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A study on histogenesis of human fetal cerebellar cortex

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Abstract

The various histological features of human fetal cerebellum in spontaneously aborted fetuses of different gestational were analyzed. The age of the fetuses was observed from 16 weeks to 36 weeks of Gestation. This study was undertaken to describe the prenatal histogenesis of human cerebellar cortex. The fetuses were divided into five gestational age groups. The dissected specimens were preserved in 10% formalin and subjected to routine histological procedure. In present study we observed the major histological changes occurred in cerebellar cortex during the 16th week as 3 layered which became 5 layered at 28 weeks and 4 layered at 31 weeks. The number of layers of cerebellar cortex changes with age. The Knowledge of cerebellar anatomy has a tremendous neurosurgical importance in posterior fossa neoplasm mainly Medulloblastoma.

Keywords: Cerebellum, Histogenesis, Purkinje cell, Fetus, External granular layer.

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1. Introduction

Cerebellum is one of the structures in the brain that has a complex development. It is the first one to differentiate, but it takes longer period for development. Cerebellar cortex is divided into 3 distinct layers as seen in adults- outer molecular layer, middle Purkinje cell layer and inner granular layer [1]. In developing cerebellum there may be two additional layers which may disappear over time, one is the external granular layer and lamina dissecans. The external granular layer is a characteristic feature of developing cerebellum. It is detectable until age of ~1 year. It is described as a thin evenly calibrated layer of germinal cells [2]. The history of development of External granular layer started with Obersteiner. Obersteiner (1880) was the first to describe it more accurately and hence it is called Obersteiner layer. Further understanding of the development of cerebellar cortex was made clear by Ramon y cajal mainly by his invention of Golgi techniques. The foetal primate cerebellar cortex is composed of 2 layers at 9-11 weeks, 5 layers at 21-31 weeks 4 layered at 31 weeks -12th postnatal month and finally acquires the adult 3 layered pattern 3 at about 12-18th Postnatal month [3]. The present study has been undertaken to elucidate the above findings.

2. Materials and Methods

25 aborted normal fresh fetuses 15 male and 10 female of different age groups ranging from 16 weeks to 36 weeks were collected from department of obstetrics and gynecology, Thanjavur medical college, Thanjavur. Ethical committee clearance and informed consent was obtained. The fetuses were products of terminated pregnancies under medical termination of pregnancy MTP act of India, 1971. Fetuses free from gross anatomical abnormality were selected for the study. The age of the fetuses was calculated from the obstetrical history and from the crown rump length. The fetuses were divided into different age groups according to their gestational age. The fetuses were dissected and fixed in 10% formalin for 15 days and processed (Fig. 1). Using automated tissue processor (Leica tp1020). Dehydrated with isopropyl alcohol in ascending grades clearing done with xylene and impregnation done with paraffin wax (melting point 550 - 600 c) using Leuckhart's L molds. The sections were cut from the blocks at 5µ thick were stained with Hematoxylin and eosin. The stained slides were studied using binocular light microscope under 4x, 10x, 40x, 100x objectives and analyzed.

3. Observations

25 fetal cerebella ranging from 16-36 weeks of gestational age were considered and classified into 5 group (Table 1). The appearance of various cortical layers and their period was noted and studied analyzed under groups (I -V):

3.1. Group I (16-20 weeks)

The external surface of the cerebellum was lined by a thin layer of cells above the marginal layer to form the external granular layer. The cells contain, scanty cytoplasm and darkly stained nuclei. At 19-20 weeks, the external granular layer contains five to six rows of cells. The cell sparse marginal layer is now referred as molecular layer well differentiated from the intermediate zone beneath it. No folia could be observed. By 16 weeks the molecular layer was undifferentiated from the intermediate layer beneath it. By end of 20 weeks, molecular layer could be differentiated. These cells were spherical but the nuclei were not as darkly stained as external granular layer. There was no evidence of lamina dissecans. Internal granular layer was seen as well dense layer below the cell sparse molecular layer. The cells were lightly stained immature cells, but not clearly demarcated (Fig. 2 & Fig. 3).

3.2. Group II (21-24 weeks)

The external granular layer contains six-nine rows of cells. The width of this layer was increasing. The molecular layer thickness was also increasing. The Purkinje cells could not be identified due to poorly formed cytoplasm. By end of 21 weeks, an acellular band was visible below the Purkinje layer and above internal granular layer. This layer made Purkinje layer look more differentiated. The internal granular layer was seen as cell dense layer. The cell bodies of Purkinje cell were arranged on the superficial layers of internal granular layer. The appearance of lamina dissecans separated the internal granular layer from Purkinje cell layer (Fig. 4).

3.3. Group III (25-28 weeks)

By 25-26th week, the width of external granular layer starts reducing. Folia could be observed. The molecular layer was even wider and the ratio between the external granular and molecular layer was 1:1.5. The Purkinje cell layer was identified by 25-28 weeks after the formation of lamina dissecans, which was layer of a cellular band of cells above the internal granular layer. This layer appeared as tightly packed cells with an oval cell body with dark staining nuclei. These cells are arranged in a 2-3 layer of cells. The presence of lamina dissecans made the internal granular layer differentiated from the Purkinje cell layer. The border between the internal granular layer and white matter, could be distinguished.

3.4. Group IV (29-32 weeks)

Width of external granular layer starts decreasing. The ratio between the external granular layer and molecular layer was 1:2. By 29-32 weeks, the Purkinje cell dendrites were visible and cell nucleus could be observed. Lamina dissecans started to decrease in thickness. In some specimens, the layer has disappeared completely.

3.5. Group V (33 - 36 weeks)

The external granular layer was thinner than internal granular layer. The width of the molecular layer was at highest. The Purkinje cell processes were visible, they were arranged in a single row and was widely spaced (Fig. 5 & 6). The cells looked elongated when compared to the rest of the cells. At certain sites these cells demonstrated well developed branching dendrites. Observations of different layers of cerebellar cortex were given in Table 2.

4. Discussions

The development of human fetal cerebellum is discussed under the following histological patterns:

4.1. External granular layer

In the present study, the external granular layer was observed from 16th week as a thin layer of spherical cells with dark staining nuclei and scanty cytoplasm. The time of appearance of external granular layer could not be ascertained, since the present study examined fetuses from 16th week The external granular layer was identified only at 18 weeks according to studies by Narasinga rao and Pramila [4]. Raaf observed that from the fifth postnatal month, until term, the width of external granular layer decrease [5]. Studies by Abraham et al observed the highest cell proliferation rate in external granular layer between 28th to 34th gestational weeks. Friede observed that the external granular layer thickness is at peak at 24 weeks and remained constant till term [6].

4.2. Molecular layer

In this study, the molecular layer could be differentiated at the end of 20 weeks as cell sparse layer containing few spherical cells with lightly stained nuclei. The molecular layer started growing rapidly after 24 weeks. According to Abraham et al, the ratio of EGL: ML at 24-28 weeks was 1.4:1 whereas the ratio of width of EGL: ML in present study was 1:1.5. John Woodard observed from his study that the molecular layer can be differentiated from 14 week onwards along with the external granular layer differentiation [6].

4.3. Purkinje cell layer

In the present study, the Purkinje cells were differentiated only after the formation of lamina dissecans by about 25 weeks. These cells were arranged as tightly packed cells with an oval cell body and dark staining nuclei. By 32-34 weeks the Purkinje cells were arranged in a single row and acquired its characteristics appearance. Studies by Krishnaveni et al., noticed the appearance of Purkinje cells at 17 weeks as multilayered in arrangement and were beginning to organize in single layer at 30 weeks and clearly organized by 36 weeks [7]. Friede observed that the bodies of Purkinje cells could be noticed at the same time when the lamina dissecans disappeared that in around 32 weeks of gestation [8]. Ashalatha et al., concluded from their studies that the Purkinje cell diameter increase as age advances and attain they adult size only by 9th postnatal month [9]. Halder et al., observed the Purkinje cell maturation in three stages. In the first stage (12–16 weeks), th differentiation of Purkinje cells and their relationship to other components is observed. The second stage lasts through the fifth, sixth and seventh fetal months (16–28 weeks).

The third stage extends throughout the remaining period of intrauterine life and the first postnatal year and continues

at a slow rate thereafter [10]. The Purkinje cells appeared during the 4th month of embryonic period according to divya et al., [11].

4.4. Lamina dissecans

In the study, lamina dissecans appeared by 22 weeks as an acellular band above internal granular layer. The thickness was at peak at 25 weeks, after which it started to decrease and finally disappeared. Rakic and Sidman did an extensive study on lamina dissecans and observed that this layer was peculiar to human fetal cerebellum. He noticed the appearance of this layer by 20 weeks and its disappearance by 31 weeks. The purpose of this layer is yet to be understood [12].

4.5. Internal granular layer

The internal granular layer was very well differentiated at 29 weeks after the layer started increasing in two folds during 33-36 weeks. According to Yamaguchi et al., the internal granular layer of cerebellum cortex is developed by 3 stages. The primary or undifferentiated stage (before 18 weeks) the secondary or intermediate stage (18-35 weeks) during this period the internal granular layer was clearly visible. The tertiary or developing stage (35-40 weeks) during which the external granular layer showed dramatic increase in thickness as the formation of cerebellar folia proceeds [13-14]. In the present study, the width of internal granular layer showed variations between the hemispheres and folia in same specimen. The width of internal granular layer at the hemispheres were only taken into account. Comparisons between studies were given in Table 3.

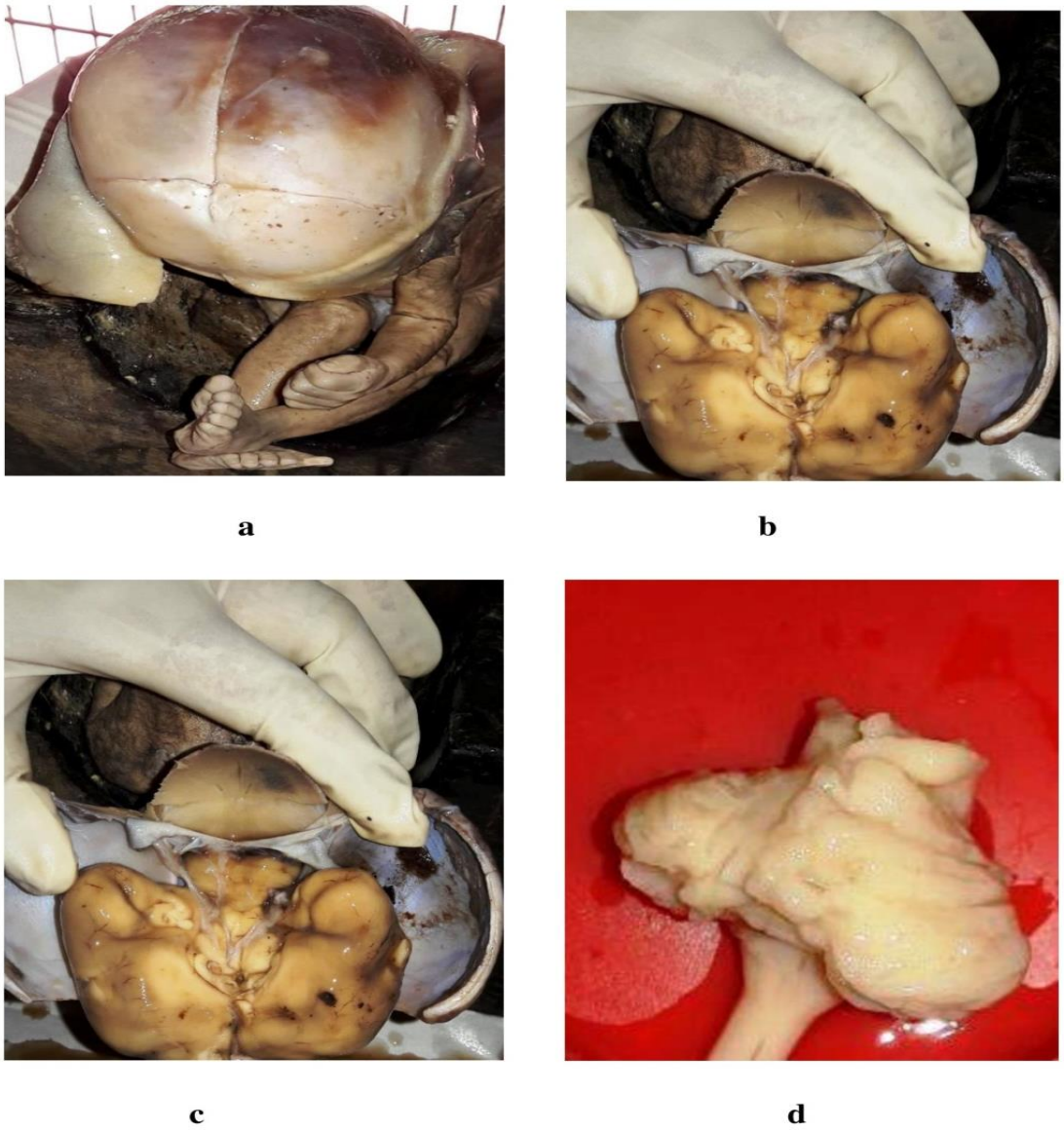

Fig. 1. Dissection of specimen.

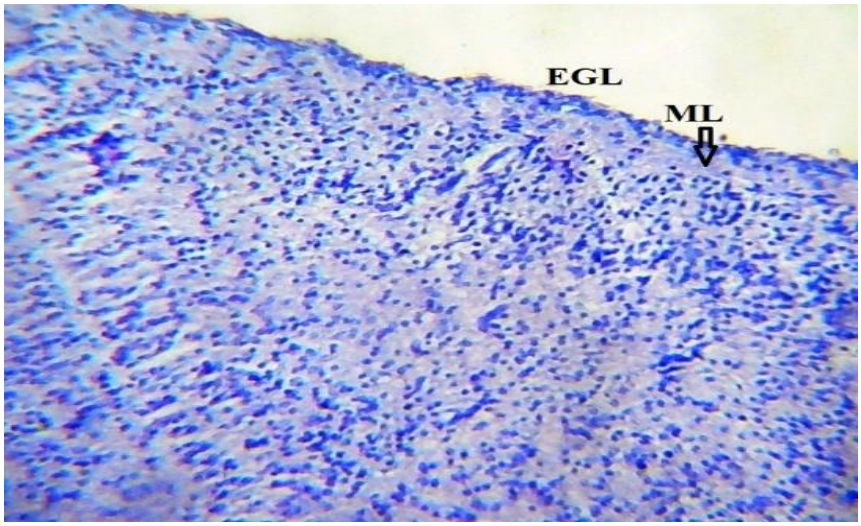

Fig. 2: 16 Weeks, cerebellum transverse section, Hematoxylin and Eosin, 100x Magnification.

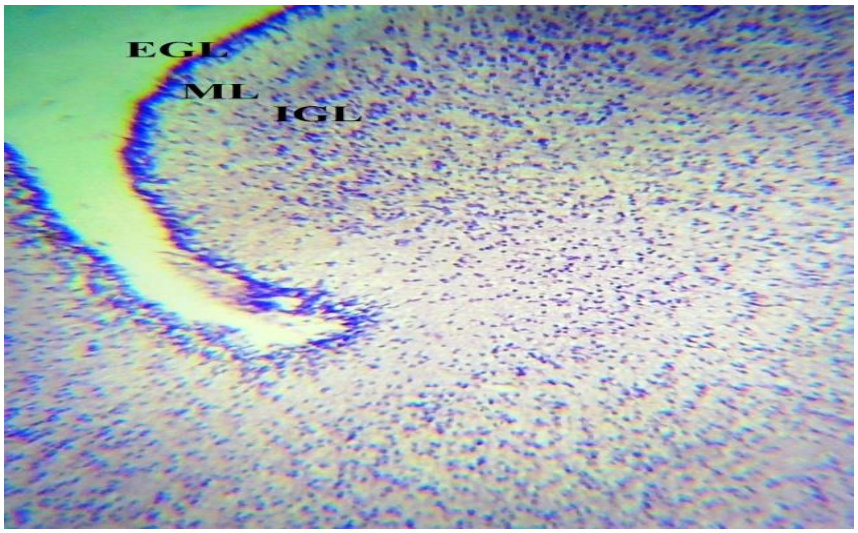

Fig. 3: 20 Weeks, cerebellum transverse section, Hematoxylin and Eosin, 100x Magnification.

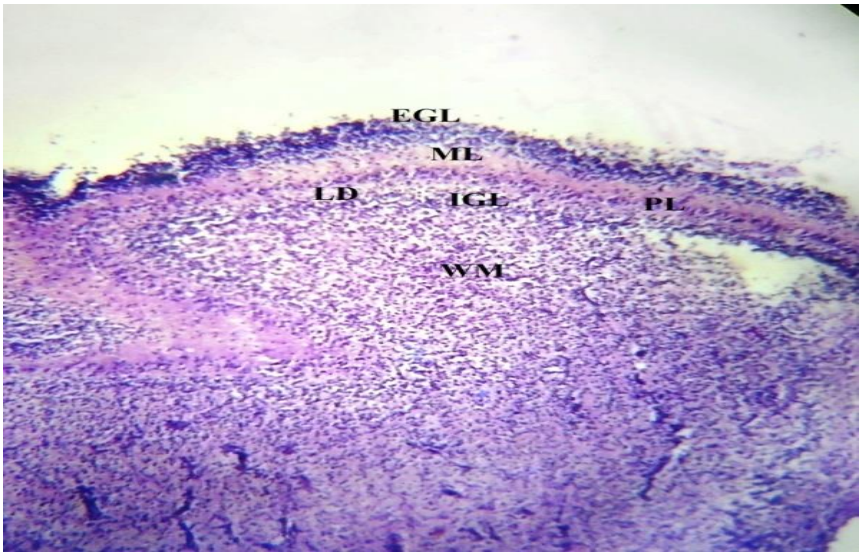

Fig. 4: 24 Weeks, cerebellum transverse section, Hematoxylin and Eosin, 100x Magnification.

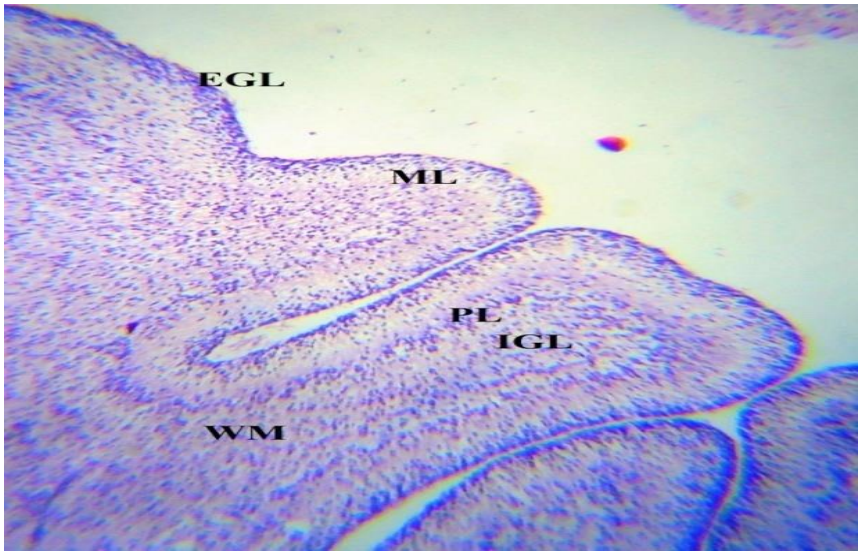

Fig. 5: 34Weeks, cerebellum transverse section, Hematoxylin and Eosin, 100x Magnification.

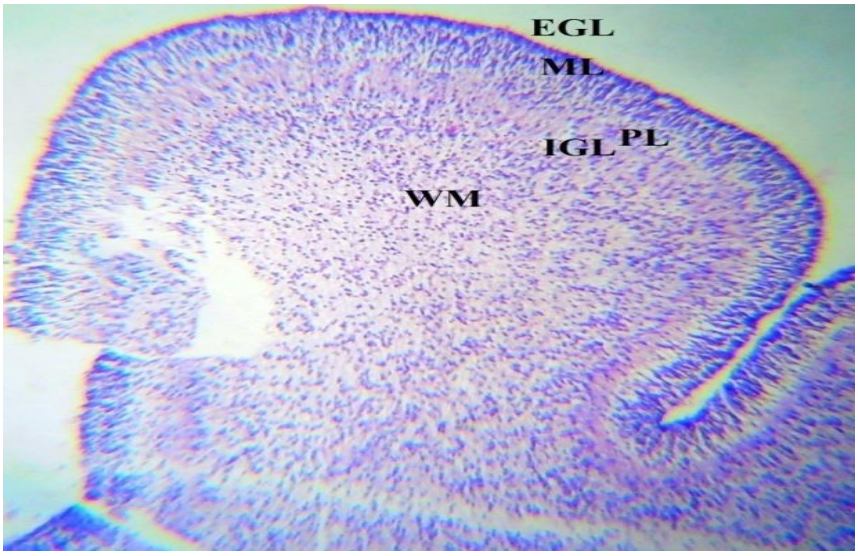

Fig. 6: 36 Weeks, cerebellum transverse section, Hematoxylin and Eosin, 100x Magnification.

Table 1: Grouping of fetuses by gestational age.

Groups	Age in Weeks	No. of Fetuses
I	16-20	5
II	21-24	7
III	25-28	5
IV	29-32	6
V	33-36	2

Table 2: Group observation of histology of cerebellum.

Parameters	Group I	Group II	Group III	Group IV	Group V
Folia	Absent	Present	Present	Present Well formed	Present
EGL	Present	Starts increasing	Thickness reduces	Present but thin	Thinner
ML	Undifferentiated	Weight increases	Weight increases	Thicker	Thickest
PL	Absent	Undifferentiated	Well differentiated Randomly arranged	Well differentiated Dendrites visible	Arranged in a single row
LD	Absent	Present	Present	Width decreases	Disappeared
IGL	Present	Present	Present	Present	Present

Table 3: Study Comparisons.

Layers of Cortex	In weeks	Abraham et al	Friede	John Raaf	Krishnaveni	Rakic and Sidman	Present Study
EGL	Appearance	-	-	12	13	11	-
	Peak (In growth)	28-34	24	28	-	21	24
	Disappearance	1 year	1 year	1 year	-	-	-
ML	Time of Differentiation	24	24	20	20	20	20
LD	Period of existence	24-31	28-32	-	-	20-31	22-31
PL	Time of Differentiation	24	24	-	17	16	25
GL	Time of Differentiation	24	30	20	20	20	29

5. Conclusions

The cells of external granular layer are highly proliferative and acts as stem cells. The Purkinje and cells of another layer are said to be derived from external granular layer. Sometimes the neurons in the external granular layer continue cell division longer than the other neurons in other parts of the brain. These cells were morphologically similar to cells of the medulloblastoma.

Conflict of interest

None

Author contributions

The authors in this article have made the following contribution: V. shanmugapriya: Conceptualization, specimen Collection, Literature research, Writing original draft; M. poongothai: Dissection of specimen, histotechniques; G. Paramesh Manuscript and Figure editing. All authors have read and approved of the final version of the article.

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