ORIGINAL RESEARCH PAPER

Medical Science

HISTOGENESIS OF SUPRARENAL GLAND IN HUMAN FETUSES AT DIFFERENT WEEKS OF GESTATION WITH IMPLICATIONS OF CADAVERIC TRANSPLANTATION OF SUPRARENAL GLAND IN PATIENTS WITH NEUROBLASTOMA AND OTHER ADRENAL TUMOURS

KEY WORDS: Mesothelial cells, Mesenchyme, Reticular zone, Neural crest cells, Pheochromocytoma.

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ABSTRACT

The suprarenal gland develops from two components: *(a)* a mesodermal portion, which forms the cortex, and *(b)* an ectodermal portion, which forms the medulla. During the fifth week of development, mesothelial cells between the root of the mesentery and the developing gonad begin to proliferate and penetrate the underlying mesenchyme. Here they differentiate into large acidophilic organs, which form the fetal cortex, or primitive cortex, of the suprarenal gland. Shortly afterward a second wave of cells from the mesothelium penetrates the mesenchyme and surrounds the original acidophilic cell mass. These cells, smaller than those of the first wave, later form the definitive cortex of the gland. After birth the fetal cortex regresses rapidly except for its outermost layer, which differentiates into the reticular zone. The adult structure of the cortex is not achieved until puberty. While the fetal cortex is being formed, cells originating in the sympathetic system (neural crest cells) invade its medial aspect, where they are arranged in cords and clusters. These cells give rise to the medulla of the suprarenal gland. They stain yellow-brown with chrome salts and hence are called chromaffin cells. During embryonic life, chromaffin cells are scattered widely throughout the embryo, but in the adult the only persisting group is in the medulla of the adrenal glands. This study will help in knowing the anatomical basis of histogenesis of adrenal gland with a view of future prospects of Cadaveric Transplantation of Suprarenal Gland in Patients of Adrenal Tumours after adrenalectomy.

INTRODUCTION-

The Anatomy of suprarenal gland was described almost 450 years ago in 1563 by Bartholomeo Eustacius and zonation of gland and its distinction from medulla were elucidated shortly thereafter. Brown-sequard demonstrated that adrenal gland was "Organs Essential for Life.

The suprarenal (adrenal) gland is a crucial component of the hypothalamic-pituitary-suprarenal axis that is responsible for coordinating mammalian stress response and metabolism. Initially, formation of the suprarenal gland is closely tied to that of the gonads, as both arise from a common region of intermediate mesoderm lying adjacent the developing kidney. Segregation of the suprarenal and gonadal primordia occurs when primordial germ cells enter the gonadal region. By the 9th week, the suprarenal primordia are completely enclosed by a capsule. As might be expected, the specification of the suprarenal primordia depends on many of the same transcription factors and signalling molecules as those involved in kidney and gonadal development (e.g., Wt1 and Wnt4). During the 5th week of development, the coelomic epithelium adjacent to the developing gonadal ridge proliferates and a subset of these cells delaminates and enters the underlying mesoderm. These delaminating cells differentiate into large acidophilic cells forming the fetal suprarenal cortical cells. A second wave of delaminating cells subsequently migrates, proliferates, and forms a thinner definitive cortex that almost completely surrounds the fetal cortex. Ultrastructurally, cells of both fetal and definitive cortical layers exhibit cytologic characteristics of steroid-producing cells. During the second trimester, the fetal cortical layer grows rapidly in size and begins secreting dehydroepiandrosterone (DHEA), a hormone converted by the placenta to estradiol, which is essential for maintaining pregnancy. Moreover, products from the fetal suprarenal cortex influence the maturation of the lungs, liver, and digestive tract and may regulate parturition. By the second postnatal month, the fetal cortex rapidly regresses and the remaining definitive cortical cells then organize into the zona glomerulosa, zona fasciculata, and zona reticularis layers seen in the adult suprarenal gland. Before being cordoned off by the formation of the suprarenal capsule, neural crest cells migrate into the suprarenal medullary region adjacent the developing fetal cortex. These neural crest cells differentiate into chromaffin cells, which are specialized postganglionic sympathetic neurons innervated by preganglionic sympathetic fibers that release Epinephrine and Norepinephrine upon sympathetic stimulation. [1]

Microscopically neuroblastic nodules and giant epithelial cells are found in the fetal adrenal glands. Beckwith and Perrin have termed them as 'in situ neuroblastoma'. As the age advanced, both disappeared. Neuroblastic nodules are consistently observed at the end of the first trimester and reach a peak number of 70 to 100 per gland between the 16th to 20th weeks of gestation. From late second trimester through the end of gestation, the nodules decrease in number so that relatively few or none are present at birth. [2]

Bronsterin et al (1993) succeeded in visualizing and detecting fetal glands by transvaginal ultrasound examinations even in the 12th week. Jeanty and Romero performed a successful transabdominal ultrasound examination in the 23rd 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 11th to 40th week of gestation.

AIMS AND OBJECTIVES-

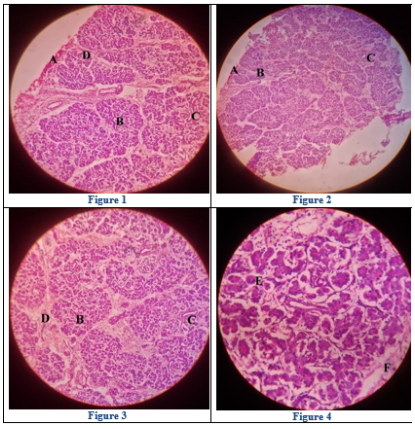
The present study aims to study histogenesis and development of human suprarenal gland in prenatal period to observe microscopic structure of adrenal gland at various gestational age groups and its future implications in cadaveric transplantations of suprarenal gland in patients with adrenal tumours like neuroblastoma and Pheochromocytoma.

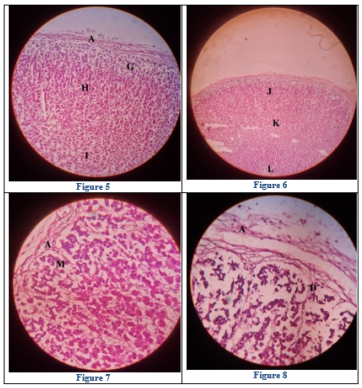
MATERIALS & METHODS-

This study was done to correlate the chronological pattern of suprarenal gland development in this geographical eastern region of India, Odisha & compare the results from other researchers nationwide & worldwide. This is a hospital based, observational,

cross sectional study conducted at Hi- Tech Medical Colleges & Hospital, Bhubaneswar, India by the Department of Anatomy in collaboration with Department of Obstetrics & Gynaecology from November 2011 to June 2013 on thirty two aborted human foetuses without obvious congenital anomaly of gestational age between 12 weeks and 36 weeks collected within 6 hours of delivery by spontaneous miscarriages & therapeutic legal abortions. Study samples were arbitrarily divided into groups of biweekly gestational age by duration of amenorrhoea from medical records & ultrasound fetometry after receipt of informed consent from mother and legal guardians. Fetuses were immediately fixed in 10% Formalin for 1-2 hrs. Suprarenal gland was dissected by Dissecting Microscope, fixed in 10% Formalin for 48-72 hrs. After fixation by formalin, the tissues were transferred to 30%, 50%, 70%, 90% and Absolute alcohol each for 30 minutes. This ascending grading of the dehydrating fluid was done because when alcohol mixes with water, it produces diffusing current which can damage the tissues. Then the tissues were put in xylol for 24 hours to clear the residual alcohol. These tissues were processed for paraffin sections by tissue blocking (Paraffin Embedding). 3 pots of hard paraffin were taken; paraffin was melted in the incubator at 56 degrees, as hard paraffin is ideal for materials which are to be cut in thin sections about 12 mu. The tissue was put in the first pot containing equal parts of paraffin and xylol and then changed to second and third pots containing only fresh melted paraffin at 90 minutes interval. Then the tissues were mounted in fresh melted paraffin with L-Block. The L-Block was then trimmed to a rectangular shape. Then the L-Block was fixed with the block holder (choke) and the block holder was clamped in the rotary microtome. 5 mu sections were cut in rotary microtome. The microtome was revolved at 40 per min and ribbon was formed. Then the ribbon was put in tissue flotation bath. Albuminised slide was then made by putting a drop of Mayor's albumin (equal parts of glycerine and egg white) and spreading it uniformly by rubbing with finger. The piece of ribbon was then taken on the slide and dried at room temperature. The slide was then put in the warming table. When the paraffin melted the slide was put into xylol for 2-3 minutes because xylol removes paraffin. Then the tissue was put in decreasing grades of alcohol (Absolute alcohol,90%,70%,50% and 30%) then was put in the prepared Harris Alum Haematoxylin (nuclear) stain for 7 minutes and lastly washed with distilled water.2-3 drops of 1% acid alcohol (1cc Hcl in 75% alcohol) was added to remove the excess stain beyond the nucleus. The slide was then put in running tap water for 30 minutes to develop haematoxylin colour (bluish). Then the slides were again dipped in ascending grades of alcohol (30%, 50%, and 70%) and then put in eosin Y (cytoplasmic) stain for 30 seconds. Then the slide was washed with absolute alcohol for a few seconds so that excess of eosin was removed and lastly the slide was placed in xylol. The slide was then taken out from xylol and then put in 1-2 drops of DPX (Adhesive agent) and a cover slip was put on it and pressed slightly so that air bubbles were removed. Sections were then seen in light microscope under low power 10X followed by high power 45X magnification. Thereafter photomicrographs were taken by camera using microscope adapter.

OBSERVATIONS-





In earlier weeks of gestation Capsule (A) is very demarcated by not thickened which was made up of collagen fibres. Deep to the capsule loose subcapsular connective tissue was seen. Fetal Cortex (B) and Blood vessels can be seen in the Medulla ©. Vascularity of the gland is seen to be increasing in the Medulla region. Trabeculae (D) were seen in later weeks of Gestation which were seen to be extending into the fetal cortex. Some Fibroblasts accompanying occasional blood vessels can also be seen to be present along the trabeculae. Very few Ganglionic cells (E) can be seen which indicates the presence of Neuroblastic nodules. Giant Epithelial Cell with degenerative changes were not seen. Medulla with seen with fibrous zone (F). In later weeks of gestation, the Definitive cortex (M) was formed which were further differentiated into Superficial Cortex (J) and Deep Cortex (K) and Medulla (L). The Definitive cortex was further divided into Zona Glomerulosa (G), Zona Fasciculata (H) and Zona Reticularis (I). The width of zona fasciculata is more. The cells are large with abundant cytoplasm and well-defined nuclei. The Zona glomerulosa is subsequently reduced in later weeks of gestation. The cells of glomerulosa are polyhedral, arranged in semi-circular groups with central sinusoids. 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; polyhedral cells are present in between anastomosing cords. Sinusoids are also seen.

DICUSSION-

According to Wendell Smith [3] after vascularization and encapsulation by surrounding mesoderm, nests of proliferating cells under the capsule form the primitive glomerular zone. From them cords grow centripetally to constitute the foetal cortex, which is bulky and responsible for the relatively large size of the suprarenal in the newborn. The foetal cortex shrinks rapidly after birth as the cords degenerate and are replaced by a definitive fascicular zone growing from the glomerular zone.

In their study, Sangma et al [4] have reported to have observed a well-defined capsule in foetal SRGs of 9-16 wks. that was well developed and completely surrounding the gland in the SRGs of 28-32 wk. foetuses. They have observed a superficial narrow zone of darkly stained cells underneath the capsule which is the permanent cortex that showed uniformly scattered cells in 16-22nd week foetuses that in later age groups clumped together in arc or acini formation and a deeper lighter zone called the foetal cortex that became bulkier with advancing age occupying 5/6th of the entire cortex in term foetuses.

Sant Ram et al [5] in their study have done histometry and reported that the capsule was identifiable & measured 59 µ at periphery & 124 µ at hilum, at 11-15 wks. of gestation & the thickness increased to 312 µ at more than 25 weeks of gestation. They observed two zones of cells in the cortex like the previous authors. They reported that the superficial strip of dark zone (permanent cortex) occupied 1/4th of the cortex at 11-15 weeks of gestation which increased to 4/5th of cortex at >25wks. They observed that the cells were present in U shaped arrangement/clusters/groups and glomerular arrangements of cells were also seen. They reported that deep to the dark zone there was a lighter zone (foetal cortex) constituting 3/4th of the cortex at 11-15 weeks

which increased to 4/5th of the cortex at >25 weeks which suggested that the foetal cortex was becoming bulkier.

Starkel et al [6] and Mc Intosh [7] attributed that the large size of the suprarenal in fetal period was due to presence of a zone present only during fetal period. This zone occupyied about 80% of the entire cortex. This zone was limited by a peripheral strip of darkly stained zone called the permanent cortex constituting about 20% of the entire cortex. However they did not measure fasiculoreticular zone and sinusoidal vessels.

Uotila [8] observed fetal cortical cells were large, eosinophilic and homogeneous in contrast to the cells of the permanent cortex which were small, basophilic and possess a darkly staining nuclei and fetal cortex further gave rise to reticular and fascicular layers. Almost, same findings were observed in the present study.

According to Hervonen [9], the adrenal medulla is derived from neural crest cells in association with the development of the rest of the sympathetic nervous system. Neuroblastic cells migrate from the neural crest which forms collections alongside the aorta which later develop into the paravertebral sympathetic ganglia. Nerve fibers extend laterally from the last eight thoracic and the first two lumber paravertebral sympathetic cells. Cells and fibers enter the adrenal primordium throughout its length, passing between the cortical cells and separating them into small groups and islands.

Sadler [10] describes the origin of the suprarenal medulla and cortex from a different source. As the medulla of the suprarenal gland, it is derived from neural crest cells these cells reach the medio-dorsal aspect of the primitive cortex at the 16 mm stage (44 days) and soon begin to invade it. Later they form a cell growth on the medial aspect of the extensive cortex. However, they are not completely encapsulated by the cortex until later in fetal life. They show histological evidence of the presence of catecholamines by the 10th week of fetal life.

Turkel and Itabashi observed that the individual neuroblastic nodule size remained essentially unchanged at about 60 x 60µ in all age groups, which indicated an optimal size for these avascular, tightly packed clusters. A cystic change within neuroblastic nodules was common and considered to be a part of the normal developmental pattern. It did not appear until early in the second trimester, reached a maximum in the 16th week and declined in the older fetuses. [11]

Ikeda et al observed that in normal fetal development, nodular collections of neuroblast cells were found in the suprarenal glands from 7th week of gestation. These nodules increased in size and number in fetuses of 14-18weeks gestation. Aggregations of nodules closely resembling neuroblastoma in situ were found. From 12 week gestation, the large neuroblastic nodules appear to split up into smaller nodules and differentiated into chromaffin cells. [12]

Khayati et al observed that migrating neuroblastic cells were seen from capsule toward medulla at 11-15 weeks of gestational age groups. At least one neuroblastic nodule was present on an average in fetuses up to 25 week gestation. No neuroblastic nodule was seen in the fetal specimens 25-28 weeks. [13] The findings of present study were similar to the previous research.

Mini Mol P. et al observed that neuroblastic cells migrate from capsule towards central blood vessels. They differentiate into the chromaffin cells and sympathetic neurons, decrease the number of neuroblastic nodules from 20 weeks onward. [14]

Guin et al worked on a combined retrospective and prospective study of incidental neuroblastoma. They found that the small lesion commonly found in the youngest patients were possibly embryologic remnants. [15]

Lonergan et al concluded that neuroblastoma, ganglioneur oblastoma and ganglioneuroma are tumors of the sympathetic nervous system that arise from primitive sympathogonia and are referred to collectively as neuroblastic tumors. [16]

Kampmeier observed that the giant epithelial cells developed with the adrenal cortex, though there is considerable variability in the size of the common adrenal cortical cells and their nuclei and possibility exists that the giant cells arise from simple enlargement. He also observed that the giant cells were not seen at periphery or subsequent glomerular area but present in the rest of cortex and medulla at the gestational age of 8-12 weeks (2-3 months) only. [17] Mesiano and Jaffe described the transitional zone as a zone of finger like columns of cells extending from definitive cortex to foetal cortex. [18]

According to Allen et al [19] the suprarenal gland in armadillo is composed of two definitive regions, an outer zone i.e the definitive cortex consisting of numerous small rounded cells and an inner zone i.e fetal cortex where two types of parenchymal cells were present throughout gestation. Increase in size of suprarenal gland was at the expense of fetal zone but the permanent zone was much smaller. The zone was similar to that observed in humans.

Iwanaga et al [20] observed a gradual, linear increase in the number of chromaffin cells of developing adrenal medulla. They observed medullary chromaffin cells as small islands of cells dispersed throughout the gland, were more numerous in the central part and adjacent to the medial border. They consisted of 5-14 cells with dark, pynotic nucleus and scarce cytoplasm. These cells were also scattered singly.

Keene et al [21] observed that degeneration of fetal cortex started during last 10 weeks of intrauterine life and was completed by the end of first year. Our study was correlated with the observations in earlier studies but Neuroblastic Nodules and Giant Epithelial cells showing degenerative changes were not seen in any gestational weeks.

CONCLUSION-

The present study is an attempt to add to the existing literature on the histogenesis of the suprarenal gland available nationwide and worldwide. Histological studies on sections obtained from neonate and infant supra renal gland specimens would show the involution of foetal cortex and the persistence of the adult cortex that is similar to the cortex of the adult supra renal gland. [22] The knowledge of the behavior of neuroblastic nodules and their fate gives an additional dimension to the histogenesis of adrenal gland. The spontaneous regression of such nodule or persistence and progression to neuroblastoma may be of great clinical significance. [23]

This study also showed that due to the presence of increasing vasculogenesis in fetal suprarenal gland, the fetal suprarenal tissue can be better transplanted in children with Neuroblastoma and other adrenal tumours because of the decreased risk of Graft Rejection. This study can be enriched further by Immunohisto che mical and Ultrastructural studies to highlight the neuroblastic nodules and their regressions in relevance to Neuroblastoma in children.

ACKNOWLEDGEMENT-

The corresponding author acknowledges the support and guidance received from Dr. Shyamal Kumar Basu.

CONFLICTS OF INTERESTS- None

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