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Original Article

Histogenesis of suprarenal gland in fetuses of different gestational age groups

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ABSTRACT

Thirty suprarenal glands (both right and left) of different gestational age groups were studied to see the cytoarchitecture using different stains. The present study was carried out in the department of Anatomy, Government Medical College & Hospital, Chandigarh. The material for the study consisted of 30 spontaneously aborted human fetal specimens from 12th to 28th weeks of gestational ages. The suprarenal glands were taken from fetal specimens for histological study. The staining was done by three different methods; Hematoxylin and eosin, Malory's Trichrome and Singh's Modification of Masson-Hamperl Argentaffin Technique. Histologically, it was observed that suprarenal gland has a superficial narrow zone of darkly stained cells underneath the capsule, which was the permanent cortex and a deeper lighter zone called the fetal cortex. The changes were seen in thickness of capsule, medulla and the layers of the cortex. The study will establish the micro development of suprarenal gland in human fetuses in North-West Indian population. The arrangement of cells in the permanent cortex changed from the discrete cells and clusters to well formed glomerulus like structure with increasing gestational period. Fasiculo reticular zone of the fetal cortex decreased in thickness as gestation advanced. The cells were arranged in columns extending deeper into the cortex towards medulla. Sinusoidal vessels increased in number and were 34 micron wide at 11-15 weeks and decreased to 15 micron at >25 weeks gestation. In the medulla, the ganglionic cells were 3-4/field initially which increased to 25-30/field in later gestation period. Few cells showed vacuolization at 11-15 weeks and there was presence of fibrous zone in the medulla consisting of collagen fibres with fibroblasts at >15-20 weeks suggesting early degenerative changes

1. Introduction

The suprarenal gland plays a vital role in survival and maintenance of internal milieu1. Microscopically the adult supra renal gland has two zones cortex and medulla. The cortex is further divided in to zona glomerulosa, zona fasciculata and zona reticularis. These zones are different both functionally as well as morphologically. Histologically, fetal suprarenal gland has a superficial narrow zone of darkly stained cells underneath the capsule, the permanent cortex and a deeper lighter zone, the fetal cortex (Sadler, Hamilton et al). As, the gestational age advances, fetal cortex becomes bulkier and before term it constitutes about 5/6th of entire cortex11. After birth, fetal cortex regresses rapidly except for its outer most layer, which differentiates into the reticular zone. Medulla is filled with large blood vessels, few large cells having abundant cytoplasm with vesicular nuclei, which stain yellow/brown with chrome salts. The chromaffin cells show presence by the 10th week of fetal life11.The medulla is completely encapsulated by the cortex till later fetal life.

Rapid growth and changes within the different zones of human fetal supra renal gland is primarily reflection of growth of the unique fetal zone (Sadler, Hamilton et al).At the end of pregnancy, the fetal zone undergoes senescence through an apoptotic process7. As the gland grows caudal part differentiates more rapidly than cranial portion of the gland.

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Therefore the structure of suprarenal gland undergoes a massive change during its development and growth. In earlier stages fetal cortex grows maximum, followed by regression which paves way for the appearance of adult cortex. In addition the changes are seen in the thickness of capsule, permanent cortex and fetal cortex

Therefore an attempt was made to see histological changes in fetal suprarenal gland.

2. Material and Methods

The study was carried out in the department of Anatomy, Government Medical College & Hospital, Chandigarh. The material for the study consisted of 30 human fetuses from 12th to 28th weeks of gestational ages which were sent by the department of Obstetrics & Gynaecology for routine fetal autopsy. All the fetal specimens obtained were the result of intrauterine death and spontaneous abortion and were apparently normal. Consent for the autopsy and relevant history from the parents was taken. The suprarenal glands were dissected out, removed and fixed in 10% formalin for 72 hours. All the fetuses included did not have any congenital deformity or malformation of the genitor urinary tract and central nervous system. The specimens were divided into four groups according to the gestational age as follows.

GROUPS	GESTATIONAL AGE	NUMBER OF FETUSES
A	11-15 weeks	5
B	>15-20 weeks	9
C	>20-25 weeks	10
D	>25 weeks	6

The suprarenal glands thus obtained were processed for histological examination. The different stains used to see the microscopic structure are as follows.

1) Hematoxylin and eosin was used to demonstrate the normal structure of the suprarenal gland.

2) Malory's Trichrome was used to demonstrate the histology of the capsule3,4 . The constituents of the stain were 0.2% acid fucshin, 1% phosphotungstic acid, 2.5 gm aniline blue, 2 gm Orange-G.

3) Singh's Modification of Masson-Hamperl Argentaffin Technique was used to demonstrate the histology of medulla in suprarenal gland3,4. The constituents were 10% aqueous silver nitrate, ammonia, 1% aqueous sodium thiosulphate, neutral red.

The prepared slides were examined under pentahead microscope. The selected sections were photographed.

3. Results:

The observations in the tissue of the different age groups will be described age wise

Group A (11 to15 weeks)

Group A (11 to15 weeks)

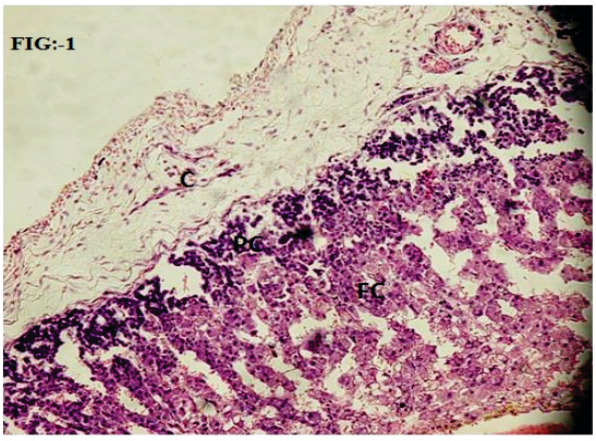
Haematoxylin and eosin- With this stain capsule was identifiable under light microscope. It was thin and made up of collagen fibres. Amongst the collagen fibres scattered fibroblasts were also seen. (fig. 1) Capsule was thicker at periphery than at hilum. Fibres were more densely packed superficially in the capsule. Deep to the capsule loose subcapsular connective tissue was also seen .

The cortex of the fetal supra renal exhibited two distinct zones. A superficial dark zone and deeper, a comparatively lighter zone.

Superficial strip of dark zone (permanent cortex) was observed. The cells in this zone were present in U shaped arrangement/ clusters (fig.1).

Deep to the dark zone there was deeper lighter zone (fetal cortex) (fig.1) . This zone could be divided in to:-

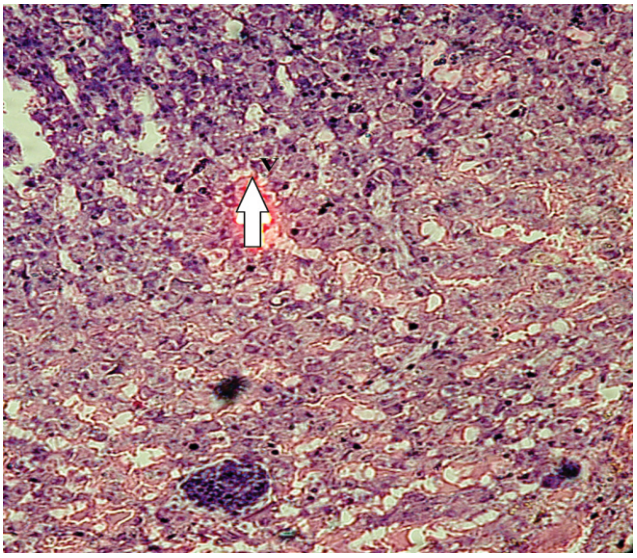
Figure 1: Capsule(c) And Permanent Cortex(pc) Showing U Shaped Arrangement Of Cells And Fetal Cortex (fc) At 15 Weeks. (h&e; X 60)



Fasiculo reticular zone- It was 6-7 layered thick. The cells were larger having nucleus but lighter stained than permanent cortex. The cells were arranged in columns extending deeper into the cortex towards the medulla. Few cells showed vacuolization in the cytoplasm showing early degenerative changes.

Cells of fetal cortex towards the medulla were arranged in anastomosing network forming reticularis layer. Cells were polyhedral in shape. Nucleus was smaller. Vacuolization was also seen in few cells (fig. 2).

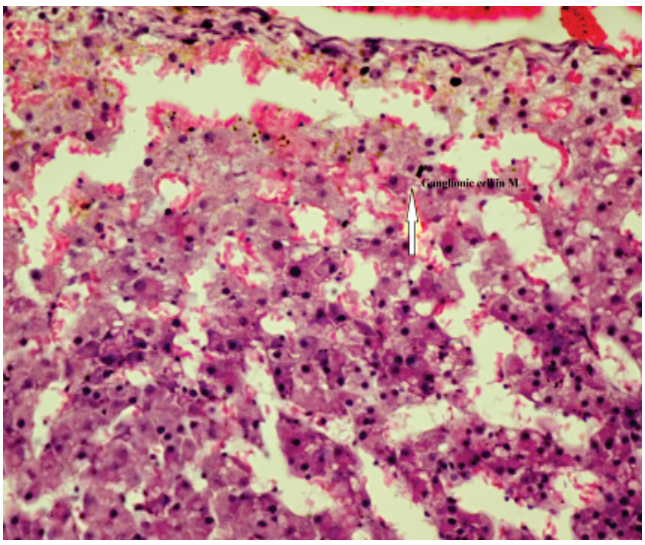
Figure 2: Fasiculoreticular Zone Few Cells Showing Vacuolization(v) In The Cytoplasm Suggesting Early Degenerative Changes At 15 Weeks (h&e; X60).



Medulla was recognized by the presence of blood vessels at the centre with a few ganglionic cells (fig. 3). The ganglionic cells were 3-4/field. The cells were oval shaped with large cytoplasmic processes. These cells also showed presence of coarse granules in them nucleolus was also visible in these cells.

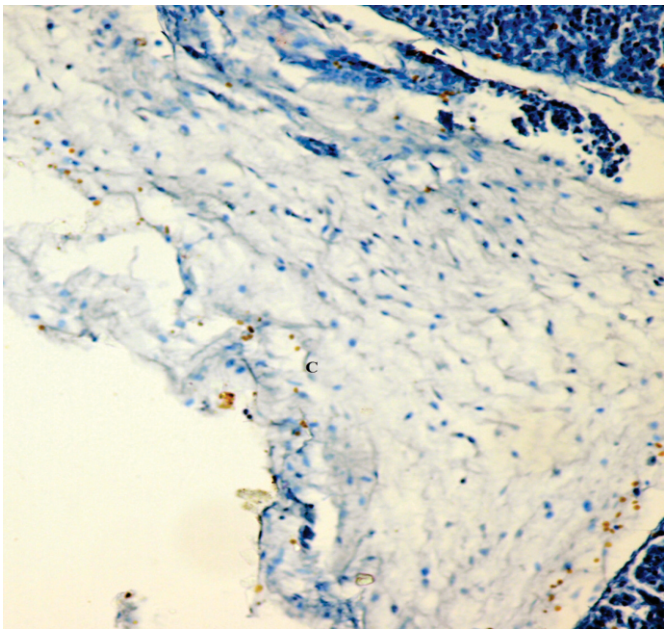
Some round to oval neuroblastic cells were also seen in medulla at this age. Cytoplasm was visible as thin rim around nucleus in these cells. Nucleus was heterochromatic with prominent nucleoli. Sinusoidal vessels were seen.

Figure 3: Medulla (m) At 15 Weeks Showing Few Ganglionic Cells And Blood Vessels (h&e; X60).



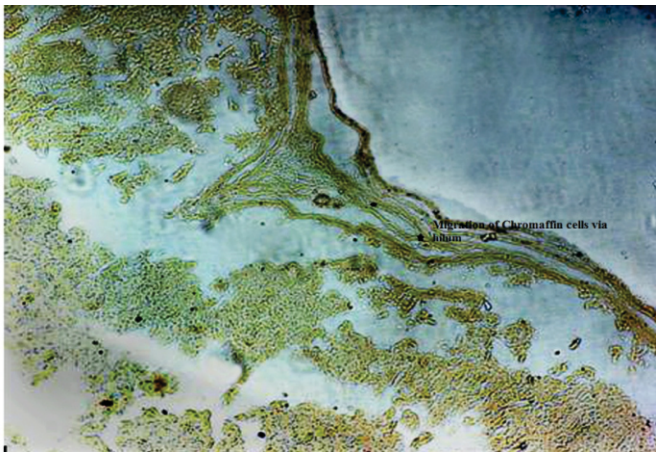
Mallory's stain:- With this stain collagen fibres of the capsule were stained blue in colour. Nucleus of fibrocytes were stained dark blue and blood vessels were stained orange in colour (fig. 4). The arrangement of the collagen fibres in capsule were in conformation with the arrangement seen in the haematoxylin eosin stain.

Figure 4: Collagen Fibres And Fibrocytes In The Capsule (c) At 15 Weeks (mallory's; X100).



Singh's modification of masson-hamperel argentaffin technique:- This stain was used to demonstrate chromaffin cells. It showed migration of cells through the hilum of capsule (fig. 5).The cells were lying singly or in groups. Each cell had single round nucleus. Most of these cells had granules in cytoplasm. Some cells did not have any granule in them .

Figure 5: Migration Of Chromaffin Cell Via Hilum Of Capsule At 15 Weeks (singh's Modification Of Masson-hamperel Argentaffin Technique; X 100)

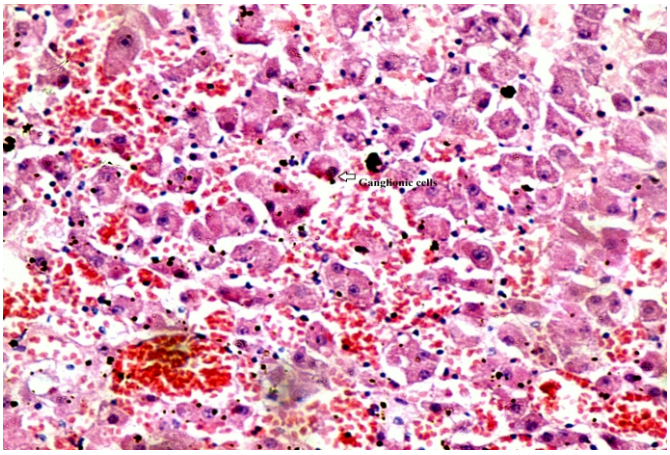


Group B (>15-20 weeks)

Capsule - Capsule was well identifiable at this stage. Amongst the two zone of cortex in superficial strip of dark zone (permanent cortex) cells were now reorganized in the form of semilunar arc/ groups.

The deeper lighter zone (fetal cortex) was bulkier than permanent cortex. Fasiculoreticular zone was 5-6 layered thick. Here the cells were larger than permanent cortex, having abundant cytoplasm with well defined nucleus. The cells were arranged in rows extending from superficial dark zone to the central region towards medulla. Cells of fetal cortex, in the areas just adjacent to the medulla, were arranged in loose network like that of adult zona reticularis layer. Cell population in fetal cortex was more than previous gestational age groups.

Figure 6: Medulla With Ganglionic Cells At 18 Weeks (h&e; X100).



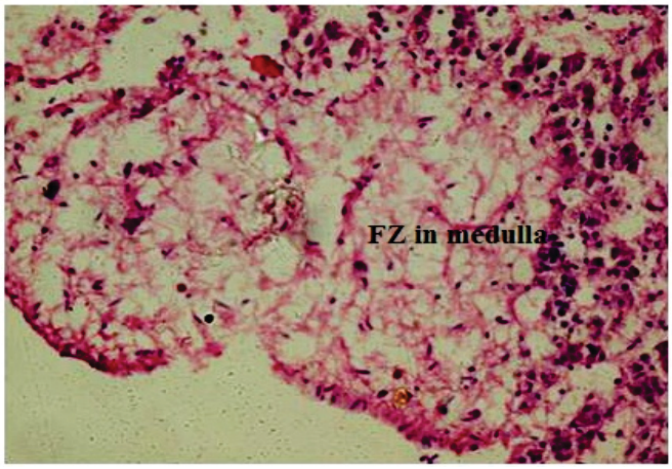
Medulla was filled with numerous blood vessels at the centre with a few ganglionic cells. The ganglionic cells were observed 2-3 cells /field (fig: 6). An indication of degenerative change in the medulla was noticed by the presence of a fibrous zone in medulla consisting of collagen fibres with fibroblasts. (fig. 7).

The round to oval neuroblastic cells were also seen. Sinusoidal spaces were better developed at this age.

Mallory's stain:- Capsule was stained blue in colour. Nucleus of fibrocytes were dark blue. There was increase in blood vessels which were stained orange in colour.

Singh's modification of masson-hamperel argentaffin technique:- The chromaffin cells were organized in trabecula /groups. The cells were in close contact with the blood vessels. The cells were present in fasiculoreticular zone also.

Figure7: Medulla With Fibrous Zone(fz) At 18 Weeks (h&e; X60).



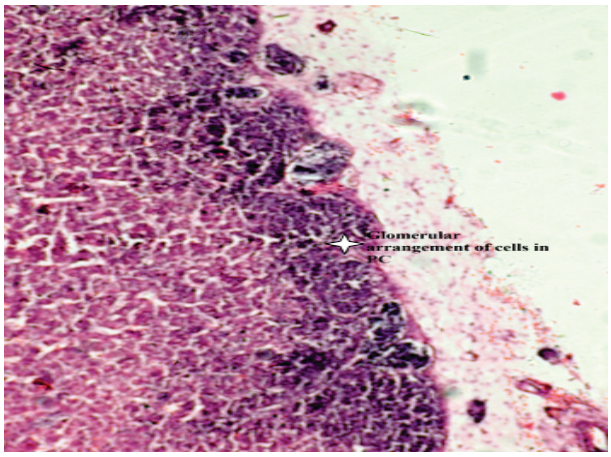
Group C (>20-25 weeks)

Capsule – The capsule was well developed at this age and it surrounded the whole of the gland. The blood vessels were seen in the capsule as compared to previous gestational age groups.

In superficial strip of dark zone (permanent cortex) cells were arranged in small groups and glomerular arrangement of cells was also seen distinctly at this age (fig. 8). Trabeculae were also seen extending from the capsule

In Deeper lighter zone (fetal cortex) the fasiculo reticular zone was 2-4 cell layered thick. Cells in this gestational group were better defined. The extracellular matrix was also well marked. There was increased peri sinusoidal space.

Figure 8: Cells Of Permanent Cortex In Glomerular Arrangement At 24 Weeks (h&e; X60).

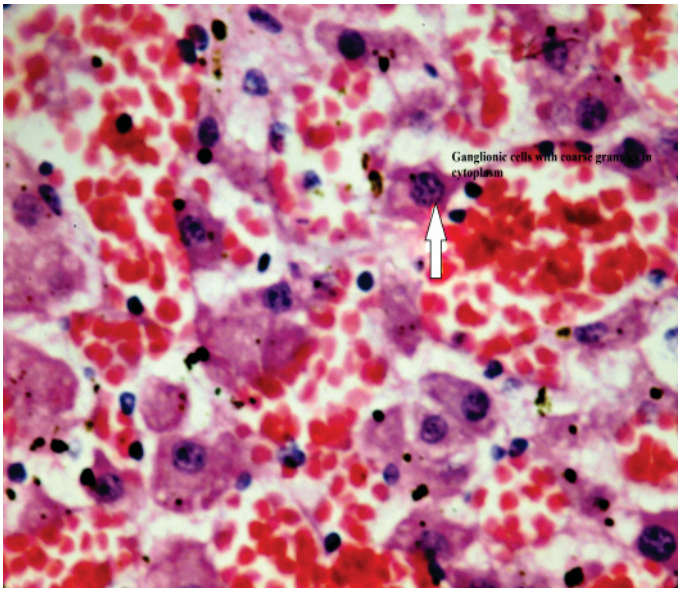


Medulla was filled with many blood vessels at the centre. The number of ganglionic cells increased compared to the previous gestational age groups. The ganglionic cells showed presence of coarse granules in the cytoplasm in almost all of the cells (fig.9). . The number of ganglionic cells increased from 2-4 in previous gestational age groups to 20-25 /field at this age

Mallory's stain:- Similar findings were observed as seen in group B.

Singh's modification of masson-hamperel argentaffin technique:-The chromaffin cells were organized in trabeculae/ groups. The cells were present in the medulla. The cells were slightly larger than the previous group.

Figure 9: Medulla With Ganglionic Cells Showing Coarse Granules In The Cytoplasm At 24 Weeks (h&e; X100).



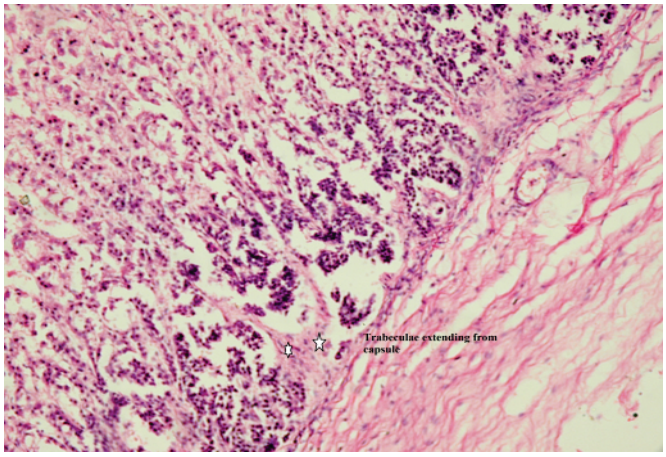
Group D (>25 weeks)

Capsule – The capsule was made up of densely packed collagen fibres. The fibroblasts were seen along the trabeculae which were extending deep to the cortex (fig.10).

In the cortex superficial strip of dark zone zone the cells were arranged in glomerular form. Trabeculae extending from capsule were better seen.

In Deeper lighter zone (fetal cortex) the fasiculo reticular zone was 1-2 cell layered thick. Cells were well defined arranged in rows like the adult zona fasciculata. The cells in inner part had reticular arrangement giving rise to zona reticularis.

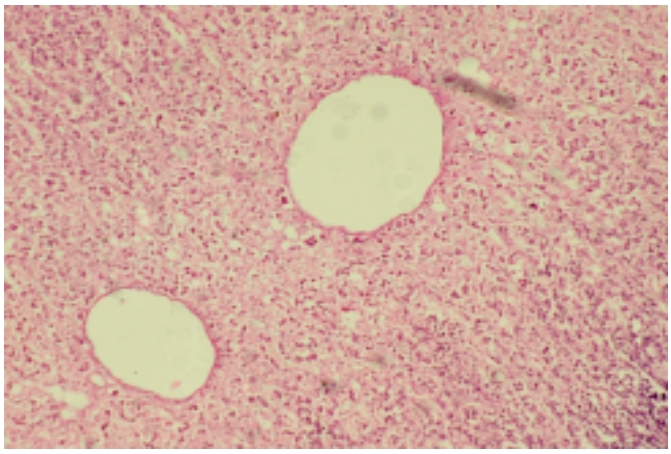
Figure 10: Trabeculae extending from capsule at 26 weeks (h&e; x60)



Medulla was full of blood vessels. Large ganglionic cells were present. They were found 25-30 /field at 100x magnification. The cells were oval in shape having big cytoplasmic processes. Nuclei was displaced towards the periphery.

Sinusoidal vessels were numerous as compared to previous gestational age groups. These sinusoids were larger in central part of the gland (fig11)

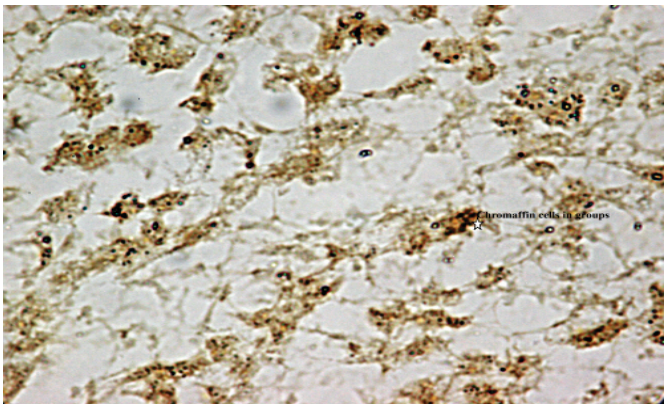
Figure 11: Large Sinusoids In Central Part Of The Gland At 26 Weeks (h&e; X60)



Mallory's stain:- Capsule becomes thick than the previous age groups.

Singh's modification of masson-hamperel argentaffin technique:- Chromaffin cells were more in number compared to previous gestational age groups. The cells were arranged in clusters/groups (fig.12).

Figure 12: Chromaffin Cells Present In Groups (singh's Modification Of Masson-hamperel Argentaffin Technique; X 100)



3. Discussion

The histogenesis of suprarenal gland has been subject of interest by large number of researchers [2,6,8,9,11,12,13]. The gland parenchyma has two parts, cortex and medulla. Both these parts differ in their development.

The fetal suprarenal cortex is mesodermal structure. It develops at 10mm CRL stage (37 days) from columnar mesothelial cells situated on posterior abdominal wall in the angle between the root of mesentry and the developing gonad. The cells proliferate and form invasive cords which soon separate from coelomic epithelium and condense in the mesenchyme of the dorsal abdominal wall to form a large eosinophilic cell mass on each side of the dorsal aorta, where they differentiate into large acidophilic cells. To its initial proliferation is added, at about the 12mm CRL stage (40 days) a second wave of smaller cells which are derived from the same general somatopleuric area and migrate to develop the first cluster forming the outer subcapsular layer. The smaller cells will become those of the definitive cortex while the initial proliferation becomes the fetal or primitive cortex which retrogresses after birth except the outermost layer which becomes zona reticularis[5,10].

The origin of the suprarenal medulla is from a different source. The medulla of the suprarenal gland is composed of sympathetic nerve cells which is derived from the the neural crest. These cells reach the medio-dorsal aspect of the primitive cortex at the 16mm 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 catecholomines by the 10th week of fetal life[5,10].

According to Allen et al[2] 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[6] 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[8] observed that degeneration of fetal cortex started during last 10 weeks of intrauterine life and was completed by the end of first year.

Sangma et al11 studied one hundred and two suprarenal glands (both right and left) of different age groups to see the cytoarchitecture using haematoxylin eosin stains. He observed that suprarenal has superficial narrow zone of darkly stained cells underneath the capsule which is the permanent cortex and a deeper lighter zone called the fetal cortex. As the age advanced, fetal cortex became bulkier and before term it constituted about 5/6th of entire cortex. The cells of permanent cortex were small with darkly stained nuclei and scant perinuclear cytoplasm .These cells were uniformly scattered from 16th to 22nd weeks. After this period they clumped together in arc or acini formation. Glomerular arrangement was noticed from 34 weeks onwards, presenting the future adult glomerulosa. Cells of fetal cortex were large, polyhedral in shape and arranged in 2 to 3 rows. Medulla was characterized as centrally placed space filled with large blood vessels and few large cells having abundant cytoplasm with vesicular nuclei.

Starkel et al[12] and Mc Intosh[9] 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[13] 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.

Uotila [13] observed that the fetal cortex of the human adrenal develops from columnar mesothelial cells in the “adrenal groove” which proliferated at 20-25 days, forming a mass of cells which soon separated from coelomic epithelium and becomes differentiated into large acidophilic cells. The permanent cortex formed from further proliferation of mesothelial cells in the same area at the age of 6weeks, forming a mass of cells on ventral surface of fetal cortex. These cells were smaller than those giving rise to the fetal cortex and have smaller nuclei. They come in direct contact with the

ventral portions of the fetal cortex where there was no capsule. They continued to multiply and spread along the surface of the gland. Subjacent to the capsule in such areas a layer of small basophilic cells collected which became the permanent cortex. The cells of permanent and fetal cortex are cytologically distinct from the time of their earliest differentiation, blood vessels first appeared in fetal cortex at 9mm CRL, and rapidly increased in number, forming a network of sinusoidal vessels in embryos of about 14mm. By the 16 to 18mm CRL stages blood vessels were seen in periphery of the gland. A primitive to zonal arrangement began to appear in the 16 to 18mm CRL stages, consisting of reticular and fascicular layers formed by the fetal cortex. On the periphery there was layer of permanent cortex. The capsule was poorly developed until 13mm CRL, after which it became more definite. Sympathetic elements appeared on the mediodorsal side of the primitive cortex in 11 to 12 mm embryos, but invasion of the cortex by sympathetic cells did not occur until the 13-14mm CRL stage. They observed invasion of the cells at 7 weeks and continued through the largest embryos. In the present study, some cells were amoeboid shape which suggested that invasion of colemic epithileum from dorsomedial aspect of the gland via loose areolar tissue at the hilum seen at 11-15 weeks.

In the present study, under light microscopy, capsule was identifiable and measured 59 μ at periphery and 124 μ at hilum at 11-15 weeks of gestation and the thickness increased to 312 micron at >25 weeks. It was made up of collagen fibres and fibroblasts. The capsule was initially thin and as the gestational age advanced it became thicker with condensation of the collagen fibres and increase in number of blood vessels. None of the authors have done micrometry to that extent; therefore no study was available for comparison.

In the present study two zones were observed like the previous authors[2,6,8,9,11,12,13]. 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 >25 weeks. The cells were present in U shaped arrangement/ clusters/groups and glomerular arrangement of cells were also seen. Deep to the dark zone there was deeper lighter zone (fetal cortex) constituting 3/4th of the cortex at 11-15 weeks which decreased to 1/5th of the cortex at >25 weeks which suggested that the permanent zone was becoming bulkier. Fasiculo reticular zone was 6-7 layered thick at 11-15 weeks and as the gestation advanced it decreased to 1-2 cell layer thick. The cells were arranged in columns extending deeper into the cortex towards the medulla. Sinusoidal vessels increased with increase in gestational age In the present study medulla was recognized by the presence of blood vessels at the centre with a few ganglionic cells which increased at >25 weeks. The cells were oval shaped with big cytoplasmic processes. These also showed presence of coarse granules in it. Nuclei with nucleolus were also seen. The ganglionic cells were 3-4/field initially which increased to 25-30/field .

In our study the chromaffin cells which were lying singly, in groups/trabeculae/clusters. These cells had single round nucleus. The cytoplasm were filled with coarse granules. Some cells did not show any granules.

In the present study, Few cells showed vacuolization at 11-15 weeks and there was presence of fibrous zone in the medulla consisting of collagen fibres with fibroblasts at >15-20 weeks suggesting early degenerative changes after that no degenerative changes were observed. Not much literature could be gathered on histological changes occurring in the medulla. In the present study chromaffin cells were studied, the cells seen migrating via different layers of gland. The cells were lying singly/groups or trabeculae. There were few cells seen at 12 weeks and increased with increase in gestation. Few cells had granules in their cytoplasm. Some cells did not have any granules suggesting presence or absence of epinephrine and nor epinephrine at this stage.

4. Conclusion

In our study we found that the capsule was initially thin and as the gestational age advanced it became thicker because of the condensation of the collagen fibres and invasion of the blood vessels. In the cortex two zones were observed -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 >25 weeks. Deep to the dark zone there was deeper lighter zone (fetal cortex) constituting 3/4th of the cortex at 11-15 weeks which decreased to 1/5th of cortex at >25 weeks, which suggested that the permanent zone was becoming bulkier.

The arrangement of cells in the permanent cortex changed from the discrete cells and clusters to well formed glomerulus like structure with increasing gestational period. Fasiculo reticular zone of the fetal cortex decreased in thickness as gestation advanced. The cells were arranged in columns extending deeper into the cortex towards medulla In the medulla, the ganglionic cells were 3-4/field initially which increased to 25-30/field in later gestation period. Few cells showed vacuolization at 11-15 weeks and there was presence of fibrous zone in the medulla consisting of collagen fibres with fibroblasts at >15-20 weeks suggesting early degenerative changes.

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