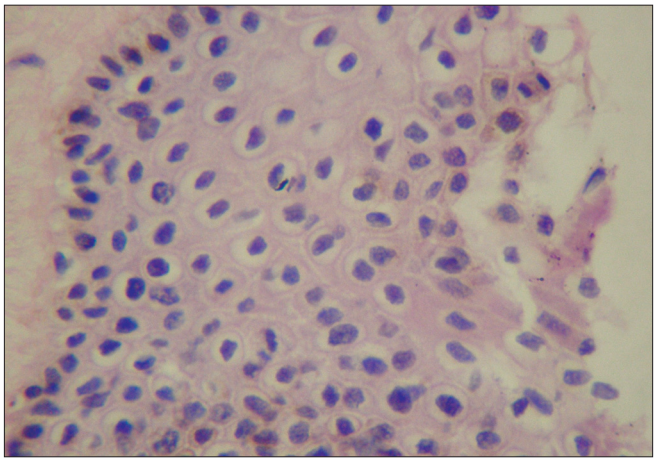

Figure 5: Photomicrograph showing pyknosis (400×)

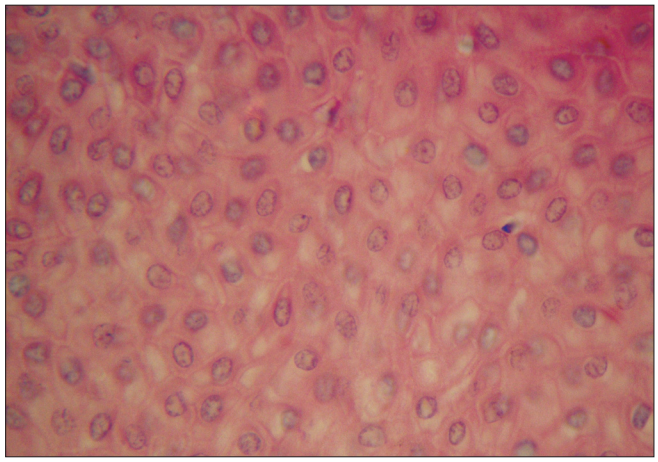

Figure 6: Photomicrograph showing nuclear vacuolation (400×)

changes visible in the cytoplasm of the dying cell, and as time passes, these changes disappear. There are two interesting points to be noted here:

- The disappearance of these two changes with the passage of time is not seen to be compensated by a proportionate increase in cytoplasmic vacuolation.
- The ante-mortem samples did not show eosinophilia and homogenization in any of the time periods. Could this be an indication of earlier onset of decomposition in these samples? This needs to be explored further by studying ante mortem samples at an earlier and more frequent time intervals.

Table 1: Comparison of total number of cells showing cytoplasmic and nuclear changes in ante-mortem samples at different time intervals

Ante mortem	0-10 hrs	10-20 hrs	>20 hrs	*P*-value
Cytoplasmic changes	57	90	106	
Nuclear changes	228	269	262	0.03
Total	285	359	368	

Table 2: Comparison of cytoplasmic and nuclear changes in post-mortem samples at different time intervals

Post mortem	0-10 hrs	10-20 hrs	>20 hrs	*P*-value
Cytoplasmic changes	320	104	86	
Nuclear changes	468	300	480	<0.001
Total	788	404	566	

In comparison to cytoplasmic vacuolation these two changes were seen in very few cells and hence, have not been considered in further comparisons.

The following nuclear changes were observed:

- Chromatin clumping was the most prominent nuclear change. It was most conspicuous in the prickle cell layer, while a few cells in the superficial layer exhibited this change. It was most significant in samples collected within 0-10 hours of death. From this we can conclude that this is the initial nuclear change visible in a dying cell.
- Pyknosis was most obvious in the prickle cell layer in samples collected at all the time intervals. Basal layer displayed the least number of cells with this nuclear change.
- Nuclear vacuolation was observed only in few cells in the prickle layer in samples collected at all the time intervals.

When we compare changes in the ante-mortem and post-mortem samples, the following observations can the made:

- In the 0–10 hour time period, many cells show pyknosis and nuclear vacuolation (statistically significant difference) in the ante-mortem than post-mortem samples.
- In the 10-20 hour time period, many show cytoplasmic vacuolation along with pyknosis in ante-mortem than post-mortem samples, but nuclear vacuolation is significantly more in the post-mortem samples.
- In the samples greater than 20 hours time period, chromatin clumping and nuclear vacuolation were more prominent in the post-mortem than ante-mortem samples (statistically significant difference) [Table 3].

Table 3: Comparison of cytoplasmic and nuclear changes in prickle cell layer at different time intervals

Time	Change	Ante mortem (n=900)	Post mortem (n=900)	*P*-value
10th hour/0-10 hrs	Cytoplasmic	57	45	0.221
10th hour/0-10 hrs	Nuclear	228	260	0.09
20th hour/10-20 hrs	Cytoplasmic	90	66	0.044
20th hour/10-20 hrs	Nuclear	269	264	0.796
>20 hrs	Cytoplasmic	92	84	0.526
>20 hrs	Nuclear	262	312	0.011

Discussion

A number of continuous cellular alterations occur in the period after death, and these vary according to the time interval and circumstances of death.[8] At the cellular level, initially, no alteration in the structure is visible. In the dying cell, respiration ceases, glycolysis proceeds for a while and a drop in pH results due to the production of lactic acid. The synthetic activities of the cell stop, but lytic destructive enzymes continue to work. These enzymes are mostly active at a low pH. The cell thus undergoes a process of autolysis.[1]

In this study, the hematoxylin and eosin stained paraffin embedded sections of gingival specimens were examined to assess the cytoplasmic and nuclear changes in the epithelial cells. Ten cells were examined in three high power fields, pertaining to the different epithelial cell layers. The number of cells in each high power field showing the above stated features were counted. Based on the degree of cellular changes, the initiation of decomposition was assessed in both the ante- and post-mortem samples.

The process of fixation was standardized such that the same preparation of 10% formalin was used in the fixation of all ante-mortem and post-mortem samples collected for the study. Since fixation artifacts resemble the morphological changes in post mortem tissue decomposition, standardization of fixation was a necessary prerequisite.

The gingival samples were not collected from individuals dying due to poisoning. Poisons have been found to cause degenerative changes in target organs.[9]

On the basis of these observations, we could probably conclude that decomposition sets early in the ante-mortem samples compared to post-mortem samples. This could probably be due to the rate of decomposition being accelerated when tissue is removed from the whole body in small bits, as was done in the case of the ante-mortem samples.

In a similar study done by Gururaj N and Sivapathasundaram B (2004)[5], 20 post-mortem gingival samples, obtained between 10-20 hours from the time of death were fixed in 10% formalin immediately, processed routinely, sectioned

and stained with hematoxylin and eosin. The gingival specimens were examined to assess the cytoplasmic and nuclear changes in the epithelial cells. Post mortem gingival samples showed nuclear vacuolation in spinous and superficial layers of the epithelium, suggestive of autolysis. They also studied 10 ante-mortem gingival samples, each divided into three parts, one-third of each gingival tissue was fixed immediately in 10% formalin, one-third after 24 hours and the rest after 48 hours. Ante-mortem gingival samples fixed immediately showed no changes in the epithelium, whereas sections fixed after 24 hours showed focal vacuolated changes indicating the progression of decomposition after death. Gingival samples fixed after 48 hours showed diffuse vacuolated changes throughout the epithelium, similar to those seen in the post mortem gingival samples. They concluded that the initiation of decomposition at the cellular level appeared within 24 hours of death and the other clinical features of decomposition occur subsequently.

However this method is hampered by lack of standardization in fixation techniques, since fixation artifacts resemble the morphological changes in post mortem tissue decomposition and the cellular changes observed in the present study were limited to a period of 30 hours from time of death.

This study demonstrates that further study and research, on a large scale, with specimens fixed at more frequent time intervals are indicated to establish the exact time of initiation of decomposition at the cellular level. Further studies, with collection of samples from dead individuals, beyond 30 hours from the time of death, is suggested to observe cellular changes as further decomposition occurs.

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