MAK HILL Publications International Journal of Tropical Medicine

Research Article
doi: 10.36478/makijtm.2025.1.24.29

OPEN ACCESS

Key Words
Cerebellum, histogenesis, purkinje cell, fetus

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Received: 10 December 2024
Accepted: 29 December 2024
Published: 31 December 2024

Citation: V. Shanmugapriya, T. Sivakami and G. Subbulakshmi, 2025. Developmental Origin of Purkinje Cells in Human Fetal Cerebellum: A Descriptive Study on Cerebellar Histogenesis. Int. J. Trop. Med., 20: 24-29, doi: 10.36478/makijtm.2025.1.24.29

Developmental Origin of Purkinje Cells in Human Fetal Cerebellum: A Descriptive Study on Cerebellar Histogenesis

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ABSTRACT
The study aims to provide a detailed description of how the cerebellar cortex develops and matures during fetal life mainly the Purkinje cells. The age range of the fetuses was ranging from 16-36 weeks of Gestation. The objective of this study was to provide a detailed description of the histogenesis of the human cerebellar cortex during prenatal development. The fetuses were categorized into five age groups based on gestation and then processed histologically using 10% formalin fixation. Around 25 weeks, Purkinje cells began to differentiate and by 32-34 weeks, they formed a single row with characteristic morphology. A comprehensive understanding of cerebellar anatomy and Purkinje cell histogenesis is crucial for elucidating the complex mechanisms underlying various neurological and developmental disorders, including, Cerebellar ataxia.

INTRODUCTION

The cerbellum is one of the complex brain's structures, undergoing a prolonged developmental period. Although it's the first structure to differentiate, its maturation takes longer[1].

In Adults, the Cortex of Cerebellum is of Three Distinct Layers:

- Outer most molecular layer.
- Middle Purkinje Cell Layer.
- Innermost Granular layer.

During Cerebellar Development, Two Additional Layers form But Eventually Disappear:

- External Granular Layer.
- Lamina Dissecans.

These transient layers play important role in the formation of the mature cortex of cerebellum[2]. "Purkinje cells are a unique type of neuron-specific to the cerebellar cortex. They are remarkable (and instantly recognizable) for their massive, intricately branched, flat dendritic trees, which give them the ability to integrate large amounts of information and learn by remodeling their dendrites. As an important part of the cerebellar circuits, Purkinje cells are necessary for well-coordinated movement and other areas of function, such as cognition and emotion[3]." Purkinje cell developmental defects in the fetus can lead to various neurological and developmental disorders, including cerebellar ataxia, characterized by impaired motor coordination, balance and posture. Early diagnosis and intervention significantly improve outcomes for individuals with Purkinje cell developmental defects[4].

MATERIALS AND METHODS

This study is a descriptive type of observational study.

Study Cohort: This study utilized 25 human fetuses (15 males, 10 females), belonging to gestational age groups from 16-36 weeks, collected from the Department of Obstetrics and Gynecology. The age of the fetuses were estimated using the obstetrical history and from measuring the crown rump length. The fetuses were categorized into five groups according to their gestational age.

Sample Size Calculation: Study was conducted over a period of six months in a tertiary care hospital, (Department of Obstetrics and Gynecology), where the population size would be around 266(delivery outcomes)/six months. Applying YAMANE sample size formula with population size =266, with marginal error of 5%. we arrived at a sample size of 25.

Collection and Selection Criteria: The fetuses are results of terminated pregnancies under the Medical Termination of Pregnancy (MTP) Act of India, 1971.

Exclusion Criteria: Fetuses with gross anatomical abnormalities .Approval from the Ethical committee clearance and informed consent were secured prior to the collection and utilization of fetal tissue samples specimens. The fetuses were dissected immediately and fixation was done with 10% formalin for 15 days (Fig. 1). The fetuses were subjected to routine histological processing. The sections were cut from the blocks at 5-7 thick were stained with Hematoxylin and eosin. The stained slides were, then analyzed using a binocular light microscope equipped with multiple objective lenses (4x, 10x, 40x,100x)[5].

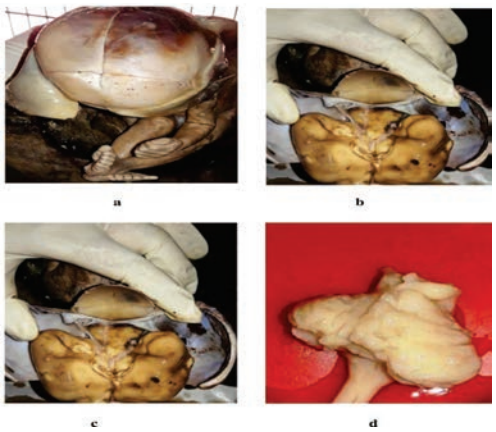

Fig. 1: Dissection of Specimen Weeks

RESULTS AND DISCUSSIONS

The period of appearance of Purkinje cell layer and its morphological features were observed and analyzed. The earlier the age of the fetuses, the Purkinje layers cannot be made out, as the cytoplasm was poorly formed. The Purkinje cells can be identified only after 24 weeks between lamina dissecans and molecular layer. The cells of this layer are arranged in different rows as tightly packed cells with an oval cell body and a dark staining nucleus. Lamina dissecans is an acellular layer between Purkinje cell layer and inner granular layer. The presence of this layer distinguishes the Purkinje cell layer from internal granular layer. This layer starts to decrease very soon and later disappears in older fetuses. The significance of this layer is unknown. By this time 25-28 weeks white matter could be distinguished. (fig 2-fig 4).

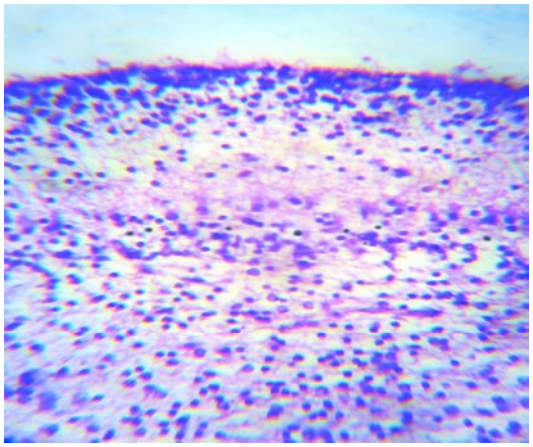

Fig. 2: Transverse Section of Cerebellum, Hematoxylin and Eosin 100x-16 Weeks Showing EGL-External Granular Layer, ML-Molecular Layer

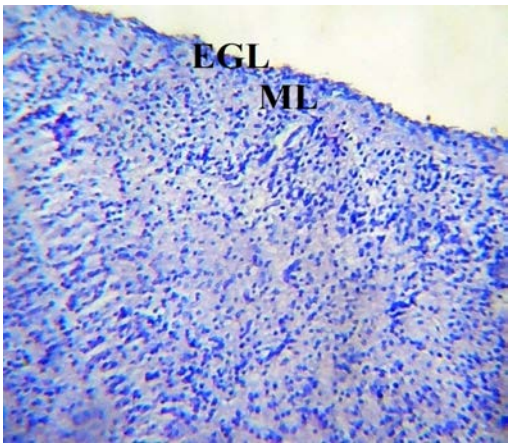

Fig. 3: Transverse Section of Cerebellum, Hematoxylin and Eosin 100x-20 Weeks Showing EGL-External Granular Layer, ML-Molecular Layer, IGL-Internal Granular Layer

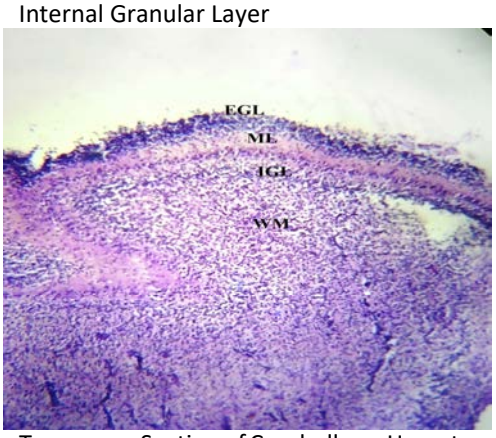

Fig. 4: Transverse Section of Cerebellum, Hematoxylin and Eosin 100x-24 Weeks Showing EGL-External Granular Layer, ML-Molecular Layer, LD-Lamina Dissecans, PL-Purkinje Layer, IGL-Internal Granular Layer ,WM-White Matter

In group IV (29-32 weeks) the Purkinje cell dendrites were observed and cell nucleus could be observed.(fig 5 and chart 1).

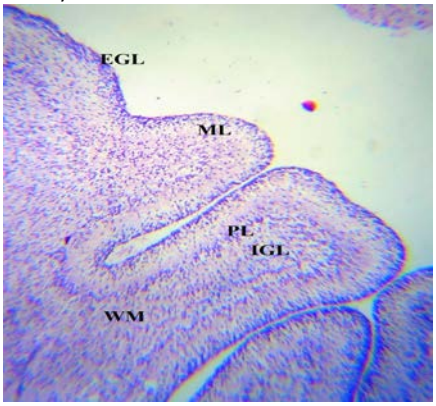

Fig. 5: Transverse Section of Cerebellum ,Hematoxylin and Eosin 100x-34 Weeks Showing EGL-External Granular Layer, ML-Molecular Layer. PL-Purkinje Layer, IGL-Internal Granular Layer, WM-White Matter

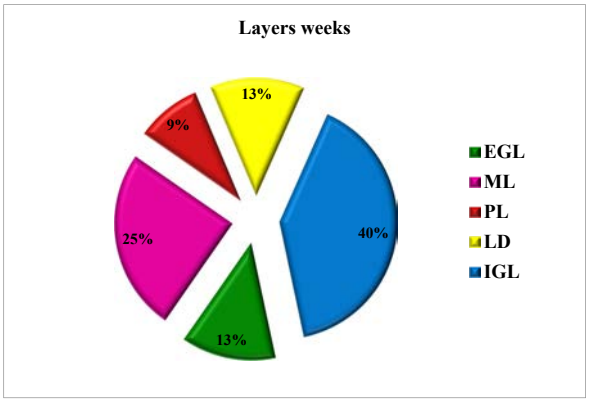

Chart 1: Percentage of Thickness of Various Layers of Cerebellar Cortex at 29 Weeks

By 34-36 weeks, the Purkinje cell becomes more differentiated, they were characterized by distinct cell processes, they were arranged in 2-3 rows and in widespread spacing.(fig 6-fig 8).

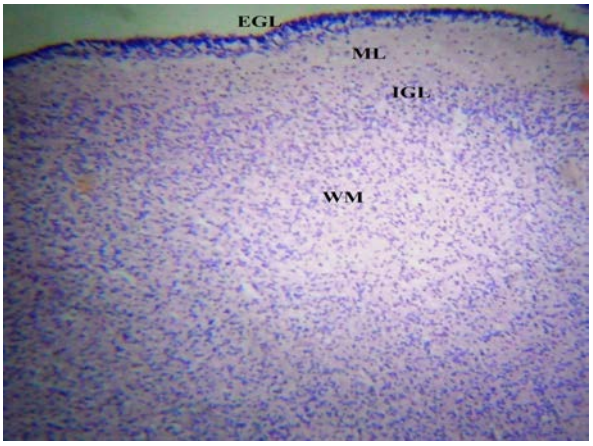

Fig. 6: Transverse Section of Cerebellum ,Hematoxylin and Eosin 100x-36 Weeks Showing EGL-External Granular Layer, ML-Molecular Layer. PL-Purkinje Layer, IGL-Internal Granular Layer, WM-White Matter

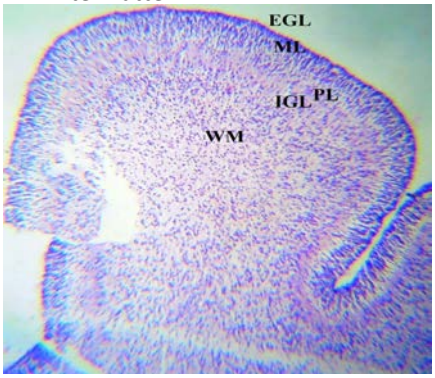

Fig. 7: Transverse Section of Cerebellum,Hematoxylin and Eosin 400x-36 Weeks Showing EGL-External Granular Layer, ML-Molecular Layer, PL -Purkinje Layer, IGL-Internal Granular Layer, WM-White Matter

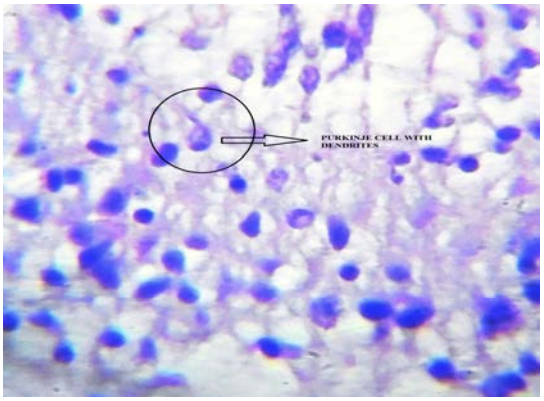

Fig. 8: Transverse Section of Cerebellum ,Hematoxylin and Eosin 1000x-36 Weeks Showing EGL-External Granular Layer, ML-Molecular Layer, PL-Purkinje Layer, IGL-Internal Granular Layer, WM-White Matter

The number of cells seems to be decreased. The Purkinje cells have now become elongated when compared to the rest of the cells. At certain sites these cells demonstrated well developed branching dendrites like a mature Purkinje cell.(Table 2 and 3).
The external granular layer is a distinguishing aspect of the developing cerebellum. It can be observed until approximately one year of age. It is described as a uniformly organized thin layer of germinal cells[6]. The lamina dissecans is a clear acellular layer found between Purkinje cell layer and inner granular layer. The lamina dissecans is a very unique feature of developing human fetal cerebellum. This layer can be first identifiable at 21-22 weeks of intrauterine life. Some authors have identified this layer in newborn whales also[7].

This Study Centers on the Purkinje Cell Layer: This layer contains highly differentiated Purkinje neurons. These neurons are very peculiar as they have huge dendritic tree. The dendritic tree is two dimensional and are arranged parallel to each other like pages of a book. The dendrites are arranged perpendicular to the folia. The cell bodies are flask shaped. They are arranged in a single layer in the deepest part of molecular layer. Each Purkinje cell is widely spaced[8]. The two large primary dendrites extend from the neck of flask shaped cell bodies .they travel superficially for some distance and then they give secondary dendrites. These dendrites appear smooth without spines. The third order will arise from second and are characterized by short, thick dendritic spines on their surfaces. The axons coming from the base of the cell body is navigating to the white matter, where they will synapse with the deep cerebellar nuclei of the brainstem.

Connection of Purkinje Cells:

- Parallel fibre bundles of granule cell.
- Climbing fibres from olivocerebellar system. One climbing fibre is restricted to single Purkinje cell on primary dendrites.
- With synaptic terminals from outer stellate cells.
- Synaptic contacts from the basket cells[9].

The cerebellum during its development is organized into three embryonic layers, near the fourth ventricle. From inner to outer, they are ventricular, mantle and marginal zones. The ventricular layer contains many neural progenitor cells that proliferates and differentiates to form various cerebellar cell types. By the third month of embryonic life, proliferating cells appear above the ventricular zone. One such group of cells gets differentiated and migrate towards the surface. This layer is identified as external granular layer. The cells of this layer are having proliferating capacity and give rise to granule cells. The granule cells will then migrate down to form the future inner granular layer. The other cells of cerebellum namely, stellate, basket, Golgi, Purkinje cells and deep cerebellar nuclei all said to be originated from ventricular layer. All of these cells will eventually reach their future positions. The formation of Purkinje cells are said to be as the same time as external granular layer, but remain undifferentiated[10]. In the current study, Purkinje cells can be differentiated only at 25 weeks, after the formation of lamina dissecans. These cells appear as closely packed oval shaped cell bodies with dark staining nuclei. By 32-34 weeks, the Purkinje cells were arranged in a single row and had their characteristic appearance. (table 2 and 3). In a study by krishna veni *et al,* the authors observed that Purkinje cells appeared at 17 weeks onwards with multilayered arrangement, starting to organize into a single layer by 30 weeks[11]. The cells achieved clear organization by 36 weeks. Studies by friede and abraham *et al.* noted the presence of Purkinje cell bodies at 32 weeks of gestation, the same time lamina dissecans began to disappear[12,13]. According to ashalatha *et al.* Purkinje cells width increases with age and only reaches adult size by ninth postnatal month[14]. Halder *et al* noted the development of Purkinje cell layer in three stages. During the first stage(12-16 weeks), the Purkinje cells differentiation and its connection to other components within cerebellum were detected. The second stage lasts through 16-28 weeks. The third stage of developments lasts for the last few weeks of fetal life and continues till the first postnatal year[15]. Study by

Table 1: Gestational Age Grouping of Fetuses

Groups	Group I	Group II	Group III	Group IV	Group V
AGE (WEEKS)	16-20	21-24	25-28	29-32	33-36
No of Fetuses	5	7	5	6	2

Table 2: Appearance of Purkinje Cells in Various Age Groups

Groups	I	II	III	IV	V
Purkinje layer	Unidentifiable	Undifferentiated	Purkinje cells-clearly defined-Randomly arranged	Well differentiated cells-Dendrites visible	Arranged in a single row

Table 3: Percentage of Presence of Purkinje Cells in Different Age Groups

Groups	Presence of Purkinje cells in different age groups
II	14%
III	80%
IV	100%
V	100%

Table 4: Time of Appearance of Purkinje Cells by Various Studies

Layer Observed	Studies By other authors	Abraham and associates	Friede and associates	Krishnaveni and associates	Rakic and associates	Present study
PL	The time of differentiation (in weeks)	24	24	17	16	25

divya *et al.* Observed the appearance of Purkinje cells began to appear by the fourth month of fetal life[16]. (table 4).

CONCLUSION

In conclusion. This study investigated the development and migration of Purkinje cells, shedding light on their critical role in shaping cerebellar circuitry and motor behavior. Our findings underscore the importance of understanding Purkinje cell biology, particularly their differential development and connectivity features. By exploring the complex processes governing Purkinje cell development, this study contributes to the ongoing quest for improved diagnostic and therapeutic strategies for neurological conditions, such as ataxia, autism and spinocerebellar ataxia[17].

Conflict of Interest: none.

ACKNOWLEDGMENTS

we would like to thank the anonymous donors who contributed specimens for this study. Their generosity and willingness to support this scientific research are greatly appreciated.

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