Original Article

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Light Microscopic Study of Developing Medulla of Fetal Adrenal Gland

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Abstract

One hundred human foetuses having crown-rump length in the range of 7–36 cm and gestational age of 12–36 weeks were subjected to morphological and histological studies to understand and observe the developing medulla of foetal adrenal glands. It is observed that from week 12 to 14, medulla is not clearly demarcated and clusters of medullary cells are found in the foetal cortex. Towards week 38, we observe that the medulla is well organized and differentiated with phaechromocytes and few ganglion cells clearly visible. The observations are important for clinicians to help in diagnosis of neuroblastomas.

Keywords: Medulla; Foetal Adrenal Glands; Neuroblasts; Phaeochromocytes.

Introduction

The prominence of the adrenal glands in the foetus and in the new-born infant has been recognized for almost two centuries [1]. During the foetal life there is remarkable increase in the size of adrenal gland mainly due to presence of foetal or provisional cortex present between the medulla and thin rim of permanent cortex [2].

The adrenal medulla is functionally related to the sympathetic nervous system. It secretes the hormone epinephrine and norepinephrine in response to sympathetic simulation. Alternatively, the adrenal cortex secretes an entirely different group of hormones called corticosteroids. In a broad sense, both the steroid hormones synthesized by the cortex and the catecholamines synthesized by the medulla mediate adaptive responses of the organism to a changing environment [3].

This makes it important to understand and observe the developing medulla. A gross examination of slices of fresh human adrenal show the outer and broader yellowish zone of cortex as a result of the presence of lipids and a thin reddish brown structure, i.e. the medulla [4].

The adrenal gland develops from two components. During fifth week of development, mesothelial cells between root of the mesentery and the developing gonad begin to proliferate and penetrate the underlying mesenchyme and differentiate into fetal cortex of the adrenal gland. Shortly after second wave of smaller cells from msothelium penetrates the mesenchyme surround fetal cortex, and later form the definitive cortex [5]. The neural crest cells from adjacent celiac ganglia [6] reach mediodorsal aspect of the primitive cortex and begin to invade it to comprise medulla. There is simultaneous development of venous sinusoids in medulla [7]. The origin of the adrenal medulla is involved with origin of a whole group of tissue masses of the same general type and potentialities i.e. chromaffin cells. Chromaffin cells are derived from the cells of sympathodrenal lineage. In adrenal medulla these cells differentiate into small neuroblasts and larger phaeochrmocyte [8].

Over the past two decades, routine prenatal ultrasound has significantly increased the rate of diagnosis of fetal neuroblastoma. More than 90% of these tumors arise in the adrenal gland, suggesting a link between perinatal tumors and the nodular collections of neuroblasts that are part of normal adrenal development [9].

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Materials and Methods

The study was performed on one hundred normal human foetuses (71 male and 29 female) in developmental week 12 to week 36. The foetuses were obtained from Medical Termination of Pregnancies and spontaneous abortions from tertiary care hospital in Mumbai.

The menstrual histories provided by the mothers were taken into consideration. The crown rump length of the foetuses ranged from 7 cm to 36 cm. The foetuses were classified according to their gestational age into groups as Weeks 12–14, Weeks 15–16, Weeks 17–18,Weeks 19–20, Weeks 21–22,Weeks 23–24,Weeks 25–26 , weeks 27-28, Weeks 29–30, Weeks 31-32 and Weeks 36–38.

Preservation

In order to minimize post-mortem changes, the fetuses were immediately preserved by injecting formalin into the abdominal cavity. Subsequently, they were immersed in formalin solution. For identification, numbered tags starting from 1 were tied to their wrists. Normal growth of the fetus was ensured by taking different external measurements and ascertaining various parameters.

Dissection and Fixation

The fetuses were dissected by taking vertical abdominal incision. Subsequently, the adrenals were removed along with the kidneys by blunt dissection. Immense care was taken during the separation of the right adrenal from the liver. The left adrenals were kept in small locked plastic pouches to differentiate from the right. Finally, the adrenals were stored in small bottles and were numbered.

Next, the large adrenals were cut into pieces with sharp razor blade. The capsule was not damaged in the process.

Finally, the tissues were fixed by immersing in SUSA solution [10] for 24–36 hours.

Dehydration

First, the tissues were immersed in 50% alcohol solution for 6–8 hours. Next, they were transferred to 70% alcohol and allowed to stay in it overnight. Subsequently, the tissues were bathed in 90% alcohol for one hour and finally they were subjected to absolute alcohol for duration of one hour each. Lastly, the tissues were dried using filter paper.

Clearing and Embedding

Post dehydration, the tissues were subjected to two–three xylene changes until they turned transparent.

Once the tissues were impregnated with xylene, they were embedded in melted paraffin at 58°–60° for duration of 5–6 hours. The paraffin blocks containing tissues were made using L-shaped molds. These blocks of paraffin were then numbered accordingly. Care was taken during the preparation of the tissues to ensure that the respective numbering was maintained.

Sectioning

The numbered blocks of paraffin containing tissues were sectioned by microtome steel blade to a thickness of 4–5 µm. The sections were floated on warm water and transferred to glass slides which were rubbed with a drop of albumin. A drop of 70% alcohol was put on a glass slide to ensure spreading of the section.

Subsequently, the slides were numbered accordingly with diamond marker and kept in over for a few seconds to fix the section on slide.

Slide Staining

Next, the slides were stained with Hematoxylin and Eosin stain. After the slides were stored in Koplin jar, the following procedure was undertaken:

1. First, two changes of xylene were given for a short period of time. Next, the slides were successively bathed in descending grades of alcohol starting from absolute alcohol (three momentary changes as absolute alcohol I, II, III) to 90% and 70% alcohol.

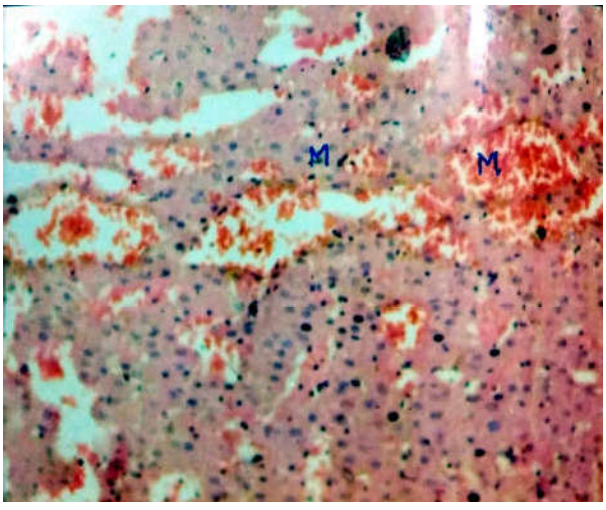

Fig. 1: Medulla showing formation of vascular pattern

2. The Koplin jars were kept under running water for 5–10 minutes. Then, the slides were stained with Hematoxylin for 10–15 minutes. After checking the intensity of staining under the microscope, the Koplin jars were kept under running water for 15 minutes,
3. Subsequently, Eosin was used as an acid dye for staining the slides for 3–5 minutes.

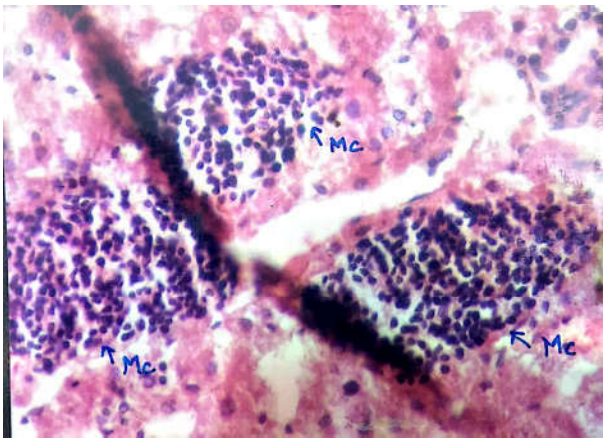

Fig. 2: Clusters of medullary cells Mc

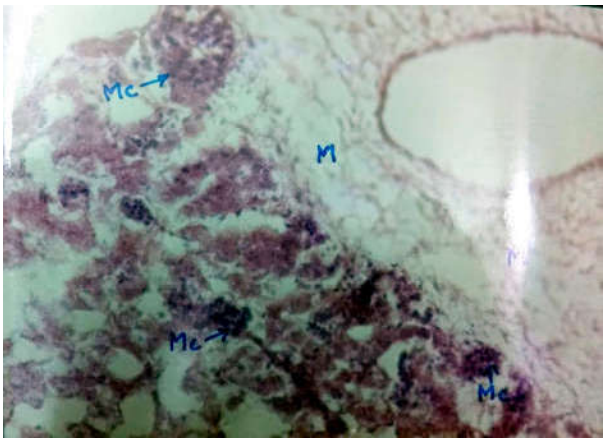

Fig. 3: Formation of connective tissue scaffolding M

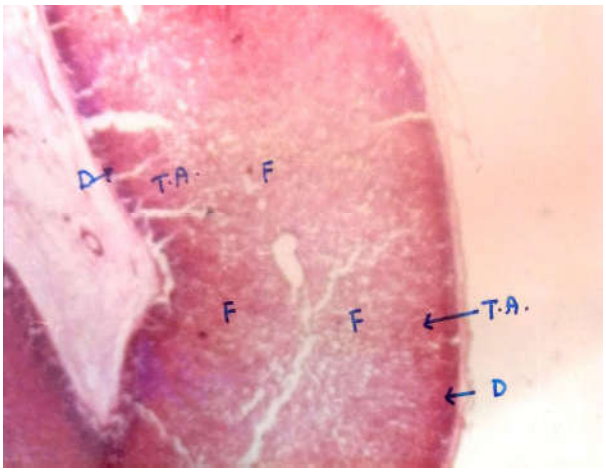

Fig. 4: Scanning view of adrenal gland at 23-24 weeks

4. After the Eosin staining, the slides were transferred from absolute alcohol I to II and III and then kept in xylene.
5. Using DPX mount, the coverslips were added. The slides were then cleaned and readied for the microscopic study.

The slides were then examined under the microscope to study the developing medulla of fetal adrenal gland under various stages. Histological slides of adrenal glands from each group were photomicrographed using trinocular Nikon microscope.

Observations and Discussion

Weeks 12–14

The medulla at this stage is not clearly demarcated. Clusters of medullary cells are found in the foetal cortex and the cells are small and basophilic in nature.

Weeks 15–16

The cortico-medullary junction is not clear. However, the medulla showed the formation of medullary sinusoidal vascular pattern. This suggests that medullary vascular pattern is developed prior to the migration and settling of the medullary cells in their definitive positions in the medulla.

Weeks 17–18

Clusters of the medullary cells are seen in the deeper part of fetal cortex as depicted in Figure 2. The cells are small and basophilic. We observe that the nuclei of most of the cells are dark. Very few cells nuclei are pale with visible nucleoli.

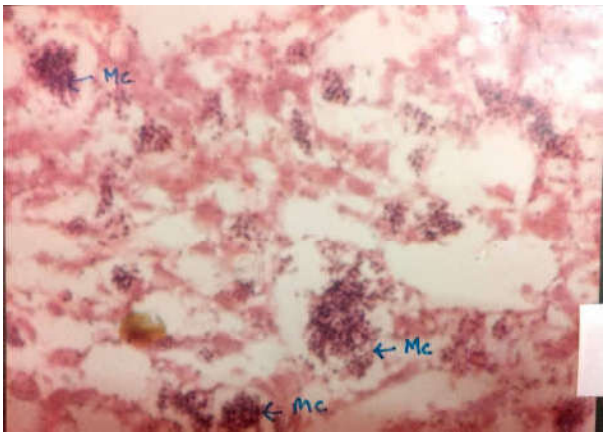

Fig. 5: Mc- Medullary cells

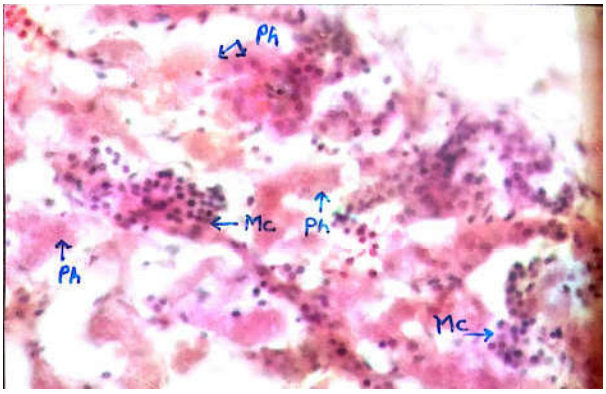

Fig. 6: Clusters of small basophilic cells Mc- neuroblasts

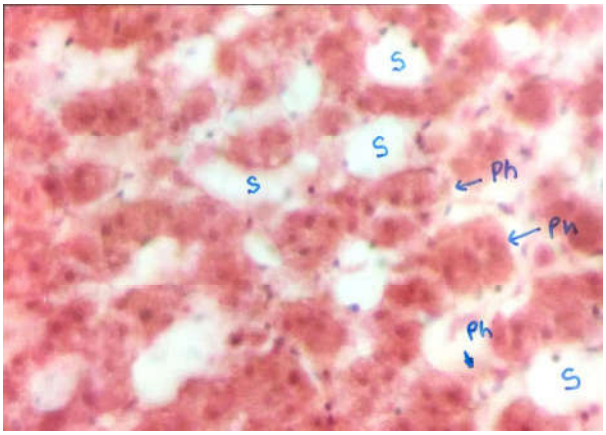

Fig. 7: Phaechromocytes Ph, with granular cytoplasm and vesicular nucleus.

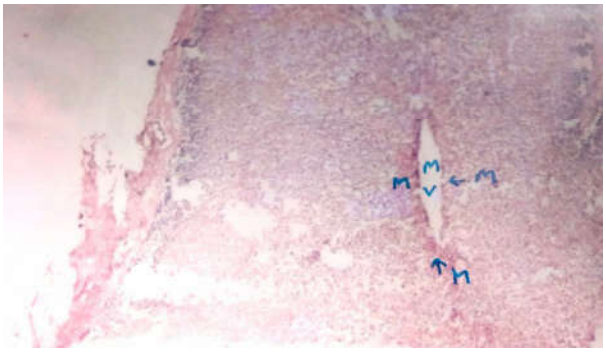

Fig. 8: Well organized medulla, M- Medulla, Mv- Medullary vein

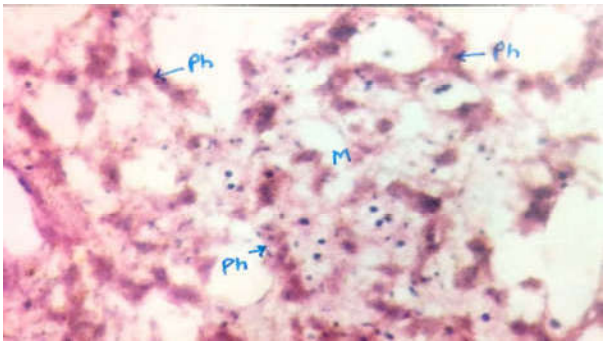

Fig. 9: Formation of groups of basophilic cells around sinusoids

Weeks 19–20

Medullary islands of small basophilic cells have increased in number and scattered. Some of them are present towards the medullary vein.

Weeks 21–22

Medulla does not reveal any change.

Weeks 23–24

The connective tissue scaffolding of medulla is formed. Clusters of medullary cells are ready to migrate as seen in Figure 3.

We observe two types of cells

1. Cells are large and lightly stained.

Nucleus is vesicular and Cytoplasm is granular as shown in Figure 7.

These can be called as phaechromocytes.

2. Clusters of small basophilic cells are neuroblasts as depicted in Figure 6.

These clusters along with phaechromocytes have arranged themselves around well formed sinusoids (Figure 5 and 6).

Weeks 25–28

Medulla does not reveal any changes during this period.

Weeks 29–30

Medulla is now well organized as shown in Figure 8. Clusters of small basophilic cells are not seen as they are mingled with few phaechromocytes (Figure 9) and are forming groups around sinusoids.

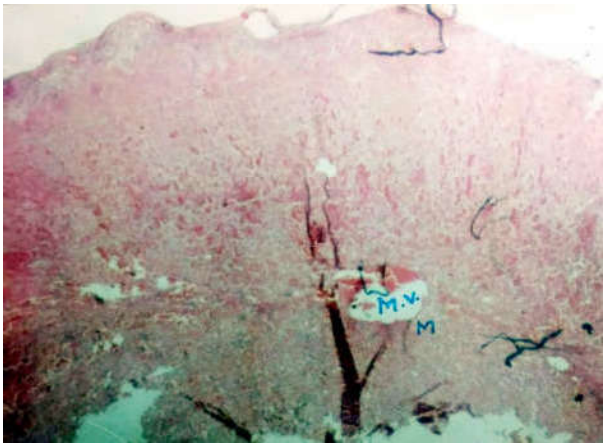

Fig. 10: Organized and differentiated medulla. Mv: medullary vein. M:medulla

Weeks 31–32

No changes are visible in the medulla.

Weeks 36–38

Medulla is well organized and differentiated (Figure 10). We can observe the phaechromocytes (Figure 11) and few ganglion cells (Figure 12).

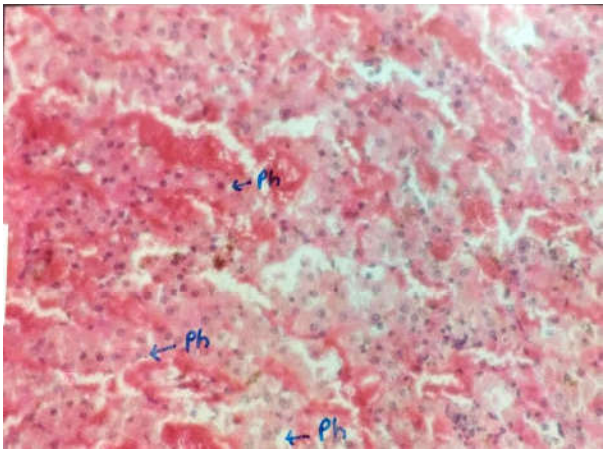

Fig. 11: Phaechromocytes can be observed. Ph: phaechromocytes

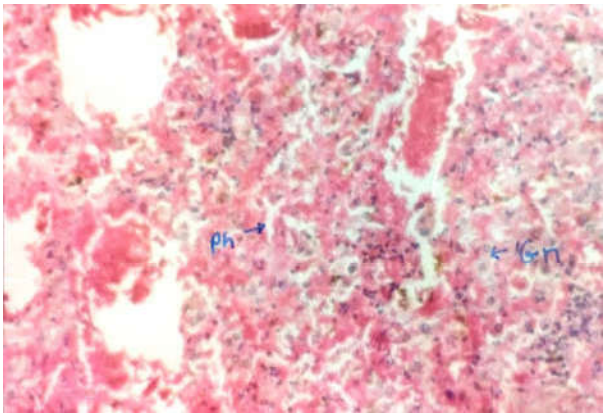

Fig. 13: Phaechromocytes - Ph, Ganglion cells-Gn

Conclusion

A prospective study of one hundred human fetuses is done (71 males and 29 females) ranging from 7 cm to 36 cm in crown-rump length and in gestational period of 12 to 36 weeks. In this study, we observe the developing medulla of the fetal adrenal gland. It is clearly seen that in the initial weeks, the medulla is not clearly demarcated, however, from the 15th week we can see the formation of medullary cells. Subsequently, the connective tissue scaffolding of the medulla is fully differentiated at 23 to 24 weeks of gestation. The neuroblasts (small darkly staining basophilic medullary cells) in the form of clusters migrate from the deeper part of fetal cortex to the medulla but don't settle in the medulla till the medullary sinusoidal pattern is well organized.

Moreover, the phaechromocytes are differentiated and can be identified at 23 to 24 weeks of gestation. Finally, this knowledge of normal development of adrenal medulla will help clinicians in diagnosis of conditions such as fetal neuroblastoma.

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