Effect of Embalming Fluid on the Histological Appearance of Organs - A Cadaveric Study

Swapna R[1], Annie Jojo[2], Tintu TS[3], Asha G[4], Pillay VV[5], Minnie Pillay[6]

[1]Lecturer, Department of Anatomy, [2]Professor, Department of Pathology, [3]Lecturer, Department of Anatomy, Amrita Institute of Medical Sciences, Amrita Vishwa Vidyapeetham, Kochi, Kerala, India, [5]Professor & Head, [6]Professor, Department of Anatomy, Amrita School of Medicine, Amrita Institute of Medical Sciences, Amrita Vishwa Vidya peetham, Kochi, Kerala, India

Abstract

Objective: Histopathological investigations with tissues from embalmed cadavers are generally not considered ideal for determining the cause of death at autopsy in forensic medicine. Instead fresh tissue fixed in 10% formalin is usually preferred. The aim of the present study was to compare the histological appearances of samples processed from routine formalin-embalmed cadaver with samples fixed in 10% formalin from a fresh cadaver.

Methods: The study was carried out on cadavers received as donation to the department of Anatomy from body donors. Tissue samples of four organs comprising kidney, lung, liver and spleen were obtained from a fresh cadaver (used as control) as well as from embalmed cadavers. The quality of the slides was graded 1 to 3 based on organ microscopic architecture, cell morphology and state of epithelium, as follows: 1 - high degree of cell distortion, 2 -moderately good sections and 3 - near normal.

Results: Kidney: Slight architectural distortion, with collapsed convoluted tubules were observed in embalmed tissue sections (grade 2). Lung: The architecture of the embalmed tissue showed alveolar spaces that were cystically dilated, but pneumocytes appeared normal (grade 2). Liver and spleen: The cell morphology and The cell morphology and architecture appeared almost as preserved as in fresh tissue. (grade 3).

Conclusion: Of the four organs studied, liver and spleen were well preserved and close to normal, whereas cytoarchitecture of lung and kidney specimens showed slight distortion. However this is a preliminary study and efforts are on to study more number of specimens with varying concentrations of formalin.

Key words: *histopathology, embalmed body, cytoarchitecture, cellular morphology.*

Introduction

Tissues from an embalmed cadaver are generally not preferred for histopathological investigations in forensic medicine, especially for determining the cause of death at autopsy. However, if only an embalmed cadaver is available then it is better to have some tissue than no tissue at all. The microscopic findings of various organs in the deceased can contribute in a major way to the forensic investigation which will enable the forensic pathologist to integrate the subjective (history) with the objective (lab investigation and post-mortem results) for reconstruction of circumstances and cause of death on legislative basis[1]. There is dearth of published literature regarding the use of tissues from a formalin embalmed cadaver for forensic histopathology, hence the need for this study, which was undertaken to compare the

Corresponding author:
Dr. Minnie Pillay
Professor, Department of Anatomy
Amrita School of Medicine, Amrita Vishwa Vidyapeetham, Kochi-682041, Kerala, India
Phone: Official: +91-484-2858101
Mobile: +919895511577, Fax: +91-484-2802020
E-mail address: minniepillay@aims.amrita.edu, pillayminnieanat@gmail.com

histological appearance of samples processed from four formalin-embalmed cadavers with histological tissue samples obtained from an un-embalmed cadaver and processed as per the standard procedure. Moreover, the study is also intended to explore the possibility of utilizing tissues from formalin-embalmed cadavers for teaching purposes, for which tissue samples are routinely taken from guinea pigs, rabbits, etc

Materials and Methods

Tissue samples were obtained from cadavers received as donation to the department of Anatomy from the period June 2018 to August 2019. The cadavers were embalmed as per the standard embalming protocol using 10% formalin. Four cadavers without any pathological findings affecting the tissues and age group between 44-89 were selected for the study. Tissue samples were obtained from four organs, i.e., kidney, spleen, liver and lung. The organs were dissected and exposed as per instructions in Cunningham's Dissection Manual[2] and the tissues were taken separately from all the four cadavers and collected in labelled containers. Fresh tissue samples from all the four above mentioned organs from an un-embalmed cadaver were used as control. These samples were fixed in 10% formalin after which they were processed as per the standard procedure and stained with haematoxylin and eosin. The sixteen tissue samples from the four embalmed cadavers, as well as the control samples from the un-embalmed cadaver, were processed directly without any additional fixation as per standard histological technique, and sections of 4 micron thickness each were obtained, which were stained using routine haematoxylin and eosin staining procedure.

The slides were examined with a light microscope and photomicrographs were taken using Carl Zeiss multiheader microscope fitted with a digital camera. The quality of stained sections was assessed as per the criteria proposed by Nlebedum et al[3].

Accordingly, the sections were assessed for

a. General organ microscopic architecture,

b. Cell morphology and state of epithelium graded on a scale of 1 to 3

Grade – 1 Refers to a high degree of distortion

Grade – 2 Moderately good sections

Grade – 3 Sections very close to normal

Observations

The sections obtained from four organs belonging to formalin-embalmed cadavers were observed and graded as per the criteria proposed by Nlebedum et al[3]. The results are depicted in Table 1. Photomicrographs of fresh samples of spleen, lung, liver, kidney are represented in Fig-1A, Fig-2A, Fig-3A, Fig-4A respectively.

With regard to spleen, cell morphology and architecture appeared almost preserved as in fresh tissue. White and red pulp, as well as the covering capsule and vascular endothelium appeared normal. All four samples were graded 3. (Fig-1B)

All four lung tissue samples presented distorted architecture, visualised as irregular distorted alveolar spaces. However at higher magnification, cell morphology appeared normal. Hence all specimens were graded 2. (Fig-2B)

Liver tissues were well preserved in three specimens. General organ microscopic architecture was mildly distorted, but cell morphology of hepatocytes and Kupffer cells was well preserved and normal. Portal triads, central veins, capsule, and sinusoids were normal. All three samples were graded 3 (Fig-3B). In one liver sample, lobular architecture, central veins and portal triads were normal, but hepatocytes and Kupffer cells were poorly preserved.

One specimen of kidney had high degree of distortion with poor cellular details and collapsed convoluted tubules and was graded 1(Fig-4B1). Three specimens had only slight architectural distortion with collapsed convoluted tubules of grade 2.The epithelial lining appeared moderately good (Fig-4B2). The capsule appeared to be well preserved (Fig-4B3).

Table 1: Histological grading of cadaveric tissues

Grade				
Organs	**Cadaver 1**	**Cadaver 2**	**Cadaver 3**	**Cadaver 4**
Kidney	2	2	1	2
Lung	2	2	2	2
Liver	3	1	3	3
Spleen	3	3	3	3

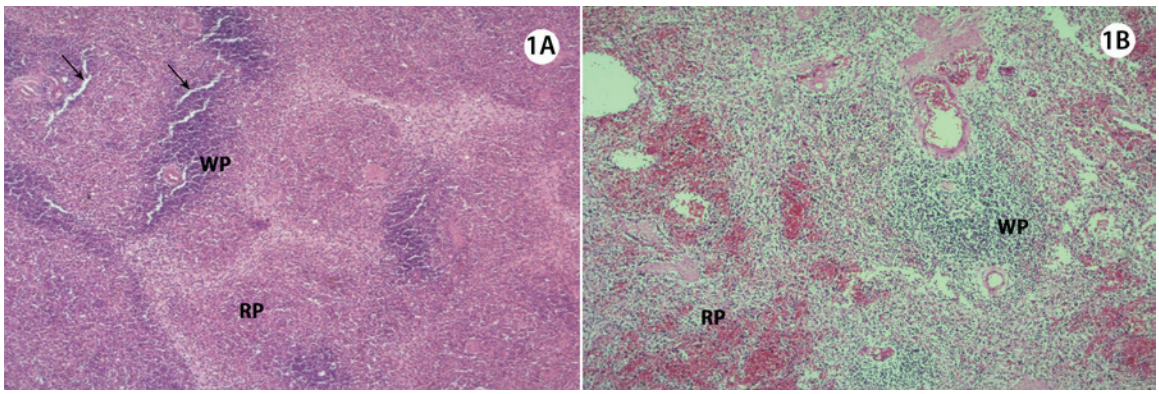

Figure 1A: Photomicrograph of spleen - Fresh sample - 100x. WP –white pulp, RP – red pulp, arrow indicates open spaces in the splenic parenchyma,1B: Photomicrograph of spleen - 100x.Sample from embalmed cadaver grade 3. WP – white pulp, RP – red pulp.

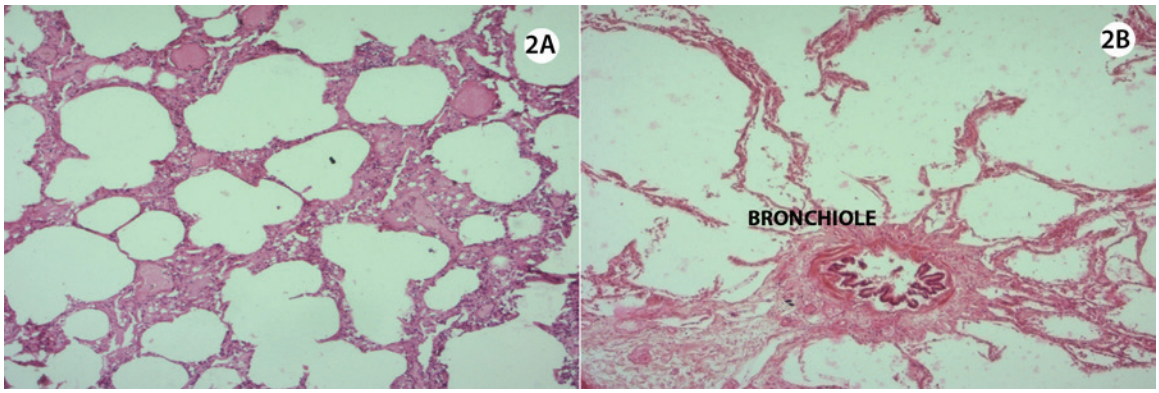

Figure 2A: Photomicrograph of lung - Fresh sample - 100x, 2B: Photomicrograph of lung -100x. Sample from embalmed cadaver grade 2.

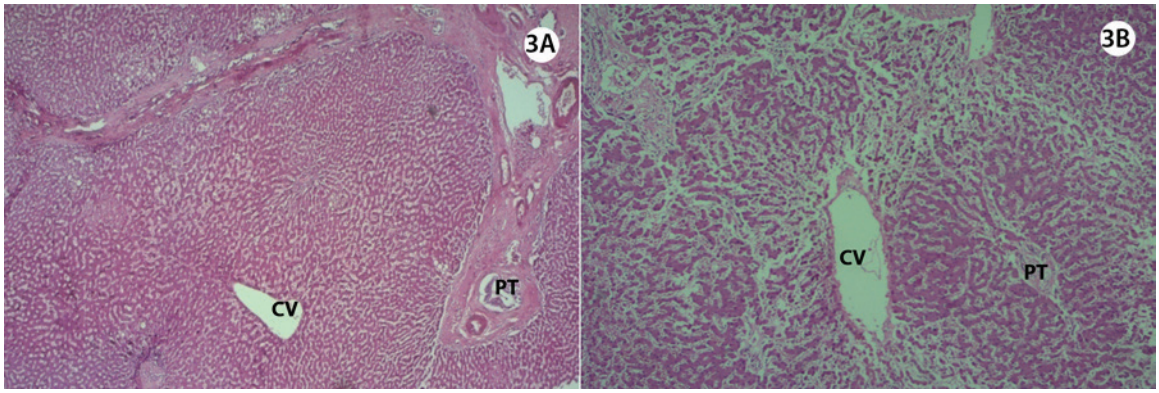

Figure 3A: Photomicrograph of liver - Fresh sample - 100x. CV - central vein, PT – portal triad, 3B: Photomicrograph of liver - 100x. Sample from embalmed cadaver grade 3.CV - central vein, PT – portal triad.

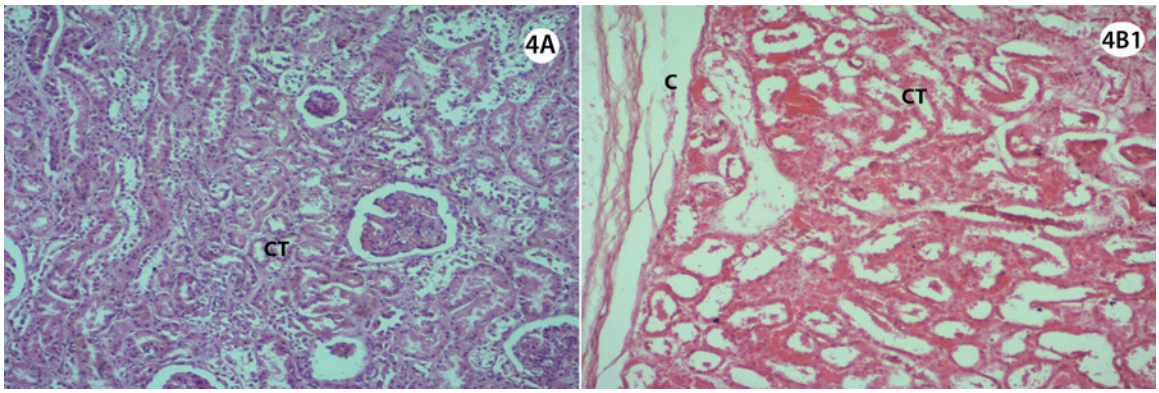

Figure 4A: Photomicrograph of kidney - Fresh sample - 100x.CT- convoluted tubules, 4B1: Photomicrograph of kidney - 100x. Sample from embalmed cadaver grade 1.C- capsule, CT- convoluted tubules.

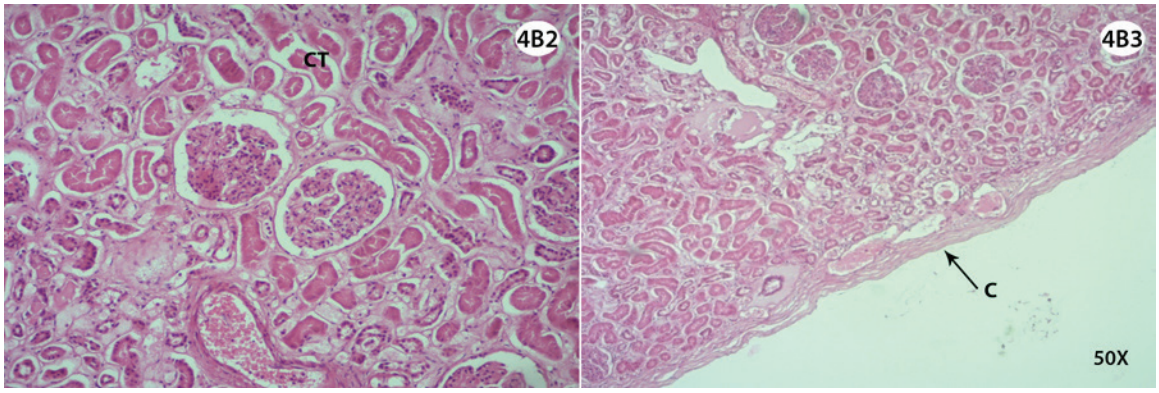

Figure 4B2: Photomicrograph of kidney - 100x. Sample from embalmed cadaver grade 2 .CT- convoluted tubules, 4B3: Photomicrograph of kidney - 50x. - Sample from embalmed cadaver grade 2. C- capsule.

Discussion

Archana Kalyankar et al[4] studied the histological samples of skeletal muscle tissue obtained from twelve embalmed human cadavers and compared them with skeletal muscle tissue obtained from guinea pig samples and processed as per standard histological technique, and their results indicated the feasibility of muscle tissue from human cadaver for teaching and research activity. They also proved that embalmed human skeletal muscle

tissue sections are better in staining and appearance than that of guinea pig slides.

Gupta and Gauba[5] conducted a study to standardize the histology of different tissue types obtained from embalmed cadavers available in the department of Anatomy. They reported that all the tissues studied including skin, muscle, cartilage, artery, spleen, brain, trachea, liver and small intestine showed excellent tissue organisation with nuclei, cell membranes and cytoplasmic details, being clear and well preserved. They concluded that human cadaver could be an ideal source of tissue for teaching and research.

In our present study, out of the four organs evaluated, kidney and lung tissues presented maximum architectural distortion. With regard to the kidney specimen, our study is in agreement with Nlebedum et al[3] with reference to collapsed convoluted tubules, but the capsule was not found detached in our specimens, and cellular morphology appeared moderately well preserved. Nicholson et al[6] compared the histological samples of heart, liver, kidney, skeletal muscle and skin obtained by embalming with four different fluids, namely formalin, traditional Dunedin mix, Michigan mix and phenoxyethanol mix. The results of the study showed satisfactory or good quality tissue, except the one embalmed with Dunedin mix.

Adequate preservation of kidney sample was also observed and mentioned as comparable to liver tissue which showed good preservation of morphology in their study.

Lung tissues presented with distorted irregular alveolar spaces, but cellular morphology was normal in all the four samples studied, and the same was observed by Nlebedum et al[3]. But unlike in his study, spleen was well preserved in all the four tissue specimens of our series, and all were graded with a score of 3. They had observed open tissue spaces in the organ parenchyma, but these could be artefacts because in certain sections of splenic tissues from fresh cadaver we did observe open spaces in the organ parenchyma. Three out of four tissue specimens of liver, presented with only mild architectural distortion, but the capsule, sinusoids, portal triads were all well preserved. Cellular morphology also appeared normal.

Nicholson et al[6] in their study mentioned good preservation of morphology in the sections of liver, which support the above. Regarding the liver samples, similar observations were reported by Nlebedum et al[3]; Gupta and Gauba[5]. Architectural distortion could be due to the lack of ability of the embalming fluid to properly fix the cells of the connective tissue stroma.

Better preservation of liver and spleen could be due to increased vascularity.

Gupta and Gauba[5] had explored the possibility of utilizing cadaveric tissue for histological studies and reported that cadaveric tissue can be considered ideal for teaching and research.

According to a study by Kalanjati et al[7], low concentration of formalin (5- 7.5%) was more suitable for processing cadavers to be used for anatomy teaching and learning than cadavers embalmed with higher concentration of formalin. Histological samples with low formalin technique should be studied so that adverse effects of formalin can be minimised. However the use of embalmed cadaveric tissue in forensic histopathology is dependent on whether the diagnosis is heavily based on cellular morphology or architectural distortion. The present study proves that the former is reliable.

Conclusion

Despite embalming, cellular morphology appears well preserved in the organs studied, and hence can be considered fairly reliable for forensic investigations. Architectural distortion is observed in tissue samples taken from cadavers embalmed using 10% formalin, and hence cannot be relied upon for forensic purposes. However, more studies are required to be undertaken with different concentrations of formalin to ascertain whether the tissue architecture can be improved upon. It is important to note that the vascularity of an organ also has a role in preserving tissue architecture. However for routine teaching purposes, the slides prepared from tissues of embalmed cadavers appear to be good enough.

Conflict of Interest - Nil

Source of Funding - Nil

Ethical Clearance - NA

Limitation of The Study

The study was carried out on cadavers meant for routine dissection by first year medical students. Hence tissues could not be taken from the same cadavers pre and post embalming as it would have interfered with their dissection protocol.

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