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Organogenesis & morphogenesis of cerebellum in human foetuses at different weeks of gestation

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Abstract
The aim of present study is to describe the prenatal histogenesis of human cerebellar cortex of various weeks of gestation. This study was done to demonstrate the various histological features of foetal cerebellum in aborted human foetuses of different gestational ages received by spontaneous miscarriages and therapeutic abortions for analysing the cerebellar histology. The dissected specimens were preserved in 10%formalin and subjected to routine histological procedure for age related histogenesis and developmental anatomy. Histogenesis of cerebellum is observed from 10 weeks to 30 weeks of gestational age by dividing the specimens into six gestational 3 age groups (group A to group C). External granular layer is observed at 13-16 weeks of gestation and Purkinje cell layer is arranged at 20 weeks as a multi-layered and single layer at 26 weeks of gestation. The knowledge of cerebellar anatomy has a tremendous role in neurosurgical importance in surgeries of posterior fossa neoplasm mainly Medulloblastoma. Future investigations might involve evaluation of the cerebellum at other gestational ages.

Keywords: cerebellum, histogenesis, purkinje cell layer, external granular layer, medulloblastoma

Introduction
Cerebellum in the brain begins first to differentiate but last to mature since its development is spread over a longer period and shows age related changes (Standring *et al*, 2008) [1]. Cerebellar cortex is divided into 3 distinct layers as seen in adults- outer molecular layer, middle Purkinje cell layer and inner granular layer. At third embryonic month External granular layer appears first on the surface of the cerebellum as a dense layer of cells. External granular was precisely described by Obersteiner who observed that the cells in the outer layer form the basal membrane and cells of the inner layer enter the molecular layer and migrate through this into the inner granular layer **[2]**. External granular layer consists of indifferent cells that may convert into either nerve cells or glial cells, (Schaper 1894) [3]. External granular layer is the precursor of Purkinje cell and internal granular cell layer, (Popoff 1895) [4].

Embryological Basis: In the embryonic life the sub ventricular zone forms at the edge of the rhombic lip. Its cells then migrate over the outermost surface of the entire cerebellum multiplying as they go, to form a transient embryonic structure containing incredibly numerous tiny cells. This layer is known as external granular layer. of the developing brainstem cells. When EGL cells undergo their final mitosis, they grow their axons and translocate their cell bodies in different cell directions. These cells grow parallel to one another and this layer is superficial to the Purkinje cell bodies which are already in place and this layer is superficial to Purkinje cell bodies. This layer constitutes of parallel fibres of granule cells and becomes molecular layer. The granule cells migrate from EGL inwards by passing the Purkinje cell bodies which are already in place and this leads to formation of IGL. They use the pre-existing radial Glial fibres as guide wires for migration. [5].

Aims & objectives
Study was done to correlate the Chronological Pattern of age related histogenesis and developmental anatomy of Cerebellum development in this geographical eastern region of India & compare the results from other researchers nationwide & worldwide.

Materials & Methods
This study was done to correlate the chronological pattern of cerebellum development in this geographical eastern region of India, Odisha& compare the results from other researchers nationwide & worldwide. This is a hospital based, observational, cross sectional study conducted at Hi- Tech Medical Colleges & Hospital, Bhubaneswar, India by the Department of Anatomy in collaboration with Department of Obstetrics & Gynaecology from November 2011 to June 2013 on thirty-two aborted human foetuses without obvious congenital anomaly of gestational age between 12 weeks and 36 weeks collected within 6 hours of delivery by spontaneous miscarriages & therapeutic legal abortions. Study samples were arbitrarily divided into groups of biweekly gestational age by duration of amenorrhoea from medical records & ultrasound fetometry after receipt of informed consent from mother and legal guardians. Fetuses were immediately fixed in 10% Formalin for 1-2 hrs. Spleen was dissected by Dissecting Microscope, fixed in 10% Formalin for 48-72 hrs. After fixation by formalin, the tissues were transferred to 30%, 50%, 70%, 90% and Absolute alcohol each for 30 minutes. This ascending grading of the dehydrating fluid was done because when alcohol mixes with water, it produces diffusing current which can damage the tissues. Then the tissues were put in xylol for 24 hours to clear the residual alcohol. These tissues were processed for paraffin sections by tissue blocking (Paraffin Embedding). 3 pots of hard paraffin

were taken; paraffin was melted in the incubator at 56 degrees, as hard paraffin is ideal for materials which are to be cut in thin sections about 12 mu. The tissue was put in the first pot containing equal parts of paraffin and xylol and then changed to second and third pots containing only fresh melted paraffin at 90 minutes interval. Then the tissues were mounted in fresh melted paraffin with L-Block. The L-Block was then trimmed to a rectangular shape. Then the L-Block was fixed with the block holder (choke) and the block holder was clamped in the rotary microtome. 5 mu sections were cut in rotary microtome. The microtome was revolved at 40 per min and ribbon was formed. Then the ribbon was put in tissue flotation bath. Albuminised slide was then made by putting a drop of Mayor's albumin (equal parts of glycerine and egg white) and spreading it uniformly by rubbing with finger. The piece of ribbon was then taken on the slide and dried at room temperature. The slide was then put in the warming table. When the paraffin melted the slide was put into xylol for 2-3 minutes because xylol removes paraffin. Then the tissue was put in decreasing grades of alcohol (Absolute alcohol, 90%, 70%, 50% and 30%) then was put in the prepared Harris Alum Haematoxylin (nuclear) stain for 7 minutes and lastly washed with distilled water. 2-3 drops of 1% acid alcohol (1cc Hcl in 75% alcohol) was added to remove the excess stain beyond the nucleus. The slide was then put in running tap water for 30 minutes to develop haematoxylin colour (bluish). Then the slides were again dipped in ascending grades of alcohol (30%, 50%, and 70%) and then put in eosin Y (cytoplasmic) stain for 30 seconds. Then the slide was washed with absolute alcohol for a few seconds so that excess of eosin was removed and lastly the slide was placed in xylol. The slide was then taken out from xylol and then put in 1-2 drops of DPX (Adhesive agent) and a cover slip was put on it and pressed slightly so that air bubbles were removed. Sections were then seen in light microscope under low power 10X followed by high power 40X magnification. Thereafter photomicrographs were taken by camera using microscope adapter.

Table 1

Crown-Rump Length & Crown-Heel Length

WEEKS	CRL (in cm)	WEEKS	CHL (in cm)
10	3.1	22	27.8
12	5.4	24	30.0
14	8.7	26	35.6
16	11.6	28	37.6
18	14.2	30	39.9
20	16.4	32	42.4
		34	45.0
		36	47.4
		38	49.8

Observations

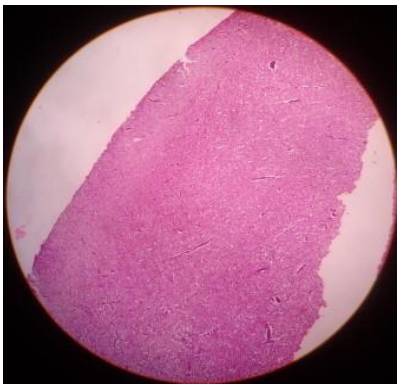

Fig 1

In 10-12 Weeks Fetus, peripheral dark zone is visible and most probably it is the external granular layer of marginal zone.

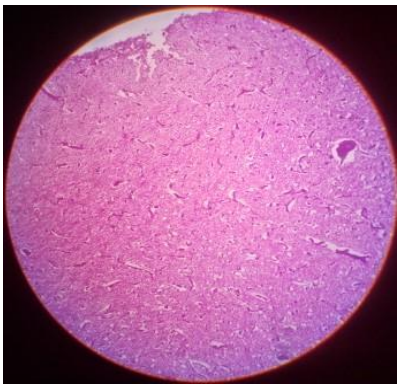

Fig 2

In 12-16 Weeks Fetus, Mantle and marginal zones could be differentiated and external granular layer is present. Thin external granular layer in the superficial part of marginal zone and thick internal granular layers in its deeper part were observed.

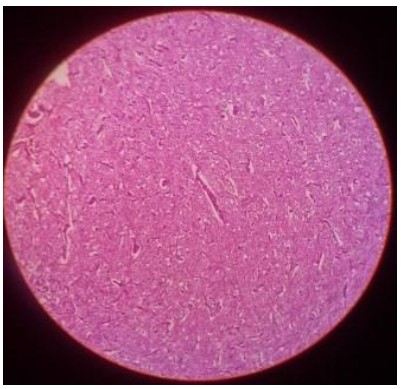

Fig 3

In 16-20 Weeks Fetus, Randomly arranged, rounded or oval shaped Purkinje cells are present in the internal granular layer with their small nucleus.

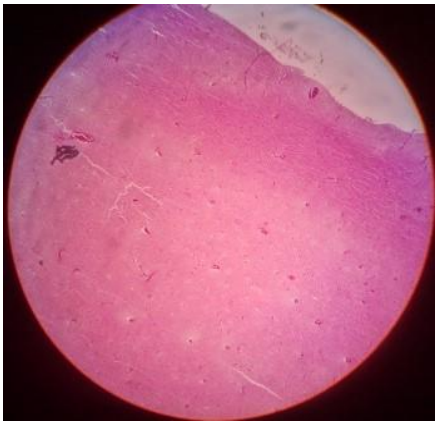

Fig 4

In 20-24 Weeks Fetus, darkly stained external granular and lightly stained internal granular layers are clearly demarcated.

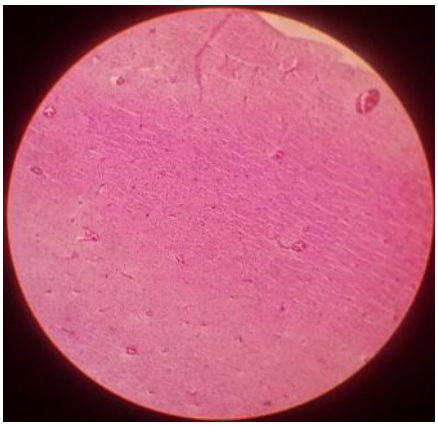

Fig 5

In 24-28 Weeks Fetus, Internal granular layer is thicker than the external granular layer.

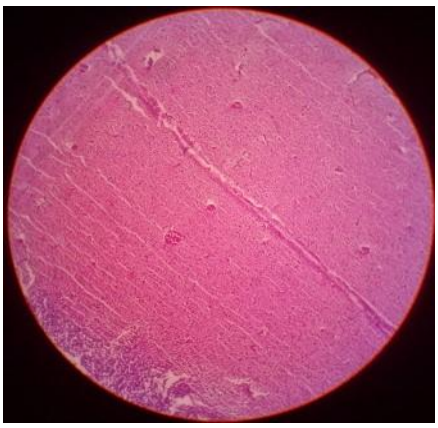

Fig 6

In 28-32 Weeks Fetus, increase in the number of Purkinje cells and their processes were clearly identified. Purkinje cell nucleus could be observed.

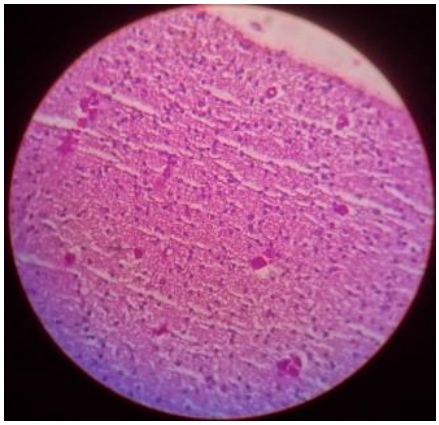

Fig 7

In 32-36 Weeks Fetus, Pear shaped Purkinje cells are arranged in single row and nucleus of the cell is vesicular.

- Up to about 10 weeks of gestation, cells proliferated only at or near the ventricular surface and migrated radially outward to occupy the full thickness of the cerebellar primordium except or an outermost cell-sparse marginal layer (2-layer stage).
- The external granular layer first appeared at 10-11 weeks while another group of cells became concentrated beneath the marginal layer (3-layer stage).
- At 20–21 weeks the lamina dissecans first became evident as a relatively acellular band in the midst of the zone of compact cells below the marginal (now molecular) layer, and for the next ten weeks the cerebellar cortex displayed this 5-layered form.
- At about 32 weeks the lamina dissecans disappeared (4-layer stage) and postnatally the external granular layer in turn disappeared as the last of its cells migrated inward (adult 3-layer configuration).
- The Purkinje cell population was established by 13 weeks, though the cerebellum was destined subsequently to increase several orders of magnitude in surface area and volume. The increase was achieved in part by cell growth, but mainly by extensive cell proliferation in the external granular layer.
- At 22 weeks, about 30% of the external granular cells incorporated thymidine-H3 upon a single supravital exposure; the external granular layer attained maximum cell number at some stage after birth.
- At the 5-layer stage from about 21 to 32 weeks, the interrelationships between various classes of young neurons in the cerebellar cortex became very complex. The Purkinje cells developed ascending branched dendritic processes with growth cones and displayed transient short cytoplasmic processes that extended from the soma in all derections. Basket cell neurons had formed but their axons appeared not to envelop the Purkinje somas as yet. Less mature, smaller cells were beginning to migrate from the external granular layer inward past the Purkinje somas. Their cell bodies in the newly forming granular were separated from the Purkinje cell bodies by a dense tangle of axons in the lamina dissecans. Many of

these axons terminated in swellings interpreted tentatively as immature mossy endings, while others passed outward to enclose the cell bodies and proximal dendrites of the Purkinje cells.

Discussion

The differentiation of Purkinje cells and their relationship to other components of the developing cerebellar cortex can be analysed by the Golgi impregnation method and by electron microscopy in human specimens of various pre- and postnatal ages. During the first stage, Purkinje cells are distributed in a layer, several rows deep. Their bipolar somas are relatively smooth and have only a few processes at the apical and basal cell poles. In the 3-month period of the second stage, Purkinje cells become gradually organized into a single row (Nada Zecevic *et al*, 2004).

According to (Pasko Rakic *et al*, 1970) external granular layer was first appeared at 10-11 weeks, Purkinje cell population was established in cerebellar cortex by 13 weeks and proved that lamina dissecans was more evident at 20-21 weeks [6]. According to (Zecevic and Rakic, 1976) Purkinje neurons were small and several rows deep between 12 and 16 weeks and they become ordered into a single row, enlarge, and develop increasing complex dendritic branches and synapses between 16–28 weeks of gestation [7].

(D. Asha Latha *et al*. 2014) observed the external granular layer as a single layer at 16 weeks and became 2 layered at 20 weeks and 3 layered at 26 weeks of gestation. External Granular layer is the precursor of the molecular, Purkinje and internal granular layers of the cerebellum [8]. According to (Yamaguchi *et al*. 1992) internal granular layer of cerebellar cortex is developed by 3 stages [9].

1. **The primary or undifferentiated stage (before 18 weeks of gestation):** In this stage the internal granular layer was hardly distinguishable from layer of immature Purkinje cell.
2. **The secondary or intermediate stage (from 18weeks of gestation to 35 weeks of gestation):** In this stage the internal granular layer was clearly visible and almost stable in thickness in all parts.
3. **The tertiary or developing stage (35 to 40weeks of gestation):** In this stage the internal granular layer showed dramatic increase in thickness as the formation of cerebellar folia proceeds.

The three stages of Purkinje cell maturation that have been previously recognized in other species are also evident in man [10]:

1. The first stage occupies primarily the fourth fetal month. During this period (12–16 weeks) differentiation of Purkinje cells and their relationship to other components is observed.
2. The second stage lasts through the fifth, sixth and seventh fetal months (16–28 weeks).
3. The third stage extends throughout the remaining period of intrauterine life and the first postnatal year and continues at a slow rate thereafter.

Conclusion

Sometimes the neurons in the external granular layer continue cell division longer than the other neurons in other parts of the brain. This leads to the tumour development called Medulloblastoma [11]. This study will help in neurosurgical surgeries with Gamma Knife and advanced research in the field of neurosciences.

Acknowledgement

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References

1. Standring S, Harold E, Jeremiah CH. Gray's Anatomy, Spain. 40th edition. Churchill Livingstone, 2008, 375-379, 297- 309.
2. Obersteiner H. Der feinese Bau der Kleinhirnridebein Menschen und bei Tieren. Biol Zentralb. 1883; 3:145-155.
3. Schaper-Alfred. Die morphologische and histologische Entwicklung des kleinhirns der Teleostier. AnatAnz. 1894; 9:489-501.
4. Popoff S, Zur Frageuber. Die histogenese der Kleinhirnrinde. Biol. Zentralb. 1895; 15:745-752.
5. Saddler TW. Langman's Medical Embryology 8th edpp, 2000, 430.
6. Pasko Rakic, Richard Sidman. Histogenesis of cortical layers in human cerebellum. Perticularly the lamina Dissecans. Journal of comparative neurology. 1970; 139:473-500.
7. Zecevic N, Rakic P. Differentiation of Purkinje cells and their relationship to other components of developing cerebellar cortex in man. J Comp Neurol. 1976; 167:27-47.
8. 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; 3:23-25.
9. Yamaguchi K., Goto N, Nara T. Development of human cerebellar granular layer: a morphometric study. No To Hattal Su. 1992; 24: 327-334.
10. Ariens kappers, Huber CG, Crosby E. The comparative anatomy of the nervous system of vertebrates, including man Vol: Mac Millan Co, New York. 1936; 696-700:775-790.
11. Altman A, Bayer SA. Time of origin and distribution of new cell type in the cerebellar cortex of rats. Exp Brain Res. 1977; 29:265- 274.