

Experimental Study on the Effect of Air-Drying on Durability of Embalmed Human Cadavers

Hayder J. Mobarak *MBChB, PhD*

Dept. of Human Anatomy, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Abstract

- Background** The embalmed human cadavers used for teaching anatomy in the medical colleges could be preserved for a very long time, the crucial factors for achievement of this prolonged preservation was not fully discussed.
- Objectives** This is an experimental study to evaluate the veracity of using paraffin sectioning feasibility as a test for the degree of damage in the embalmed cadaveric tissue affected by recurrent and prolonged air drying in anatomy laboratories.
- Methods** Routine paraffin sections were done for tissues obtained from cadaveric organs exposed to recurrent and prolonged air drying and tissues from cadaveric organs preserved most of the time in a hydrated condition.
- Results** The results of this study showed unblemished paraffin sectioning of the embalmed cadaveric tissue preserved with good hydration. The cadaveric tissues exposed to recurrent air drying could not be sectioned properly, the sectioned tissues were hard and brittle.
- Conclusion** The experts dealing with the embalmed cadavers evidently necessitate maintenance of cadaveric hydration as a requisite to prolong durability of the demonstrative details in the dissected cadavers. Accordingly; the comparably more proper paraffin sectioning of the well hydrated cadaveric tissue may be considered as a sign of more durable embalmed cadaveric organs.
- Keywords** cadaver, paraffin, anatomy, fixation, formalin.

Introduction

The human cadavers in medical colleges usually were preserved for a very long time in a fixative solution which contains formalin as a major constituent, in addition to alcohol, phenol, water, and glycerin⁽¹⁾.

Routinely, many of the laboratories in the departments of human anatomy underestimated the harmful effect of air dryness on the cadaveric samples. The human cadavers are not easily provided for teaching purposes in many countries, a fact that encourages this study.

The available references fulfilled no simple description to the process of tissue fixation, and the aims of good fixation are not fully fulfilled with any of the fixative materials used routinely. The routine methodologies for tissue fixation could preserve the histological criteria as much as it is necessary^(2,3).

The effects of tissue fixation with a low-formalin embalming fluid on the histology of organs obtained from embalmed cadavers were debatable. Many histopathologists believed that the use of specimens from embalmed cadavers is good enough for investigative research and forensic medicine, especially in determining the cause of death at

autopsy⁽⁴⁾. Hardening is one of the difficulties predictable if the embalmed cadaveric tissues were processed for paraffin sections, the formalin fixatives used overharden the cadaveric tissue due to the long time of exposure⁽⁵⁾.

In the ultimate methods for histological preparations, the tissues should be fixed directly and completely from the living status. This is rarely accomplished in practice due to many factors. The extracted tissues often exposed to anoxia for a period, and the penetration of the fixatives requires relatively long duration⁽⁶⁾. Anoxia brings about changes which are visible with electron microscope within minutes⁽⁷⁾, the enzymes are lost within few hours of anoxia⁽⁸⁾.

The postmortem tissues have various adverse factors disturbing the use of these tissues for paraffin histological sectioning. There might be agonal changes in tissue, the body is usually left for some time at room temperature before it is transferred to the mortuary and refrigerated. These factors might give rise to artefacts. The tissues will undergo drying if left in air before fixation, also the tissue will undergo shrinkage and bacteriological changes with autolysis. There are many damaging chemical reactions taking place by the cellular enzymes within the tissue⁽⁹⁾.

This study is an experimental appraisal of utilizing the paraffin sectioning feasibility as an indicator for the severity of the damaging effect resulting from prolonged and recurrent air drying on the embalmed cadavers.

Methods

The cadaveric tissues used for this experimental study were obtained from the Department Of Human Anatomy, College of Medicine, Al-Nahrain University. All the cadaveric tissues were primarily embalmed by the same procedure and materials⁽¹⁾, also all these tissues were preserved in the same constituents of the preservative solution since 2-3 decades.

The tissue used for this study were macroscopically intact and not affected by fungal infection, cadaveric organs having a damaged gross anatomical configuration were excluded.

Small pieces of 0.5 cubic centimeter size were taken from the tissues of the quadrate lobe of the liver, the descending colon, and the left ventricle of the heart. The tissues used in this experimental study were of two groups:

Group A: Include tissues taken from extracted cadaveric organs that were routinely preserved in containers filled with the preservative solution immediately after each anatomy session, which usually lasting about 2-3 hours. Three specimens were taken from each of the organs used, two extracted organs of each type were selected.

Group B: Include tissues taken from organs that were part of the whole cadaver. These cadavers were frequently exposed to recurrent and prolonged air dryness (specially during the hot seasons) because it is difficult to return the whole cadavers in the preservative solution daily. These cadavers were placed routinely on the bench in the laboratory of human anatomy for a week or more, and thus they were exposed to dryness due to the long time exposure to atmospheric temperature that may reach up to 45 °C in summer. The poor ventilation in the anatomy laboratory exaggerated the effect of high temperature on the cadaveric tissue.

Also, three specimens from each of the organs used were taken, and two cadaveric organs of each type were selected.

The tissues of both group A and B were placed in the preservative solution routinely used in the anatomy laboratory for preservation of the cadaveric organs. The tissues were stored in this solution at room temperature for one week in order to ensure proper hydration. The tissues were then transferred into three changes of 70% ethanol (two days for each), after that, the tissues were dehydrated by higher concentration of ethanol alcohol, cleared in xylene, and impregnated in a 58 °C

melted paraffin and paraffin blocks were prepared. The wax impregnation was used in three changes with a total impregnation time of three hours. The paraffin blocks sectioned using a rotary microtome (Riechert Jung) at 7, 10, 14, and 20 microns tissue thickness. Sections were float in an albumen solution on a glass slides. The sections were stained with hematoxylin and eosin ⁽¹⁰⁾.

Results

The process of sectioning the paraffin blocks obtained from the two groups of cadaveric tissues were compared. The paraffin blocks of group B could not being sectioned easily. The tissues were hard and brittling of the tissues occurs during sectioning. Few of the sections of this group contain pieces of the tissues, most of the sections show holes in the strips of the paraffin at the region of the falling brittled tissues. The sectioned liver tissue produced few fairly intact sections.

The hematoxyline and eosin staining of the tissues obtained from group B showed hazy histological boundaries of the cells and tissue fragmentation with complete loss of the histological tissue architecture of each type of tissue. The low power microscopic examination of the tissues of group B showed irregular section margins (Fig. 1).

The sectioning of the paraffin blocks of group A was much easier in comparison to group B. All the paraffin strips of group A sectioned contain intact tissues.

The histological examination of the sections of group A showed delineated cellular boundaries, however; the histological criteria of each tissue were distorted and the microscopic features of each type of tissue were not ideally demonstrated. The low power microscopic examination of these sections showed a linear cutting margin compared to that of section of group B (Fig. 2 and 3).

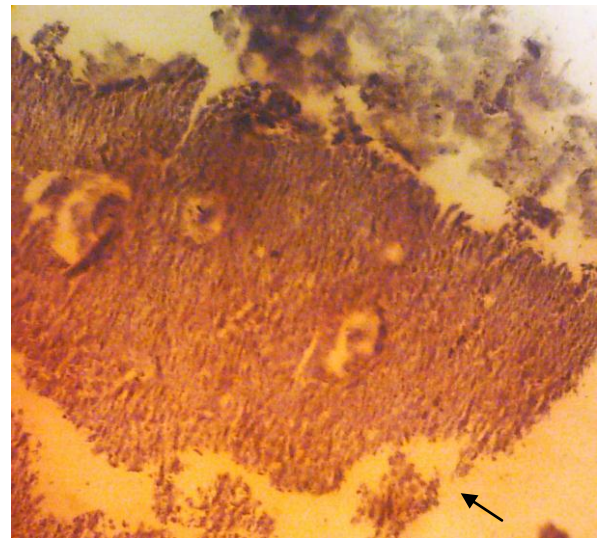


Fig. 1. The paraffin section of liver tissue, group (B). The section showed irregular margins (arrow). H&E 100X.

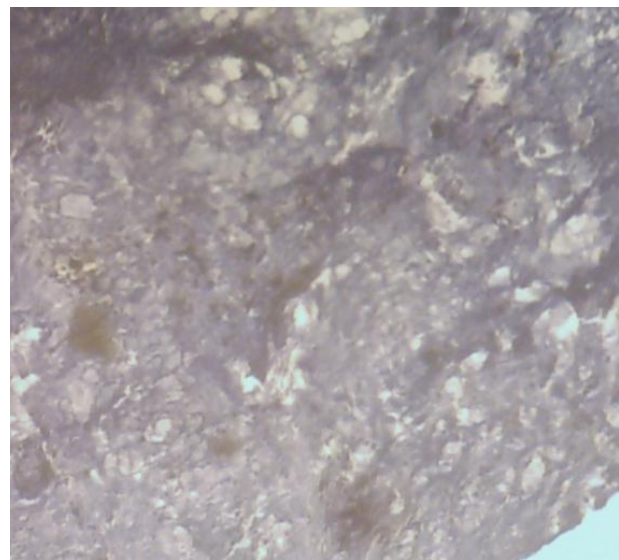


Fig. 2. Paraffin section of cardiac muscle tissue from group (A). H&E 400X

Discussion

It was logically concluded from the practice in anatomy teaching that preserving the extracted human cadaveric organs in the preservation solution after each anatomy session kept the samples beneficial for teaching purposes for a much longer duration. This logic conclusion was not previously tested experimentally, a theme which this study established.

Therefore; the results of this study may suggest that the comparably more easy and the proper paraffin sectioning of the tissues obtained from the extracted cadaveric organs that were mostly kept hydrated in the preservative solution (group A) could be considered as a sign for the relatively more durable and sound tissue.

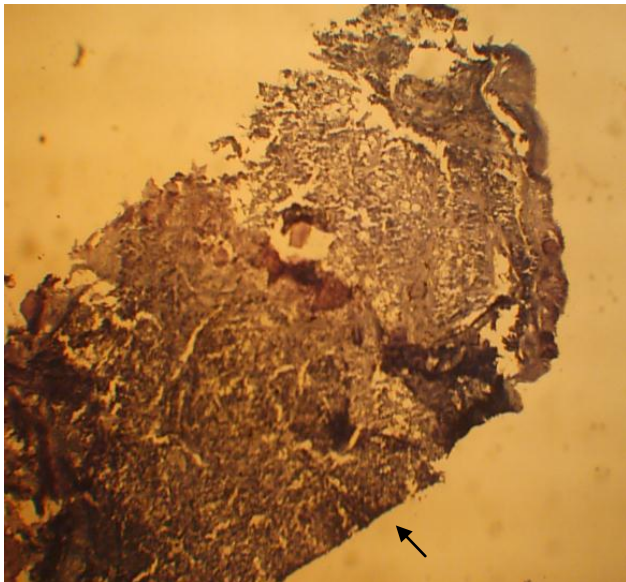


Fig. 3. Paraffin section of cardiac muscle tissue from group (A) stained with hematoxylin and eosin. Linear cutting margins are seen (arrow). 50X.

The comparison of normal histology of the human liver, colon, and heart ⁽¹¹⁾ with histological features of the sections obtained from the extracted organs of group (A) markedly proved distorted morphology. This distortion may be attributed to the effect of the embalming solution on these tissues. The embalming solution was not prepared for histological tissue processing as it contains materials that do not fit with the criteria of proper tissue fixation ^(1,10). The prolonged exposure of the extracted organs of group A to preservative solution (for 2-3 decades) may also be a contributing factor to the distortion of the histological features of these tissues. This interpretation about the effect of the composition of embalming solution on histological quality of the specimens was a

supportive conclusion to the previously reported experimental studies ⁽¹²⁾.

The histologist and pathologist were always obsessive from the bad processing of the samples as the proper tissue processing usually leads to easy and good paraffin sectioning and produces comprehensive histological details ⁽¹³⁾. This attentiveness was the bases of the proposal done in this study considering the feasibility of paraffin sectioning as an experimental procedure that could evaluate the extent of tissue damage by the effect of exposure to air drying.

The traditional practice in teaching anatomy in the laboratories of human anatomy considered an implication that the dried human cadaveric tissue could be returned back to its beneficial status by returning the cadaver into the preservative solution. The results of this study may present an evidence that recurrent air drieriness of the cadaveric tissue results in its advanced damage. This assumption was established from the difficulties noticed during paraffin sectioning of the cadaveric tissue of group B compared to that of group A. Also, the loss of the histological appearance of the sections of group B with loss of cellular boundaries and tissue fragmentation may be considered as feature of the evident tissue damage by the effect of recurrent air drying.

The laboratory in the Department of Human Anatomy in the College of Medicine Al-Nahrain University stored the cadaveric samples for many years with a minimal tissue damage and with minimal loss of the cadaveric organs that kept the details of the anatomical descriptions. This prolonged period of preserving the anatomical samples is a fact that may be considered as a supportive evidence to the results of this study as the staff members in the department used to routinely protecting the cadaveric samples from being left exposed to air for a long time.

In agreement with results of this study, it was reported that the aim of embalming is to achieve perfusion of the fixative solution throughout all parts of the body. The low

formalin fluid used for embalming is designed to preserve the tissue, made the cadaver suitable for dissection, and prevent bacterial and fungal growth. The low formalin embalming fluid made the cadavers suitable for histological sectioning, however; some organs showed distorted microscopic architecture that is considered to be more close to normal architecture than others⁽⁴⁾.

The maintenance of the embalmed human cadaveric organs used for teaching for a longer duration carries an importance in many various considerations as financial, humanitarian, and scientific respects. The results of this study may objectively provided a technical method to evaluate the durability of the apparently intact cadaveric organs and detecting the organs exposed to neglect, application of this method may designate the embalmed cadaveric organs that are damaged should to be replaced within a short period.

Aknowlegments

I would like to express my gratefulness to the members of the Department of Human Anatomy for their help and for keeping the human cadaveric sample in a very organized way.

Conflict of interest

The author disclose no any financial and personal relationships with other people or organizations that inappropriately influence (bias) out work.

Funding

The research working funding was provided by the authors.

References

1. Hamash MH, Al-Salihi AR. Embalming and preservation of the cadavers and the anatomical

materials used for teaching and scientific purposes. Al- Nahrian University, College of Medicine. Saadon printing Company, 2000.

2. William JH, Beverley AW. Tissue fixation and the effect of molecular fixatives on downstream staining procedures. *Methods*. 2014; 70: 12-9.
3. Benerini Gatta L, Cadei M, Balzarini P, et al. Application of alternative fixatives to formalin in diagnostic pathology. *Eur J Histochem*. 2012; 56: e12. doi: 10.4081/ejh.2012.e12.
4. Uchenna N, Ekele I, Okechukwu N, et al. Effect of embalming fluid on histological appearance of organs from embalmed West African dwarf goat cadavers. *HISTOLOGIC*. 2013; 10: 1-4.
5. Coleman R, Kogan I. An improved low-formaldehyde embalming fluid to preserve cadavers for anatomy teaching. *Journal of Anatomy*. 1998; 192 (3): 443-6.
6. Mythily S, Daniel S, Scott J. Effect of fixatives and tissue processing on the content and integrity of nucleic acids. *Am J Pathol*. 2002; 161: 1961-71.
7. Yukari T, Makoto N, Youkichi O, et al. Ultrastructural changes during in situ early postmortem autolysis in kidney, pancreas, liver, heart and skeletal muscle of rats. *Legal Med*. 2014; 4: 25-31.
8. Joseph M, Jie L, Karen FT, et al. Robust Immunohistochemical Staining of Several Classes of Proteins in Tissues Subjected to Autolysis. *J Histochem Cytochem*. 2007; 55: 597-606.
9. Lee MG. Early post-mortem changes and stages of decomposition in exposed cadavers. *Exp Appl Acarol*. 2009; 49: 21-36.
10. Kiernan J. *Histological and histochemical methods: theory and practice*. 4th ed. London: Scion Publishing; 2008. p. 141-75.
11. Junqueira LC, Carneiro J. *Basic histology text and atlas*. 11th ed. USA: McGraw Hill; 2005. p. 212-89.
12. Nicholson HD, Samalia L, Gould M, et al. A comparison of different embalming fluids on the quality of histological preservation in human cadavers. *Eur J Morphol*. 2005; 42: 178-84.
13. Sano M, Harada T, Sakagami M, et al. Pathological specimens of rat temporal bone-comparison between different fixative solution. *Med J Osaka Univ*. 1993; 41: 17-22.

E-mail: hayder_67_67@yahoo.com

Tel.: + 964 7904772919

Received 26th Mar. 2015: Accepted 4th Jun. 2015.