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Morphometry and Histological Architecture of 7 days Human Neonate cerebral cortex (A Comparative study of Frontal and Occipital lobe)

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ABSTRACT

Four samples of brain at age 1-7 days postnatally were obtained, fixed by 10% neutral bufferedformalin immediately after removal from the skull, for 24 hrs. Specimens from frontal and occipital lobes were taken and immersed in formalin 10% for 48 hrs. and processed histologically to obtain slides at 6 micrometer thickness and stained with hematoxylin and eosin (H&E).

The present results clarify that the mean brain measurement from the frontal to occipital pole was177.0 mm, while the measurements of the frontal and occipital cortices were 2.680 & 2.903 mm respectively.

The histological technique was done to obtain slides for examination under light microscope. The result indicated that the frontal and occipital region of cerebral cortex formed by six layers, molecular, external granular, external pyramidal, internal granular, internal pyramidal and multiform layers.

Keywords: *Brain cortex; Brain development; gyrification; Brain*

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الهيكلية النسيجية والشكلية لعمر ٧ أيام في قشرة دماغ حديثي الولادة للإنسان

(دراسة مقارنة في الفص الجبهي والفص القفوي)

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الملخص

أخذت أربع عينات من الدماغ بعمر ١–٧ أيام بعد الولادة ، ثبتت بالفورمالين ١٠% لمدة ٢٤ ساعة بعد إزالتها من الجمجمة مباشرتاً.

أخذت عينات من فصي الجبهة والقفوي مباشرتاً حيث ثبتت في ١٠ % فورما لين لمدة ٤٨ ساعة ثم عوملت نسيجياً للحصول على شرائح نسيجية بسمك ٦ ما يكروميتر والصبغ بواسطة الهيماتوكسلين والأيوسين. بينت الدراسة الحالية بأن قياسات الدماغ من القطب الجبهي إلى القطب القفوي كانت ١٧٧ ملم ، بينما قياسات قشرتي الجبهي والقفوي كانت ٢.٦٨٠ و٢.٩٠٣ ملم بالتعاقب .

أجريت التقنية النسيجية للحصول على شرائح نسيجية للفحص تحت المجهر الضوئي. أشارت النتائج النسيجية إلى إن مناطق قشرة الدماغ في الفص الجبهي والقفوي مؤلفة من ست طبقات هي الطبقة الجزيئية ، الحبيبية الخارجية ، الهرمية الخارجية ، الحبيبية الداخلية ، الهرمية الداخلية وطبقة متعددة الأشكال.

الكلمات الدالة: قشرة الدماغ ؛ تطور الدماغ ؛التلافيف ؛ الهيكل النسيجي للدماغ ، حديثي الولادة

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1. INTRODUCTION

Cerebral cortex is the most highly developed part of human brain, and it is the most recent structure in the history of brain evolution[1].The character and timing of gyral development is one of the manifestation and complex orchestration of human brain development. The ability to quantify these changes would not only allow for deeper understanding of cortical development, but also conceivably allow for pathology improved detection[2].The central nervous system appear at the beginning of the third week of gestation as a slipper- shaped plate of thickened ectoderm, the neural plate, in the mid- dorsal region infront of the primitive node. Its lateral edges soon elevate to form the neural folds[3] .

Neocortex was transformed from the single layer of output pyramidal neurons of the dorsal cortex of earlier ancestors to the six layers of all present- day mammals[4],[5].

In the human brain, the cortical folding occurs early during brain development [6],[7] . Alterations of the architecture of cerebral white matter in the developing human brain can affect cortical development and result in functional disabilities[8].

The laminar structure of the cerebral cortex is encoded early in development. By neuroblasts begin to differentiate into either specific neuronal cell types or macrogila, depending on their location within a complex topographic matrix of molecular gradients in the ventricular zone layer. Postmitotic cells migrate out of this layer to form cortical laminae in an ‘inside–out' manner in which deeper cortical layers are formed before more superficial ones[9]. Most postmitotic neurons travel along radial glial cells that serve as guides on the path of neurons to their final destination [10].

The subplate is thicker than all other cortical layers between gestational age (GA) 18 and 22 weeks, when it is up to five times thicker than the cortical plate. Similar to the mature cortex, the developing cortical plate consists of 3–6 cellular layers [11]. Depending on the specific cortical region, cortical layer I is formed between GA weeks 24 and 34, and layers III and IV appear between GA weeks 32 and 34 .Each layer contains a distinct array of cells type, the morphology, and laminar location of which dictate the pattern of local and distant projections that each cell may send or receive[12],[13].

Cells in adjacent vertical lamina are organized into functional radial ontogenetic columns, each of which consists of many smaller minicolumns with their longest axes arranged perpendicular to the cortical surface, Pyramidal neurons are clonally derived from radial glial

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cells and can reside in a number of layers within the same minicolumn. As they mature, beginning approximately in the late third trimester, these clonally derived cells preferentially form functional excitatory synaptic connections with one another, rather than with adjacent cells that are not derived from the same progenitor [14]. This layer contains local inhibitory and excitatory interneurons, and it receives input from subcortical and other cortical layers. The presence and extent of cellular differentiation in layer IV reflects the evolutionary origin and complexity of cortical tissue across the cortical surface. Isocortical regions contain a granular layer IV and are typically involved in higher-order processing. transitional regions have a less developed layer IV, whereas allocortical regions lack layer IV. The layer V contains the largest pyramidal cells, similar to layer VI and to more superficial layers, layer V also receives and sends other excitatory and inhibitory projections [15].

The cerebral cortex is the outer covering of gray matter over the hemispheres. This is typically 2- 3 mm thick, covering the gyri and sulci. Certain cortical regions have somewhat simpler functions, termed the primary cortices. However, there are phylogenetically older areas of cortex termed the allocortex [16]. In turn, the allocortex has two components: The paleocortex includes the piriform lobe, specialized for olfaction, and the entorhinal cortex. The archicortex consists of the hippocampus, which is a three-layered cortex dealing with encoding declarative memory and spatial functions.

The neocortex represents the great majority of the cerebral cortex. It has six layers and contains between 10 and 14 billion neurons. The six layers of this part of the cortex are numbered with Roman numerals from superficial to deep. Molecular layer II, the external granular layer; III the external pyramidal layer; IV the internal granular layer; V the internal pyramidal layer; and VI the multiform, or fusiform layer. Each cortical layer contains different neuronal shapes, sizes and density as well as different organization of nerve fibers[17].

Aim of the study

The aim of the present study was designed to describe the morphometry and histological architecture of the human neonate cerebral cortex at the first 7 days after birth of the frontal and occipital lobe and to measure the initial cerebral cortex parameters of the whole layers with description of its histological architecture.

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2. Materials and Methods

Four male and female human neonate term samples at one to seven days of age, were obtained from Baghdad forensic hospital. A coronal incision, anterior and posterior separation was made through the skull, and then the brain was carefully removed. The samples were fixed in 10% neutral buffer formalin for 48 hours, and were washed with tap water for 2 hours to remove the excess formalin and debris. Measurement of brain samples were made using electronic digital caliber, to determine the cerebral cortex thickness.

Brain specimen were taken from frontal and occipital lobes at 0.5 cm^3 then passed through graded alcohol solution from 70,80, 90 and100 % for dehydration, followed by clearing in xylene and infiltered with paraffin wax for 24 hrs. Blocking in wax and finally sectioning at 6 µm thickness were made. Staining was carried out using routine haematoxylin and eosin[18]. Specimens were then digitally photographed.

3. Results

The brain was formed by two cerebral hemispheres indicated by the presence of longitudinal cerebral fissure, which was extended from the of frontal pole to the occipital pole . The frontal lobe was well recognized and indicated by the presence of inferior, middle and superior gyri, each of which was tortuous and covered by the pia matter which pass into the shallow sulci. Superio - posteriorly, there was indication of the occipital lobe and its gyri and sulci. Laterally, the temporal lobe was present by the superior, middle and inferior gyri and superior and inferior sulci. The insular gyri detected and located in the floor of the longitudinal cerebral fissure of the posterior halve .The morphometry and thickness of the various brain parts are shown in Table (1).

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Table(1): Morphometry (thickness) of brains human neonate cerebral cortex at one to seven days

Brain Sample	Measurement of brain from frontal to occipital pole (mm)	Frontal cortex (mm)	Occipital cortex (mm)d
1	145.42 mm	2.770 mm	2.280 mm
2	182.5 mm	2.980 mm	3.460 mm
3	190.0 mm	2.800 mm	2.540 mm
4	190.0 mm	2.170 mm	3.330 mm
Mean± S. E	177.0±10.667	2.680±0.176	2.903±0.290

The difference in the mean values of the two gropes (Frontal cortex, Occipital cortex) is not great enough to reject the possibility that the difference is due to random sampling variability. There is no statistically significant difference between the input groups (P= 0.0.05).

5.1 Male at 1 day

Histological description , Frontal Lobe , Layers of Cerebral Cortex

1. Molecular layer was containing a network of nerve fibers in a spongy form with the presence of neuronal and glial cells.

2. External granular layer was occupied by small size pyramidal cells and some medium size, also associated with nerve fibers and glial cells.

3. External pyramidal layer was enriched with pyramidal cells of medium and large cells, most of these cells had spherical nuclei with clear nucleoli.

4. Internal granular layer Fig. (1) was formed by many small pyramidal cells and also glial cells, it was possible to detect a micro blood vessels extended toward the white matter.

5. Internal pyramidal layer was associated with the presence of medium size pyramidal cells, glial cells and microglial cells.

6. Multiform layer was containing different types of neuronal cells (Pyramidal cells) and non neuronal (glial cells). The whole lamine were occupied with a network of nerve fibers extended to the white matter, with a spaces of different size and shapes of spaces surrounded by glial cells inside, associated with nerve fibers.

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Occipital Lobe (male at 1 day)

Layers of Cerebral Cortex

1. Molecular layer was formed by individual cells of an different types (Neuronal and glial) .

2. External granular layer was wider and also containing an individual cells of undifferentiated types, a minute blood vessels Fig. (2).

3. External pyramidal layer, the third layer formed by pyramidal and glial cells but the pyramidal cells were very small in size with some medium size cells .

4. Internal granular layer was enriched with a great number of cells of pyramidal and glial cells .

5. Internal pyramidal layer which contained pyramidal cells of medium small size cells, surrounded by vacuoles and spherical nuclei associated with microglial cells.

6. Multiform layer was containing medium size pyramidal cells and individual of large cells and microglial cells.

5.2 Male at day 5

Histological description , Frontal lobe , Cortex

1. The first layer was molecular layer which was containing many neuronal cells surrounded by zone of white color .

2. The second layer was the external granular layer containing a greater number of cells which some of them clumped and surrounded by a many cavities or white zone with glial cells and network of nerve fiber .

3. The third layer was the external pyramidal layer, there was an large pyramidal cells, inside of each one there was an a large nucleus .Each cell surrounded by large zone also. The pyramidal cells of medium size also surround by white zone, there was an network of nerve fibers containing glial cells .

4. The fourth layer was the internal granular layer was wider zone with a high number of neurons with glial and microglial cells and a nerve fibers of different directions.

5. The fifth layer , the internal pyramidal layer the layer was containing a large pyramidal cells with great zone surrounded with whitish color , there was individual cells of supporting glial cells embeded in the network of nerve fiber Fig. (3).

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6. The sixth layer, the multiform layer, this layer have an great amount of nerve fibers with a different shapes and sizes of cells, also there was an a small blood vessels in this layer, with a certain number of red blood cells . there was an a great pyramidal cells in between the other type of cells which was mostly glial cells .

Occipital lobe (male at 5 day)

Layers of Cerebral Cortex

1. The surface of cortex looked like smooth surface, below the surface, there was an molecular layer with a scantly neurons and glial cells , below it, external granular layer had an a population of cells of pyramidal form and glial cells in between, also there was an a longitudinal extension of micro blood vessels up to the deeper layer.

2. The third lamina, external pyramidal layer had containing a large and medium size pyramidal cells extended in white zone containing a larger spherical nuclei with it, prominent nucleoli , most of these cells were surrounded by whitish color or zone.

3. the fourth layer was internal granular layer which also have pyramidal cells, small and medium size and single cells of large size (pyramidal) .

4. The fifth layer was internal pyramidal layer the pyramidal cells in this area were of great size cells with certain number of glial cells.

5. The sixth layer was multiform layer which was formed by multiple cells shapes mostly of pyramidal and glial cells associated with nerve fibers extended in the different zones at the middle of brain which was containing a great number of glial cells embeded in the network of nerve fiber and contained different pattern of compartments Fig. (3).

5.3 Female at 5 day of age

Histological description , Occipital Lobe (Female at 5 day)

The pia matter was formed by two layers of membrane which were delicate Fig. (4) in between these membranes, there was blood vessels, inner layer of membrane was adherent to the surface of cerebral cortex.

Layers of Cerebral Cortex

1. The Molecular Layer : was identified as a narrow zone of the cells which were uniform in size.

2. Second layer was external granular layer present below this layer more in number and individual in size but rarely seen the pyramidal cells , below this layer III- was the pyramidal

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cells which was containing some of pyramidal cells associated with glial cells and the pyramidal cells were containing circular, nucleus and darker cytoplasm.

3. The fourth layer which was internal granular layer, this layer formed by great number of cells, some of these were pyramidal cells, medium size and large size.

4. The fifth layer was containing more number of pyramidal cells Fig. (5), surrounded by vacuoles intermingling with glial cells.

5. Sixth layer which was multiform layer, were containing many types of cells also containing blood vessels of great, medium size of artery (muscular artery), these cells were facing the white matter which had characteristic feature of it.

Frontal Lobe (Female 5 day)

Pia matter was formed by a delicate form of membrane appeared separated from the brain tissue, also there was blood vessels in this area, it was containing red blood cells (RBC) also were empty in others.

The layers in the cerebral cortex ;

Layers of Cortex

1. Molecular Layer : was present periphery for the cerebral cortex and cells were very few (Figure 5) .

2. external granular layer was present more obvious than the first layer and the cells were of small size.

3. The third layer was external pyramidal layer was contained larger cells number, some of them were larger in size and contained pyramidal cells, the microglial small cells were in between .

4. The internal granular layer were containing the medium size and larger cells, some of them well pyramidal shaped.

5. The fifth layer was having predominant with pyramidal shaped cells and most apices of these cells directed toward the cerebral outer surface.

6. Sixth layer The multiform Layer was containing larger size of cells of different shapes.

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4. Discussion

Dietrich RB, et al (1988) demonstrated that, the white matter (WM) provides the bulk of the brain volume and hence brain growth over the first 2 years. This increase is accompanied by an increase in head circumference at birth of about 0.5cm/week for the first 3 months and then at decreasing rates to 43–45cm length brain from frontal to occipital pole at 1 year. The result of the present study showed that, white matter appeared containing great cavities surrounded by glial cells associated with stroma of brain tissue. These cavities formed by great compartments on empty spaces from any object surrounded by a micro nerve fibers and form a network containing small amount of glial cells. The result of the present study disagree with **Dietrich RB, et al (1988)**.

The morphometry and thickness of the various brain parts are shown in Table (1).

Early dissimilarities were outlined among newborns birth[20]. The cortical thickness measurement might point towards a structural abnormalities, and represent an early marker for the later appearance of functional disturbance and may be in part responsible for the lower measures in children born with intrauterine growth restriction [21] .This study disagree with kandel, Schwartz, James, Jessell and Thomas[22]. They found that, the thickness of the cerebral cortex was about 2-4 mm. The study also disagreed with Nieuwenhuys, Donkelaar, Nicholson; who found the thickness of about 2.3- 2.8 mm. This study was carried out for the first time in Iraq as far as the authors aware of.

5. Conclusions

The present work conduced that the brain of neonate at 1-7 days was formed by 4 parts of lobes which are the frontal cortex was measured by digital Verna and its measurement main 2.68 mm, 2.9mm. The occipital cortex was the histological study of both neonatal lobes to be presence of six layers which was molecular, external granular, external pyramidal, internal granular, internal pyramidal and multiform layers.

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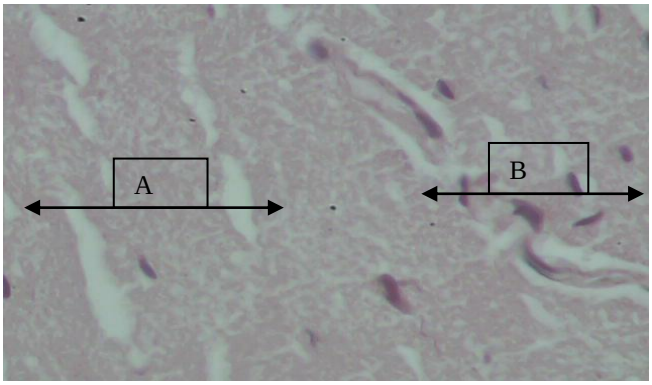

Fig. (1): Occipital lobe : molecular layer **A;** External granular layer **B ;** of the first seven days neonate (H&E X40).

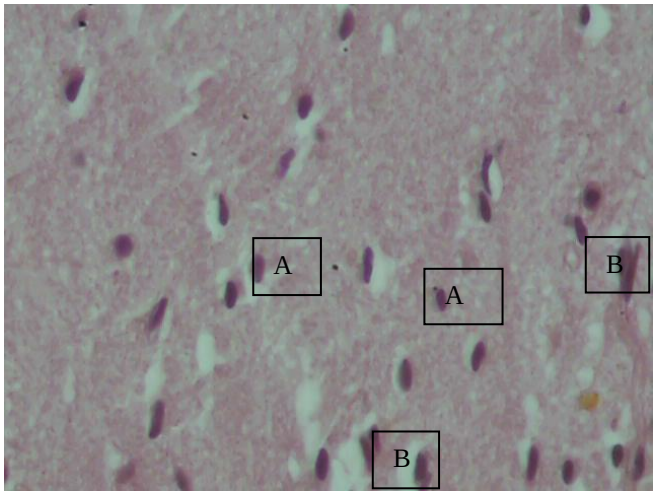

Fig. (2): Frontal lobe : External pyramidal layer, glial cells **A;** pyramidal cells **B;** of the first seven days neonate (H&E X40).

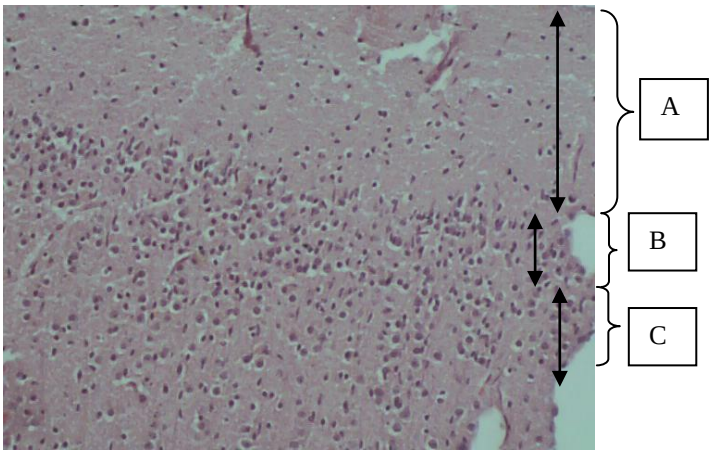

Fig. (3):Frontal lobe : Internal granular layer **A**; Internal pyramidal layer **B;** Multiform layer **C** (H& E X10).

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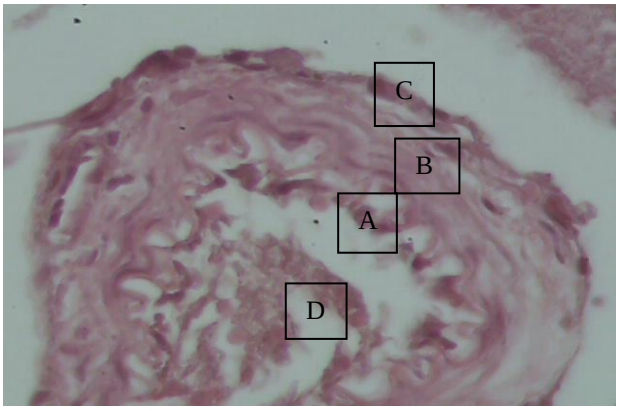

Fig. (4): Occipital lobe: Meningeal artery; Internal elastic lamina **A;** Tunica media **B;** External elastic lamina C; Blood clot **D,** (H&E X40).

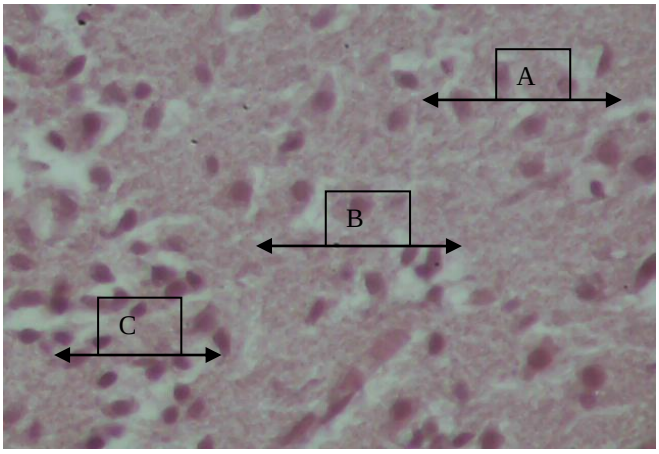

Fig. (5):Occipital lobe : Internal granular layer **A;** Internal pyramidal layer **B;** Multiform layer **C;** of the first seven days neonate (H&E X40).

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