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Prenatal Cerebellar Ontogenesis

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Abstract: ***Although the ontogeny of the cerebellum has been studied with cytological techniques for more than two centuries, it is still of extended clinical importance in the basic neuroscience as the explanation of the mechanisms driving cerebellar neurogenesis is relevant to clinical neuroscience. This study is an attempt to perform a thorough morphological investigation of the cerebellum development during the prenatal period. The rat was the animal model of this work and the embryonic period covered in it,was from the embryonic day 15 till birth. We used the E-designation system to determine the relevant developmental stages of (62) rat embryos. For the general histological study, serial sections of paraffin blocks of the embryonic tissues were stained with H&E stain and for the neurohistological study, they were stained with Nissl and Bielschowsky's Silver stains. The results of the present study revealed that major morphogenetic events take place during prenatal cerebellar ontogenesis including the formation of the Rhombencephalic demilune, external granular layer, migration and settlement of the Purkinje cells, primitive cerebellar cortex and 1st stage of the foliation. This work concluded that hallmark of the major morphogenetic events that take place during prenatal cerebellar ontogenesis is the migration of Purkinje and granular cell precursors.***

Keywords: Rhombencephalic demilune, primitive cerebellar cortex, granular cellprecursors, Purkinje cells.

1. Introduction

The ontogeny of the cerebellum has been studied with cytological technique for about 200 years. Contributions made to the subject in the 19th century by Cajal and Retzius[1]. It was generally accepted by the turn of the century that the cerebellum originates from bilateral eminences in the metencephalon, the cerebellar plates and it was assumed that the isthmus, neural tissue at metencephalic- metencephalic boundary has an inductive activity for both midbrain and cerebellum [2, 3]. Although, the cerebellum accounts about 10% of the total volume of the brain, its neurons represent about 80% of the total number of the neurons of the brain[4]. Of them, the Purkinje and granule cells, are essential for the normal development and functioning of the cerebellum. Purkinje cells, inhibitory gamma- butyric acid (GABAergic) output neurons, are the key neurons of the cerebellar cortex which are responsible for the proliferation of granule cells and the establishment of crude projection maps with extracerebellar afferent fibers. The granule cells, are the smallest and most abundant glutamatergic interneurons in the body[4]. In the same context, it was established that the cerebellar neurons are produced sequentially, the output neurons being first and the interneurons last during late embryonic and early postnatal life. Neurons that are born before birth,are generated from two germinal zones in the cerebellar primordium that are the ventricular neuroepithelium zone (VZ) and rhombic lip (RL) [5, 6]. At late embryonic age, such germinative zones disappear and the progenitor cells emigrate either into the cerebellar white matter or along the pial cerebellar surface, where RL-derived progenitors form a secondary germinal zone, the external granular layer (EGL). The picture that emerged during prenatal period was that the Purkinje cells migrate from the region of the 4th ventricle outward in a course perpendicular to the surface using Bergmann glial fibers as scaffold(radial migration) and granular cells progenitors follow a migration path parallel to the surface away from the RL(tangential migration)[7].

2. Materials and Methods

Postcopulatory age of the experimental animals:
This work wascarried out on thirty albino mature female rats (Rattusrattusnorvegicusalbinus) collected from the animal house in the AL-Nahrain university at 2016 AD. Females weighing 300 ± 30 g were housed individually in breeding cages each with one male in a controlled room temperature (25±2o°C) at 12 hr light/dark cycle (lights on 7.00 am).They were provided with water and food ad libitum. The females were daily examined early in the next morning between 8:00-9:00 am. In this work, the day on which vaginal plug and/or spermatozoa in a smear of the vaginal contents were observed in situ, was regarded as an indication for copulation and was considered as the gestational day zero. The female was then transferred to anisolated cage and chronologically labeled for day post coitum (dpc) which is a day that follows the day of copulatory plug observation.

Retrieved of Embryos and Samples preparation:
At the appropriate post- copulatory age, the pregnant females were sacrificed by deep anesthesia with chloroform. The abdomen was opened and the two cornua of the uterus were dissected out. The gestational sacs were incised and the embryos were extracted from the gestational sacs, rinsed in normal saline and examined under the dissecting microscope. It was recommended that measurements of the Crown-Rump Length (CRL) of embryos to be made after two weeks of fixation[8]. Therefore, at least two embryos for each particular embryonic age were transferred to 10% neutral buffered formalin for this purpose. Heads of the 15, 16 & 17 days old embryos (E15-17) were decapitated and were fixed in 10% neutral buffered formalin for 36 hours. While heads of 18 days old and more(E18-21)were first decapitated and were fixed in 10% neutral buffered formalin

for 24 hours, then under dissecting microscope, the calvaria was removed, the hindbrain was mobilized and delivered out by transecting the tectum of the mesencephalon. Only the hindbrain was immersed in the 10% neutral buffered formalin for 24 hours.

Developmental staging:
Several developmental staging systems for rodent embryos wereused by different investigators to standardize the embryological materials[9]. In this work, we depended on the E-designation system. This system refers to a specific development stage of the rat embryos by letter E and followed by a numerical value which indicates a particular embryonic day. This system includes several parameters such as postcopulatory age, CRL, Theiler's stage and Carnegie's stage [(10, 11)].

General histological preparations:
For the general morphological study, embryonic samples were dehydrated, cleared and paraffin embedded then serial sections of (6u) thickness were achieved from tissue blocks in sagittal orientation. H&E staining of selected sections was prepared at appropriate intervals to get a morphological viewpoint[12]. Sets of serial sagittal sections were classified according to E-designation, which was done on delegate embryos achieved from each set of embryos, recovered from the particular pregnant female.

Neurohistological preparations:
For visualization of the developing neurons and their processes in this work. We used two stains;Cresyl Fast Violet (Nissl) and Bielschowsky's Silver stains. After dewaxing with xylene, the slides were coved with filtered cresyl fast violet stain for 10–20 minutes (prepared by mixing 0.5 g cresyl fast violet with 100 ml distilled water), rinsed in distilled water and differentiated in 0.25% acetic alcohol(prepared by mixing 250 µl glacial acetic acid with 100ml alcohol) for 4–8 seconds. The slides were dehydrated, cleared and mounted with Eukitta mounting media and cover-slipped[12]. The second stain was Bielschowsky's silver stain. We followed the protocol of Abcam Company[13] to stain our slides. This stain is the best tool to detect and stain axons and neurofibrils. It is a silver staining method used for histochemical visualization of nerve Fibers and axons because nerve fibers are sensitive to the silver solution. When the sections are treated with ammoniacal silver, it reduced by tissues to metallic state producing an opaque, usually black deposit[14].

3. Results

On E15, the dorsal portion of the rhombencephalon, the roof plate is made of thin layer of cells stretched over the myelencephalic part of the ventricular cavity caudally and a compact layer of neuroepithelium forming the dorsal metencephalic anlage (DMA) rostrally. The part of DMA in the midline is continuous dorsomedially with the isthmus of the mesencephalon and caudally with the choroid plexus. The post-isthmal recess of the fourth ventricle underlays the midline component of the DMA (Fig. 1).

On E16, the development of the DMA is well ahead and stratification of cells and nerve fiber was observed. From ventral to dorsal direction, there are; a layer of neuroepithelium lines the ventricular cavity, a thick layer of elongated spindle-shaped cells (Purkinje cell migration wave), a fibrous layer which is the deep plexiform layer, a cellular layer forms the nuclear migration wave and another fibrous layer has extreme superficial dorsal location which is the superficial plexiform layer (Fig. 2).

On E17, the beginning of cerebellar corticogenesis is marked by two events. The first event is the development of Rhombencephalic Demilune (Rh.D) and subsequent expansion of a canopy of multiple cell layer over the surface of the developing cerebellum, the external germinal layer (EGL). The second event is the migration and settlement of Purkinje cells. Rh.D is a wedge-shaped zone of proliferative activity seen at caudal part of the DMA that appears triangular in the sagittal plane and has three prongs; horizontal prong (ventricular prong), inferior prong (choroidal prong) and third prong is the superior prong (the germinal prong) which is the most significantly developed prong that gives rise to the EGL. EGL is composed of 3-4 rows of closely packed cells that are darkly stained with prominent nuclei and there is an organized and distinct cell poor fibrous layers underlying the EGL. Synchronous with the formation of the EGL, Purkinje cells migrate from the Purkinje cell migration wave (which is the distinctive morphological feature of the developing cerebellum on E16) to the dorsal surface of the developing cerebellum in a radial course forming a new cellular layer underlying the fibrous layer. Thus the formed primitive cerebellar cortex (PCC) consists of three elements. The EGL, fibrous layer beneath and the layer of settling Purkinje cells (Fig. 3).

On E18, two events appeared. There is an increase in size and thickness of the Rh.D. It superimposes the primitive fourth ventricular cavity forming the post isthmal recess roof. The cells become more darkly stained, more packed together with indistinct cellular boundaries and the prongs become more prominent and more darkly stained and more cellular. The EGL expands over the dorsal surface of the developing cerebellar hemispheres in a lateral- to- medial direction. The cells become darker and more closely packed and arranged in multiple layers. The second observed event on E18, the cells of nuclear migration wave sink toward the depth of the developed cerebellum. (Fig.4). The Purkinje cells are seen radially advancing toward the spreading EGL. Embedded in the matrix are the leading fibers of many Purkinje cells that are still in the process of migration and a peculiar fuzzy appearance is distinguishing feature of early settling Purkinje cells (Fig.5) and (Fig.6).

On the successive embryonic days (19-21), certain striking histological features were identified. The first one was the appearance of the principle fissures. On E19, outer cerebellar surface was nearly smooth with two slight indentations representing two principal fissures: preculminate and primary. By E20, the third principal fissure (secondary fissure) was appeared. At E21, the fourth principle fissure (postero- lateral) also was evident and the other fissures become deeper. The five cardinal lobes which seen between the principal fissures, were apparent at E21. From anterior to posterior, they are called the anterobasal, anterodorsal, central, posterior and inferior lobes (Fig.7),

(Fig.8) and (Fig.9). We observed also the regression of ventricular neuroepithelium and Rh.D with further development of the EGL that cover the whole surface cerebellar hemisphere and extend rostrally to cover the anterior vermis. On E21, the PCC rostral to early developing preculminate fissure is established.

To follow the time sequence of morphological organization of PCC at anterior vermis, we compared different lobes in sections of same embryo at E 21. The fissura prima and preculminate fissures were used as landmark for division of anterior vermis. Midsagittal and parasagittal sections were obtained. In the region caudal to the primary fissure, we noticed that the EGL is formed of 2-3 rows of darkly stained cells. Next is a cell spare layer consisting few darkly stained rounded cells with abundant deeply stained ECM. The purkinje cells layer consists of the crowded rounded cells with interposing fibrous mesh (Fig.10). In the region between the primary fissure and preculminate fissure, we observed that the EGL segregates into two ill-defined components; a superficial one of densely packed 2-3 rows of cells and a deeper component is made of elongated almond shape cells with their long axis mostly toward the primitive molecular layer. They seem to be sinking into the fibrous layer beneath the EGL. The primitive molecular layer is intermingled with the EGL above it because of the sinking cells and is continuous with the fuzzy matrix below. It has a weak staining property to Nissl stain. The settling purkinje cells layer is wider and the cells are more rounded in shape with vesicular nuclei and scanty cytoplasm. The fuzzy matrix enlarges tremendously and become more abundant along the side of the primitive layer (Fig.11). In the region rostral to the preculminate fissure, we diagnosed that the EGL consists of 3-4 rows of darkly stained cells similar in morphological characteristics to those observed in the same region earlier at location caudal to primary fissure . The cells seem to flow from lateral to medial forming the caudo-lateral gradient of the PCC formation in the anterior vermis. The primitive molecular layer is lightly stained with Nissl stain or not at all. Few darkly stained small cells are seen in this layer. The settling purkinje cells form a well characterized layer. The cells are large elliptical cell or pyramidal in shape with an apical directed towards the primitive molecular layer. Their nuclei are large and vesicular and cytoplasm forms a rim and becomes abundant at the apical region. Nissl stain shows a lightly stain nuclei and faint basophilia of the cytoplasm. Around these settling cells, the ECM stains with Nissl stain but no distinctive morphological features can be resolved by LM. These are features of the immature cells. (Fig.12).

From the above description, we realized that the final stages of PCC histogenesis in the region caudal to the primary fissure occur at E19-20, while early settling and configuration of PCC at more advanced stages of organization of PCC is identified in the region between the primary fissures and preculminate fissure at E20. By E21, the early events in formation of the PCC is found in the region rostral to preculminate fissure. Thus the process of corticogenesis of PCC follows caudal – to – rostral gradient in vermis.

Figure 1: E15, Lateral parasagittal section through DMA: AQ, aqueduct; IC, isthmal canal; Lr, lateral recess of the 4th ventricle. H&E, 100X

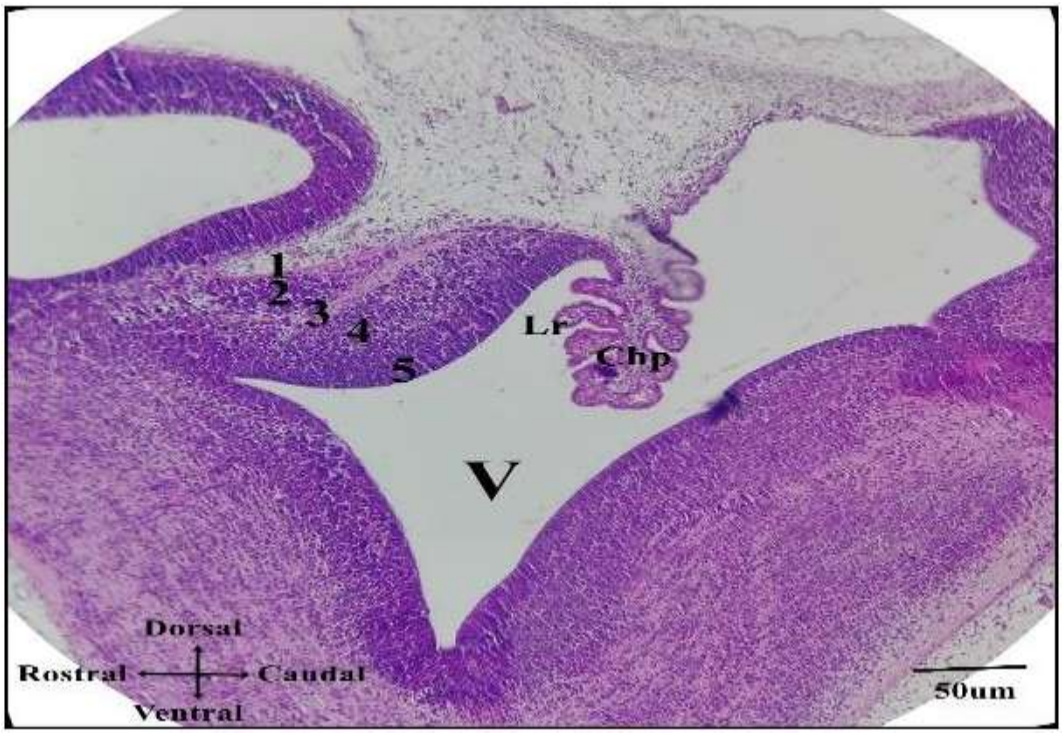

Figure 2: E16, Lateral parasagittal section through DMA: 1, superficial plexiform layer ; 2, nuclear migration wave; 3,deep plexiform layer; 4,Purkinje cell migration wave; 5, neuroepithelium; Lr, lateral recess of the fourth ventricle; Cp, choroid plexus; V ,primitive 4th ventricle. H&E, 100X

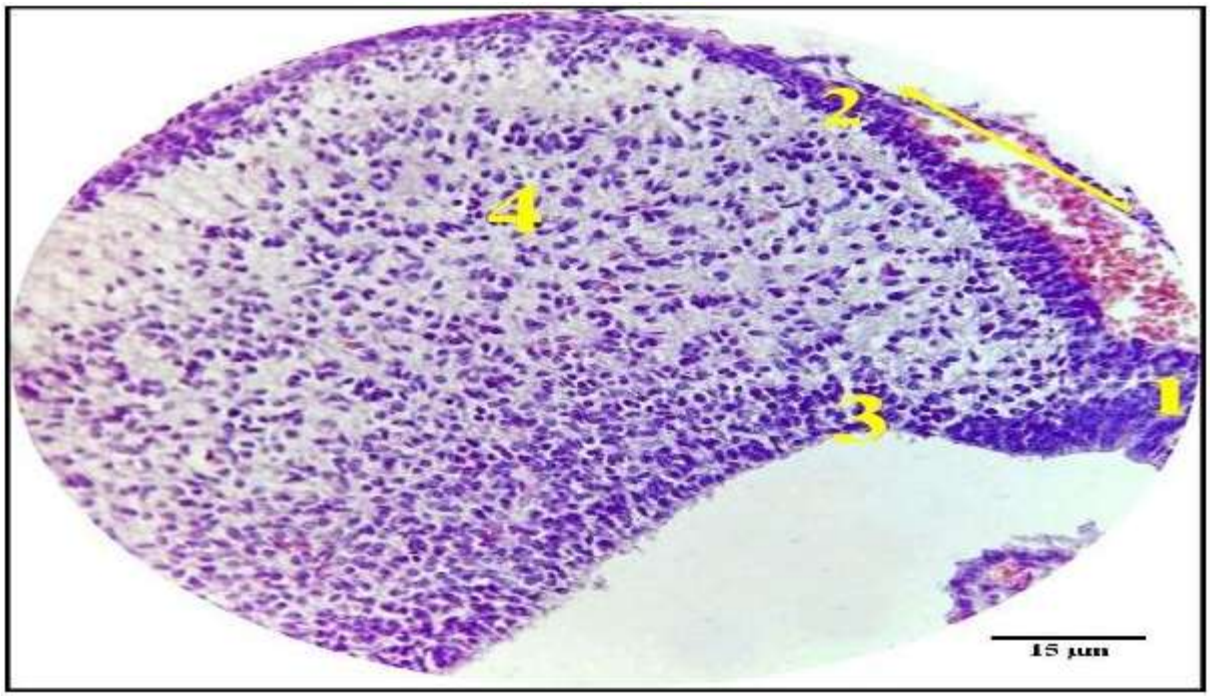

Figure 3: E17, mid-sagittal section through developing cerebellum Showing the pronges of the Rh.D: 1, Rh.D; 2, the superior (germinal) prong; 3, the inferior (ventricular) prong; 4, Purkinje migration wave; arrow, the direction of spread of the external germinal layer over the dorsum of the cerebellum. H&E, X 100

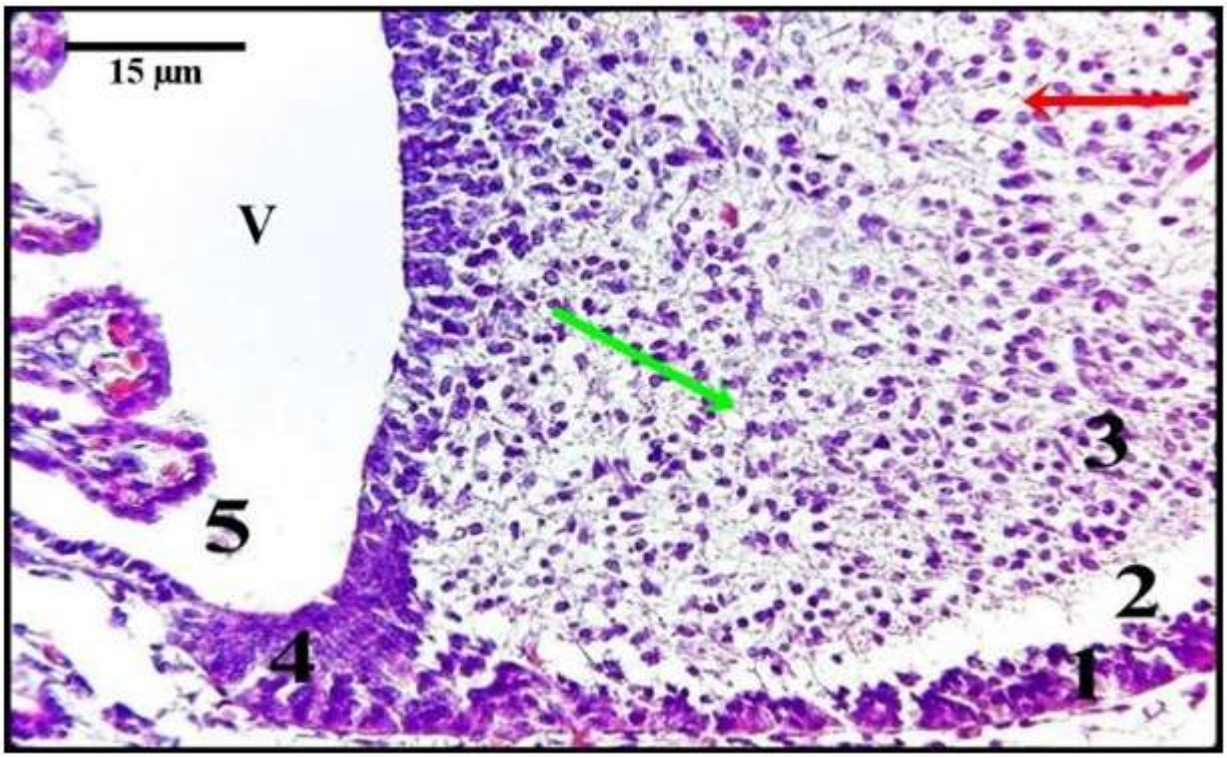

Figure 4: E18, mid-sagittal section through developing cerebellum showing the components of PCC. 1, EGL; 2, fibrous layer; 3, settling Purkinje cells; 4, Rh.D; 5, post-isthmal recess of the 4th ventricle ; red arrow , sinking cells of nuclear migrating wave; green arrow, migrating Purkinje cells. H&E, X 100

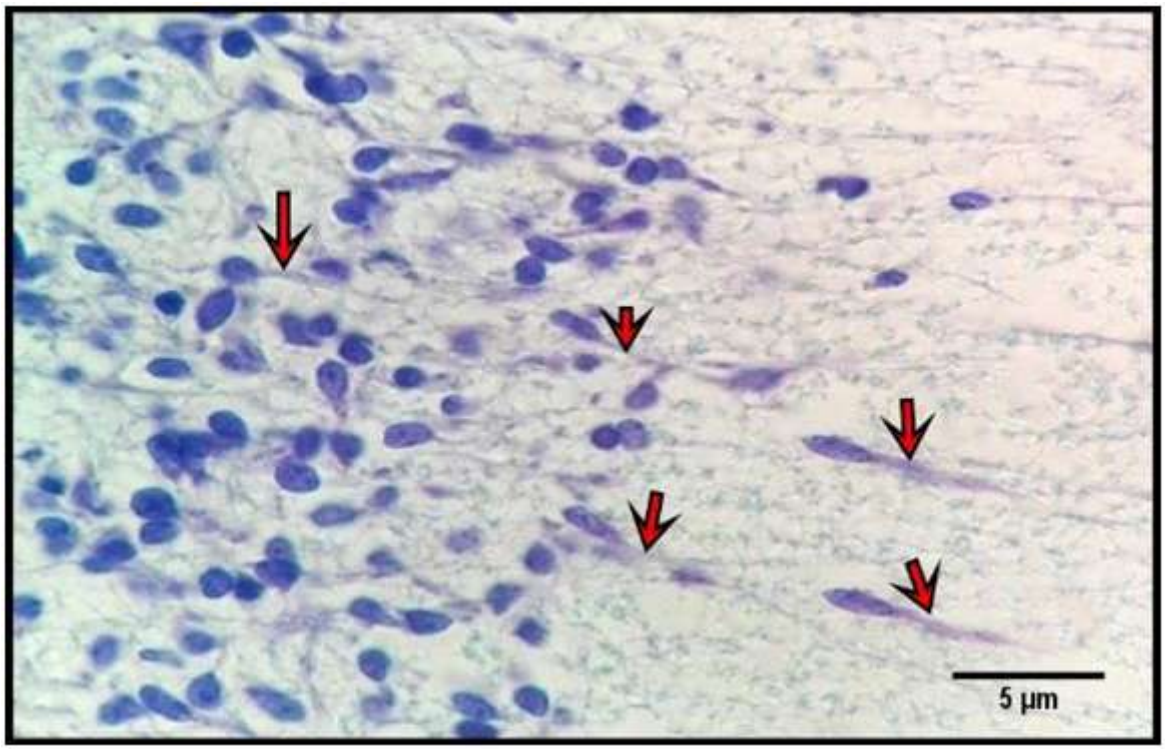

Figure 5: E18, mid-sagittal section through developing PCC showing migrating Purkinje cells. Arrows indicate the leading processes of Purkinje cells facing towards their destination; notes the fuzzy matrix around migrating Purkinje cells. Nissl stain, X 1000

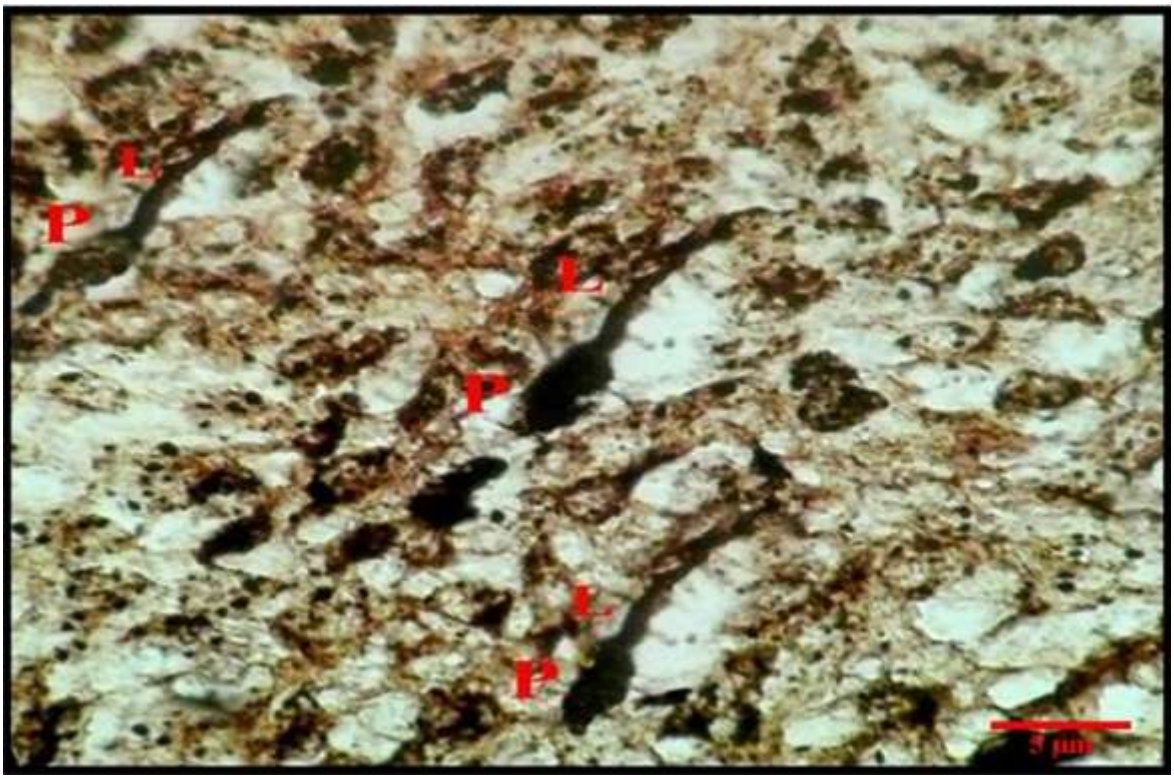

Figure 6: E18, mid-sagittal section through developing PCC showing migrating Purkinje cells; L, leading fibre; P, perikaryon. Bielschowsky's silver stain, X 1000

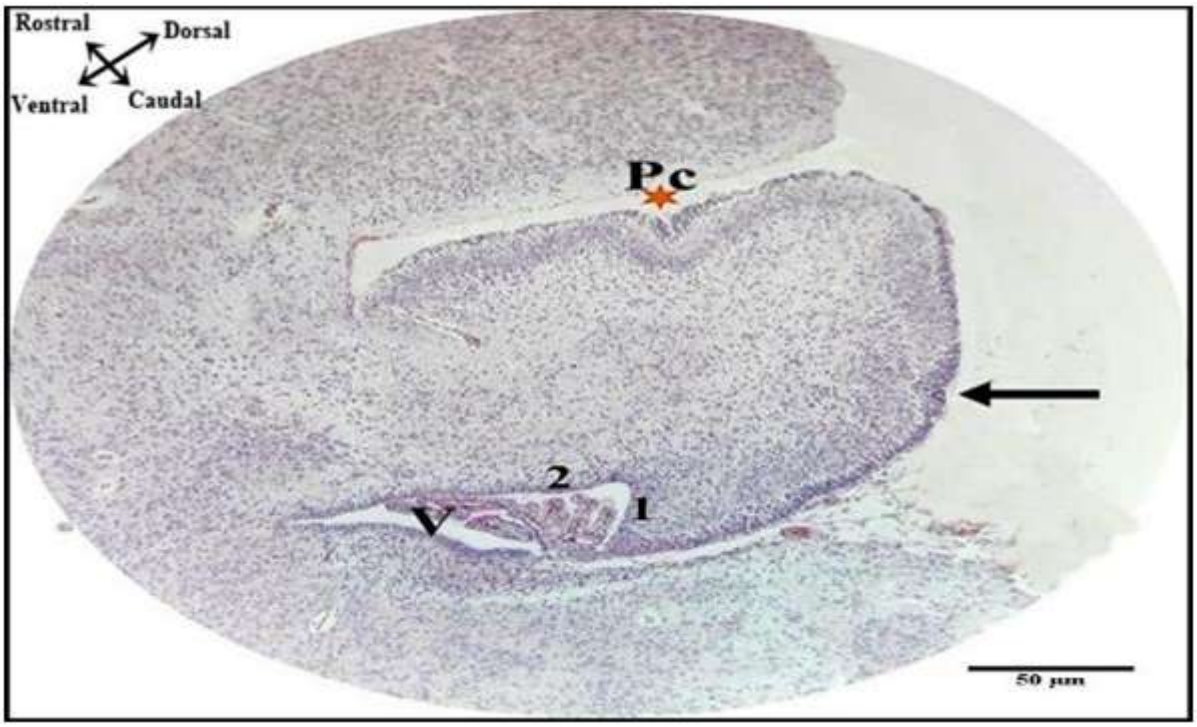

Figure 7: E19, parasagittal section through developing cerebellum showing formation of principle fissures. Pc (asterisk), preculminate fissure; arrow, indentation of primary fissure; V, fourth ventricle. Paraffin, H&E, X 100

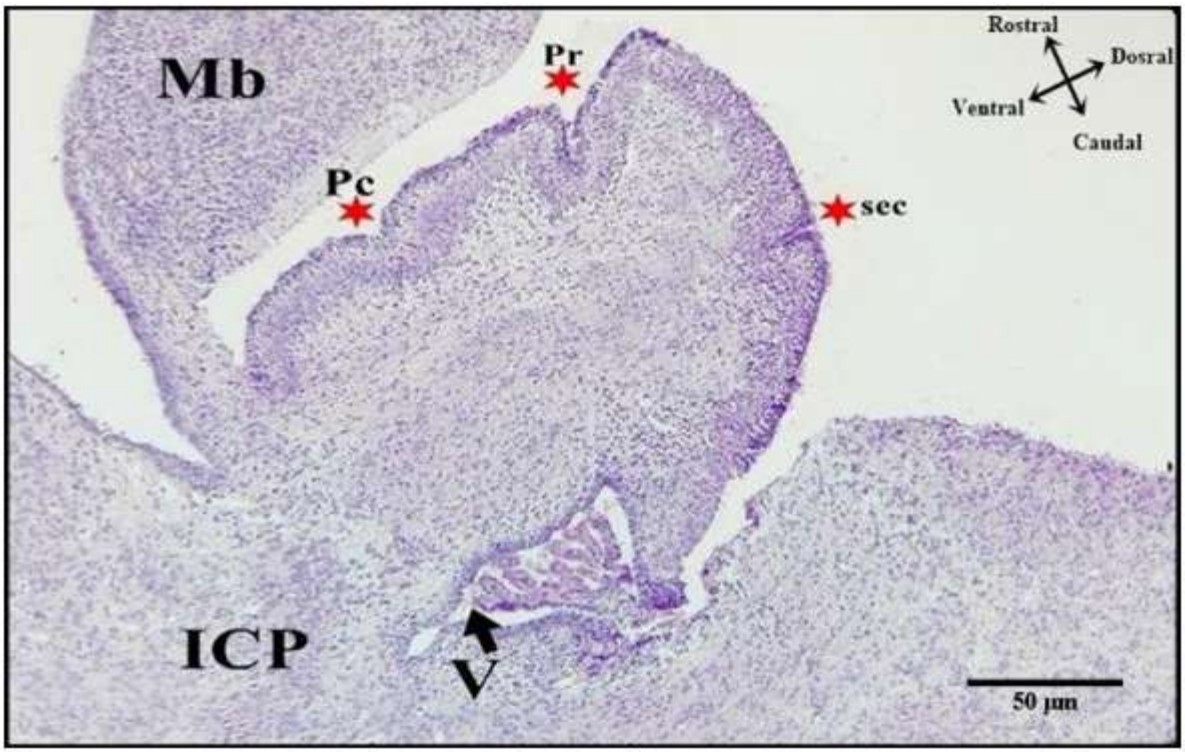

Figure 8: E20, parasagittal section through developing cerebellum showing formation of principle fissures. Pc, preculminate fissure; Pr, primary fissure; sec, secondary fissure; V, fourth ventricle; ICP, inferior cerebellar peduncle. Paraffin, H&E, X 100

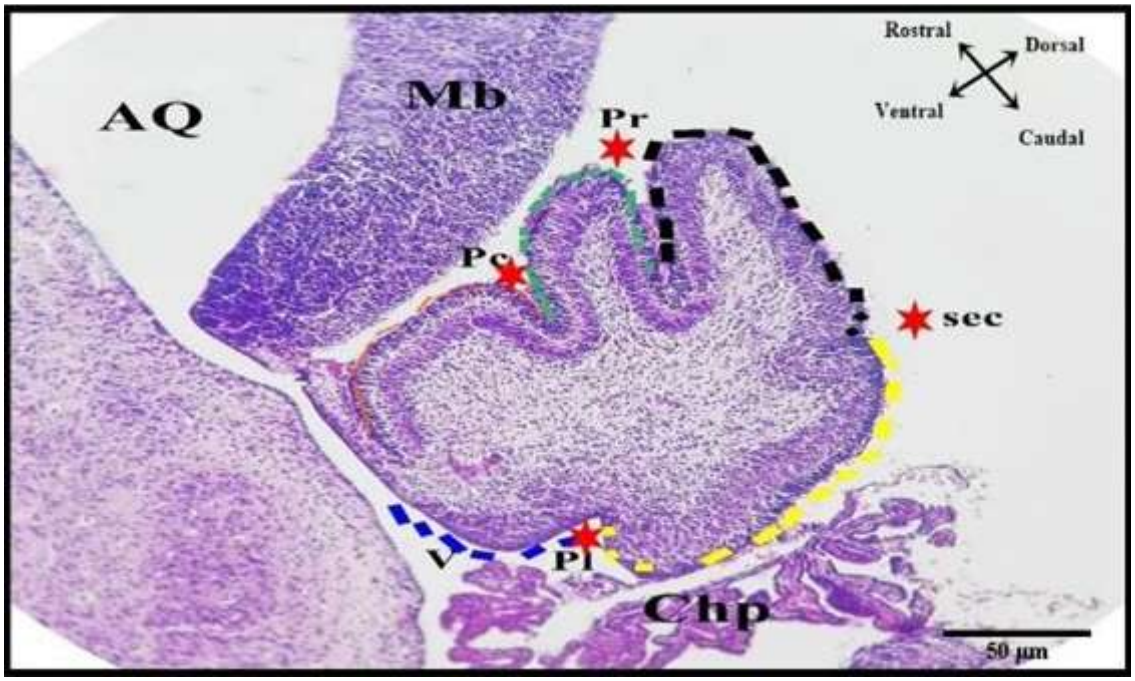

Figure 9: E21, midsagittal section through developing cerebellum Showing formation of the four principal fissures (asterisks) and five cardinal lobes (dotted outlines).The principle fissures are designated as Pc, preculminate; Pr, primary; sec, secondary; Pl, posterolateral fissure; The cardinal lobes are designated as anterobasal (red outline), anterodorsal (green outline), central (black outline), posterior (yellow outline) and inferior (blue outline);AQ, aqueduct; V, fourth ventricle; Chp, choroid plexus . Paraffin, H&E, X 100

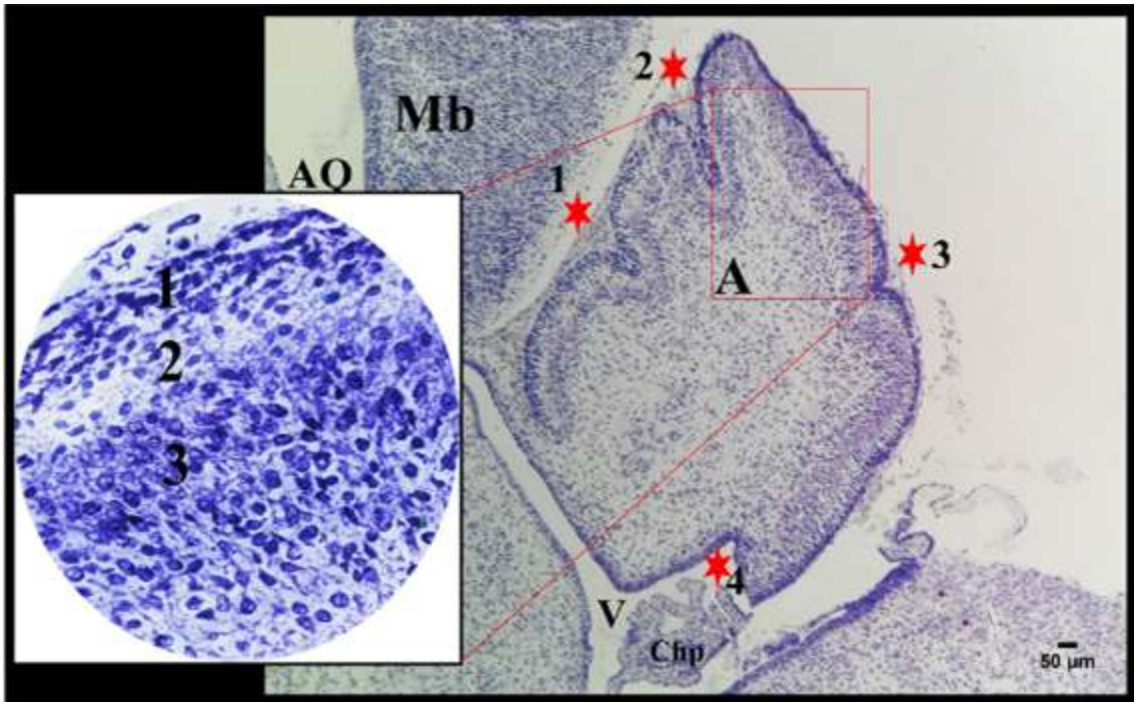

Figure 10: E21, mid-sagittal section through developing cerebellum: shows histogenesis of PCC in the region caudal to the fissura prima. Asterisk (1),preculminate fissure; asterisk(2), primary fissure; asterisk(3), secondary fissure; asterisk(4), posterolateral fissure; Mb, midbrain; AQ, aqueduct; Chp, choroid plexus; V, primitive 4th ventricle. Nissel stain, 100X. Box (A) is shown at higher magnification. 1, EGL; 2, primitive molecular layer; 3, settlement of purkinje layer. Nissl stain, 1000X

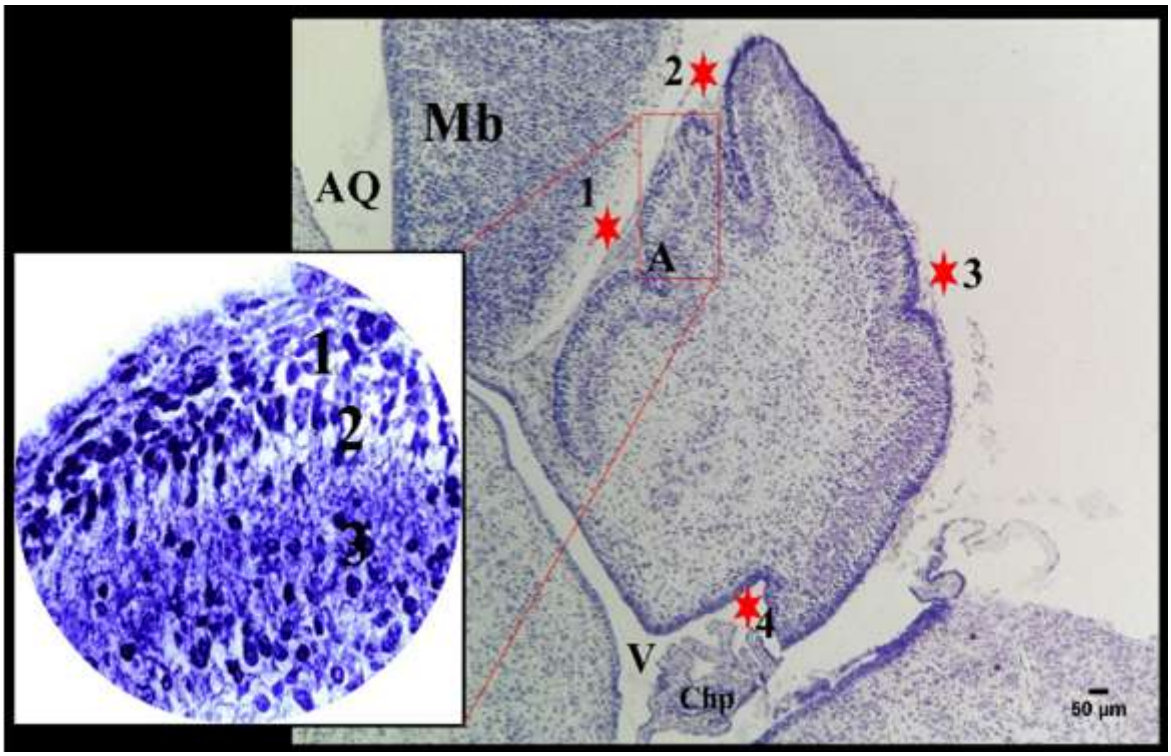

Figure 11: E21, mid-sagittal section through developing cerebellum: shows histogenesis of PCC in the region between the primary fissuresand preculminate fissure. Asterisk (1),preculminate fissure; asterisk(2), primary fissure; asterisk(3), secondary fissure; asterisk(4), posterolateral fissure; Mb, midbrain; AQ, aqueduct; Chp, choroid plexus; V, primitive 4th ventricle. Nissel stain, 100X. Box (A) is shown at higher magnification. 1, EGL; 2, primitive molecular layer; 3, settlement of purkinje layer. Nissl stain, 1000X

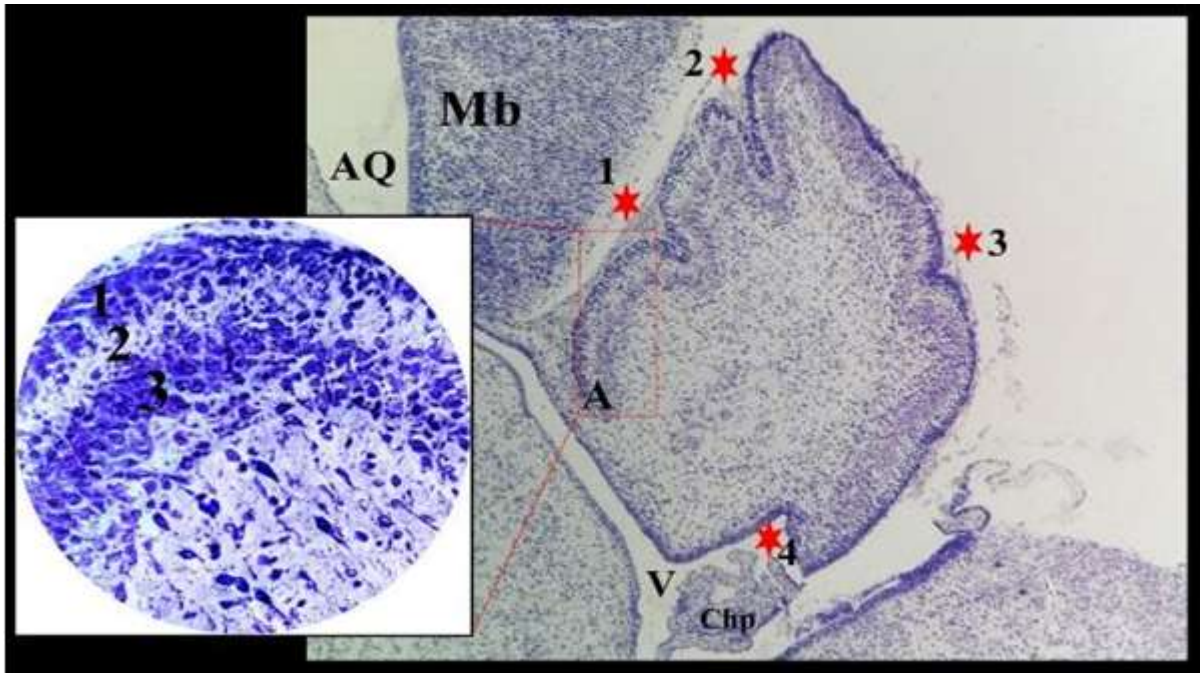

Figure 12: E21, mid-sagittal section through developing cerebellum: shows histogenesis of PCC in the region rostral to the preculminate fissure. Asterisk (1),preculminate fissure; asterisk(2), primary fissure; asterisk(3), secondary fissure; asterisk(4), posterolateral fissure; Mb, midbrain; AQ, aqueduct; Chp, choroid plexus; V, primitive 4th ventricle. Nissl stain, 100X. Box (A) is shown at higher magnification. 1, EGL; 2, primitive molecular layer; 3, settlement of purkinje layer. Nissl stain, 1000X

4. Discussion

The results give a morphological description and delineation of the dorsal metencephalic anlage (DMA). Instead of talking about cerebellar primordium, which we regard as retrospective consideration of development. Dorsal metencephalic anlage (DMA) is more precise description. The metencephalon has a floor plate and a roof plate, the cerebellum develops "in" the dorsal metencephalon.This description matches with much of the old and modern articles but the difference is in the designation[15, 16, 17, 17].

The results give a morphological description and delineation of the Rhombencephalic Demilune (Rh.D) as transient proliferative zone appeared at caudal part of the DMA at E17- E21. In the sagittal plane, it appears triangular in shape but in a three-dimensional, it surrounds the DMA in half a circle from caudal to anterolateral direction. It has three prongs; ventricular, choroidal and germinal prongs. Ventricular and choroidal prongs are appeared before the germinal prong but germinal prong is the most significantly developed one that gives rise to the EGL. This description matches with much of the old and modern articles but the difference is in the designation[19, 20]. Therefore it is more precise to delineate it as Rh.D, instead of talking about rhombic lip or germinal trigone.This is in agreement with the terminology used by (*Al-Anbaki, 2006*)[21]. Many authors have described Rh.D as the "Rhombic lip" and they differed in their description. Some of them described it as an upper rhombic lip [14, 22], others called it as rostral rhombic lip[23] and another described it as anterior rhombic lip[24]. Wilhelm His (1891) was the first author who used the term "Rhombic lip" in human material to encompass the cerebellar primordium[19]. The later interpretations and view of the rhombic lip proposed by many authors is not identical with the original description. Other group of authors have used the term "germinal trigone" referring to the Rh.D in their studies of the prenatal development of the mammalian cerebellum [11, 19,25]. This designation was made up first by Altman and Bayer in the developing rat cerebellum[15].

The result of this work conceptualize the histogenetic organization of various cell population in the DMA as a layer of neuroepithelium lines the ventricular cavity; ventricular zone (VZ). The longitudinally oriented cells are Purkinje cells generated from the neuroepithelium on E15 which form Purkinje cell migration wave (PcMi). The horizontally oriented cell are deep nuclei neurons generating from the neuroepithelium on E14 which form nuclear migration wave (NMi), finally a superficial plexiform layer (SPlx) occupies the dorsal part of the anlage. We postulate that deep nuclei neurons take a roundabout path and easily disperse medially in the traffic- free superficial part of DMA. The migration of Purkinje cells that commences caudally and is directed toward the lateral part of the developing cerebellum will not interfere with neither early migration of deep nuclei neurons nor with their subsequent translocation toward the roof of the 4th ventricle. This description matches with much of the old and modern articles but the difference is in the designation[2, 7, 26]. Instead of talking about differentiation and transitory zones, while these cell wave are migrating cells not cells waiting in a transitory zone for differentiation. It is more precise to use nuclear migration wave and Purkinje cell wave. This is in agreement with the terminology used by (*Al-Salihi, 1995*)[11].

The result of this work conceptualize the histogenetic organization of foliation pattern of the rat cerebellum when the cardinal lobes and fissures appeared at E19-E21. The surface of developing cerebellum initially smooth, then four

shallow fissures (preculminate fissure, primary fissure, secondary fissure, and posterolateral fissure) start to form in the vermis sequentially in restro-caudal direction producing the five cardinal lobes (anterobasal lobe, anterodorsal lobe, and central lobe, posterior and inferior lobes).This is in agreement with the terminology used by[27]. We postulate that the underline mechanisms behind foliation are inward thickening of the EGL and Purkinje cells positioned at base of fissures to anchor via their axons to the DCN. This is in agreement with the explanation made by (*Anamaria and Alexandra, 2007*)[28]. *(Hassan et al., 2015)* divide the stages of foliation pattern of the cerebellum in to two stages. The first stage occurs during embryonic development which is the formation of five cardinal lobes, and the second stage occurs during early postnatal development which is the formation of ten distinct lobules at the vermis and lobules formation is completed by P15[18].

In view of our overall result, we can put forward the following as regards the mechanism of formation of the PCC. First, the proliferation and spread of the EGL. Second, Purkinje cell migration. These two events occur simultaneously (over time) and sequentially (over place) in a spatio- temporal organized manner resulting in the formation of PCC. Therefore, the PCC is not a stationary structure but the process of cortical layering is a dynamic one. PCC starts to form on E17 where three distinctive layers are observed. The superficial EGL, primitive molecular layer and Purkinje settlement layer. Of note, the internal granular layer is yet present in PCC of the rat.

The result of this work illustrates that the EGL, a derivative of Rh.D is a multiplying proliferative layer which covers the most caudal and lateral parts of developing cerebellum during E17. On E18, it creeps to cover the future cerebellar hemispheres in a lateral- to- medial direction. During E19-E20, It extends rostrally to cover the anterior vermis. On E21, the EGL of the anterobasal lobe, rostral to the early developing preculminate fissure is established. Thus the process of corticogenesis of the PCC follows a lateral –to-medial gradient in the future cerebellar cortex and caudal –to- rostral gradient in the vermis. At this point, our result are identical to many of the findings of the current authors since these authors concluded the same results. The following are examples of these studies. (*Haiwei et al., 2013)* said that the external germinal layer (EGL) is a transitory population of proliferating cerebellar cells which locates at the subpial surface of the developing cerebellum[29]. Furthermore, (*Butts et al., 2014*) claimed that the granule cell precursors in EGZ are anchored to the basal lamina of external limiting membrane (ELM) subpialy and this attachment to the basal membrane of ELM is a mitogenic factor for proliferation of the granule cells precursors in the EGL[20]. In addition to that, (*Willi et al., 2002*) reported that the pia mater plays a critical role in granule cells proliferation and in inward migration along Bergmann glia3[0]. (*Angela and Abraham, 2013*) reported that granule cells precursors undergo extensive proliferation in the EGL which after its formation, splits into two layers; an upper layer containing dividing GNPs and a lower layer containing differentiating granule cells. In addition to that, granule cells precursors of the outer half of the EGL are vigorously proliferate and express markers, such as RU49 and Zic1 which are vital for regulating the rate of cell division[2].

As regards Purkinje cells, our results reveal that two phases of histogenesis of PCC were distinguished. First, an initial phase is associated with the early settlement of Purkinje cells and with conspicuous appearance of the just-settling Purkinje cell (fuzzy appearance). An appearance is characteristic of this phase where the leading fibres of many Purkinje cells embedded in the matrix are observed. Second, a late phase, less morphological distinct associated with the completion of the process of the settlement is observed. We postulate the conspicuous appearance of purkinje cells during their early settlement is due to the increased metabolic activity of the cells during their migration-settlement stage. The migration of Purkinje cells as followed in Nissl and Bielschowsky's silver stains throws light on the mode of cells movement. Purkinje cell seems to move by nuclear translocation (Nucleokinesis) where the nucleus with its surrounding cytoplasm move through the cell body following a leading fiber toward the PCC and leaving a trailing fiber toward the ventricular side. On the other hand, we do not observe such configuration of leading and trailing fibers in the creeping EGL. This reflect a different mode of movement is taken by the EGL. According to these finding, We postulate that Purkinje cells migrate to their destination using radial migratory pathway as they move in trajectory, parallel to the ventricular surface and they have a bipolar morphology(leading and trailing processes). While the granule cells precursors in the EGL take a tangential migratory pathway as they have path that is perpendicular to the ventricular surface, have a unipolar morphology and they also move in the field of concentration gradients of the laminin and fibronectin as we will see in the further discussion. At this point in the discussion, our result are identical to many of the findings of the old and current authors since these authors concluded the same results. The following are examples of these studies. Bellamy reported that during development, the cerebellar neurons delivered from multiple germinal sites and move to their destination using radial or tangential migratory pathways[31]. Wingate said that the rhombic lip derivatives travel tangentially autonomous to the glial guidance in subpial pathway and they display a characteristic of the unipolar morphology where a single leading process seems to guide their migration[16]. Moreover, (*Gilthorpe et al., 2002*) aided with time-lapse experiments in chicks demonstrated that granule cell precursors migrate tangentially over the surface of cerebellar anlage forming EGL and these progenitors have a unipolar morphology and do not follow radial glia[25]. In addition to that, Chédotalcited that the granule cell precursors in the EGL begin to proliferate by E10 in mice and by E15 they migrate tangentially to cover the subpial surface of the developing cerebellum following lateromedial and posteroanterior paths[32]. In contrast to that, the Purkinje cells are born from E11 - E13 and migrate radially along radial glia fibers to their final destinations clustering in multiple layers below the EGL and as development proceeds, they distribute into a monolayer (*Barbara et al., et al., 2008*)[33]

5. Conclusion

Three conclusions are explorated. First, the embryonic day 17(E17) is a peculiar day during the histogenesis of DMA when major morphogenetic events take place such as: formation of the Rh.D, sinking of the deep nuclei to their settlement near the ventricular roof and beginning the process of corticogenesis (formation of PCC) which follows a lateral- to – medial gradient in the future cerebellar cortex and caudal – to—rostral gradient in the vermis. The two important events contributing in the formation of PCC are the proliferation and tangential migration of the granule cells precursors and radial migration of the Purkinje cells. Second, cerebellar primordium and DMA are synonyms, but from the morphological point of view, the use of DMA is more accurate. Rhombic lip, germinal trigone and Rh.D are synonyms but from the morphological point of view, the use of Rh.D is more accurate. Third, the first step of the foliation begins prenatally on E19- E21 by formation of five cardinal lobes and four fissure.

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