Journal of Morphological Sciences
J Morphol Sci
Brazilian Society of Anatomy

Original Article
J Morphol Sci 2023;40:101-104

A Study on Histogenesis of Human Fetal Cerebellar Cortex

Shanmugapriya V[1], Sivakami T[2], Sasikala P[3]

[1,3]Department of Anatomy, Swamy Vivekanandha Medical College Hospital and Research Institute, Namakkal-637205, Tamilnadu, India
[2]Department of Anatomy Thanjavur Medical College, Thanjavur -613004, Tamilnadu, India

Disclose and conflicts of interest: none to be declared by all authors

ABSTRACT

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.

Aim: The present study was conducted to study the histogenesis and histodifferentiation of external granular layer of human fetal cerebellar cortex.

Materials and Methods: 25 normal fresh fetuses 15 male and 10 female aborted fetuses of various gestational ages were collected. The specimens collected were ranging from 16-36 weeks of gestation and were divided into 6 age groups. The dissected brain specimens were preserved in 10% formalin, subjected to routine histological processing and hematoxylin and eosin staining. Thickness of layers of cerebellar cortex was measured using micrometry.

Results: The external granular layer started to appear in the first trimester, showed a gradual increase in thickness in the second trimester peak being at 24 weeks and started to decrease in the third trimester.

Conclusion: The knowledge of histogenesis can be applied to diagnose certain cerebellar tumors like medulloblastoma

Keywords: Cerebellar cortex; External granular layer; Fetus; Medulloblastoma.

Introduction

The cerebellum consists of an outer cortex of gray matter and an inner central core of white matter. There are three distinct cell layers in cerebellar cortex from superficial to deep. Outer molecular layer contains predominantly unmyelinated nerve fibers derived from the axons of granule cells, cell bodies and processes of stellate cells and basket cells, dendritic processes of purkinje cells along with blood vessels and other supporting cells. Middle Purkinje layer consists of a single layer of cell bodies of Purkinje cells placed perpendicular to the long axis of folium. Inner Granular layer consists of densely packed cell bodies of granule cells, Golgi cells and axons of purkinje cells[1,2]. In fetus, an additional layer called, external granular layer is also seen which is said to be the precursor of Purkinje cell layer and internal granular cell layer[3].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[4].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. Numerous studies have noted a similarity in the histological appearance of external granular layer and the cells appearing in medulloblastoma. So the persistence of external granular layer may be responsible for the formation of meduloblastoma[4].So, the aim of this study is to analyse the histogenesis of human fetal cerebellar cortex and to study the development of external granular layer.

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 were calculated from the obstetrical history and from the crown rump length. The fetuses were divided into different age groups according to their gestational age as mentioned in Table 1.

The fetuses were dissected and were subjected to routine histological processing. The sections were cut from the blocks at 5 - 7thick were stained with

Table 1. Division of fetuses into different age groups according to their 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

DOI: 10.51929/jms.40.101.2023
ISSN 2177-0298

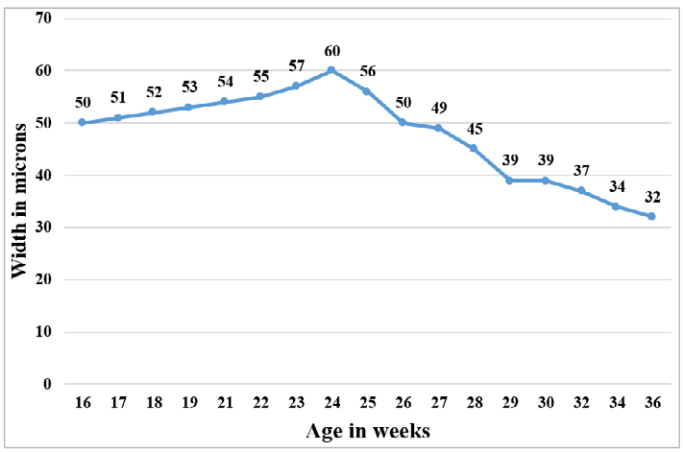
Hematoxylin and eosin. The stained slides were studied using binocular light microscope under 4x, 10x, 40x, 100x objectives and analyzed. The width of various layers of cerebellum was measured using micrometry.

Results

External Granular Layer

The width of external granular layer(in microns) at different gestational age groups were represented in figure 1.

Figure 1. Width of external granular layer in different age (chart).



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 as seen in figure 2 & 3. The cell sparse marginal layer is now referred as molecular layer well differentiated from the intermediate zone beneath it. The thickness of external granular layer at 16 weeks was 50. No folia could be observed.

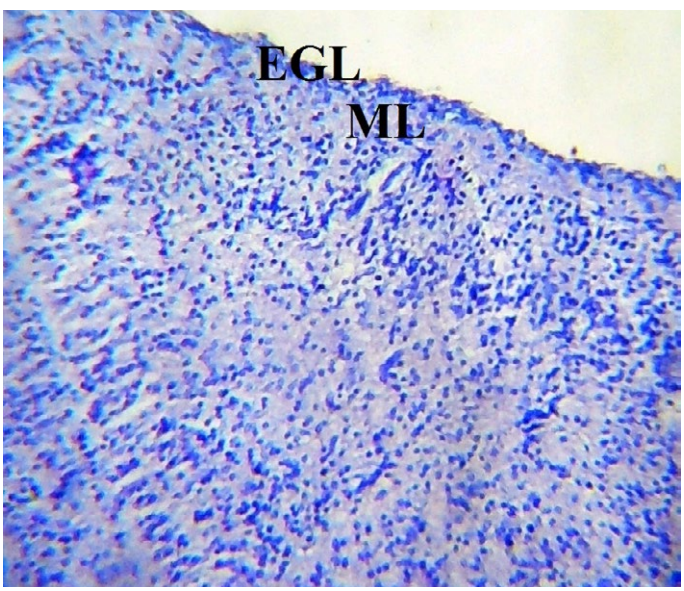


Figure 2. Cerebellum Transverse section Hematoxylin & Eosin 10x - 16 Weeks showing EGL - external granular layer, ML - molecular layer.

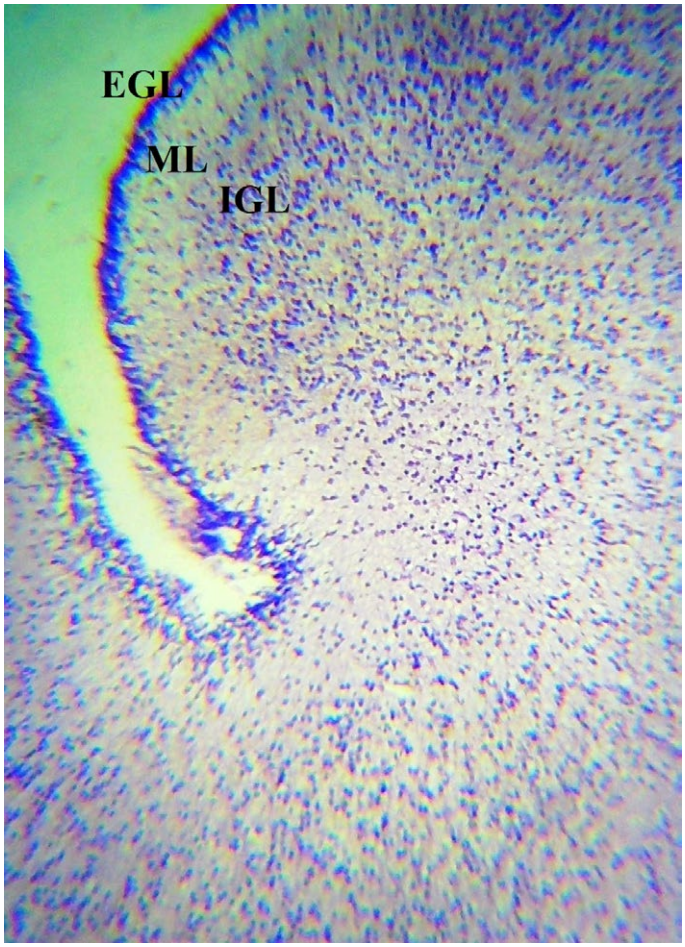


Figure 3. Cerebellum Transverse section Hematoxylin & Eosin 10x - 20 Weeks showing EGL - external granular layer, ML - molecular layer, IGL - internal granular layer.

Group II (21-24 weeks) the external granular layer contains six-nine rows of cells as seen in figure 4. The width of this layer was 54-60. The cells were spherical with a darkly staining nucleus completely filling the cell.

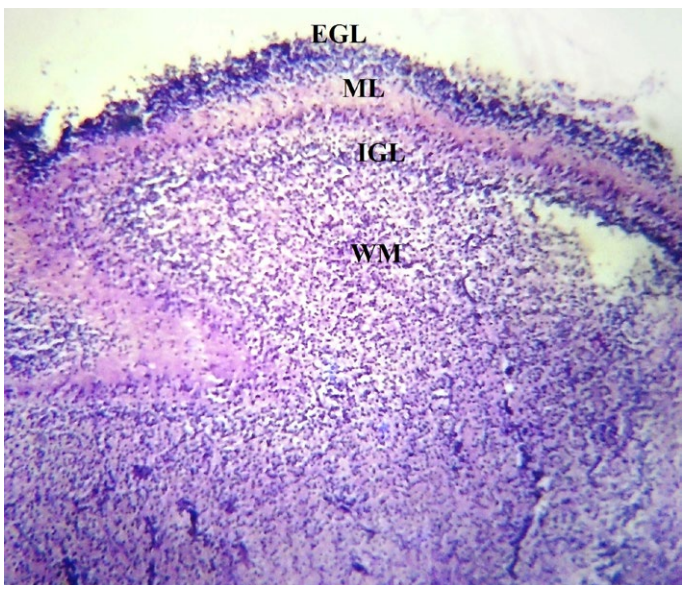


Figure 4. Cerebellum Transverse section Hematoxylin & Eosin 10x - 24 Weeks showing EGL - external granular layer, ML - molecular layer, IGL - internal granular layer, WM - white mater

Group III (25-28 weeks) by 25-26th week, the width was 50 consists of six-seven rows of cells. By 27-28th week, the thickness reduced to 45. Folia could be observed.

Group IV (29-32 weeks) Width of external granular layer was 39-37. The layer starts decreasing.

Group V(33 - 36 weeks) the width of external granular layer 34-32. The external granular layer layer was thinner than internal granular layer as seen in figure 5 & 6.

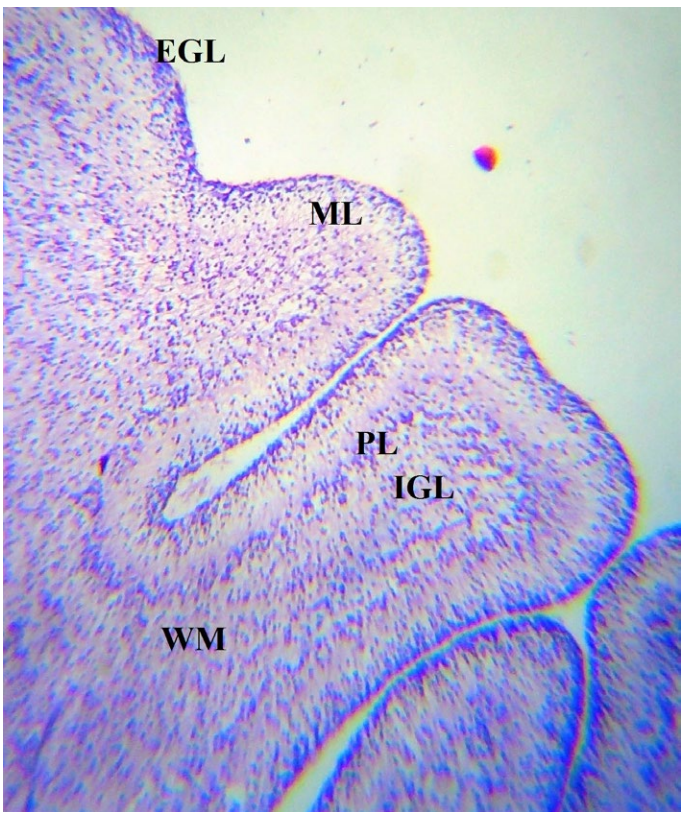


Figure 5. Cerebellum Transverse section Hematoxylin & Eosin 10x - 34 Weeks showing EGL - external granular layer, ML - molecular layer, PL - Purkinje layer, IGL- internal granular layer, WM - white mater.

Discussion

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 width of external granular layer was highest at 24-25 weeks of gestation after which it started decreasing, which coincides with Raaf[5].

Raff observed that from the fifth postnatal month, until term, the width of external granular layer decrease.

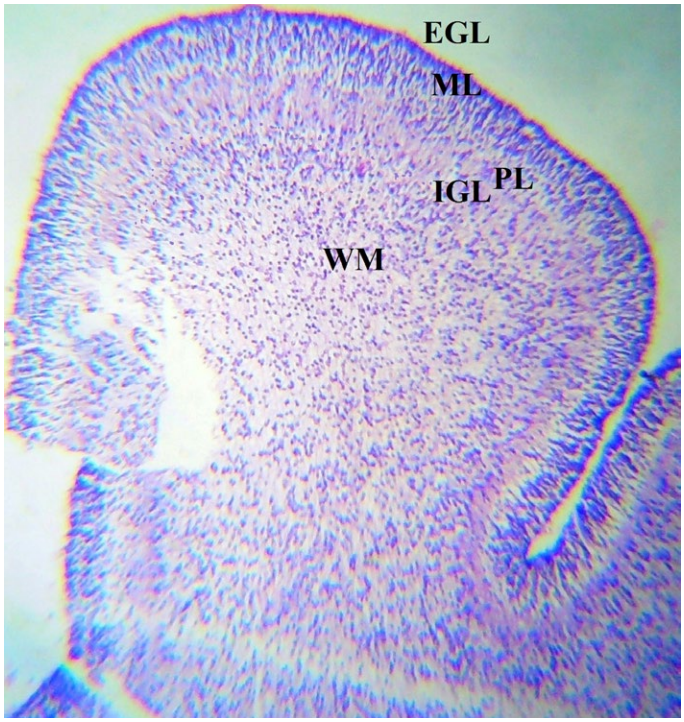


Figure 6. Cerebellum Transverse section Hematoxylin & Eosin 10x - 36 Weeks showing EGL - external granular layer, ML - molecular layer, PL - Purkinje layer, IGL - internal granular layer, WM- white mater.

Studies by Abraham *et al* observed the highest cell proliferation rate in external granular layer between 28th to 34th gestational weeks[6]. Friede observed that the external granular layer thickness is at peak at 24 weeks and remained constant till term[7].

The external granular layer was identified at 18 weeks according to studies by Narasinga Rao and Pramila[8].

Ashalatha *et al*, observed the external granular layer appears as a single layer at 16 weeks and bilayer at 20 weeks and tri-layered at 26 weeks of gestation. She also stated that external granular layer is the originator of the molecular, Purkinje and internal granular layers of the cerebellum. The EGL gradually decreases as the age of fetus increases and is completely lost after postnatal 9th month[9].

Divya C *et al*(2020) observed the presence of external granular layer in 1st trimester[10].

Krishnaveni *et al* observed the first appearance of external granular layer at 13 weeks as a thin layer and found to persist until 36 weeks[11].

According to Halder *et al* the external granular layer first appeared at 10-11 weeks and started to decrease in width by 24-28 weeks and disappeared postnataly[12]. Comparison among various studies were represented in Table 2.

Table 2. Study Comparison.

Authors		Abraham *et al*	Friede	John Raaf	Krishnaveni	Rakic and Sidman	Ashalatha	PresentStudy
EGL (In weeks)	Appearance	-	-	12	13	11	16	-
	Peak(In growth)	28-34	24	28	-	21		24
	Disappearance	1 year	1 year	1 year	-	-	1 year	-

Conclusion

The external granular layer could be observed at 16 weeks with another group of cells below this layer the molecular layer. The width of external granular layer was uniform throughout the cerebellum. The clinical implications of this study provide the basis for the knowledge of medulloblastoma. Histopathological findings of classical medulloblastoma contains densely packed cells with oval to round ,hyper chromatic nuclei, scanty cytoplasm with prominent mitotic activity resembling the cells of external granular layer, suggesting the tumor cell origin. The individuals in whom the persistence of external granular layer after 18-20 postnatal months suggest they are prone for medulloblastoma[13].

References

1. Standring S. Gray's Anatomy 40th Edition; UK: Churchill Livingstone/ Elsevier: 2008:297-310.
2. Victor P Eroschenko. DiFiore's Atlas of Histology with Functional corelations, 13th Edn. New Delhi India: Wolters Kluver. 2017. p.194-195.
3. Popoff S. Regarding the histogenesis of cerebellum. Biol. Zentralb, 1895; 15: 745-752.
4. Kershman J; The medulloblastoma, a study of human embryos. Arch Neurol and Psychiatry, 1938; 40: 937-967.
5. Raaf J, Kernohan JW. A study of the external granular layer in the cerebellum. The disappearance of the external granular layer and the growth of the molecular and internal granular layers in the cerebellum. American Journal of Anatomy. 1944; 75(2):151-72.
6. Abraham H, Tornóczky T, Kosztolányi G, Seress L. Cell formation in the cortical layers of the developing human cerebellum. International journal of developmental neuroscience. 2001; 19(1):53-62.
7. Friede RL. Dating the development of human cerebellum. Acta neuropathologica. 1973; 23(1):48-58.
8. Rao BN, Padmini MP. Prenatal morpho-histogenesis of human cerebellum. National Journal of Basic Medical Sciences.2007;56(1)249-277
9. Asha Latha D, Deena Usha K, Siva Prasad GV, Ravindra kishore, Lakshmi Kumari K. Histogenesis of Foetal Cerebellar Cortex. Journal of Dental and Medical Sciences. 2014 (3):23-2
10. Divya C, Gupta C, Tewari S, Kalthur SG, Palimar V, Gupta C, Tewari S, Kalthur SG, Palimar V. Histogenesis and Histomorphometric Study of Human Foetal Cerebellar Cortex. Online Journal of Health and Allied Sciences. 2020; 19(1):1-3.
11. Veni SK, Sugavasi R, Devi VS. Histogenesis of human foetal cerebellar cortex. Anatomy Journal of Africa.2015 4(2):598-603
12. Haldar A, Sahoo S, Chakraborty S, Banerjee P, Basu D. Organogenesis & morphogenesis of cerebellum in human fetuses at different weeks of gestation. Organogenesis. 2019 Mar; 4(2).
13. Millen KJ,Millonig JH,Wingate RJ,Alder J,Hatten ME.Neurogenetics of the cerebellar system. Journal of child neurology.1999; 14(9)574-81.

Mini Curriculum and Author's Contribution

1. Dr. T. Sivakami, M.S. - Profesor at Government Thanjavur Medical College, Thanjavur,Tamilnadu, India. Contribution: Conception, Design, Supervision, Writing, Critical Review and final approval. Contact: sivakamimeera@gmail.com

2. Dr.Sasikala.P, M.D. – Professor at Vivekanandha Medical College Hospital & RI, Elayampalayam, Tiruchengode, Namakkal. Contribution: Conception, Design, Supervision, Writing, Critical Review and final approval. Contact: kalamohan77@gmail.com

3. Dr. V.Shanmugapriya, M.D. – Assistant Professor at Swamy Vivekanandha Medical College Hospital And Research Institute, Tiruchengode, Namakkal. Contribution: Conception, Design, Supervision, Writing, Critical Review and final approval. Contact: dr.shanpriyaangel@gmail.com ORCID: 0000-0001-9412-5688

Received: November 11, 2022
Accepted: December 21, 2022

Corresponding author
Shanmugapriya V`
E-dr.shanpriyaangel@gmail.com