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Study of Histology of Adrenal Glands in Human Foetuses of Different Gestational Ages

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***Abstract*:** *Study of suprarenal glands at the histological level is of great importance as it affects the metabolism immensenly and more so in a growing foetus, the maturation of various foetal organs. And the effects in hypertension and diabetes mellitus associated prergnancies make a special note. The present work is done in 40 human foetus of different gestational ages (12 to 40 weeks). The foetuses were fixed in formalin, dissected, suprarenals extracted, fixed, processed, sectioned, stained with haematoxyline and eosin and photographed. The two regions of the gland- definitive cortex and medulla are identified. The parenchymal cells and ganglionic cells were differentiated by 28th week.*

Key words: *Adrenal gland, cortex, medulla, sympathochromaffin tissue, graded alcohols, xylene, haematoxyline and eosin.*

I. Introduction

Study of histogenesis of adrenal glands in human foetuses of different gestational ages, is of great utility as it involves several separate endocrine glands within one anatomical structure. The internal environment of the human body is influenced by the hypothalamo – pituitary adrenal axis, renal angiotensin system by the glomerulosa, catecholamines by the medullary zone.

The suprarenal gland or adrenal gland derives its name due its situation atop the kidneys. It is divided into two distinct zones – cortex and medulla.

Suprarenal gland affects normal as well as risk pregnancies especially pregnancies associated with hypertension and diabetes mellitus.

Bronsterin et al (1993) succeded in visualizing and detecting fetal glands by transvaginal ultrasound examinations even in the 12^{th} week. Jeanty and Romero performed a successful transabdominal ultrasound examination in the 23 rd week gestation.

Jennings et al (1993) diagnosed even medullary tumors (NEUROBLASTOMA), L jubic Petkovic S, Radenovec N, of Serbia reported on visualization and growth of fetal adrenal glands in the period from the 11^{th} to 40^{th} week of gestation. Thus in view of various interesting changes found in the histology and in the developmental and functional aspects of adrenal glands in the embryonic life and their further development in adult form of a human has prompted me to undertake the study of histogenesis of adrenal gland. In the present study the existing literature on the fetal adrenals has been thoroughly reviewed and observations have been made after study of 40 fetal adrenals of different gestational ages (12 to 40 weeks).

II. Materials And Methods

The present work is the result of study of human fetal suprarenals 40 in number obtained from RIMS general Hospital, Srikakulam from department of Obstetrics and Gynaecology . Age of the fetuses ranged from 12 to 40 weeks. The fetuses of earlier weeks could not be procured. The age of the fetus is judged by Crown Rump Length.

Thereafter all these 40 fetuses were fixed in 10% formalin for 10 days. Then the fetuses were subjected to dissection. The anterior abdominal wall is dissected, the liver and Gastrointestinal tract are removed to view the suprarenals in its natural location for proper recording.

The specimens of the human fetuses utilized in the present study were categorized into three groups. 1^{st} group–12 weeks, 2^{nd} group – 13^{th} to 24 weeks and 3^{rd} group 25^{th} to 40 weeks.

Histological study of Suprarenals

Fixation: The tissues of 5 mm thickness were taken and preserved in freshly prepared solution of 10% formal saline fixative for 7 days.

Tissue processing : 5 mm thick suprarenal tissues of 12 weeks, 16 weeks, 18 weeks, 24weeks, 36 weeks and 40 weeks gestation have been taken and thoroughly passed through graded alcohols of 50%, 70%, 80%, 90% and absolute alcohol as follows .

50% alcohol	-	12 hours duration
70% alcohol	-	4 hours duration
80% alcohol	-	2 hours duration
90% alcohol	-	2 hours duration
100% alcohol	-	1 hour each with two changes.

Clearing has been done in xylol for 1 hour each for two changes. Embedding of tissues is done in paraffin having a melting points of are 56^0 C for 1 hour with two changes. The paraffin blocks are made with the help of 'L' blocks and they are left overnight in the refrigerator.

Sections of 5 microns size are cut with rotary microtome and mounted on slides treated with egg-albumin. The slides are allowed to dry at room temperature for 18 to 24 hours.

Staining Procedure

Haematoxyline and Eosin method

Paraffin is removed with xylol after treating the slides for 3 to 5 minutes. Xylol is removed with graded alcohols namely 100%, 90%, 80%, 70%, 50%. They are then washed with water and stained with haematoxyline solution for 5 minutes. Sections are then kept in running tap water till light blue color appears. They are then counter stained with eosin solution for 30 to 40 seconds. They are then washed with alcohol, cleared with xylol and mounted with D.P.X. mount.

Composition of Haematoxyline Solution

Haematoxyline	2 Gms
Absolute alcohol	100 ml
Glycerine	100 ml
Distilled water	100 ml
Glacial acetic acid	100 ml
Potash Alum in excess	

Composition of Eosin

1 Gm of eosin is dissolved in 100 ml of distilled water.

The microphotographs of sections stained with H&E were studied on SAMTRON computer having a closed circuit camera and an adaptor fixed to LYNX (Lawrence and Mayo) trinocular research microscope. The stained sections were examined with 10x and 40x Magnifications.

The close circuit camera with adaptor is attached to one of the eyepieces of trinocular microscope.

With manipulations and fine adjustments of camera as well as that of microscope, pictures having magnifications of 10x and 40x have been obtained with good resolution on computer screen and this has been utilized for taking microphotographs of various sections .

Chromaffin Tissue

The fresher the material the sharper the chromaffin reaction, though staning may be obtained in specimens fixed 24 to 48 hours postmortem (Schmrol).

Slices of the organ are placed in 3% Potassium dichromate for 7 days. Then transferred to an 8% solution for 10 days. Washed in running water for 24 hours. Rapid dehydration is adopted after which clearing and embedding is completed 15-20 microns thick sections showed the yellow reaction best after counter staining with haematoxyline.

Schmorl's adaptation of Giemsa's stain procedure

It gives good differentiation of cortex and medulla.

1. Fix material in 3% potassium dichromate 8 parts, 40% formaldehyde, 2 parts for 24 hours.
2. Wash in running water
3. Either make frozen sections (said to be preferable) or embed in paraffin.
4. stain sections for 1-24 hours in Giemsa's stain (1 drop of Giemsa's stain for every ml of distilled water)
5. Rinse in water
6. Blot
7. Dehydrate with acid free acetone and control differentiation with the staining microscope
8. Clear in xylene

9. Mount and examine in Canada balsam or D.P.X.
Medullary cells – bright red to violet
Cortical cells – blue to blue violet
Red blood – corpuscles – pink

III. Observations

Microscopic Appearance

Capsule of the gland has been seen right from the 12th weeks onwards bilaterally.

12 weeks of gestation: the supra renal gland and its capsule are well identifiable. The cortex showed a superficial zone which is darker having a thickness of ¼ of total cortex. A deeper zone constituting remaining ¾ of other zone. Medulla is very much ill defined but it is identified as having very few cells at this stage.

16 Weeks of Gestation: The Superficial zone is differentiated into a deep stained area having cells arranged in closed packed nests and however, they do not show further differentiation. The superficial zone also showed cells arranged in vertical columns constituting the second differentiated layer of the cortex. The deeper part of the cortex is undifferentiated having sinusoids and cells arranged along the sinusoids. Cellular configuration is not well marked out at 10x10 magnification. anlage of medulla is seen.

At higher magnification, cells in the superficial zone are uniformly arranged. Small groups of cells, in semilunar areas are also seen. The other details of the cells are not clear.

18 weeks of gestation: Cortex is well differentiated into zona glomerulosa, zona fasciculata. The width of zona fasciculata is more. The cells are large with abundant cytoplasm and well defined nuclei. Medulla showing clusters of cells which are the analge of ganglionic cells.

24 Weeks of gestation: Further demarcation of deeper zone into anastamosing cords of cells and increasing number of ganglionic cells have been observed.

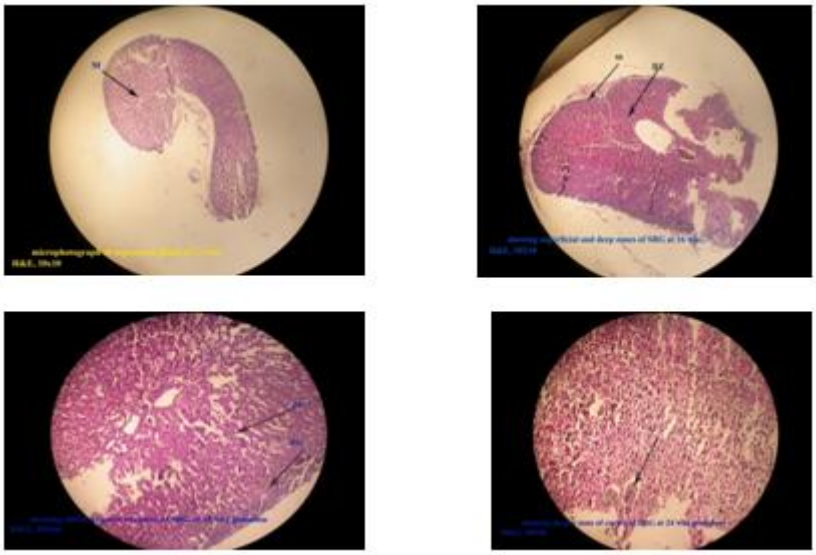
36 Weeks of gestation: Polyhedral Cells with a central nucleus are clearly seen cell population is dense. There are trabeculae extending from the capsule. Extra cellular matrix showed increased perisinusoidal spaces. Medulla of 36 weeks showing intensely developed chromaffin cells with increased number of blood vessels. Cortex and medulla are indistinct.

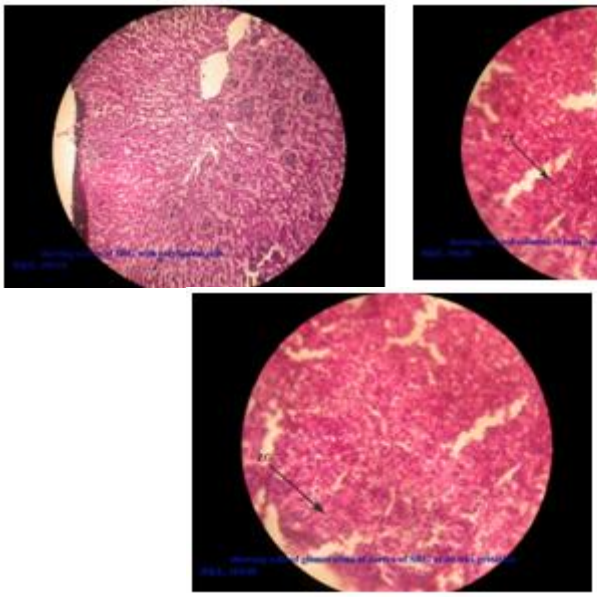
40 Weeks of gestation: There is a dense capsule and fibroblasts having been seen along the trabeculae extending deep into the cortex. The Zona glomerulosa is reduced. The earlier demarcation of superficial and deep zones has been disappeared. The cells of glomerulosa are polyhedral, arranged in semicircular groups with central sinusoids. Nucleus of cell is clearly demarcated. Zona fasciculata showing single cellular line of vertical columns of cell which are polyhedral with definite nucleus and the cytoplasm is acidophilic.

A well defined zona reticularis is also seen, the cells are small; polyhedra cells are present in between anastomosing cords. Sinusoids are also seen.

Medulla is showing larger cells, identified a chromaffin cells. There is no distinct boundary between cortex and medulla.

FIGURES 1 TO 7





IV. Summary And Conclusions

Summarizing the following conclusions is drawn out.

- Suprarenal gland is developed from the mesoderm of posterior abdominal wall in the angle between the root of mesentery and developing gonad and also from neural crest.
- Foetal suprarenals are relatively large. At 16th week of gestation the glands are large than the kidneys.
- The reduction in the size of the gland has been noticed by the time of birth.
- The fetal cortex that undergoes involution and necrosis with a net loss of 50% of gland within 2-3 weeks.
- In the present study the left suprarenal glands are large than right suprarenal glands confirming the observations of earlier authors.
- A slow increase in the size of suprarenal glands has been observed confirming in the reports of earlier authors.
- In the microscopic study the two regions of definitive cortex with two types of parenchymal cells have been identified as is said by GTN Sangma et al (2008).
- The development, differentiation and changes is the cortical zone of the foetal cortex into inner and peripheral cortex and later changes relative to the foetal age have been found and confirmed in the present study.
- The chromaffin cells have been well differentiated as early as 12th week in the present study.
- Ganglionic cells have been well differentiated by 28th week of gestation.

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