

Histology slides from cadaveric tissue- can it be an alternative?

Pranita R. Viveki^{1*}, M. Pranay Kumar¹ and R.G. Viveki²

¹Department of Anatomy, Prathima Institute of Medical Sciences, Nagunur, Karimnagar-505417, Telangana, India and ²Department of Community Medicine, Belagavi Institute of Medical Sciences, Dr. B.R. Ambedkar Road, Sadashiv Nagar, Belagavi-590001, Karnataka, India

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Abstract: *Background:* Current trends in medical curricula are shifting from teaching histology and pathology as stand-alone disciplines. Histological slides are routinely prepared by using tissues from surgically removed specimens which are an unreliable source for a normal histology, with associated with ethical issues. An alternative tissue source is animal tissue which is easily available, but it has different histological details as compared to the human tissue. Therefore, it would be useful to examine the potential value of integrating these into the anatomical dissection experience. *Objectives:* To standardize the histology of different tissue types of the embalmed human cadavers. *Materials and Methods:* Twenty two human cadavers (14 Males & 8 females) received to the department of anatomy through body donations were selected for sample collection. The taken tissues were fixed into 10% buffered formalin, processed and stained with usual H and E staining. The prepared slides were studied. Using predefined criteria, the quality of the samples was evaluated by two pathologists of the institute and each slide was categorized as good, satisfactory or poor. *Results:* All post embalmed sections were better in staining and appearance. The nuclei, the cell membranes and the cytoplasmic details were clear and well preserved. Majority (54.17%) of the slides were found to be good quality, followed by satisfactory quality (39.59%). The Fisher's exact test showed statistically significant difference in the slide quality of various tissues ($P < 0.01$). *Conclusion:* Most of the tissues acquired from the embalmed cadavers were of good or satisfactory quality. It indicates the beneficial use of histological tissue from cadavers for educational purposes. Future research into how these findings translate into meaningful medical education would be beneficial.

Keywords: Cadaveric tissue, Embalmed cadavers, Histology, Medical education.

Introduction

The integration of clinical medicine with the basic science-oriented background has allowed medical students globally to have an increased ability to appraise the pathophysiological concepts with clinical manifestations and management of diseases [1-2].

However, the availability of normal human tissue is a major limiting factor. Most of the tissues which are available for histology are taken from the periphery of the tissue specimens which are removed for some pathological indication. It is not only difficult, but also unethical to get normal tissue for histological studies. Thus, the normal histology slides which are available for study and comparison are made either from animal tissues or from normal tissues which are incidentally removed during surgical procedures [3-4].

Apart from their limited availability, the cost of procuring good teaching slides may be prohibitive for many teaching departments. Furthermore, cadavers are considered medical students' "first patient," as they work through their anatomy and encounter existing pathology during dissections, in reference to the documented clinical data and the cause of death. However, the use of embalmed tissue for histological and histopathological teaching purposes has been limited [5].

The present study was undertaken to determine the histological details of various tissues which were taken from cadavers. Our aim was to establish whether the cadaveric histology could replace the in vivo histology for normal tissue related teaching and documentation and thus provide the clinicians

and pathologists with a practically unlimited source of normal histological tissue for comparison with the pathological specimens.

Material and Methods

This study included all formalin-fixed cadavers available for dissection (N = 22) at the Department of Anatomy, Prathima Institute of Medical Sciences, Karimnagar, Telangana State. All 22 cadavers were kept in cold storage before embalming and were embalmed within an average of 4.20 days, with the earliest being 2 days and the latest being 7 days. Each cadaver was embalmed with embalming fluid (10% formalin, 10% ethanol, 20% glycerin, 5% phenol, thymol crystals and Magnesium chloride) as per gravitation method [2].

As body donation is already a legal procedure, no consent was taken from the relatives. After getting approval from Institutional Ethics Committee and permission from head of the institute for conducting the study, the tissues were taken from such cadavers for the study. As all human body organs are made up of only four basic tissue types namely epithelium, connective tissue, muscle tissue and nerve tissue, we deliberately selected skin, cartilage, muscle, artery & Nerve tissues as together they encompass all the above mentioned tissue groups and their subtypes.

The histological status of all these types of tissues in the human cadavers were studied. All cadaveric tissue samples were collected within six weeks of embalming. The cadavers were kept in cadaver tank after embalming. Twelve samples of every tissue were taken randomly from the available 22 cadavers. The transverse as well as longitudinal sections of skeletal muscles and peripheral nerves were analyzed. Thus total 96 slides were prepared solely for the purpose of this study under the supervision of an anatomist and a pathologist using standard autopsy techniques and studied thoroughly.

1. Tissue Preparation: After getting the details of cadavers like age, sex, cause of death, medical history, etc at the time of the cadaver selection, each tissue type was taken from the cadavers with no history of involvement of study tissues with a disease process. Fixation of the tissue sample was done to preserve the tissue structure for

subsequent treatments with 10% Formalin as a fixative. Then the tissue was infiltrated with the paraffin as an embedding medium that allowed it to be thinly sliced. The specimen was washed after fixation. It was dehydrated in a series of alcohol solutions of ascending concentration up to absolute alcohol to remove water from the tissue. Xylene (an organic solvent) being miscible in both alcohol and paraffin, was then used to remove the alcohol prior to infiltration of the tissue with melted paraffin.

Once the melted paraffin became cool and hardened, it was trimmed into an appropriate sized block. After mounting the block, the sections of 4 micron thickness each were obtained by using the Spencer type microtome. To stain the tissue section, paraffin was dissolved with Xylene and the slide was rehydrated through a series of solutions of descending alcohol concentration.

The tissue on the slide was then stained with Haematoxylin in water. Counterstaining was done with Eosin. After the staining, the specimen was passed through Xylene to a non-aqueous mounting medium and it was covered with a cover-slip. The quality of stained sections was assessed as per the criteria proposed by Nlebedum et al [6].

2. Microscopy: The prepared slides were examined and thoroughly studied under a trinocular light microscope with a camera. Histological slides were independently evaluated by two pathologists of the institute with >6 years of experience in anatomical pathology and both the evaluators were not involved in the sample collection or slide preparation and were blinded to each other's evaluations.

Three characteristics of the cells and extracellular matrix were reviewed for evaluating the slides-

- (i) Be clearly identified at low magnification and when using 200 and 400 objective magnifications,
- (ii) Have a sharp definition of cell size and nuclei and
- (iii) Have sharp boundaries with adjacent connective tissues.

Rating of slides quality of tissues [7]: Each slide was rated as;

- a) *Good (Score 2)*: Met all the aforementioned criteria without any cellular definition limitation at different magnifications or limitation to definition of structures, boundaries and surrounding tissue;
- b) *Satisfactory (Score 1)*: The slide was mostly adequate but there was some limitation in one of the criteria
- c) *Poor (Score 0)*: The slide had a significant limitation in two or more of the criteria.

The average score of the 2 evaluators was calculated for categorization as “Good” if the overall average score was 2, as “Satisfactory” if the score was <2 but >1 and as “Poor” if it was ≤1.

3. Data Storage and Compilation: Photographic data: The microphotographs of each tissue were taken in three different resolutions – 4X, 10X and 40X using Carl Zeiss multiheader microscope fitted with a digital camera. They were stored into the computer data base for further assessment and comparison. The cellular details and the architecture of the tissue were recorded and compiled separately.

4. Comparison: Each tissue subtype was compared with a standard histological slide of the

same tissue obtained from the guinea pig which was stained similarly.

Statistical analysis: Statistical tests were conducted using Statistical Package for the Social Sciences (Version 21.0). The inter-evaluator reliability was measured using two-way mixed effects, absolute agreement and multiple rater intraclass correlation. The frequency of overall slide quality rating and the slide rating among different tissues was measured. A comparison of the slide rating of various tissues was conducted using Fisher’s exact test. P value < 0.05 was considered statistically significant.

Results

The final rating of each evaluator was evaluated where inter-evaluator reliability was found to be 0.91, which is indicated to be an excellent level of reliability [8].

The nuclei, the cell membranes and the cytoplasmic details were clear and well preserved. Majority (54.17%) of the slides were found to be good quality, followed by satisfactory quality (39.59%) as seen in Table-1. The Fisher’s exact test showed statistically significant difference in the slide quality of various tissues (P < 0.01).

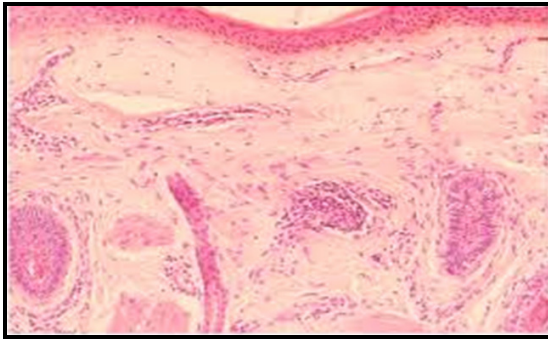
Table-1: Rating of slides quality of the study tissues

Sr. Nos.	Name of the tissues	Good (%)	Satisfactory (%)	Poor (%)
1	Thin Skin	5 (41.67%)	7 (58.33%)	0%
2	Thick skin	10 (83.33%)	2 (16.67%)	0%
3	Elastic cartilage	8 (66.67%)	4 (33.33%)	0%
4	Skeletal muscle (L.S)	6 (50.0%)	5 (41.67%)	1 (08.33%)
5	Skeletal muscle (T.S)	7 (58.34%)	4 (33.33%)	1 (08.33%)
6	Muscular Artery	5 (41.67%)	7 (58.33%)	0%
7	Peripheral Nerve (L.S)	4 (33.33%)	6 (50.0%)	2 (16.67%)
8	Peripheral Nerve (T.S)	7 (58.34%)	3 (25.0%)	2 (16.67%)
Total = 96		52 (54.17%)	38 (39.59%)	6 (06.24%)
L. S.- Longitudinal section T.S. – Transverse section				

1. Skin [Fig-1]: Both thick and thin skin tissues were taken for the study. The stratum spinosum and stratum corneum were thicker in the thick skin as compared to those in the thin skin. Sebaceous glands and hair follicles were

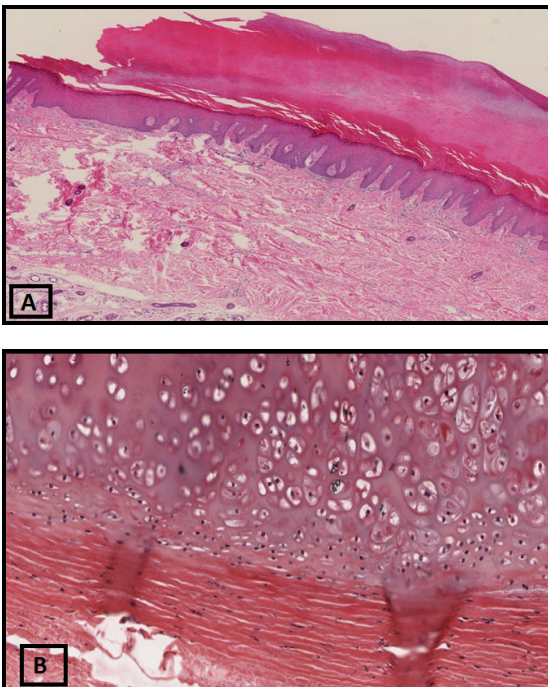
abundantly seen in the thin skin. The dermis showed irregularly arranged coarse collagen bundles. Sweat glands and their ducts were also seen.

Fig-1: The photomicrograph of the section of the thin skin- H &E staining (10X).



2. Elastic cartilage [Fig-2]: Ear pinna was used for elastic cartilage. The cartilage was much more cellular. Each pinna showed a single large chondrocyte. The surrounding matrix was deep staining. The perichondrium was present. Branching elastic fibers were seen in the matrix on higher magnification.

Fig-2: The photomicrograph of the section of (A) Thick skin and (B) Elastic cartilage - H &E staining (10X)

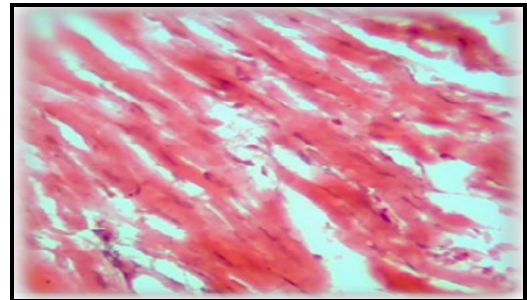


3. Skeletal Muscle: The skeletal muscles were studied in the transverse as well as longitudinal sections.

A. Transverse section [Fig-3A]: The muscle fibers appeared as polygonal profiles with flattening of the adjacent cells with multiple peripheral nuclei. The perimysium was seen

as a large amount of connective tissue which separated the bundles of muscle fibers containing the small blood vessels. The wide endomysial spaces represented shrinkage artifacts.

Fig-3A: The Photomicrograph of a longitudinal section of the skeletal muscle- H &E staining (10X)



B. Longitudinal section [Fig-3B (i) & (ii)]: The muscle fibers were arranged parallel to each other. The fibers were elongated and cylindrical, with multiple peripheral nuclei. There was shrinkage between the muscle bundles.

Fig-3B (i): The Photomicrograph of a longitudinal section of the skeletal muscle -H &E staining (40X)

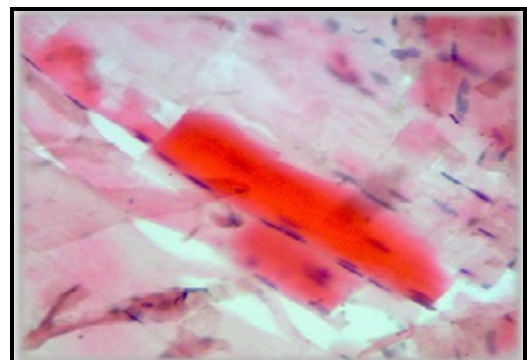
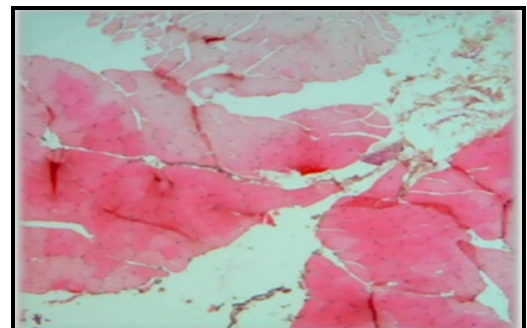
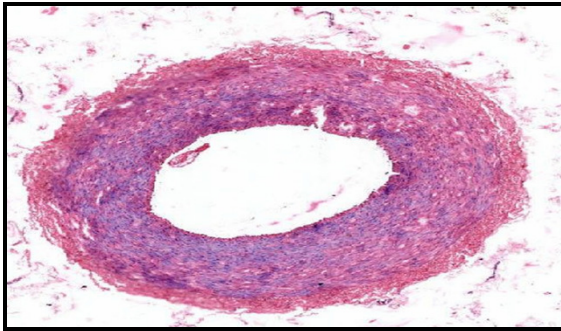


Fig-3B (ii): The Photomicrograph of Transverse section of skeletal Muscle - H &E staining (10X)



4. Artery [Fig-4]: The specimen was taken from a muscular artery. The endothelium was lined by squamous cells with very clear internal elastic lamina. The tunica media predominantly consisted of smooth muscle fibers. The irregularly arranged collagen bundles of the tunica adventitia were well defined.

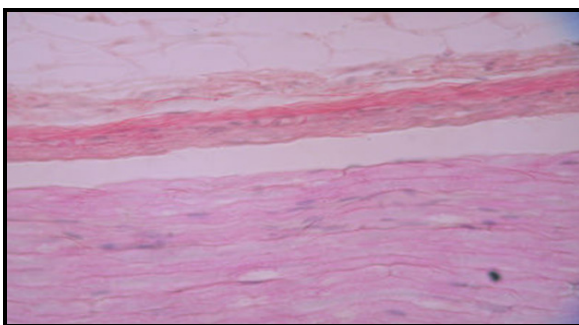
Fig-4: The Photomicrograph of section of muscular artery - H &E staining (10X)



5. Nerve: The tissue sample was taken from the sciatic nerve.

A. Longitudinal section [Fig-5A]: The nerve fascicles were seen enclosed in the perineurium. The fascicle contained many nerve fibers with nuclei in between. These nuclei were mostly Schwann cell nuclei, but some flat, elongated nuclei of the endoneurial fibroblasts were also seen.

Fig-5A: The Photomicrograph of a longitudinal section of the peripheral nerve H &E staining (40X)



B. Transverse section [Fig-5B (i) & (ii)]: The complete nerve architecture in the form of the epineurium, perineurium and the endoneurium was seen. Transversely cut axons with poorly preserved myelin were seen. Several nuclei were seen as in the longitudinal section.

Fig-5B (i): The Photomicrograph of Transverse section of the peripheral nerve - H &E staining (10X)

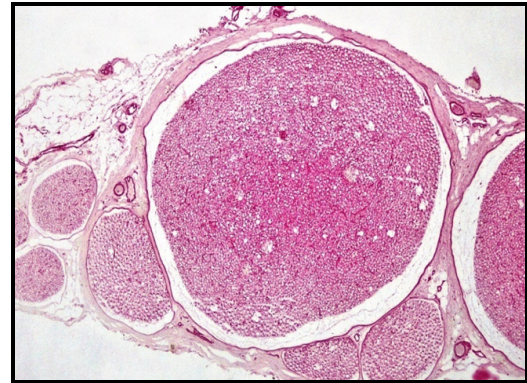
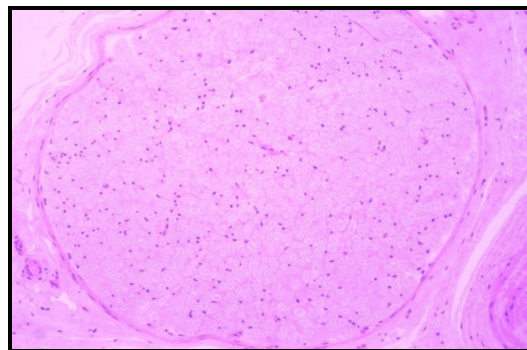


Fig-5B (ii): The Photomicrograph of Transverse section of the peripheral nerve H &E staining (40X)



The slide quality rating across the various tissues is shown in Table 1. Thick skin and Elastic cartilage had the highest percentage of “good” rating, with 83.33% and 66.67%, respectively. Skeletal Muscle & nerve tissues had the highest percentage of “poor” rating, with of 8.33% and 16.67%, respectively.

Discussion

The preparation and teaching of histological slides is an integral and important part of undergraduate and most of the postgraduate curriculum. Cadaveric tissue is an ideal source for teaching and research purpose. It does not require additional ethical clearance. In some institutes, it was considered a novel method of preparing a histological slide to integrate the teaching of gross and microscopic anatomy [9].

Lovering and Russ [10] described the fiber type composition of the cadaveric human rotator cuff muscles. McGrath et al [11]

utilized four large cadaveric tissue blocks to investigate the morphology of the long posterior sacroiliac ligament (LPSL) and its potential relationship to the adjacent structures in the posterior sacroiliac region. Dagain et al [12] did an immunohistochemical and ultrastructural study of the junction between the great cerebral vein and the straight sinus in 25 human cadaveric brains.

Andrew W. quoted that Chapman J.A. from university of Tasmania, Australia studied the suitability of human cadaveric tissue for the generation of Histology teaching slides. He obtained tissues such as skin, liver, pancreas, kidney, lung, lymph node and ureter from the one female and five males' embalmed cadavers. All tissues placed into 10% buffered formalin, processed, embedded into paraffin wax, sectioned and stained with H and E staining. Only certain tissues obtained from the dissected cadavers appears to be suitable for generating tissue sections and high enough quality for using them as teaching slides [4].

In our study the tissues obtained from cadavers after embalming needed less fixation time for the tissue in the embalming fluids as compared to tissue that availed from guinea pig and mice sample. The reason may be embalming of the cadaver added fixation properly of cadaveric tissues to preserve the morphology and chemical composition of the tissue but at the same time hardening of cadaveric tissue get doubled as that of guinea pig tissue. So the cadaveric tissue blocks prepared was getting difficulty to take serial sections [6].

Gupta and Gauba [13] had explored the possibility of utilizing cadaveric tissue for histological studies and reported that cadaveric tissue can be considered ideal for teaching and

research. Kalanjati et al [14] found low concentration of formalin (5-7.5%) which 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 minimized. 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.

Limitations ad Recommendations: A major limitation of this study was the lack of exact information about the clinical history of the body donors and their timing of death. Though the number of cadavers used in this study could provide useful data, a study using a larger number of cadavers with a detailed clinical history would have allowed for a better extrapolation of results. Future studies can include more organs not highlighted in the literature such as pancreas, testes, ovaries, brain, spleen, etc.

Conclusion

The present study observed that almost all the tissues taken from cadavers were either of good or satisfactory quality, with thick skin and elastic cartilage being the best rated. These findings serve as the first step in establishing that embalmed cadaveric histological slides can be useful additional educational tools in medical curricula. The cadaver is an ideal and unlimited source of tissue for research and teaching. Cadaveric tissue histology need to be standardized.

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Conflicts of interest: There are no conflicts of interest.

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*All correspondences to: Dr. Pranita R. Viveki, Associate Professor, Department of Anatomy, Prathima Institute of Medical Sciences, Nagunur, Karimnagar-505417, Telangana, India. E-mail: prviveki@gmail.com