Journal of the Anatomical Society of India 62 (2013) 73–78

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage:www.elsevier.com/locate/jasi

Original article

Neuroblastic nodules and giant epithelial cells in suprarenal gland in fetuses of different gestational age groups

Khayati Sant Ram[a,*], Mahesh Sharma[b], Anshu Sharma[c]

[a]Assistant Professor, Department of Anatomy, Hind Institute of Medical Sciences, Lucknow, Uttar Pradesh
[b]Associate Professor, Department of Anatomy, Government Medical College and Hospital, Chandigarh
[c]Assistant Professor, Department of Anatomy, Government Medical College and Hospital, Chandigarh

KEYWORDS

Giant epithelial cell, Neuroblastic nodule, Suprarenal gland.

ABSTRACT

Introduction: Microscopic neuroblastic nodules are found in the suprarenal gland of newborns and young infants. The purpose of this study was to determine the morphology and derivation of neuroblastic nodules and giant epithelial cells of human fetal suprarenal gland. *Materials and methods:* The study consisted of 30 spontaneously aborted human fetal specimens from 11th weeks to 28th weeks of gestational ages. The suprarenal glands were taken from fetal specimens for histological study. The staining was done by hematoxylin and eosin. The following points about the neuroblastic nodule and giant epithelial cells were noted: number, location, size, any specific change like cystic degeneration with increasing age in the cells, distribution pattern of neuroblasts and giant epithelial cells, and their relation to the capsule, cortex, and central vein. *Results:* The neuroblastic nodules and giant epithelial cells increased in number as the gestation advanced but after 20 weeks of gestation, their number started decreasing. At the gestational age 25–28 weeks, both the giant epithelial cells and neuroblastic cells were absent. *Conclusion:* Both giant epithelial cells and neuroblastic cells are an integral part of the normal development of the suprarenal gland and they disappear with the advancement of the gestational age. These cells are important because some authors consider them to be the precursor of malignancy in later life.

1. Introduction

The adult suprarenal gland has two zones: cortex and medulla. The cortex is further divided into zona glomerulosa, zona fasciculate, and zona reticularis. These zones are different both functionally and morphologically. Histologically, the fetal suprarenal gland differs from the adult suprarenal gland. It has a superficial narrow zone of darkly stained cells underneath the capsule, the permanent cortex, and a deeper lighter zone, the fetal cortex. As the gestational age advances, the fetal cortex becomes bulkier and before term it constitutes about 5/6th of entire cortex. After birth, the fetal cortex regresses rapidly except for its outermost 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 life.[1] The medulla is completely encapsulated by the cortex till later fetal life.

Microscopic neuroblastic nodules are found in the suprarenal gland of newborns and young infants. Beckwith and Perrin have termed them 'in situ neuroblastoma'.[2] These cell aggregates were common in the development of suprarenal gland. So an attempt was made to investigate their derivation. The other cells which were observed in the developing suprarenal gland were giant epithelial cells. As the age advanced, both of them disappeared. Not much has been commented upon the neuroblastic nodules and giant epithelial cells. Therefore, an attempt was made to see their derivation and histological changes as the gestational age advanced.

*Corresponding author: Tel: +91 (0) 8004003534, 9161888138
E-mail address: khayati_santram@yahoo.com. (Khayati Sant Ram)

2. Material and methods

The present study was carried out in the Department of Anatomy, Government Medical College and Hospital, Chandigarh. The material for the study consisted of 30 aborted human fetal specimens from 11th weeks to 28th weeks of gestational ages. The gestational age was determined by the ultrasound report and in case the ultrasound report was not available, the age was told by the parents. The specimens were provided by the Department of Obstetrics and Gynaecology Government Medical College and Hospital, Chandigarh, for routine fetal autopsy. All fetuses were the result of the intrauterine death or spontaneous abortion. Consent for autopsy was taken from the parents.

The fetuses were divided into four groups according to the gestational age shown in Table 1.

Table 1 – Division of fetuses into four groups according to the gestational age.

Groups	Gestational age	Number of fetuses
A	11–15 weeks	5
B	15–20 weeks	9
C	20–25 weeks	10
D	25–28 weeks	6

The fetuses were preserved in 10% formalin. They were examined for any congenital deformities with special attention given to the cardiovascular, urogenital, and musculoskeletal systems. The suprarenal gland was exposed and removed after 72 hours of fixation. Every section was embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The prepared slides were examined under a penta head microscope. The selected sections were photographed. Various measurements were taken according to software Image Pro Plus 6.0.

All neuroblastic nodules/fields and giant epithelial cells/fields within the suprarenal glands were counted and measured. The following points about the neuroblastic nodule and giant epithelial cells were noted: number, location, size, any specific change like cystic degeneration with increasing age in the cells, distribution pattern of neuroblasts and giant epithelial cells, and their relation to the capsule, cortex, and central vein.

3. Observations and results

Histology of the suprarenal gland was done to see the changes in the neuroblastic nodule and giant epithelial cells during different gestational age groups. The stain used for this purpose was hematoxylin and eosin.

3.1 *Group A (11–15 weeks)*

The pathway of migration of the neuroblastic cells was seen from the capsule toward medulla at this gestational age group. Some neuroblastic cells were ameboid in shape. This suggested invasion of colemic epithelium by the neuroblastic cells from the dorsomedial aspect of the gland via loose areolar tissue at the hilum (Fig. 1). There was the presence of clusters of neuroblastic cells as well as nodules inside the parenchyma of the gland. Two types of nodules were observed: superficial neuroblastic nodules which were seen deep to the capsule and deep neuroblastic nodules present in the glomerular zone of permanent cortex of the fetal suprarenal gland. They were surrounded by a thin capsule made up of collagen fibers. Some nodules were present in the fetal cortice's fasciculoreticular zone also. These nodules were oval in shape and consisted of densely packed basophilic cells.

The number of the nodule was 2–3/field at 60× magnification. The nodules were interconnected by fine nerve fibers. Single discrete neuroblastic cells dispersed in the cortex were also found. Clusters of neuroblastic cells were also present directly on the wall of sinusoids. The maximum average size of individual nodule was 214 × 170 μ and the minimum size was 13 × 20 μ (Fig. 2).

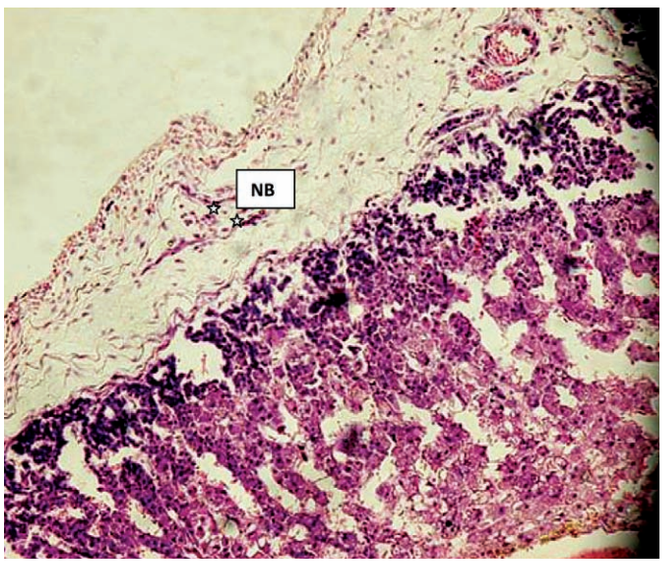

Fig. 1 – Migration of neuroblastic (NB) cells via hilum of the capsule at 11 weeks (H&E; ×60).

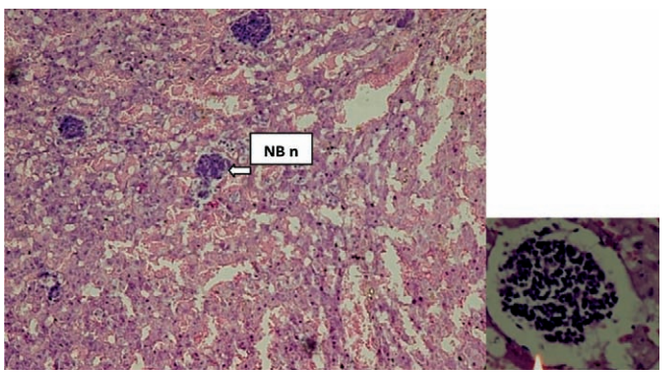

Fig. 2 – Neuroblastic nodule (NB n) traversing via cortex toward medulla present directly on the wall of sinusoids at 15 weeks (H&E; ×60).

Giant epithelial cells were seen scattered throughout the fetal and permanent cortex of suprarenal gland cortex. These cells were large and polyhedral measuring on an average 15 × 8 – 14 × 10 μ. They were found 8–10/field 60× magnification. The nucleus was eccentric in position with prominent nucleoli (Fig. 3). The giant epithelial cells were seen more in the permanent cortex.

3.2 *Group B (15–20 weeks)*

At this gestational age, superficial and deep neuroblastic nodules were seen. Nodules were present in the fasciculoreticular zone of the fetal cortex, migrating to medulla. These were oval in shape and consisted of densely packed basophilic cells. Few cells showed cystic changes giving the cells eosinophilic appearance (Fig. 4). Small groups of several neuroblasts and occasional single cells were dispersed throughout the cortex. Maximum numbers of neuroblastic nodules were seen in this gestational age; they were observed 7–8/field. The maximum average size of nodule was 224 × 215 μ and minimum was 20 × 30 μ.

Giant epithelial cells were also seen scattered throughout the cortex. These cells measured 17 × 10–20 × 8 μ. They were found 8–10/field. They were polyhedral in shape. Nucleus was eccentric in position. Some cells had a binucleate nucleus with prominent nucleoli and were heterochromatic (Fig. 5).

3.3 *Group C (20–25 weeks)*

In this age group, the only deep neuroblastic nodules were present mainly in medulla. The number of nodules decreased and was 2–3/field. The maximum average size of individual nodule was 214 × 271 μ and the minimum size was 20 × 30 μ (Fig. 6).

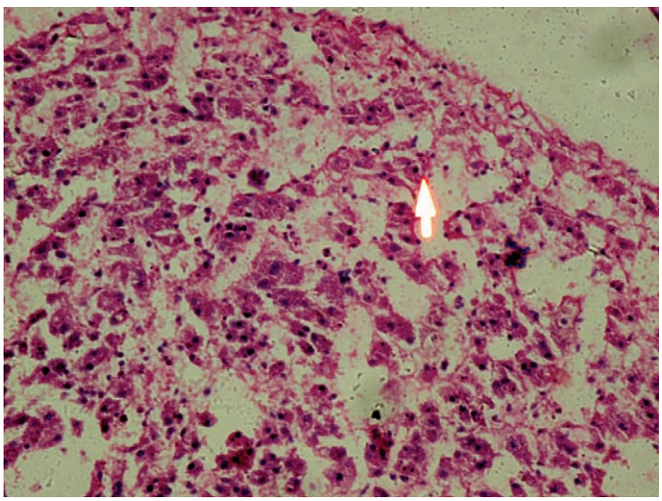

Fig. 3 – Giant epithelial (GE) cells scattered throughout the cortex at 15 weeks (H&E; × 60).

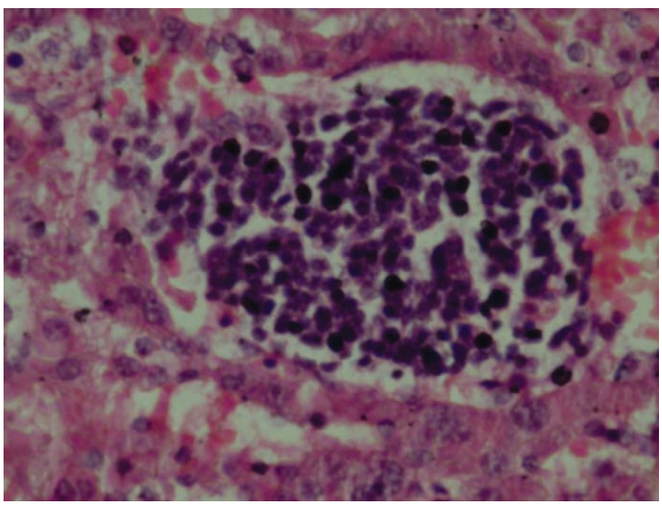

Fig. 4 – Neuroblastic nodule consisting of densely packed basophilic cells and few eosinophilic cells (showing cystic changes) at 18 weeks (H&E; ×100).

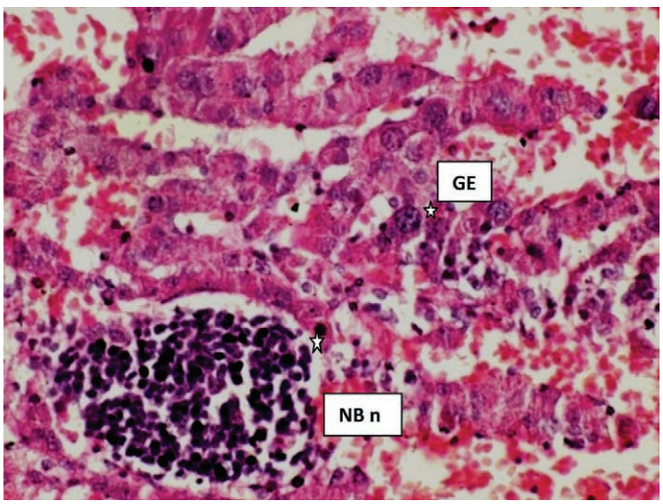

Fig. 5 – Giant epithelial cells (GE) with neuroblastic nodule (NB n) in the cortex at 18 weeks (H&E; ×100).

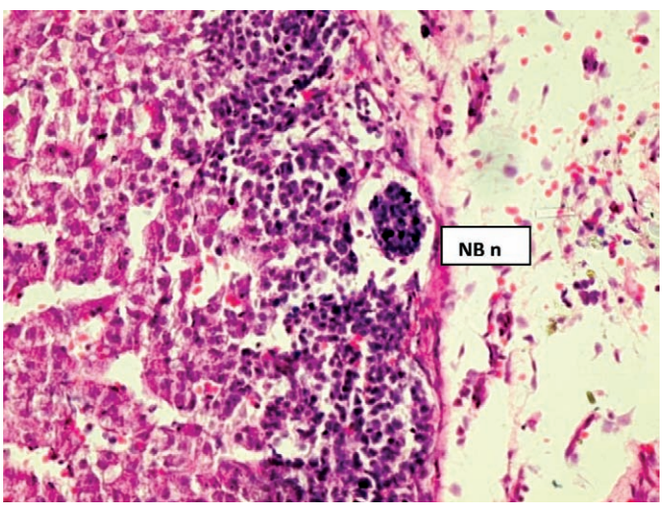

Fig. 6 – Neuroblastic nodule (NB n) below the capsule at 24 weeks (H&E; ×60).

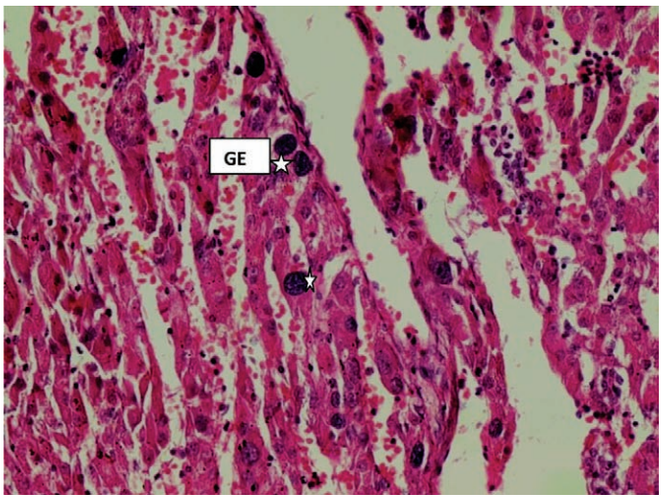

Fig. 7 – Giant epithelial (GE) cells with darker acidophilic cytoplasm having coarse granules encircling the nucleus at 24 weeks (H&E; ×60).

Giant epithelial cells were few in number present in the cortex but were more seen in the medullary region with a range of cell size from 19 × 12 μ to 24 × 10 μ. These were polyhedral in shape and were found 4–5/field at 60× magnification. Nucleus was eccentric in position. Few cells were binucleate with prominent nucleoli. The cytoplasm of cells displayed a darker acidophilic reaction, having coarse granules encircling the nucleus. The cells were lying adjacent to the sinusoids (Fig. 7).

3.4 *Group D (25–28 weeks)*

No giant epithelial cells were seen at this gestation. No neuroblastic nodule was present in this age group.

4. Discussion

Sadler describes the origin of the suprarenal medulla from a different source, different from the suprarenal cortex.[1] As the medulla of the suprarenal gland is composed of sympathetic nerve cells, it is derived from the neural crest. 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.

According to Hervonen,the adrenal medulla is derived from cells of the neural crest in association with the development of the rest of the sympathetic nervous system.[3] 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 lumbar 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.

In the present study, the pathway of migration of the neuroblastic cells was seen from the capsule toward medulla at 11–15 weeks of gestational age group. Some neuroblastic cells were ameboid in shape. This suggested invasion of colemic epithelium by the neuroblastic cells from dorsomedial aspect of the gland via loose areolar tissue at the hilum.

After postnatal degeneration of the fetal cortex, the support to the neuroblastic nodules by cords of cortical cells and reticulum of intervening sinusoids is lost, and the neural tissue settles against the central veins. At first the nerves, chromaffin cells, and neuroblasts are loosely intertwined among the remaining cortical cells and debris. When the reticular zone begins to form at 12–18 weeks, cords of cortical cells again become mixed among groups of chromaffin cells, and the medulla is no longer discrete. The same observations were observed in our study.

In the present study, superficial neuroblastic nodules were seen deep to the capsule till the age of 20 weeks. These were surrounded by a thin capsule made up of collagen fibers. The pathway of migration of the neuroblastic cells was seen from the capsule toward medulla. These nodules were present throughout the glomerular and fasciculoreticular zones in early age while situated deep in older age group. These were oval in shape interconnected by fine nerve fibers and consisted of densely packed basophilic cells. Single discrete neuroblastic cells dispersed in the cortex were also found. Clusters of neuroblastic cells were also present directly on the wall of sinusoids. At least one neuroblastic nodule was present on an average in fetuses up to 25 week gestation. The maximum size of individual nodule was 214 × 271 μ and the minimum size was 13 × 20 μ. No neuroblastic nodule was seen in the fetal specimens 25–28 weeks.

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.[4] These nodules increased in size and number, and in all specimens from fetuses of 14–18 week gestation, aggregates of nodules closely resembling neuroblastoma in situ were found. From 12 week gestation, the large neuroblast nodules appear to split up into smaller nodules and differentiation into chromaffin cells takes place. The mean nuclear size of neuroblasts at all stages of development studied was statistically significantly less than the mean nuclear size of the cells measured in seven neonatal neuroblastomas. Measurement of nuclear size may be a way of distinguishing a true potentially malignant neuroblastoma in situ from a normal benign residual neuroblast nodule.

Turkel and Itabashi observed that the individual neuroblastic nodule size remained essentially unchanged at about 60 × 60 μ in all age groups, which indicated an optimal size for these avascular, tightly packed clusters.[5] Cystic change within neuroblastic nodules was common and considered to be 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. Superficial nodes were found to decrease with age. This decline in superficial nodules could reflect either the inward migration of nodules, their attrition, or the relatively greater growth of the cortex. Even though no nodules were found in direct contact with the capsule in the oldest group, they were found throughout the gland. Neuroblastic nodules are a normal part of the fetal adrenal gland which may be present until birth or early infancy.

Beckwith and Perrin have interpreted the nodules as normal variants, whereas others have considered them to be small malignancies.[2]

Guin et al did a combined retrospective and prospective study of incidental neuroblastoma.[6] They found that the small lesions commonly found in the youngest patients were possibly embryologic remnants. Their second group was borderline, and the largest lesions, found in the third group, appeared to be true, small intra-adrenal neuroblastomas. Their first group included the six youngest cases. The lesions in this group were all of minute size, consisting of discrete aggregates of neuroblasts found within the medullary compartment. Some aggregates of neuroblasts showed extension into the zona reticularis with compression of adjacent tissue. There were two cases in their second group, which they termed the 'intermediate stage in incidental neuroblastoma'. In this group, the adrenals were not grossly enlarged, but neuroblasts filled and expanded the medulla with irregular extension and compression of the cortex. Their last group included three cases in which the suprarenal were grossly enlarged, with tumor replacing and expanding the medulla and compressing the cortex. Gross and microscopic cystic spaces, multifocal hemorrhage, necrosis, and fibrosis were found.

Jennings et al observed fetal neuroblastomas detected by routine prenatal sonography.[7] All were adrenal tumors diagnosed between 26 week and 39 week gestation. All five tumors were completely resected postnatally and the patients have remained disease-free from 2 months to 10 years after resection without adjuvant therapy. A literature review collated 16 other cases of fetal neuroblastoma detected by sonography between 29 week and 38 week gestation. These cases included 1 cervical, 1 thoracic, and 14 adrenal tumors. Thirteen neonates had Evans stage I or II tumors, and three had more advanced disease. Eleven mothers did not have hypertension or pre-eclampsia during the pregnancy, and all the neonates had stage I or II disease. Four mothers had hypertension or pre-eclampsia. Three of these neonates had stage IV or IVS disease with liver metastases, and all three had fetal hydrops. Review of the congenital neuroblastoma literature documented 71 cases diagnosed soon after birth, and several of these cases had unusual features that could have been detected by prenatal ultrasound. Four of the tumors were so large that dystocia resulted and fetal dismemberment was required for delivery. Eight of the tumors metastasized to the placenta, and one metastasized to the umbilical cord with subsequent fetal death. We conclude that fetal neuroblastoma can be diagnosed by prenatal sonography. Accurate staging is difficult by sonography, but in mothers with no pre-eclamptic symptoms, the chance of widely disseminated disease is small.

Lonergan et al concluded that neuroblastoma, ganglioneuroblastoma, and ganglioneuroma are tumors of the sympathetic nervous system that arise from primitive sympathogonia and are referred to collectively as neuroblastic tumors.[8] They arise wherever sympathetic tissue exists and may be seen in the neck, posterior mediastinum, adrenal gland, retroperitoneum, and pelvis. The most benign tumor is the ganglioneuroma, which is composed of gangliocytes and mature stroma. Ganglioneuroblastoma is composed of both mature gangliocytes and immature neuroblasts and has intermediate malignant potential. Neuroblastoma is the most immature, undifferentiated, and malignant tumor of the three. Neuroblastoma, however, may have a relatively benign course, even when metastatic. Thus, these neuroblastic tumors vary widely in their biologic behavior. Features such as DNA content, tumor proto-oncogenes, and catecholamine synthesis influence prognosis, and their presence or absence aids in categorizing patients as high, intermediate, or low risk. Neuroblastoma remains a relatively lethal tumor, accounting for 10% of pediatric cancers but 15% of cancer deaths in children.

Kampmeier said that the giant 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.[9] We have no decisive observations of such metamorphosis. But gives conclusive information that giant cells themselves proliferate by division and division occurs by the mitotic process. Kampmeier observed that the giant cells were not seen at the 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.

In the present study, at 11–15 weeks, giant epithelial cells were seen scattered throughout the cortex. These cells were polyhedral in shape measuring 14 × 10–19.4 × 20 μ. They were found 8–10/field and gradually decreased to 4–5/field at 20–25 week gestation and were absent in >25 week gestation. Nucleus was eccentric in position with prominent nucleoli. These were seen more in permanent cortex. As the gestation increased, they were seen in medulla. At 15–20 weeks, some of the cells were binucleate with prominent nucleoli and heterochromatic. At 20–25 weeks, the cytoplasm of cells displayed a darker acidophilic reaction, having coarse granules encircling the nucleus. The cells were lying adjacent to the sinusoids. At 25–28 weeks, no giant cells were observed.

5. Conclusion

Both giant epithelial cells and neuroblastic cells are an integral part of the normal development of the suprarenal gland and they disappear with the advancement of the gestational age, as earlier it was thought to be metastatic tumor deposits during development. The knowledge of histology of gland is important in many cases of female virilization, hirsutism,

pseudohermaphroditism, and hypertension in association with suprarenal cortical and medullary tumors.

REFERENCES

1. Sadler TW, Langman J. *Medical Embryology* 11th edn, Baltimore: Lippincott Williams and Wilkins, 2010:322–3.
2. Beckwith JB, Perrin EV. In situ neuroblastomas: a contribution to the natural history of neural crest tumors. *Am J Pathol* 1963;43:1089–104.
3. Hervonen A. Development of catecholamine-storing cells in human fetal paraganglia and adrenal medulla. A histochemical and electron microscopical study. *Acta Physiol Scand Suppl* 1971;368:1–94.
4. Ikeda Y, Lister J, Bouton JM, et al. Congenital neuroblastoma, neuroblastoma in situ, and the normal fetal development of the adrenal. *J Pediatr Surg* 1981;16:636–44.
5. Turkel SB, Itabashi HH. The natural history of neuroblastic cells in the fetal adrenal gland. *Am J Pathol* 1974;76:225–44.
6. Guin GH, Gilbert EF, Jones B. Incidental neuroblastoma in infants. *Am J Clin Pathol* 1969;51:126–36.
7. Jennings RW, LaQuaglia MP, Leong K, et al. Fetal neuroblastoma: prenatal diagnosis and natural history. *J Pediatr Surg* 1993;28:1168–74.
8. Lonergan GJ, Schwab CM, Suarez ES, et al. Neuroblastoma, ganglioneuroblastoma, and ganglioneuroma: radiologic-pathologic correlation. *Radiographics* 2002;22:911–34.
9. Kampmeier OF. Giant epithelial cells of the human fetal adrenal. *Anat Rec* 1927;37:95–102.