Original Article

Anatomy Section

Cadaveric Tissue Histology: A Viable Alternative

TULIKA GUPTA, KRISHAN GAUBA

ABSTRACT

Background: 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.

Aim: Therefore, this study was conducted to standardize the histology of different tissue types of the human body, which were obtained from the embalmed cadavers which were available in anatomy departments.

Methods: Histology slides were made the following standard protocol, with haematoxylin and eosin staining. The study material was selected to include all the tissue types.

Results: Histologically, all the tissues showed excellent tissue organization. The nuclei, the cell membranes and the cytoplasmic details were clear and well preserved.

Conclusions: This study showed that cadaveric tissue is suitable for histological studies. A cadaver is an ideal source of tissue for the purpose of teaching and research.

Key Words: Cadaveric tissue, Histology, Histology teaching

KEY MESSAGE

- The cadaveric tissue is suitable for histological studies.
- The cadaver is an ideal and unlimited source of tissue for research and teaching.
- There is a need to standardize the cadaveric tissue histology.

INTRODUCTION

Knowledge of the histology of normal tissues is an essential requirement for establishing parameters for diagnosis of pathological conditions. Thus, teaching histology is an important and integral part of the undergraduate and most of the post graduate curriculums. 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. Surgically removed tissues have the drawbacks of erratic supply as well as ethical clearance, which is becoming increasingly difficult. Apart from their limited availability, the cost of procuring good teaching slides may be prohibitive for many teaching departments.

An alternative method to obtain normal tissue for histology is from cadavers. Once a cadaver has been obtained for anatomical studies by following the standard procedures, there are no ethical issues which are involved with it. Thus, cadaveric tissue could be an ideal source of tissue for bio-medically related structural research, without the constraints of additional ethical approval. This can provide a ready source of material for getting sufficient body tissues for studying the normal human histology.

There are sporadic reports on the cadaveric histology of different tissues like ligaments and muscles, but there is no comprehensive study which is available on the importance and the utility of the cadaveric histology. 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 histopathologists with a practically unlimited source of normal histological tissue for comparison with the pathological specimens.

METHODS

This study was carried out in the Department of Anatomy at the Institute of Dental Sciences and Hospital, Punjab University, Chandigarh. The tissues were harvested from a cadaver which was available in the Department of Anatomy at the HSJ Institute of Dental Sciences and Hospital, Punjab University, Chandigarh. The cadaver was embalmed about forty-eight hours after death. The body was kept in a refrigerated chamber before embalming. The embalming was done by using a 40% of formaldehyde solution

which was mixed with adjuvants. As this cadaver had been procured by following a standard ethical protocol, additional ethical clearance was not required to harvest the tissues.

Despite their disparate structure and physiological properties, all the organs are made up of only four basic tissue types, the epithelium, connective tissue, muscle tissue and nerve tissue. The following tissues were selected deliberately, as together they encompass all the above mentioned tissue groups and their subtypes. This enabled us to study the histological status of all the types of tissues in the human cadaver.

1. Skin
2. Muscle
3. Cartilage
4. Artery
5. Brain
6. Trachea
7. Liver
8. Spleen
9. Nerve
10. Small intestine

Five samples of every tissue were taken from the same cadaver.

1. **Tissue Preparation**

   - **Cadaver selection:** The following parameters were recorded at the time of the cadaver selection: age, sex, cause of death, medical history and the time lapse between the death and embalming. The cadaver was embalmed by using a perfusion and local injection method. The embalming fluid contained 10% formalin, 10% ethanol, 20% glycerin, 5% phenol, thymol crystals and magnesium chloride. The embalming was done within 48 hours of death.
   - **Tissue harvesting:** Each tissue type was taken from the same cadaver, with no history of involvement of the above tissues with a disease process.
   - **Fixation of the tissue sample:** It was the first step in the tissue preparation. It was done to preserve the tissue structure for subsequent treatments. 10% Formalin was used as a fixative.
   - **Paraffin embedding:** The tissue was infiltrated with the embedding medium that allowed it to be thinly sliced. The specimen was washed after fixation and it was dehydrated in a series of alcohol solutions of ascending concentration up to absolute alcohol to remove water from the tissue. An organic solvent, (Xylene), which is miscible in both alcohol and paraffin, was then used to remove the alcohol prior to infiltration of the tissue with melted paraffin.
   - **Block making:** When the melted paraffin was cool and hardened, it was trimmed into an appropriate sized block.
   - **Sectioning:** The block was mounted and sliced by using the Spencer type microtome.
   - **Staining:** Haematoxylin and Eosin (H and E) staining was done. 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..As an Eosin stain is more soluble in alcohol than in water, the specimen was again dehydrated through a series of alcohol solutions of ascending concentration and was stained with Eosin in alcohol.
   - **Mounting:** After the staining, the specimen was passed through Xylene to a non-aqueous mounting medium and it was covered with a cover-slip.

2. **Microscopy:** The prepared slides were examined and thoroughly studied under a trinocular light microscope with a camera. Each slide was seen by two different observers. These slides are available in the Department of Anatomy. A detailed histological description of each tissue was recorded.

Each tissue was examined for its histological details. The tissue architecture, including the thickness and the condition of various layers in the given tissue were checked. The cellular details like the cell size, the cell membrane and the nuclear details, as well as the intercellular spaces were noted. As the histological specimens were from a cadaveric tissue, special attention was paid to identify the features which indicated cell death.

3. **Data Storage and Compilation: Photographic data:** Microscopic photographs were taken for each tissue. The microphotographs were taken in three different resolutions – 4X, 10X and 40X. They were directly stored into the computer data base for further analysis and comparison. The objective data for each slide which described the cellular details and the architecture was recorded and compiled.

4. **Comparison:** Each tissue subtype was compared with a standard histological slide of the same tissue which was similarly stained, but was made from the tissue which was obtained from a live human body. One point each was awarded for the following details and a total score of 10 was devised for the perfect match.

   - **Tissue architecture:**Arrangement of various cellular elements in relation to each other.
   - Sharpness of the cellular outline.
   - Cell size.
   - Intra-cellular details at the given magnification.
   - Size and position of the nucleus.
   - Staining properties of the nucleus.
   - Intra-nuclear features like nucleoli and the arrangement of chromatin.
   - Connective tissue: state and staining.
   - Absence of the intra-cellular spaces which indicated shrinkage.
   - Absence of the features which indicated cell death

RESULTS

The histological specimens were obtained from a cadaveric tissue and therefore, special attention was paid to identify the features which indicated cell death. Cell death can be of two types: (1) Cell necrosis: Cell swelling and cell lysis are two characteristic features of this process and (2) Apoptosis: The characteristic features are- DNA fragmentation (the nucleus divides into several discrete fragments which are bounded by the nuclear membrane), decrease in cell volume, loss of mitochondrial function, membrane blebbing and formation of the apoptotic bodies. In the present study, there was no evidence of necrosis or apoptosis. Histologically, all the tissues showed excellent tissue organization and cellular detail. The nuclei, the cell membranes and the cytoplasmic details were clear and well preserved.

1. **Skin [Table/Fig-1a and b]:** The thick skin and the thin skin tissues were both taken for the study. The epidermis showed excellent cell detail. All the epithelial layers could be clearly appreciated: the stratum basale showed columnar cells with oval nuclei, the stratum spinosum was seen to be having polyhedral cells, the stratum granulosum was one to two cells thick and the keratohyaline granules were clearly visible and the stratum corneum was seen as the top most layer which consisted of cells which had lost their nuclei (keratinized cells). As expected, the layers, the stratum spinosum and the

stratum corneum were thicker in the thick skin as compared to those in the thin skin. Hair follicles and sebaceous glands were abundantly seen in the thin skin. The dermis showed a preserved tissue architecture- a papillary dermis with fine interlacing collagen fibers and normal neurovascular structures was seen, while the reticular dermis showed coarse, irregularly arranged collagen bundles. Sweat glands and their ducts were seen.

Comparison score: 10. These slides were compared with those which were made from the tissues which were obtained from the live human body. The tissue architecture was very well preserved. The cellular outlines were sharp, with clear nuclear details. The basophilia in the basal layer and in the stratum granulosum was distinct.

2. **Muscle:** The skeletal and the smooth muscles were studied in the longitudinal as well as in the transverse sections. The cardiac muscle tissue could not be studied.

   a. **Skeletal muscle:**
      i. **Longitudinal section [Table/Fig-1 C1]:** The skeletal muscle fibers were arranged parallel to each other. The fibers were elongated and cylindrical, with multiple peripheral nuclei. The endomysial connective tissue could be seen in between the fibers. There was shrinkage between the muscle bundles.
      ii. **Transverse section [Table/Fig-1 C2]:** It clearly showed a delicate endomysium in between the individual muscle fibers. The muscle fibers appeared as polygonal profiles with flattening of the adjacent cells. The cross-sectional area of each fiber was approximately the same. Multiple peripheral nuclei were present. The wide endomysial spaces represented shrinkage artifacts. The perimysium was seen as a large amount of connective tissue which separated the bundles of muscle fibers which contained the small blood vessels.

      Comparison score: 7. The tissue architecture was well preserved, but shrinkage artifacts in the form of increased tissue spaces were seen between the individual muscle fiber and the fiber bundles. The tissue was disrupted at one place. The intra-nuclear features were not so distinct. These changes might be due to tissue mishandling or they may indicate a poor fixation of the tissues.

   b. **Smooth muscle:**
      i. **Transverse section:** The cut sections of varying diameters were seen. The nucleus was not present in all the sections and when it was present, it was single, central and of different sizes. The spindle shape of the muscle fibers as well as their nuclei, were sectioned at different levels, resulting in transverse sections of different sizes of muscle fibers and their nuclei. The endomysium was clearly visible.
      ii. **Longitudinal section [Table/Fig-1D]:** Spindle shaped muscle fibers were seen. In the centre of the myocyte, a single, central and fusiform nucleus was present.

      Comparison score: 8. The fiber outline was sharp and the nuclei were distinct, but the intranuclear details were not clear and the nuclei were over stained.

3. **Cartilage:**

   a. **Hyaline cartilage [Table/Fig-2A]:** Chondrocytes were present in groups, with a darker territorial matrix surrounding these isogenous cell nests. Each chondrocyte was in the vacuole and it showed a distinct, small nucleus. The perichondrium was distinct and the morphological gradation of the chondrocytes extended from the inner region towards the outer region, in such a way that the outer most cells were like the fibroblasts.

      Comparison score: 9. The tissue architecture is well preserved, with good cellular details. The intra nuclear chromatin pattern was not discernible.

   b. **Elastic cartilage [Table/Fig-2B]:** This was taken from the ear pinna. The cartilage was much more cellular then the hyaline cartilage. The cells were not arranged in groups or as cell nests. Mostly, a single chondrocyte was seen in each lacuna. The surrounding matrix was deep staining. The perichondrium was present. On higher magnification, branching elastic fibers were seen in the matrix.

      C**omparison score: 9.** The intra-nuclear chromatin pattern was not discernible.

4. **Artery [Table/Fig-2C and D]:** The specimen was taken from a muscular artery. The endothelial lining which consisted of squamous cells, was seen. The internal elastic lamina was very distinct. The tunica media predominantly consisted of smooth muscle fibers. The irregularly arranged collagen bundles of the tunica adventitia were well defined.

   Comparison score: 9. The tissue was torn at one place, with the rent passing from the endothelium to the tunica media. This was possibly the result of tissue mishandling rather than a postmortem change.

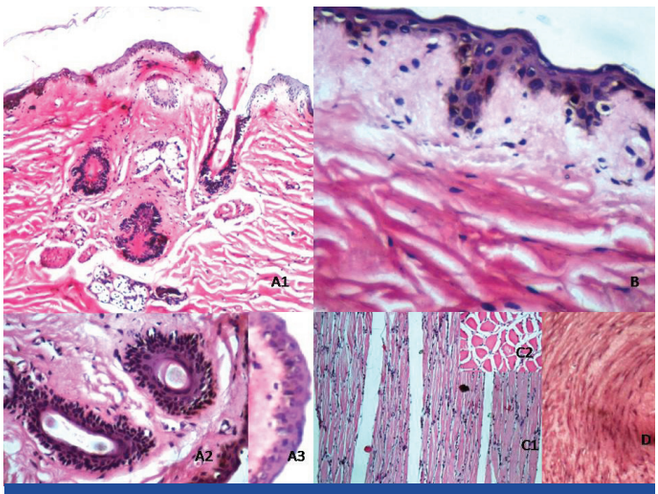
5. Nerve: The tissue sample was taken from the ulnar nerve.

   a. **Longitudinal section [Table/Fig-3 A1] :** 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.
   b. **Transverse section [Table/Fig-3 A2]:** 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.
      Comparison score: 8. The myelin preservation was not optimal and enlarged tissue spaces were observed.

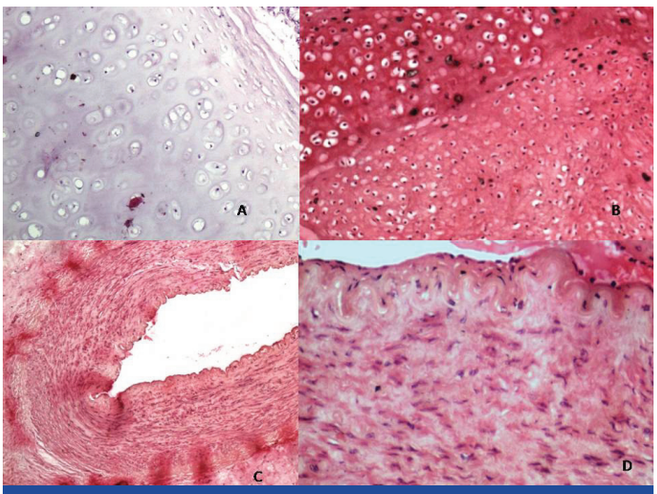
6. **Brain [Table/Fig-3 B1 and B2]:** The specimen was taken from the cerebral hemisphere. The outermost molecular layer with predominant horizontal fibers was clearly demarcated. The subsequent layers had characteristic pyramidal cells as the main cell type. The size of the pyramidal cells increased progressively in layers 2, 3, 4 and 5. In these layers, the fibers were predominantly vertically disposed. Numerous stellate cells were seen, especially in layer 4.

   Comparison score: 9. Clear areas were seen around some neuronal bodies; probably there were artifacts which were caused due to shrinkage during the tissue processing.

7. **Trachea [Table/Fig-4A]:** The wall of the trachea consisted of the mucosa, the submucosa, the hyaline cartilage and the adventitia. The mucosa showed a typical pseudostratified ciliated columnar epithelium with goblet cells, which was resting on a thick basement membrane. Beneath the basement membrane, the lamina propria was highly vascular



[Table/Fig-1]:
A. (1) The photomicrograph of the section of the thin skin at (10x) magnification showing clear details of epidermis and dermis. (2) The cut sections of the hair follicles. x 40. (3) The keratinized stratified squamous epithelium is shown under high power.
B. The photomicrograph of the thick skin. x 40. The epithelial details are clear and show stratum basale, stratum spinosum and stratum granulosum.
C. (1) The Photomicrograph of a longitudinal section of the skeletal muscle. x 10. (2) The skeletal muscle is shown in transverse section. x 40.
D. The Photomicrograph of a longitudinal section of the smooth muscle. x 10.



[Table/Fig-2]:
A. The photomicrograph of the hyaline cartilage taken from the trachea. x 10. The isogenous cell nests with the territorial and inter territorial matrix are seen. Perichondrium is visible on the right side.
B. The photomicrograph of the elastic cartilage taken from the ear pinna. x 10.
C. The photomicrograph of a transverse section of a muscular artery. x10.
D. The photomicrograph of the wall of a muscular artery with clear depiction of the endothelium, internal elastic lamina and tunica media. x 40.

and it showed diffuse lymphatic tissue. The loose submucosa contained numerous mixed seromucous glands. The hyaline cartilage was surrounded by a perichodrium which merged with the submucosa on one side and the adventitia on the other side.

Comparison score: 9. The tissue architecture was well preserved in all the layers. The glands showed mixed sero-mucous acini. The nuclei of the chondrocytes were clearly visible in the lacunae, with a clearly demarcated territorial and inter territorial matrix.

8. **Liver [Table/Fig-4 B1 and B2] :** The normal liver architecture in the form of the classical liver lobule, with a central vein and peripheral portal tracts, was appreciable. Hepatic cells were present as radiating plates which branched and anastomosed. The haepatocytes were polygonal and varied in size. Portal venules, branches of the hepatic artery and bile ductules were clearly seen in the portal tracts.

   Comparison score: 10. The cellular details were well preserved, with the haepatocytes depicting a round nucleus with peripherally dispersed chromatin and prominent nucleoli.

9. **Spleen [Table/Fig-4 C1,C2 and C3]:** Under low magnification, the dense connective tissue capsule was found to enclose the spleen, with the trabeculae extending into the substance of the spleen. The white pulp constituted of the typical lymphatic nodules, with each nodule containing an eccentric arteriole. Under high magnification, the details of the red pulp in the form of splenic cords and intervening sinuses could be appreciated.

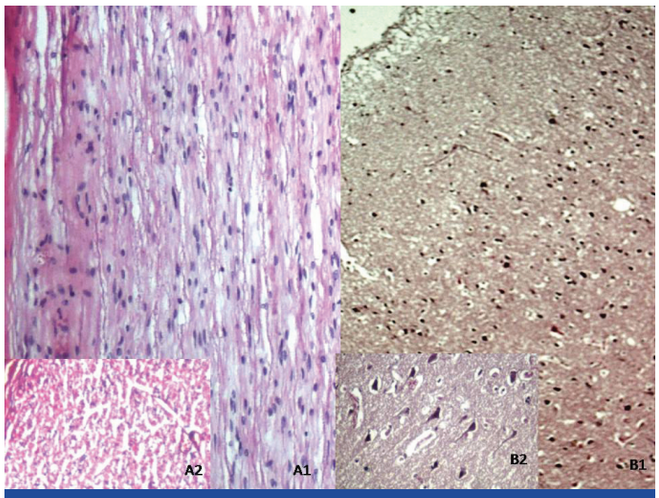
   Comparison score: 10. The nuclear details were clear in the lymphocytes. All the layers of the arterioles were clearly demarcated.

10. **Small intestine [Table/Fig-4 D1 and D2]:** The mucosa showed distinctive villi, the lamina propria and the musularis mucosae which separated the mucosa from the underlying submucosa. Tall columnar enterocytes and goblet cells covered the intestinal villi. The core of the villi was the extension of the lamina propria and it consisted of loose connective tissue. The muscularis externa and the serosa were seen.

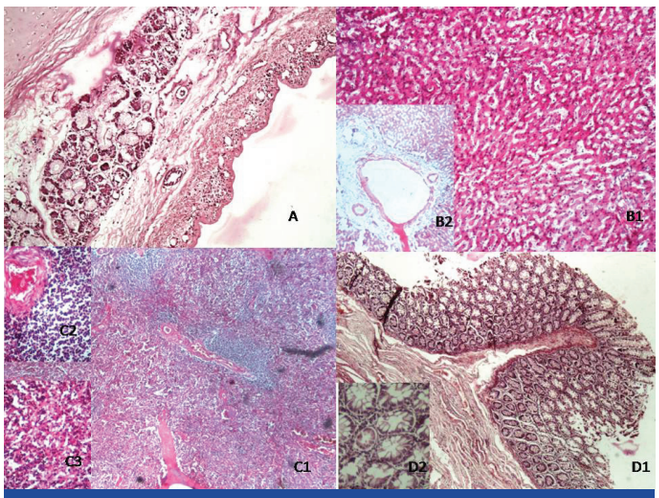
    Comparison score: 8. The cellular details were seen, but the nuclear details were not very clear. Increased tissue spaces were seen at a few places.

DISCUSSION

Histology teaching is an important and integral part of the anatomy curriculum. The histological slides are usually prepared by using tissues which have been incidentally removed during surgical procedures, which have the obvious drawback of being limited and therefore, being an unreliable source. An alternative tissue source is animal tissue which is easily available, but it has different histological details as compared to the human tissue. Cadaveric tissue histology is not used in routine practice. Therefore, this need based study was proposed to standardize the histology of different tissue types of the human body which were obtained from the embalmed cadavers which were available in anatomy departments. There are sporadic reports on the use of cadaveric tissue for histology, especially for the study of specific disease processes. However, a detailed study of the cadaveric histology which encompasses all the tissue types which are present in different organs has not been published before. Alonso et al. (2009) reported the quantitative evaluation of inflammatory cells in the human temporo-mandibular joint tissues from patients with and without implants, by using normal cadaveric tissues for the control group [1]. An anatomic and histological study of the coraco-humeral ligament (CHL) on cadaveric shoulders by Yang et al. (2009) determined the CHL's histological features in comparison with the joint capsule and the coraco-acromial ligament [2]. Lovering and Russ, (2008) described the fiber type composition of the cadaveric human rotator cuff muscles [3]. To study the connective tissue degeneration in elderly strabismic



[Table/Fig-3]:
A. (1) The photomicrograph of the longitudinal section of a peripheral nerve. x10. (2) The photomicrograph of a transverse section of a peripheral nerve. x 4.
B. The photomicrograph of a section of the cerebral cortex. 1. x 10 and 2. x 40.



[Table/Fig-4]:
A. The photomicrograph of a transverse section of the tracheal wall. x 4.
B. The photomicrograph of a section of the liver. 1. x 4 and 2. x 10.
C. (1) The photomicrograph of the spleen showing the capsule, trabeculae with part of the red pulp and the white pulp. x4 (2) The splenic follicle. x40 (3) The splenic red pulp. x 40.
D. (1) The photomicrograph of a transverse section of the small intestine. x 4. (2) The intestinal glands shown in the transverse section. x 40.

patients, Rutar and Demer, (2009) compared MRI scans with the histology of four cadaveric orbits [4]. McGrath et al. (2009) 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 [5]. Dagain et al. (2008) did an immunohistochemical and ultrastructural study of the junction between the great cerebral vein and the straight sinus in 25 human cadaveric brains [6].

All these sporadic studies authenticate the value of the cadaveric histology and underline the need to standardize it. In the present study, the integrity of each tissue type was assessed in detail. The tissue could be handled well during fixation and block making, as well as during staining. Histologically, all the tissues showed a normal tissue organization and cellular detail. The nuclei, the cell membranes and the cytoplasmic details were clear and well preserved, as has been detailed in the Results section. There was no evidence of necrosis or autolysis. This study showed that cadaveric tissue is suitable for histological studies. The cadaver is an ideal and unlimited source of tissue for research and teaching. The routine use of cadaveric tissue for the preparation of histology slides would be of immense benefit to the anatomy departments for teaching students.

REFERENCES

[1] Alonso A, Kaimal S, Look J, Swift J, Fricton J, Myers S, et al. A quantitative evaluation of the inflammatory cells in human temporo-mandibular joint tissues from patients with and without implants. *J Oral Maxillofac Surg*. 2009; 67:788-96.
[2] Yang HF, Tang KL, Chen W, Dong SW, Jin T, Gong JC, et al. An anatomic and histologic study of the coracohumeral ligament. *J Shoulder Elbow Surg*. 2009; 18: 305-10.
[3] Lovering RM, Russ DW. The fiber type composition of the cadaveric human rotator cuff muscles. *J Orthop Sports Phys Ther*. 2008; 38:674-80.
[4] Rutar T, Demer JL. "Heavy Eye" syndrome in the absence of high myopia: A connective tissue degeneration in elderly, strabismic patients. *J AAPOS*. 2009; 13: 36-44.
[5] McGrath C, Nicholson H, Hurst P. The long posterior sacroiliac ligament: a histological study on the morphological relations in the posterior sacroiliac region. *Joint Bone Spine*. 2009; 76: 57-62.
[6] Dagain A, Vignes JR, Dulou R, Dutertre G, Delmas JM, Guerin J, et al. The junction between the great cerebral vein and the straight sinus: an anatomical, immunohistochemical, and ultrastructural study on 25 human brain cadaveric dissections. *Clin Anat*. 2008; 21: 389-97.

AUTHOR(S):
1. Dr. Tulika Gupta
2. Dr. Krishan Gauba

PARTICULARS OF CONTRIBUTORS:
1. M.D., Department of Anatomy, Institute of Dental Sciences & Hospital, Punjab University, Chandigarh, India.
2. MDS, Department of Oral Health Sciences, Post Graduate Institute of Medical Sciences & Hospital, Chandigarh, India.

NAME, ADDRESS, TELEPHONE, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Tulika Gupta,
Department of Anatomy, Institute of Dental Sciences & Hospital, Panjab University, Chandigarh, India.
Phone: 91 172 2724912; Fax: 91 172 2724912
E-mail: tulikag11@yahoo.com

DECLARATION ON COMPETING INTERESTS:
No competing Interests.

Date of Submission: **Jul 28, 2011**
Date of peer review: **Sep 15, 2011**
Date of acceptance: **Oct 10, 2011**
Date of Publishing: **Dec 25, 2011**