

Histogenesis of neuroblastic nodules and giant epithelial cells of fetal adrenal glands

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Abstract

The development of adrenal gland is complex and fascinating. The cortex shows migrating neuroblastic nodules derived from neural crest cells which are described in literature as “in situ neuroblastoma”.

Aim: The present study was undertaken to highlight neuroblastic nodules and giant epithelial cells in fetal adrenal glands at different gestational weeks.

Materials and Method: Adrenal glands were dissected in thirty spontaneously aborted normal human fetuses fixed in 10% formalin with gestational age ranging from 11 weeks to 35 weeks. After tissue processing, paraffin blocks were prepared and sections stained with Hematoxylin and Eosin. Multiple serial sections were observed to note average number of neuroblastic nodules per field, size, location and degenerative changes if any in the nodules.

Observations and Results: The developing adrenal gland showed parenchyma with a thick fetal cortex showing migrating neuroblastic nodules and giant epithelial cells in the background. It was seen that they increased in number as the gestational age advanced but after 20- 21 weeks of gestation their number went on decreasing.

Conclusion: Knowledge of the behaviour 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.

Keywords: Neuroblastic nodule, Giant epithelial cells, Fetal adrenal glands.

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Introduction

The adrenal gland plays a vital role in survival and maintenance of internal milieu. Microscopically the adult adrenal gland has two distinct zones: cortex and medulla. The cortex is further divided into zona glomerulosa, zona fasciculata and zona reticularis. These zones are different both functionally as well as morphologically. Histologically, the fetal adrenal gland shows superficial dark narrow zone beneath the capsule called permanent cortex and a deeper lighter zone called the fetal cortex. As the gestational age advances fetal cortex becomes bulkier and before term it constitutes about the deeper 5/6th of the entire cortex. 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 or brown with chrome salts.⁽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 of them disappeared.⁽²⁾ 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 advances.

Embryology of the adrenal gland and “neuroblastoma in situ”:

The coelomic epithelium close to the root of dorsal mesentery opposite the sixth to twelfth thoracic segments proliferates into the underlying mesenchyme giving rise to the cortex of adrenal gland and migrating sympathochromaffin cells from the neural crest give rise to the medulla. A combination of migration and proliferation of these cells form distinct neuroblastic nodules within the adrenal gland. 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.^(3,4)

Materials and Method

Thirty spontaneously aborted dead human fetuses with gestational age ranging from 11 weeks to 35 weeks were collected from Bharati Vidyapeeth Medical College and Hospital Sangli, and other private hospitals with the kind permission of the concerned authorities, consent of parents and ethical approval. Twins and fetuses with gross congenital anomalies as well as mothers having metabolic or hormonal disorder were omitted. The gestational age was estimated by using Crown Rump Length and confirmed by referring LMP

(Last Menstrual Period). These fetuses were divided into five groups according to their gestational age, as shown in Table 1.

Table 1: Groups according to gestational age

Groups	Gestational age	Number of fetuses
I	11-15 Weeks	03
II	16-20 Weeks	12
III	21-25 Weeks	09
IV	26-30 Weeks	04
V	>30 Weeks	02

These 30 fetuses were fixed in 10% formalin for 10 days. The fetuses were dissected by opening anterior abdominal wall. The liver and gastrointestinal tract was removed to view the adrenal glands in their normal location. Both glands were removed. The tissue slices of 3-4mm thickness were taken and fixed with Bouin's fluid for 24 hours. The tissue was passed through graded alcohols (50%, 70%, 80%, 90% and absolute alcohol) for dehydration. Clearing was done by using xylene for 1 hour each for two changes. Embedding of tissue was done in paraffin wax having melting point 56°C for 1 hour with 2 changes. The paraffin blocks were made by using L moulds. The sections were cut at 5 micron thickness by using rotary microtome. The ribbons of sections were thrown on the surface of warm water in tissue floating bath and then sections were taken on slides coated with egg-albumin. Slides were kept for drying on a hot plate at 40^o- 50^o C for 2 hours or more as per requirement.

Staining Procedure

Haematoxylin and Eosin: Paraffin was removed with xylene followed by graded alcohols namely 100%, 90%, 80%, 70% and 50%. Then the sections were stained with haematoxylin solution. Excess stain was removed by dipping into acid alcohol for a few seconds followed by bluing. Counter staining was done with eosin. They were then washed with alcohol, cleared with xylene and mounted with DPX.

The prepared slides were observed under microscope and the following points about the neuroblastic nodules and giant epithelial cells were noted:

- Location- Superficial nodules were considered to be those in contact with the capsule of the adrenal gland or immediately below it. All other were classified as deep.
- Size was measured by using ocular and stage micrometer scale at 45x magnification.
- Cystic degeneration with increasing gestational age.
- Distribution pattern of neuroblast in nodules and giant epithelial cells and their relation with capsule, cortex and central vein.

- The selected sections were photographed by using CCD camera with their software.

Observations and Results

Histology of adrenal gland was studied in different gestational age groups to see the changes in the capsule, cortex, medulla, neuroblastic nodules and giant epithelial cells.

Group I (11-15weeks): Capsule was well defined, thin and made up of collagen fibers. Deep to the capsule loose subcapsular connective tissue was seen. Cortex of the fetal adrenal gland showed two distinct zones, a superficial dark zone called permanent or definitive cortex and deeper comparatively light zone called fetal cortex. Medulla was recognized by the presence of blood vessels at the centre with a few ganglion cells. At this gestational age, the migration pathway of the neuroblastic cells was observed from the capsule towards the medulla. (Photograph 1A)

The parenchyma showed presence of clusters of neuroblastic cells and nodules. Neuroblastic nodules were surrounded by a thin capsule made up of collagen fibers. They were oval in shape and consisted of densely packed basophilic cells interconnected by thin nerve fibers. (Photograph 2) The number of the nodules was 2-3/ low power field. Single discrete neuroblastic cells were dispersed in the cortex. Clusters of neuroblastic cells were also found directly on the wall of sinusoids. The maximum average size of nodule was 211 x 162 μ and minimum size was 35 x 29 μ .

Giant epithelial cells were scattered throughout the fetal and permanent cortex of adrenal gland. These cells were large, polyhedral with eccentrically placed nucleus with prominent nucleoli and were 8-10/low power field.

Group II (16-20 weeks): At this gestational age, migrating nodules present in the fetal cortex which were migrating towards medulla region. (Photograph 1B) Few cells showed cystic degenerative changes giving the cells eosinophilic appearance. Maximum numbers of neuroblastic nodules were seen in this gestational age, with average number of 6-7/ field at 10x magnification. (photograph 1A) The maximum average size of neuroblastic nodule was 290 x 280 μ and the minimum size was 32 x 26 μ . Giant epithelial cells were scattered throughout the cortex of adrenal gland. These cells were large and polyhedral with eccentrically placed nucleus with prominent nucleoli. Some cells were binucleated with prominent nucleoli and were heterochromatic.

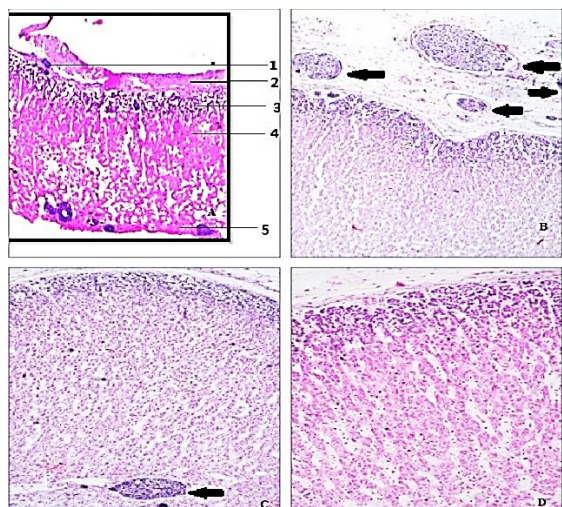


Fig. 1A: Photomicrograph showing Suprarenal gland at 13 weeks capsule (2) and Neuroblastic nodules (1) migrating via cortex [Primitive cortex (3), Fetal cortex (4)] towards medulla (5) (H & E, 10x), **B:** Photomicrograph showing large number of neuroblastic nodules consisting densely packed basophilic cells and few eosinophilic cells at 18 weeks (H & E, 10 X), **C:** Photomicrograph showing neuroblastic nodule in the medulla at 25 weeks (H & E, 10X), **D:** Photomicrograph showing Clusters of Neuroblastic cells at 26 weeks (H & E, 10X)

Group III (21-25 weeks): At this gestational age, the number of neuroblastic nodules was decreased and showing degenerative changes. Only deep neuroblastic nodules were observed mainly in medulla. (Photograph 1C) The maximum average size of neuroblastic nodule was $280 \times 250\mu$ and the minimum size was $60 \times 40 \mu$. Giant epithelial cells were few in number and were 4-5 /field at 10 x magnifications. These cells were large and polyhedral with eccentrically placed nucleus with prominent nucleoli. Some cells were binucleated. The cytoplasm of cells displayed a darker acidophilic reaction, having coarse granules encircling the nucleus. The cells were seen lying adjacent to the sinusoids and some giant epithelial cells show degenerative changes. (Photograph 4)

Group IV (26-30 weeks): At this gestational age, neuroblastic nodules were not found, but a few clusters of neuroblastic cell were seen. The giant epithelial cells were few and show degenerative changes. (Photograph 1D)

Group V (>30 weeks): At this gestational age, neuroblastic nodules as well as giant epithelial cells were not observed. (Photograph 5)

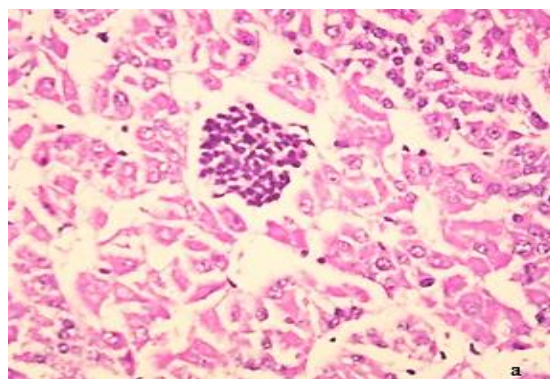


Fig. 2: Photomicrograph showing neuroblastic nodules consisting densely packed basophilic cells and few eosinophilic cells at 18 weeks (H & E, 45X)

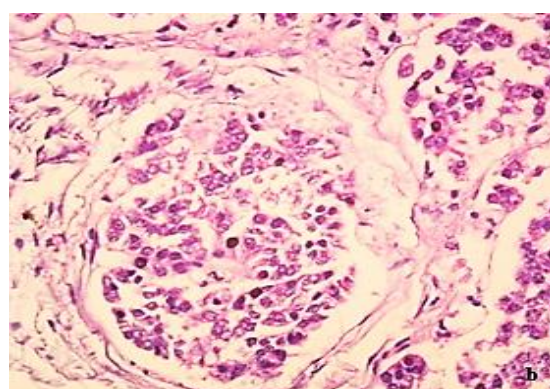


Fig. 3: Photomicrograph showing cystic degenerative changes in Neuroblastic nodule at 25 weeks (H & E, 45X)

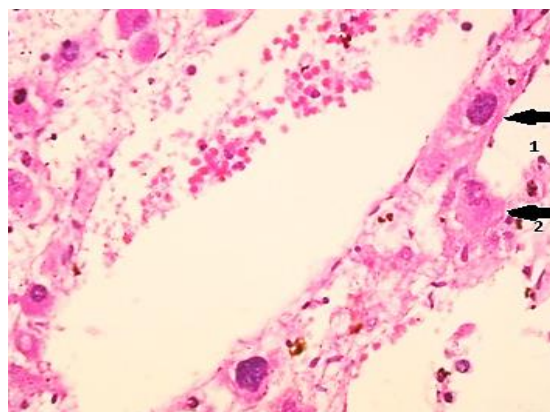


Fig. 4: Photomicrograph showing normal Giant epithelial cell (1) and that showing degenerative changes (2) at 24 weeks (H & E, 45X)

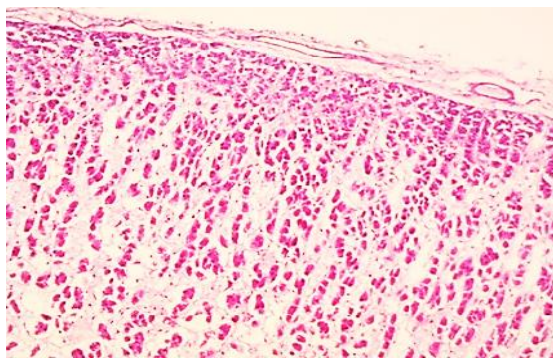


Fig. 5: Photomicrograph showing adrenal cortex with medulla seen at 35 weeks. Neuroblastic nodules and giant epithelial cells were not seen. (H & E 10X)

Discussion

According to Hervonen, 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 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.⁽⁵⁾

Sadler 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.⁽¹⁾

Turkel and Itabashi observed that the individual neuroblastic nodule size remained essentially unchanged at about 60 x 60 μ 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.⁽³⁾

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 14-18 weeks 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.⁽⁴⁾

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.⁽⁶⁾

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.⁽⁷⁾

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.⁽⁸⁾

Loneragan 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.⁽⁹⁾

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.⁽¹⁰⁾

In the present study, at 11-15 weeks, giant epithelial cells were scattered throughout the fetal and permanent cortex of adrenal gland. As gestational age advanced, they are seen in medulla and show degenerative changes. The giant epithelial cells are absent after 30 weeks gestation.

Conclusion

The number and size of neuroblastic nodules increased up to 20 weeks after which regression in size and number was noted and after 30 weeks neuroblastic nodules were not observed at all. Giant epithelial cells were observed up to 25-30 weeks after which no giant epithelial cells were noted. Giant epithelial cells and neuroblastic nodules/cells are an integral part of the normal development of the adrenal gland.

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.

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