

Study on the gestational age-related histogenesis of human fetal suprarenal glands



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ABSTRACT

Background: Fetal suprarenal gland consists of three zones. They are the outer definitive zone or neocortex (DZ) that forms adult Zona Glomerulosa (ZG), the centrally located fetal zone and a third transitional zone (TZ) the future adult Zona Fasciculata (ZF) which lies just between the neocortex and fetal zone (FZ). The inner ZF that consists of large eosinophilic cells, and the outer definitive zone (DZ) consists of small, densely packed basophilic cells. As early as in 6th week of gestational Age, pheochromoblasts derived from the neural crest migrate through the fetal cortex to form the medulla at a later stage of development. The medulla unrecognizable as a distinct structure in the suprarenal gland throughout most of the gestation, except for small clusters or nests of chromaffin cells scattered throughout the body of the cortex. **Aims and Objective:** The study was designed and aimed to understand the early developmental histology of suprarenal gland. **Materials and Methods:** Study conducted at the Department of Anatomy, SV Medical College, Tirupati, Andhra Pradesh, India. The study was carried out by processing representative samples of fetal suprarenal tissues of 13 weeks, 17 weeks, 24 weeks, 25 weeks and 38 weeks gestational age. While processing, we observed the standard dissection procedure and histopathological tissue processing for the human samples. **Results:** Peripheral narrower and darker definitive zone or true cortex and an inner wider and lighter fetal zone was observed at 13, 17, 24 weeks with 10x objective. The demarcation between cortex and medulla could be seen clearly at 24 weeks. At 25 weeks capsule is very thick and vascular. A well-differentiated gland tissue with clear demarcation of cortex and medulla and a large central vein was observed. At 38 weeks degeneration of fetal zone with a thick-walled central vein was observed at lower magnification (10x objective) **Conclusion:** Observations on prenatal microscopic structure and gestational age-related changes in suprarenal glands form the basis for understanding structural changes and a foetal database for the local population.

Key words: Suprarenal gland (SG); Definitive zone (DZ); Fetal zone (FZ)

INTRODUCTION

The developing adrenal gland has both an exciting origin and an intriguing role in the harmonious development of the fetus as a whole. It is peculiar in that it undergoes structural modification both in prenatal and post-natal life to reach the adult status. Studies on fetal suprarenal glands facilitate understanding its development and also

contribute to future studies in obstetrics, perinatology and fetal pathology.¹

During the prenatal period, the bulk of the gland consists of the fetal cortex, and the morphology of the adrenal cortex remains relatively constant. Two primordia form suprarenal glands of higher vertebrates. The cortex and medulla are of different embryological origin. Cells of the

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human adrenal cortex arise from the coelomic epithelium covering intermediate mesoderm, the cells of medulla are derived from the neural crest.

The earliest recognizable developmental component of the adrenal gland is called “the adrenal blastema” or “the adrenal primordium”, this appears distinct from the surrounding structures at 33 days/4th-week post-conception lying posteromedial to the urogenital ridge.²⁻⁸

During the 4th week, the cells of coelomic epithelium between the root of the mesentery and the developing gonads proliferate into the underlying mesoderm that is differentiating to form adrenal cortical mass and surrounds it developing the definitive or reliable cortex. Later these cortical masses are invaded on their medial aspect by sympathochromaffin cells migrating from the nearby sympathetic ganglia.^{2,5,9,10,11} The adjacent mesenchyme forms the capsule of the gland. Miriam (1940) reported a narrow outer band of cells in the form of acini near the capsule and a more columnar arrangement near the central part.¹²

During 6th-week definitive cortex is subcapsular, and its cells are smaller in size with basophilic cytoplasm and intensely staining nuclei. The cells of the fetal cortex are larger with eosinophilic cytoplasm and contain vesicular nuclei.^{2,3,5,7,9,10,13} Those cells that have proliferated from the coelomic epithelium form the fetal cortex and they regress after birth.

During 6th to 7th week of gestation, pheochromoblasts derived from the neural crest reach the dorsomedial aspect of the primitive fetal cortex and migrate through it running between cortical cells and finally reaches the central part of gland to form the medulla at a later stage of development.^{2,7,9,10,11,13,14,15,16}

The larger size of the suprarenal gland in the fetal period is due to the presence of fetal cortex which constitutes 80% of the cortex. The definitive or permanent cortex contributes 20% of the entire cortex. The increase in the size of the fetal adrenal gland is predominantly due to significant enlargement of the central fetal zone.^{11,17-20}

MATERIALS AND METHODS

One hundred and twenty-two suprarenal glands (both right and left) dissected from 61 normal human fetuses of different gestational ages (13 weeks to 38 weeks) were utilized for the present study. Fetuses were collected from Government Maternity Hospital, Tirupati and preserved

in 10% formalin for two weeks. Then, the fetuses dissected, and suprarenals observed for their relation with kidney before removal. After studying morphology and morphometry, sections prepared for histological study from representative samples of 13 weeks, 17 weeks, 24 weeks and 38 weeks age groups. These sections were processed and stained by routine hematoxylin and eosin stains and special reticulin stain.

RESULTS

In the 2nd trimester group, histological sections of 13wks, 17wks and 24wks were observed. At the lowest magnification the capsule at 13 weeks is thicker than that at 17, and 24 weeks (Figures 1,4,5) All sections in the 13 wks group presented cortex and central vein. In 13 and 17 weeks sections, the capsule showed blood vessels and a small ovoid nodule containing cortical tissue (Figure 2). The nodule is enclosed by a capsule that is continuous with the capsule of the gland (Figure 2).

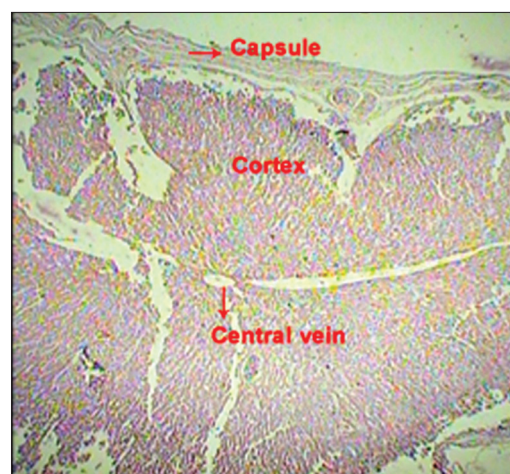


Figure 1: H&E-13weeks-4x

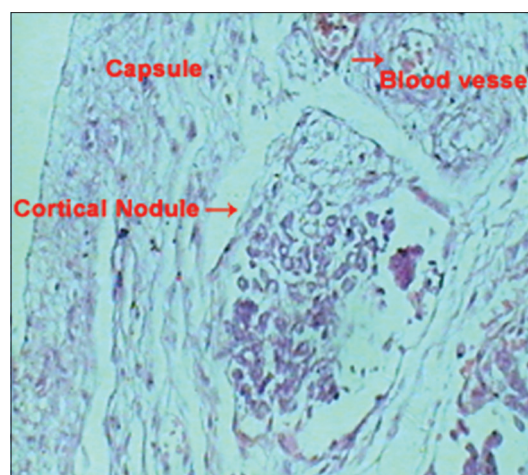


Figure 2: H&E- 13 weeks- 10x

Peripheral narrower and darker definitive zone (DZ) or true cortex and an inner wider and lighter fetal zone (FZ) can be identified at 13, 17 and 24 weeks with 10x objective (Figures 3-5). Darker peripheral zone is 1/4th of the cortex and is forming a thin subcapsular band. The lighter inner zone of fetal cortex occupied 3/4 of the cortex (Figures 3-5) with an increase in their width with the increase in gestational age.

In the lighter fetal zone the cells at the periphery are arranged in the form of cords with sinusoids in between (Figures 3 and 4). This type of appearance was not present in the central vein. These could be identified more clearly at 17 wks. In the fetal zone, small masses of darkly stained cells presenting a rosette appearance observed at 4x and 10x magnification (Figures 1 and 4) at 13 and 17 weeks. These represent the invading neuroblasts. The deeper fetal zone nearer to the medulla presented rosette formation, and the vesicles lined with basophilic cells and filled with a coagulated fluid at 17 weeks.

At higher magnification (40x) in the slides of 13, 17 and 24 weeks the cells of the darker zone and fetal zone could be differentiated by characteristic basophilic appearance of scattered darker cells that were small in size (Figures 6-8) in the darker zone from the more giant, eosinophilic cells that are organized in the form of fascicles with sinusoids in between (Figure 6) in the lighter zone. From 13 to 24 weeks, there is a progressive increase in the size and population of these cells. The nuclei of darker cells are dark in appearance, whereas those of lighter cells are vesicular (Figures 6-8).

The demarcation between cortex and medulla could be seen clearly at 24 weeks though it was not present at 13 weeks (Figure 1) and slightly differentiated at 17 weeks.

At 13 weeks medulla is represented by a blood vessel at the centre with fewer cells that could not be differentiated and plenty of sinusoids. At 17 weeks, the thick-walled blood vessel in the centre and rosette arrangement of sympathetic cells were observed. The vascularity towards medulla is more than that nearer the capsule.

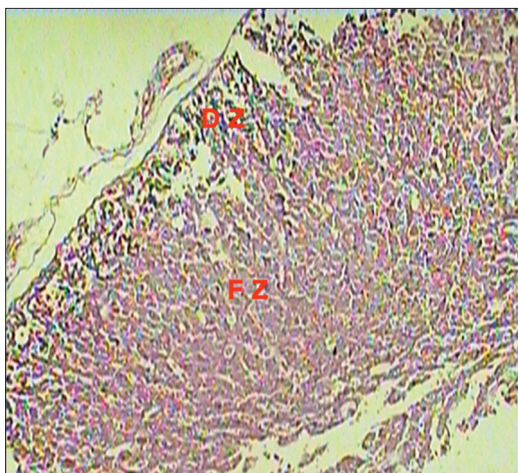


Figure 3: H&E: 13 weeks: 10x- Darker zone –(DZ) Fetal zone(FZ)

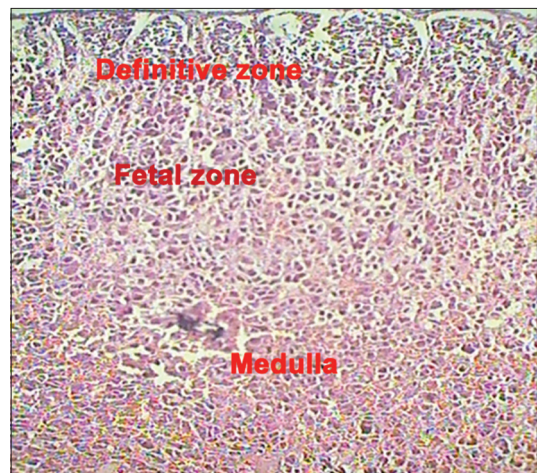


Figure 5: H&E -24 weeks- 10x

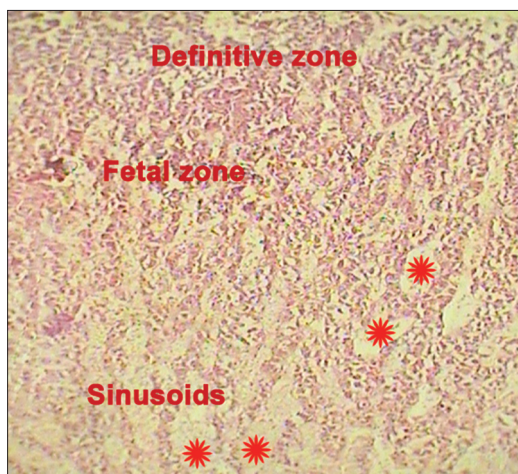


Figure 4: H&E: 17 weeks: 10x: Cortical zonation, *Sinusoids.

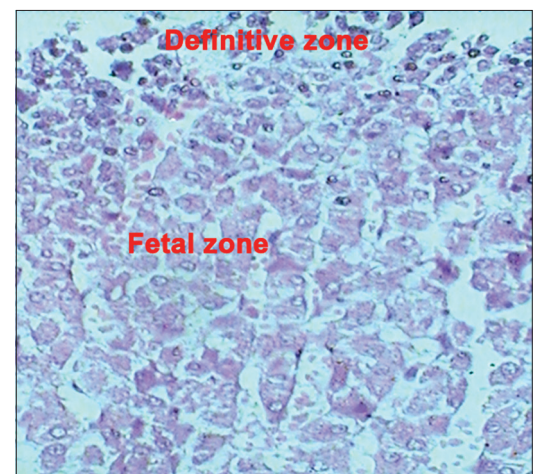


Figure 6: H&E: 13 weeks: 40 xs, DZ &FZ: Appearance of cells

At 24 weeks smooth transition between cortex and medulla could be observed at lower magnification (Figure 5). At higher magnification, the cells of lighter fetal cortex with acidophilic cytoplasm and vesicular nucleus in the form of cords interspersed with scattered large polygonal basophilic cells at cortico-medullary transition observed. The medulla at higher magnification presented aggregations of large, polyhedral, basophilic cells with an eccentric nucleus and plenty of sinusoids.

In the 3rd trimester group, histological sections of 25wks and 38 wks were observed. At 25 weeks capsule is very thick, vascular and well-differentiated, clear demarcation of cortex and medulla with a central vein that is large was noticed. At higher magnification of 40x the cells of the definitive zone (DZ) presented aggregations of small, dark basophilic cells with a condensed nucleus that can be very well differentiated when compared to 2nd trimester was observed (Figure 9). Further, the cells of fetal zone (FZ) are also very well-differentiated when compared to 2nd trimester and presented large,

acidophilic cells with a clear vesicular nucleus (Figure 10). Two types of well-differentiated cells were observed in the medulla. One is small in size and plenty in number, arranged in groups and presented basophilic cytoplasm with rounded centrally placed nucleus (Figure 11) and represents sympathoblast. The other type of cell is larger in size, polygonal in shape and scattered in distribution with slightly acidophilic cytoplasm and eccentric nucleus (Figure 11).

Degenerating fetal zone with a thick-walled central vein was observed at 38 wks (10x). At higher magnification, the definitive cortex presented vesicles filled with slightly basophilic material (Figure 12) and the fetal zone nearer to the capsule is showing signs of congestion and degeneration and lost its cellular details whereas at deeper zones presented the cord-like arrangement with less vascularity (Figure 12). In medulla increased number of fat cells and compressed medullary neuroblasts and polygonal, scattered sympathoblasts (Figure 13) observed.

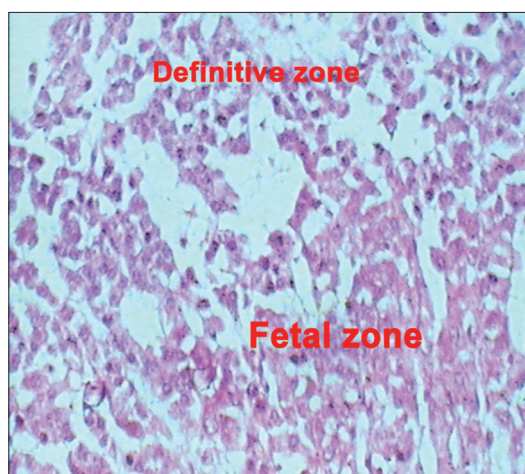


Figure 7: H&E -17 weeks: 40x: DZ &FZ

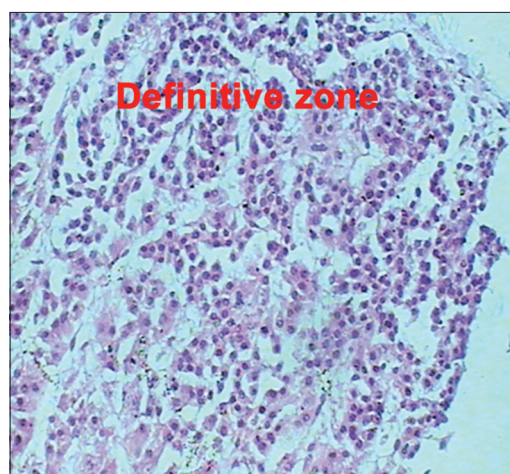


Figure 9: H&E 25 weeks -40 x: DZ cells

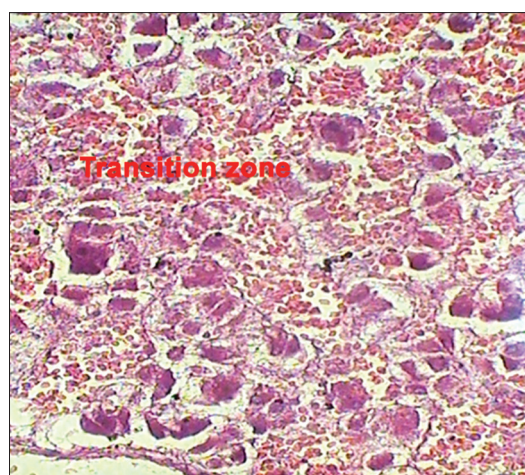


Figure 8: H&E: 24 weeks -40 xs: DZ-FZ transition

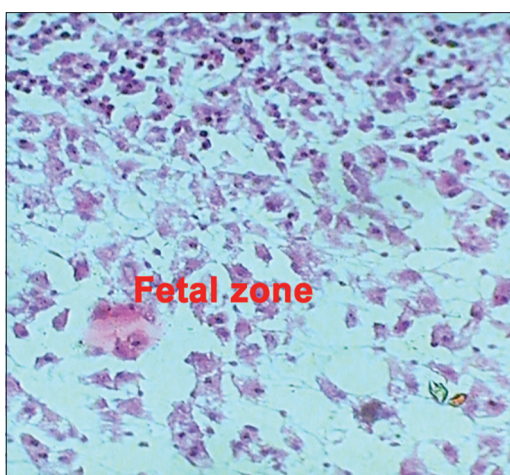


Figure 10: H&E: 25 weeks: 40x: FZ cells

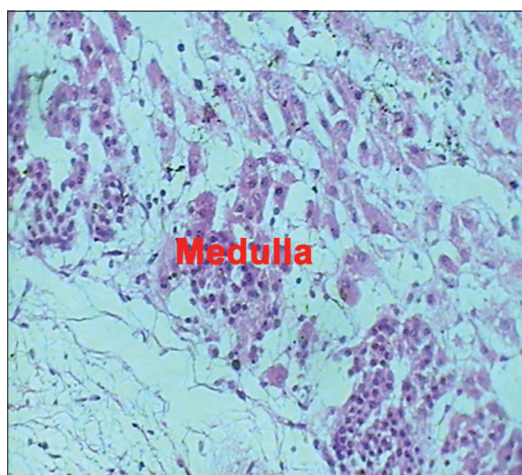


Figure 11: H&E: 24 weeks: 40x: Medulla sympathoblasts

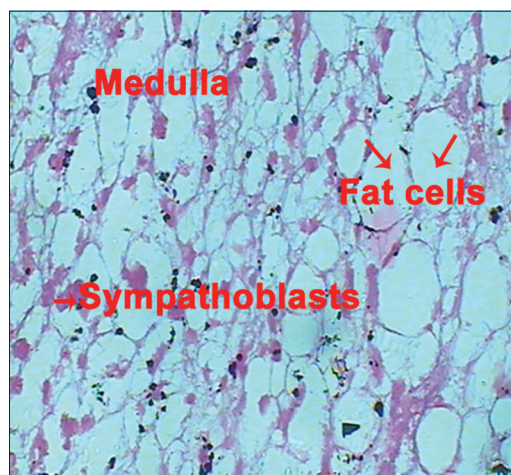


Figure 13: H&E: 38 weeks -40x- Fat cells and sympathoblasts

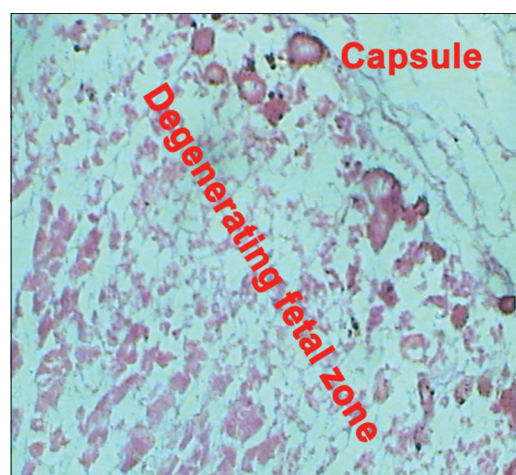


Figure 12: H&E- 38 weeks 40 x- near capsule: Degenerating fetal zone

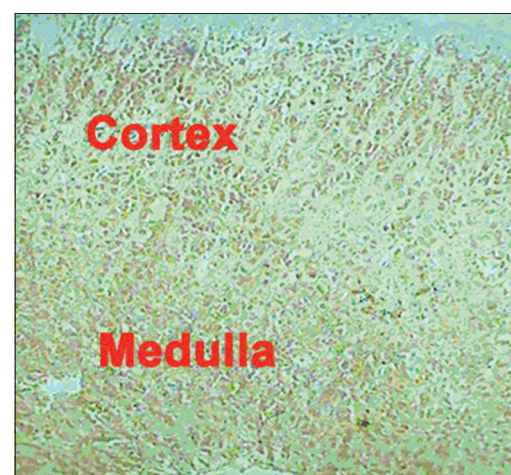


Figure 14: Reticulin stain- 24 weeks -10x.

Reticulin stain was done on 24 weeks fetal suprarenal gland section, which revealed that reticular fibres are thin and sparsely distributed (Figure 14).

DISCUSSION

In the present study, the smallest section observed for microscopy is that of 13 weeks fetus. A thin capsule made up of collagen fibers, peripheral narrower and darker definitive zone (DZ) and an inner wider and lighter fetal zone (FZ) were identified at 13 weeks. Khayati Santram et al. 2013²¹ reported that at 11-15 weeks. Between 20-25 weeks, the capsule was well developed surrounding whole of the gland with blood vessels in it. Darker peripheral zone is 1/4th of the cortex and is forming a thin subcapsular band. The lighter inner zone of fetal cortex occupied 3/4 of the cortex. The cells of the darker zone presented scattered distribution of small and basophilic cells with a dark nucleus. The cells of the fetal zone started arrangement in the form of fascicles that is more pronounced as age

advances with few capillaries in between. The cells of the fetal zone are large, acidophilic with vesicular nuclei. Rosette appearance of invading neuroblast were seen at 13 and 17 weeks. The deeper fetal zone nearer to the medulla presented rosette formation and vesicles lined with basophilic cells, and filled with a coagulated fluid at 17 weeks. Ravindra Kumar and Subhadra Devi 2019,²² reported these changes in the early embryonic stage, i.e. Carnegie stage 16 and 6-7 weeks of post-conception and Carnegie stage 17 and 8-10 weeks of post-conception.

From 13 to 24 weeks, there is a progressive increase in the size of the fetal cortex. The demarcation between cortex and medulla could be seen clearly at 24 weeks. The cells of the lighter zone are more considerable, acidophilic with a vesicular nucleus. The sinusoids are few and sparse in distribution. Similar findings are also reported by Ravindra Kumar and Subhadra Devi, 2019.²² The cortex of fetal suprarenal gland exhibited two distinct zones, a superficial dark zone and deeper comparatively lighter zone.²¹

Table 1: Human fetal suprarenal gland histogenesis- comparison of different studies in India.

Author	Population	0-12 weeks	12-25 weeks	Gestational Age related histogenesis observations	25-36 weeks	More than 36 weeks
Sangma GT, et al, 2008 ¹¹	Eastern India	Suprarenal gland and its capsule are well identifiable—cortex exhibit two distinct zones. The medulla is ill-defined.	The capsule is thicker and better defined with blood vessels in it. Cortex- superficial ¼ darker zone inner ¾ lighter zone. The medulla is better defined.	The capsule is well developed with increased blood vessels. Cortex cells are better differentiated. The medulla is well defined, and chromaffin cells are better developed.	The capsule is dense with fibroblasts. Cortex- Superficial 1/6 zone deeper 5/6 zone. Medulla- Chromaffin cells are increased.	
Khayati Santram, et al, 2013 ²¹	North India		The thin capsule made up of collagen fibres. Cortex exhibits two distinct zones.	The capsule is dense. Cortex- Superficial zone cells are arranged in glomerular form. Deep lighter fasciculo-reticular zone. Medulla- large ganglionic cells. Definitive cortex increases. Fetal zone starts degenerating.		
Mini Mol P, et al, 2014 ²⁵	Central India	Thin capsule with blood vessels. Cortex- Superficial 1/6 th dark zone. Capsule and Suprarenal gland are well identifiable.	Capsule thicker at the periphery than at hilum. Cortex- two distinct zones.	Cortex- well-differentiated glomerular cells. Medulla – more chromaffin cells.		
R Mallieswar Rao, et al, 2015 ²⁰	South India	Capsule appeared at 12 weeks. Zonation of cortex observed at 8 weeks. Sympatho-chromaffin bundles appeared at 6 weeks.	Cortex- well differentiated into Zona glomerulosa and zona fasciculata.	Dense capsule extending along trabeculae into cortex. All the three zones are well differentiated. Medulla- large chromaffin cells.		
Ravindra Kumar, et al, 2019 ²²	South India		Gland is very vascular. Both small and large vessels have extremely thin walls. Sympatho-chromaffin elements pass in with blood vessels.	Marked fibrous capsule. Demarcation of fetal cortex to medullary zone. Active medullary migration.		
Present study	South India	The thick capsule was observed in 13 weeks. small ovoid nodule containing cortical tissue. this nodule enclosed by a thick capsule with profound vasculature. Peripheral definitive zone (DZ) inner fetal zone (FZ) identified. These zones increased with gestational Age. 17 weeks small masses of darkly stained cells presenting a rosette appearance was observed. 13 to 24 weeks there is progressive increase in the size and population of cells. 24 weeks Demarcation between cortex and medulla observed. 17 weeks well vascularized medulla. 24 weeks transition between cortex and medulla.		Twenty-five weeks capsule is very thick, vascular and well-differentiated. demarcation of cortex and medulla with a central vein. the cells of fetal zone (FZ) are also very well differentiated.	Thirty-eight weeks degeneration of fetal zone with a thick-walled central vein. definitive cortex with vesicles filled with slightly basophilic material. the fetal zone nearer to the capsule is showing signs of congestion and degeneration and lost its cellular details deeper zones presented the cord like arrangement with less vascularity. In medulla increased number of fat cells and compressed medullary neuroblasts and polygonal, scattered sympathoblasts with blood vessel.	

At 13 weeks medulla is represented by a blood vessel at the centre with few cells that could not be differentiated and plenty of sinusoids, this is in agreement with that reported by Jiang Fengrong et al., (1998).^{16,21} At 17 weeks the medulla presented a thick-walled blood vessel in the centre. The vascularity towards medulla is more than that nearer the capsule at 17 weeks. The medulla at higher magnification showed aggregations of large, polyhedral, basophilic cells with an eccentric nucleus and plenty of sinusoids at 24 weeks.²¹

A cortical nodule enclosed by a capsule of fibrous tissue that is continuous with the capsule of the gland can be observed in 13 and 17 weeks sections. According to Ernest et al., (1996)⁷; Dobbine JW, (1969);²³ Hedeland et al., (1968);²⁴ Ravindra Kumar and Subhadra Devi (2019)²² these are observed with increasing frequency in aged glands and also in hypertension, diabetes mellitus.

From 25 weeks to term, there is progressive thickening and vascularity of the capsule with well-differentiated connective tissue. Well-differentiated cortex and medulla were observed. Two types of cells were observed in the medulla at 25 weeks. One is small in size and plenty in number, arranged in groups and presented basophilic cytoplasm with the rounded centrally placed nucleus and it represents sympathoblasts. The other type of cell is larger in size, polygonal in shape and scattered in distribution with slightly acidophilic cytoplasm and eccentric nucleus. The results of the present study are in agreement with earlier studies by Jiang Fengrong et al., (1998)¹⁶, Ravindra Kumar and Subhadra Devi (2019).²²

At 38 weeks to full term, there is degeneration of fetal zone, vesicles filled with slightly basophilic material in the definitive zone, signs of congestion and deterioration, with loss of cellular details in the superficial fetal zone and reduced vascularity. In medulla increased number of fat cells, compressed medullary neuroblasts and thick-walled central vein with thick muscular wall identified. Similar findings were reported in the literature Jiang Fengrong et al., (1998)¹⁶, Dobbine JW, (1969)²³; Ravindra Kumar and Subhadra Devi (2019).²²

Table 1 shows an analysis of various studies of Human fetal suprarenal gland histogenesis reported in India.

CONCLUSION

A progressive change in the growth and differentiation of capsule, definitive and fetal zones, medulla, cortico-medullary differentiation, central vein and vascularity were observed in the present study. At 38 weeks signs

of involution presented as degeneration of fetal zone, the appearance of vesicles filled with slightly basophilic material in the definitive zone, signs of congestion and degeneration, with loss of cellular details in the superficial fetal zone and reduced vascularity and increased number of fat cells and compressed medullary neuroblasts and thick-walled central vein with the thick muscular wall in the medulla. Observations on age-related changes in the microscopic structure of prenatal suprarenal sections form the basis for understanding structural changes and a database for the local population.

The findings of histogenesis of the human fetal suprarenal gland of the present study are compared and correlated with findings of studies in other regions of India.

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MR- Concept and design of the study; interpreted the results, prepared first draft of manuscript and critical revision of the manuscript; **RK-** Histological findings analyzed and interpreted; reviewed the literature and manuscript preparation; **SD-** Design of the study, histological findings analyzed and interpreted, preparation of manuscript and revision of the manuscript; **SR-** Concept and coordination of the overall study.

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