ORIGINAL ARTICLE

Documentation of postmortem changes in salivary gland architecture and staining characteristics

Swati Agarwal,
Minal Chaudhary,
Madhuri Gawande,
Puneet Gupta[1]
Department of Oral Pathology and Microbiology, Datta Meghe Institute of Medical Sciences, Wardha, Maharashtra,
[1]Department of Oral and Maxillofacial Surgery, Surat Municipal Institute of Medical Education and Research, Surat, Gujarat, India

Address for correspondence:
Dr. Swati Agarwal,
Department of Oral Pathology and Microbiology, Datta Meghe Institute of Medical Sciences, Wardha - 442 004, Maharshtra, India.
E-mail: drswati2108@gmail.com

Abstract

Context: Estimation of time passed since death continues to be a major problem for the forensic pathologist and its determination plays an important and vital role in medico-legal cases. The histological studies on various tissues after death have been mostly confined to single organ or tissue by individual workers at different atmospheric conditions. **Aims:** The aim of this study is to determine the best rehydrating solution for dehydrated tissues in postmortem examination. **Settings and Design:** This study was specific to salivary gland tissues and certain pattern of changes were determined during postmortem time intervals using hematoxylin and eosin stain and special stains like mucicarmine and alcian blue. **Materials and Methods:** The study was divided into two groups. (1) Group A: Normal tissue samples (twenty normal salivary gland tissue samples left without fixation for varying periods of time). (2) Group B: Control group (twenty normal salivary gland tissue samples immediately fixed in formalin). The three different rehydrating agents used in this study were glycerol, normal saline and modified Ruffer solution. **Statistical Analysis Used:** Not required. **Results:** Modified Ruffer solution is the best when compared to glycerol and normal saline for rehydration of dehydrated tissues. **Conclusions:** Thus in our study we conclude that the tissue which had been dehydrated at the crime scene for a fairly long period showed better rehydration with modified Ruffer solution and yield good cellular and nuclear details.

Key words: Alcian blue, forensic, modified Ruffer solution, mucicarmine, postmortem, stain

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 multicellular 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] Estimation of time since death is one of the

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most important objectives of postmortem examination. Estimation of time passed since death continues to be a major problem for the forensic pathologist and its determination plays an important and vital role in medico-legal cases.[5] During last decades, many postmortem interval studies referring to the establishing of biochemical, histological, histochemical, and ultrastructural changes in different tissues and organs have been done.[6] The body continues to change after death and understanding these changes is of major importance in medico-legal practice. These changes include autolysis, algor mortis, rigor mortis, livor mortis, postmortem clotting putrefaction, and adipocere.[7] Attempts have also been made to determine time passed since death by studying biochemical changes in blood, cerebrospinal fluid, and intraocular fluids. The biochemical methods have been found to be of not much use once the decomposition changes start. Time bound histological and histochemical study of degenerative changes in various organs and tissues may yield better information. The histological studies on various tissues after death have been mostly confined to single organ or tissue by individual workers at different atmospheric conditions.[5] However, histologic studies are technique sensitive and as by nature postmortem tissue tends to be dehydrated. The processing of such tissues has an important role to play in the final information available.

The aim of this study is to determine the best rehydrating solution for dehydrated tissues in postmortem examination. This study was specific to salivary gland tissue, and certain pattern of changes was determined during postmortem time intervals using hematoxylin and eosin (H and E) stain and special stains like mucicarmine and alcian blue.

Materials and Methods

This study was conducted on the normal salivary gland tissue taken from the resected specimens of the oral squamous cell carcinoma patients. The consent from the patient was taken.

The study was divided into two groups:

- Group A: Normal tissue samples (twenty normal salivary gland tissue samples left without fixation for varying periods of time). Group A was again subdivided into five groups depending upon time interval from living to removal state:
  a. 0–6 h
  b. 0–12 h
  c. 0–24 h
  d. 0–48 h
  e. 0–72 h.

- Group B: Control group (twenty normal salivary gland tissue samples immediately fixed in formalin).

Following procedure was conducted:

- Step 1: Group A tissue samples were dehydrated for a fixed period and then rehydrated for the similar period i.e.,
  0–6 h
  0–12 h
  0–24 h
  0–48 h
  0–72 h.

The three different rehydrating agents used in this study were glycerol, normal saline and modified Ruffer solution.

- Step 2: The above rehydrated samples were then fixed in formalin and were subjected to regular histological processing
- For the control group, step 1 was omitted.

Different rehydrating solutions used in our study were:

- Modified Ruffer solution: The Ruffer rehydration method is attractive because of its simplicity and the availability of the solution constituents. Ruffer's original formula called for 30 cm^3 of alcohol, 50 cm^3 of water, and 20 cm^3 of a 5% solution of carbonate of soda. This solution was slightly modified by Walker *et al*. In that study, the solution consists of:
  - 10 g of Na_2CO_3 (sodium carbonate)
  - 316 mL of 95% ethanol
  - 684 mL of distilled water.

Because it provided more specific descriptions of the solution constituents, we employed the Walker *et al*. adaptation.[8]

- Glycerol: It is a colorless, odourless and viscous liquid that can be used as a rehydrating solution
- Normal saline: It is the most commonly used rehydrating agent and is a sterile, nonpyrogenic solution for fluid and electrolyte replenishment and contains no antimicrobial agents. Also, it contains 9 g/L sodium chloride
  - The sections were then embedded in paraffin wax and subjected to routine H and E stain and special stains such as alcian blue and mucicarmine
  - Procedure for mucicarmine staining.

Hematoxylin (20 min) and water wash
↓
1% acid alcohol (1 dip) and water wash (5 min)
↓
Mucicarmine (30 min) and water wash
↓
Rinse with absolute alcohol
↓

Air dry, clearing in xylene and mounting.

- Procedure for alcian blue staining

Acetic acid 1 dip (pH 2.5)
↓
Alcian blue (5 min)
↓
Blott and air dry
↓
Neutral red (5 min) and then water wash
↓
Rinse with absolute alcohol
↓

Airdry, clearing in xylene and mounting.

- The obtained slides were examined under light microscope for studying the various histological changes that take place in salivary gland tissue at different time intervals from living to removal state.

Results

The various gross changes in the tissues when kept in different rehydrating agents were [Figure 1].

All the tissue sections grouped under Group A and Group B were stained with H and E stain and the various morphological changes, staining characteristics and the architecture were observed by light microscope.

Under H and E stained sections the various changes observed at different time intervals were:

- Control group (tissue immediately fixed in formalin) [Figure 2]
- The various changes observed at 6 h of dehydration and rehydration are shown in Figure 3
- The various changes observed at 12 h of dehydration and rehydration are shown in Figure 4
- The various changes observed at 24 h of dehydration and rehydration are shown in Figure 5
- The various changes observed at 48 h of dehydration and rehydration are shown in Figure 6
- The various changes observed at 72 h of dehydration and rehydration are shown in Figure 7.

To summarize, changes observed under H and E stained sections are shown in Table 1.

Thus, when seen under H and E stained sections, it can be said that rehydration in modified Ruffer solution yields the best result when compared to normal saline and glycerol.

- Also, special stains were used such as mucicarmine, which had shown expressive staining on immediately formalin fixed salivary gland tissue [Figure 8]
- Thus, it can be summarized, that poor staining characteristic was seen in tissue stored in normal saline, better for glycerol and best for modified Ruffer solution [Figures 9-12]
  Another special stain, alcian blue was applied to the respected samples which is a copper phthalocyanin dye and shows positive staining for sulfated and carboxyl radicals of the acid mucin.
- Thus, we can summarize that glycerol till the end had shown bright staining and less shrinkage compared to normal saline and modified Ruffer solution [Figures 13-15 and Table 2].

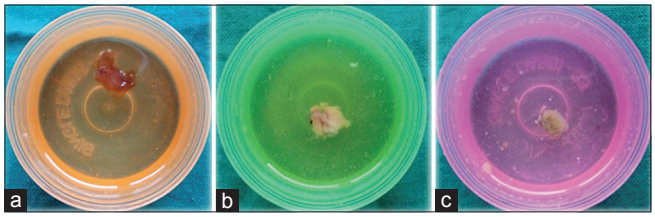

Figure 1: (a) Glycerol: Tissue was darker in color. (b) Normal saline: There was fraying of tissue. (c) Modified Ruffer: Tissue was compact without change in color

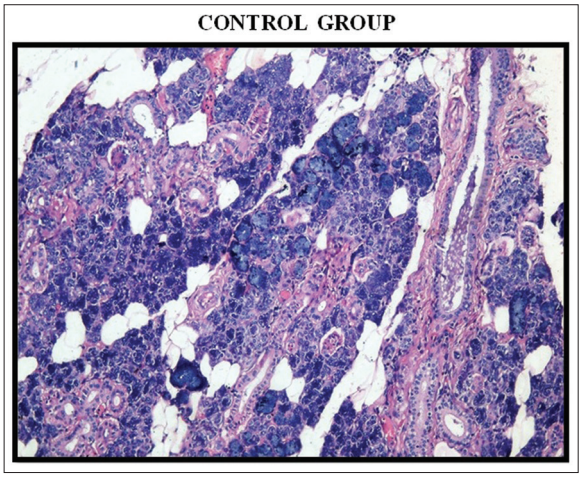

Figure 2: (i) Distinct cellular and nuclear morphology. (ii) Good staining characteristics. (iii) Maintained architecture

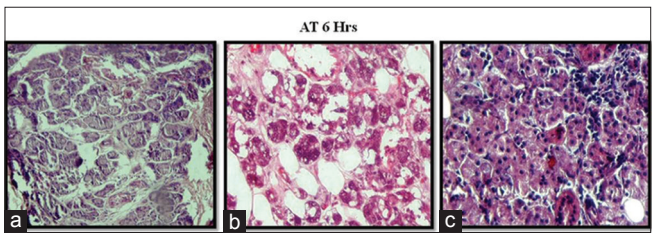

Figure 3: (a) Glycerol: There was slightly dark hematoxylin. (b) Normal saline: There were indistinct nuclear morphology and dark eosin. (c) Modified Ruffer: Staining characteristics were similar to control group

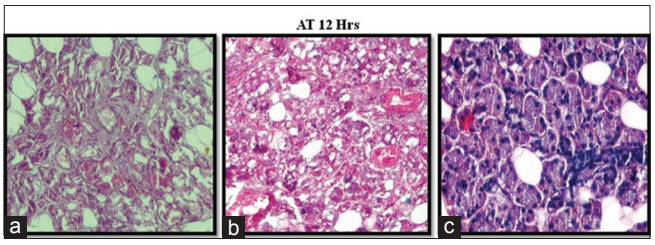

Figure 4: (a) Glycerol: Staining characteristics were faint. (b) Normal saline: There was indistinct nuclear morphology and hematoxylin was faint. (c) Modified Ruffer: Staining characteristics were similar to control group

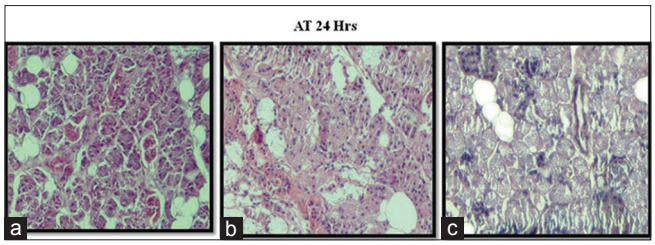

Figure 5: (a) Glycerol: Staining characteristics were faint. (b) Normal saline: There was indistinct cellular and nuclear morphology. (c) Modified Ruffer: Eosin was faint

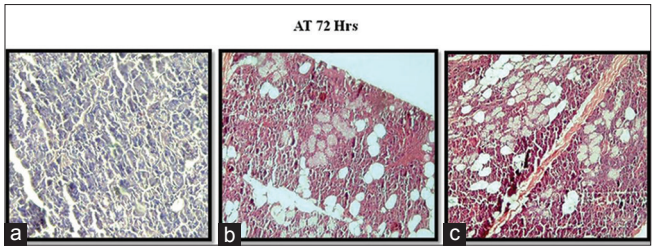

Figure 7: (a) Glycerol: There was loss of architecture. (b) Normal: There was loss of architecture. (c) Modified Ruffer: Architecture was somewhat maintained

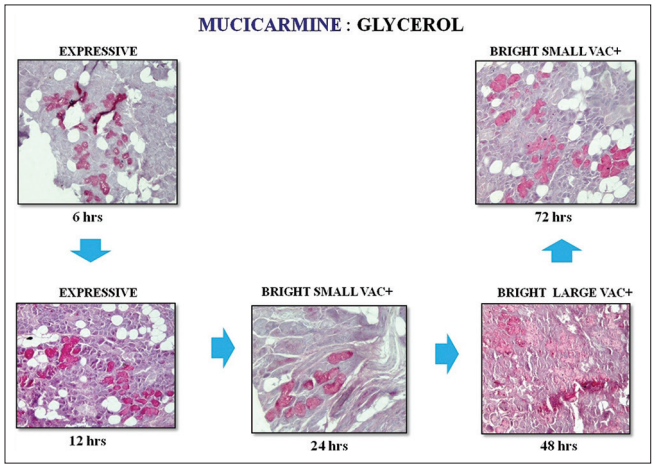

Figure 9: The staining characteristics again decreases as the time elapses, i.e., it remained expressive at 6 h, changed to bright at 12 h and finally changed to poor since 24 h. Also, there was presence of vacuolation since 12 h

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. 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.[7] Histomorphologically, the autolysis represents the intravital or postmortal disintegration of living structures and biochemically corresponds to a loss

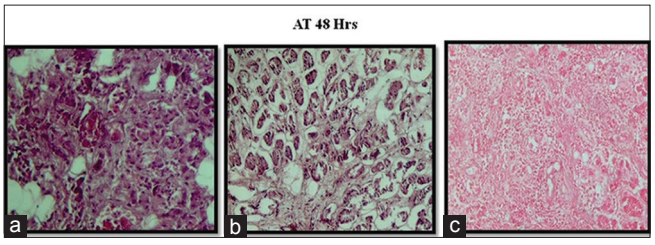

Figure 6: (a) Glycerol: There was indistinct cellular and nuclear morphology. (b) Normal saline: Complete destruction of the tissue was seen. (c) Modified Ruffer: Hematoxylin was faint but architecture was maintained

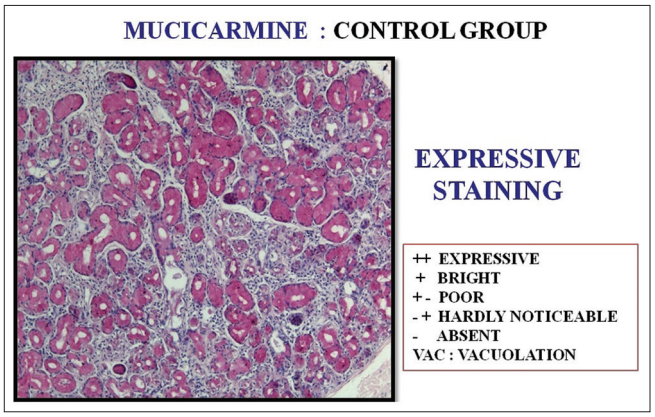

Figure 8: The staining characteristics decreases as the time elapses i.e., changing from expressive to bright. Also, there was presence of vacuolation since 24 h

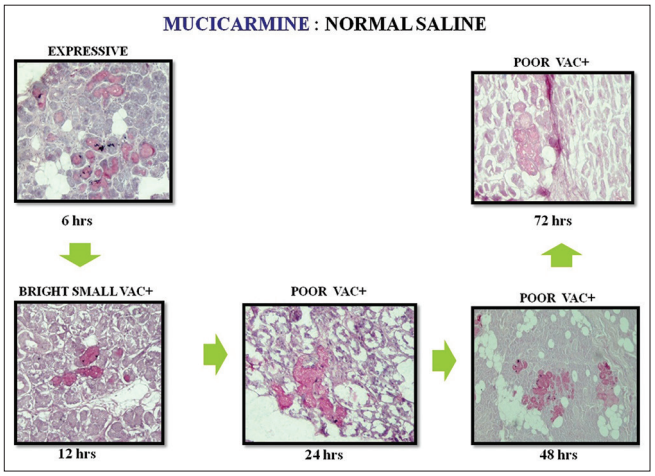

Figure 10: The staining characteristics decreases as the time elapses but it remained expressive up to 24 h and then finally changed to bright. Also, there was presence of small vacuolation since 24 h

in the system of metabolic balance with demotion of the metabolic substance which results in energy and material loss. Autolysis matches with the activity of certain enzymes, called autolytical enzymes, proved to exist in lysosomes of living cells, which after death lead to the destruction of their own cell components. Those enzymes disintegrate intracellular material, including organelles very quickly, so the cytoplasm takes dark stain, which culminates with a loss of cell details and tissue architecture.[6]

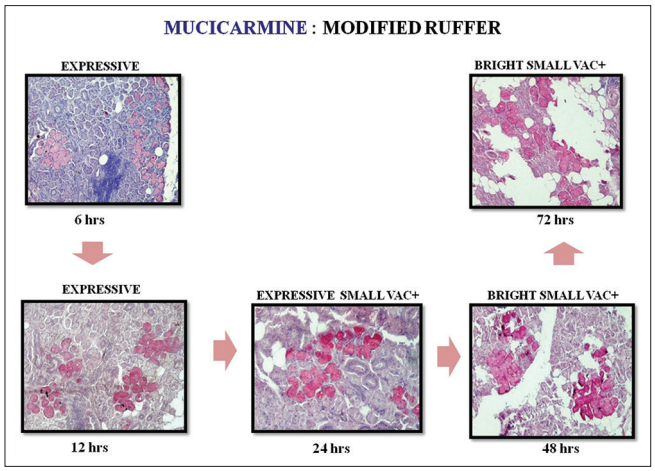

Figure 11: Mucicarmine of modified Ruffer solution

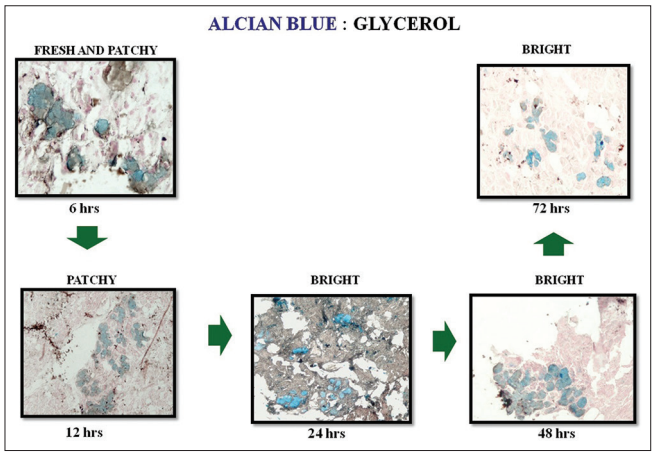

Figure 13: Fresh and patchy staining characteristics were present up to 12 h and turns to bright since then

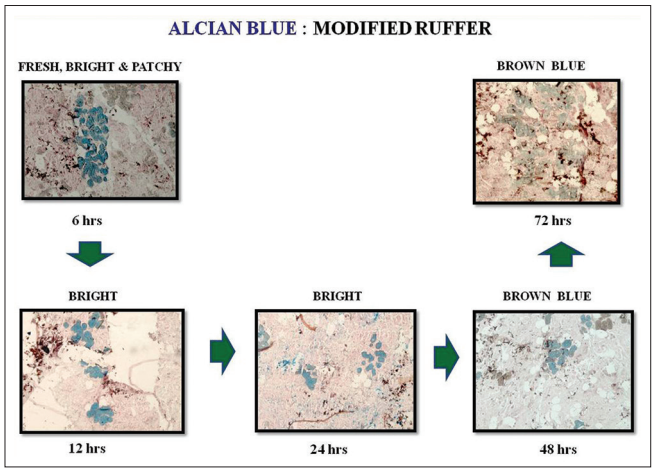

Figure 15: Staining becomes faint with time starting from fresh, bright to brownish blue in color

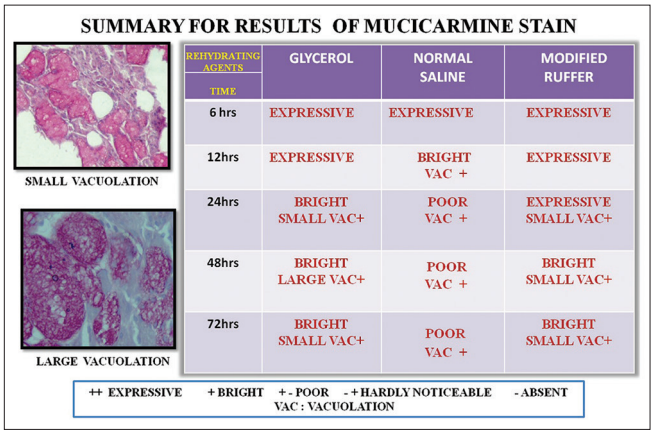

Figure 12: Results of mucicarmine stain

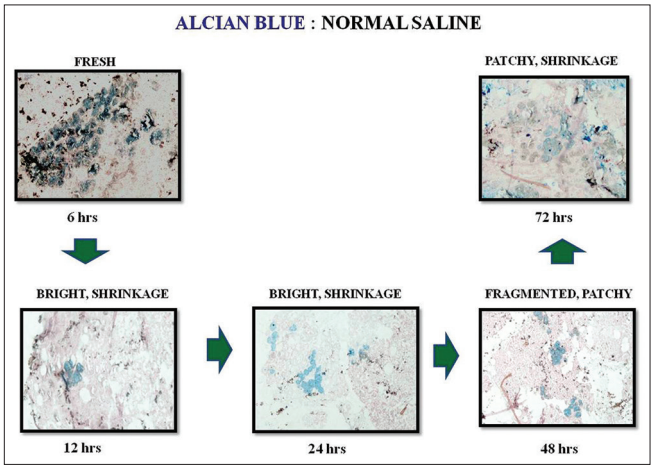

Figure 14: There is tissue shrinkage and staining becomes faint as the time elapses

Table 1: Summary results

SUMMARY FOR RESULTS OF H&E STAIN

REHYDRATING AGENTS / TIME	GLYCEROL	NORMAL SALINE	MODIFIED RUFFER
6 hrs	Slightly darker hematoxylin.	Indistinct nuclear morphology and slightly dark eosin .	Staining characteristics similar to that of control group.
12hrs	Faint staining characteristics	Indistinct nuclear morphology and faint hematoxylin.	Staining characteristics similar to that of control group.
24hrs	Faint staining characteristics	Indistinct cellular and nuclear morphology	Faint eosin stain
48hrs	Indistinct cellular and nuclear morphology with altered staining characteristics.	Almost complete destruction of the tissue.	Faint hematoxylin but maintained architecture.
72hrs	Loss of architecture	Loss of architecture	Architecture is somewhat maintained.

In this study, when observed in tissues stained with H and E, tissue architecture was well-maintained at least up to 2 days in case of modified Ruffer solution. Thus modified Ruffer solution had given fine details for a longer postmortem period compared to glycerol and normal saline.

Table 2: Results of alcian blue stain

SUMMARY FOR RESULTS OF ALCIAN BLUE STAIN

REHYDRATING AGENTS / TIME	GLYCEROL	NORMAL SALINE	MODIFIED RUFFER
6 hrs	FRESH & PATCHY	FRESH	FRESH, BRIGHT & PATCHY
12hrs	PATCHY	BRIGHT , SHRINKAGE	BRIGHT
24hrs	BRIGHT	BRIGHT , SHRINKAGE	BRIGHT
48hrs	BRIGHT	FRAGMENTED , PATCHY	BROWN BLUE
72hrs	BRIGHT	PATCHY, SHRINKAGE	BROWN BLUE

When observed under mucicarmine, Saline shows large vacuolation after 12 h and poor staining characteristics after 24 h which may be due to loss of fluid from the intracellular compartment leading to shrinkage.

Results from alcian blue stained at pH 2.5 revealed that tissue rehydrated in glycerol retained sharp staining characteristics for a prolonged period. This may be as a result of decrease in pH due to autolytic enzymes resulting from degeneration of the acini. Decrease in pH enhances the staining characteristics of the alcian blue stain.

Conclusions

Thus, we conclude that for tissue dehydrated at the crime scene for a prolonged period showed optimum rehydration with modified ruffer solution and yielded good cellular and nuclear details on stains. Also, morphological detail along with characteristics of special stains can help in estimation of the time elapsed after death.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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