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Histomorphological and Comparative Study for Cerebral Cortex Thickness and Length of Brain in Neonate of Human (Statistical and histomorphological investication)

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Abstract

Twenty five specimens of tissue brain at age 1-28 days after birth, were used, put in 10% formalin for fixation , then excised from the skull, for twenty hour . Samples of 0.5 cm3 thickness from frontal ,parietal ,temporal and occipital lobes are put in the fixative formalin ten percentage and then obtained for histological technique and finally stained by (H&E). The presented results were based on the analysis of a samples of 25 neonatal corps. The samples were further classified into 2 age groups, first 2 weeks of life with a sample size of 10 brain and the second was 15-28 days of age with a sample size of 15 brain. The antero-posterior cranial length was measured. In addition of the thickness of each of the 4 brain lobes were measured in triplicates and the mean of these 3 samples repeated measurements was used for comparison. The mean frontal lobe thickness ranged between 2.42 to 3.72 mm in the first group or younger age and between 3.11 to 4.28 mm in the second group or older age. The mean of frontal lobe thickness was significantly higher in older age (3.67 mm) compared to younger age (3.05mm). The mean difference in frontal lobe thickness of (0.62 mm) was evaluated as a large difference (Cohen's d > 0.8). The histological result demonstrated that the parietal and frontal lobes were lo ated at the anterior and lateral cerebral hemisphere and the brain cortex composed by six layers and these are outer molecular and granular . external and inner pyramidal neurons external pyramidal, internal granular, internal pyramidal and polymorphic layers.

Keywords: Cerebral Cortex, Layers Of Grey Matter, Developmental Brain Anatomy

Introduction

The brain is invested by three connective tissue membranes. The dura mater was the outer membrane and was close adhered to the inside surface of the skull. It is so tough and in elastic. Deep to that, is the arachnoid mater a thin and looser layer. The pia is the inner and thin and vascular zone, which was closely adherent to the surface of the brain and following it's gyri and fissures (Matsuzawa et al., 2001; Beal, 2013).

Development of The Central Nervous System (CNS)

Developing of the central N.S present in many epochs, each with many and various factors of morphogenic development. The brain gyri appeared at the imitation of the third week as a and present in the forntal and pons and the ectoderm of the neural crest shaped plate of thickened ectoderm, the neural plate, in the middorsal region in front of the primitive node. Its lateral edges soon elevate to form the neural folds (Sadler, 2010).

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The posterior portion of the neural canal of spinal cord. Is thin in Between 4 to 5.5 gestational week, the neuroepithelial mantle of the spinal cord usually pass for proliferation and arrive a peak. The cavity of of the neural canal form a cylindrical shape and then triangle, and its terminal end expand to form the end sinus (Valdes-Dapena, 2000).

O'Rahilly & Muller (2008) attributed that the neuroepithelial matter multiplicate and gave rising to neuronal ridge and preliminary cells that transformed into nervous and supporting cells brain and spinal cord according to the various grades of growing in different regions of the neural tube, this tube will be developing for three regions: the midbrain curvature, cervical curvature and pons flexure. The midbrain flexure is the initial ventral curvature [n between the forebrain and the midbrain; the 2nd ventral curvature is located in between the hindbrain and spinal cord (cervical flexure); and the 3rd, dorsal flexure (pontine flexure) developed in between the above two ventral flexures (O'Rahilly & Muller, 2008; Zhang & Sejnowski, 2000).

Studies of preterm born children by Peterson et al (2000) have showed a tendency for impairment of cortical gray zone (GM) growth in infancy, which is still observable in childhood and adolescence as an increase of the white matter (WM) volume whereas cortical thickness changes minimally. Therefore, one may not immediately conclude that the reduced GM volume observed in preterm-born individuals can be attributed to a reduction in cortical thickness (Peterson et al., 2000).

The neocortex of the baby is 4 mm thickness, also the layers of braib are formed by zones, also the cells of the gyri are initiated by neural crest with presence of brain ventricles, the brain ventricles are producing the cerebral fluid produced bt choroid plexus is approximately between 2 – 4 (mm) thick and consists many layers. The layers develop in a way that neural crest migrating from the periventricular germinal zone s (Bayer, 2002; Shaw et al., 2006).

Cortical Layers of Neonate

The cerebral cortex attributed cellular feature at birth and are well formed this concept was documented by Shaw et al. (2006) and the nervous cells in the cortex are mature and emigrating the nerve cells and supporting cells which were demonstrated in the stratified fields for the 1st many months postnatally. The plate is disappearing by the 1st postnatally months and the mantle neurons persisting in adult brain white core matter as intermingling excitatory nerve cells. As active neurogenic activity is remaining in adulthood under ventricular zone around the lateral ventricles. The midbrain, pons and medulla are fully activated and morpho neurogenesis is not demonstrated by the end of first postnatal month (Bayer, 2002; Shaw et al., 2006).

The brain cortex developing from the most rostral part of the neural lamia, a special part of the ectodermal embryo (Shaw et a., 2006).

Larsen (2001) indicating the presence of the plate lamina was seen that the neurogenesis of the neural tubes are initiated the t ths that the neural lamina or plate en folds and approaches to form the neural tunnel cavity are coming from the the cavity in the neural tube which developing the ventricular apparatus and from the neuroepithelial cells of its walls, the pyramidal neurons and micro glia of the nerve system. The great anterior part of the neural laminar plate.

According to many investigator which they revealed that the gyri of bran are present in the form grey mater possised by multiple layers of granular cells which are present nearby the meninges then the granular external zone which have fsclcles of nerve myelinated and unmyelinated nerve s which are ensheathed with myelin and nonmyelinated nerce due to containing lipoprotein , so the cerebral microblood vessels are pirces the brain gyri to the medulla which is occupied by the fascicles of nerve fibers and surrounded by the micro glial cells which consider non excitable cells, but the brain cortex iscontaining withexcitable cells which transport the sensory and motor signals the outermost structure of neural of the brain , in human being and other mammalian . It covers the cerebrum, and is subdivided into two cortices, along the mantal plate, involvrd with covering of, the left and right two cerebral hemispheres (Snook et al., 2005). The medial longitudinal cerebral sulcus is a deep fissure that insulate these two hemispheres (Schmithorst et al., 2002). The neocortex which is the great area of the cortex, consisting of up to six cortical layers, each with a various component in terms of neurons a. According to Smithers et al. (2005) the thickness of cortical brain is two to four millimeters thickness. Refereeing to as grey matter as it composing of c soma l bodies and capillaries and contrasts with the underlying white matter, that containing mainly of the white sheath of myelination s of neuronal axons (Kandel et al., 2000).

Growth of the Brain at Birth

Cerebral cortex is remain primary neocrtical plate and this area consider as thw base for future development of the neonate brain formation, so multiple lines will be nintiate for the formation of cortical layes refelected by formation of gyri and sulci and these will be establish for the maturity of nerveous system, also the cortex as primitive will advance in its development under the bony tissue which is the skullas well as invading by multiple venous plexus pass from the meninges, cortex and finally collect the blood from the medull and cortex which were well related with the blood brain barrier in associated with protoplasmic astrocytes still rather primitive, neonates come into the world with some very useful survival reflexes, the cerebral cortex is responsible for all of our conscious thoughts, feelings, memories and voluntary actions.." (McManus et al., 2004; Paus et al., 2001).

Objectives of the present study

This study was performed to, diagnose the differences of cerebral cortex thickness and length of brain from the frontal to occipital pole in human neonate (1-28) days of age, through the following objectives: -

1. To demonstrate the early postnatal growth of brain for the neonate (1-28 day)
2. To measure the thickness of brain cortex in both sexes at early stages of it.
3. To measure the length of brain cortex in both sexes at early stages of it.

To illustrate the morphological aspect of brain at (1-28 day) postnatally.

Methods

Dissection of Brain Specimens

Twenty five brain specimens were obtained from Baghdad forensic hospital. The specimens were at age of (1- 28 day) postnatally. The skull of each specimen was dissected by inducing a coronal incision Anterio – posteriorlly in order to remove the brain carefully (Tank, 2013), followed by immersion the brain of each one in 10 % formalin for 24 hour, then washed with running water for two hours to remove the debris materials and blood clot. All samples of brain arranged in two age groups and classified into ten of samples of age 1-14 days and fifteen of samples 15-28 days.

Histological Technique

For histological observation, 6 micrometer thickness sections were cut with the help of rotary microtome after different steps of histological processes included, washing, dehydration, clearing , embedded and mounting the sections were stained with H&E (Bancroft & Stevens, 2018). The thickness of the gray matter obtained by using electronic digital caliper.

Analytical statistical data

Informative resulation were obtained its data and input into a computerized database in order ti investigate the full cortical thickness and then comparison with that of other neonate in different states and covernorate, the ocular micrometer and morphologic measurments by using calibrated tape was utilized to determine the perfect thicknee of lobes of brain, the whole output of the results were recorded to show any different regions of braon neonate composition. The database was analyzed for faulty using range and logical information punitive methods were residue. An expected statistical advice was seen for. Statistical analyses were done by applying SPSS software (Statistical Package for Social Sciences) in association with Microsoft Excel 2010.

The output qualitative and quantitative variables were normally distributed and were thus conveniently demonstrated by mean, SD (standard deviation) and SE (standard error), and the parametric statistical tests of significance were used. Repeated measures analysis was used to assess the statistical significance of paired differences in mean brain lobes thickness between the 4 different lobes. When the P value for repeated measures analysis shows a statistically significant difference, further exploration of the statistical significance of difference in mean between each combination of 2 brain lobes was assessed. P value less than 0.05 was considered statistically significant.

The statistical significance, direction and strength of linear correlation between 2 quantitative normally distributed variables was measured by Pearson’s linear correlation coefficient. A simple linear regression model was used to study the effect of age on brain lobe thickness.

The 95% confidence interval is a statistical procedure to anticipate or predict the expected range of possible values of the calculated sample estimate of any statistic in the reference population with 95% confidence.

Cohen’s is recorded the data base for whole measuring involved with the present study so, the whole results were inout and done by drawing the output data in the form of histographa standardized measure of effect size for difference between 2 means, which can be compared across different variables and studies, since it has no unit of measurement. Cohen’s d = (mean1-mean2) / matched standered error SD of the 2 group Cohen’s d < 0.3 small effect, 0.3-0.7 (medium effect), while 0.8 and higher is a large effect Sorlie (1995) and Kleinbaum et al. (2007).

Results and Discussion

The difference between 2 age groups

The AP cranial length ranged between 75.52 mm to 200 mm in the younger age to group and between 74.05 to 220 mm for the older age group. There was no important or statistically significant difference in mean AP length between the 2 age groups, table 1 and figure 1.

The mean frontal lobe thickness ranged between 2.42 to 3.72 mm in the younger age to group and between 3.11 to 4.28 mm in the older age group. The mean frontal lobe thickness was significantly higher in the older age group (3.67 mm) compared to younger age group (3.05). The mean difference in frontal lobe thickness of 0.62 mm was evaluated as a large difference (Cohen's d > 0.8) (Table 2, figure 2).

shown in the table 2 and figure 2, the mean occipital lobe thickness ranged between 2.54 to 3.46 mm in the younger age to group and between 2.91 to 4.5 mm in the older age group. The mean occipital lobe thickness was significantly higher in the older age group (3.59 mm) compared to younger age group (3.09). The mean difference in occipital lobe thickness of 0.5 mm was evaluated as a large difference (Cohen's d > 0.8).

The mean temporal lobe thickness ranged between 2.12 to 4.16 mm in the younger age to group and between 2.77 to 4.94 mm in the older age group. These are results involved with variation of the cortica gyri of neonate There was no important or statistically significant difference in mean temporal lobe thickness between the two age groups (3.73 mm for the 1-14 days age group and 3.55 mm for the 15-28 days age group). The difference observed in mean temporal lobe thickness between the 2 age groups was evaluated as a small difference (Cohen's d <0.4).

The mean parietal lobe thickness ranged between 3.25 to 4.54 mm in the younger age to group and between 3.08 to 4.1 mm in the older age group. There was no important or statistically significant difference in mean parietal lobe thickness in between the t2 of that age groups (3.66 mm for the 1-14 days age group and 3.53 mm for the 15-28 days age group). The difference observed in mean parietal lobe thickness between the 2 age groups was evaluated as a small difference (Cohen's d <0.4).

The difference in mean thickness between the 4 brain lobes

There was a statistically significant difference in mean thickness between the 4 examined brain lobes among subjects examined in the first age group (1-14 days). The mean, thickness was (significantly higher) in the temporal and parietal lobes (3.73 to the 3.66 mm respectively) compared to frontal and occipital lobes (3.05 and 3.09 mm respectively), table 3 and figure 3.

As shown in table 4 and figure 3 there was no obvious or statistically in significant difference in mean thickness between the 4 examined brain lobes among subjects examined in the second age group (15-28 days).

The variability of the 3 repeated measurements

The thickness of each of the 4 brain lobes were measured in triplicates. The variability of the 3 repeated measurements was assessed as the standard deviation and the mean of this variability was compared between the 2 age groups for each of the 4 brain lobes.

There were no significant statistical difference in standard deviation of 3 repeated measurements of frontal and occipital lobe thicknesses between the 2 age groups. The variability was, however significantly higher in the younger age group for temporal and parietal lobe measurements (Table 5).

Linear correlation between thickness measurements

As shown in figure 5, the frontal lobe thickness showed a statistically significant strong positive linear correlation with occipital lobe thickness. Similarly temporal lobe thickness demonstrated a statistically significant moderately positive linear correlation with parietal lobe thick, (figure 6).

Using age as a predictor for brain lobe thickness

The age in days showed a statistically significant strong 3 positive linear correlation with frontal lobe thickness (fig. 7). Similarly age in days showed a statistically significant moderately to strong positively linear correlation with occipital lobe thick (fig. 8).

For each one day increase in age, the mean frontal lobe thickness is expected to increase by a mean of 0.03 mm. The model of frontal lobe thickness based on age was statistically significant and able to interpret 41.9% of output data variation in the dependent variable (frontal lobe thickness) (Table 6).

For each one day increase in age the mean occipital lobe thickness is expected to increase by a mean of 0.02 mm. The suggested model predicting occipital lobe thick based by age was statistically significant and perform to explain 29.9% of present data variation in the dependent (occipital lobe thickness) (Table 7).

The simple regression models using age as a predictor for temporal and parietal lobe thickness were not significant statistically and failed to demonstrate a reasonable predictive ability for age in these instances, (Tables 8 and 9).

Table 1. The difference in mean AP length between the 2 age groups

	Age group (days)		
	1-14 days	**15-28 days**	**P**
AP length (mm)			0.26[NS]
Range	(75.52 to 200)	(74.05 to 220)	
Mean	172.32	150.55	
SD	35.92	51.45	
SE	11.359	13.285	
N	10	15	
Effect of older age compared to younger age			
Difference in mean		-21.77	
Cohen's d		-0.47	

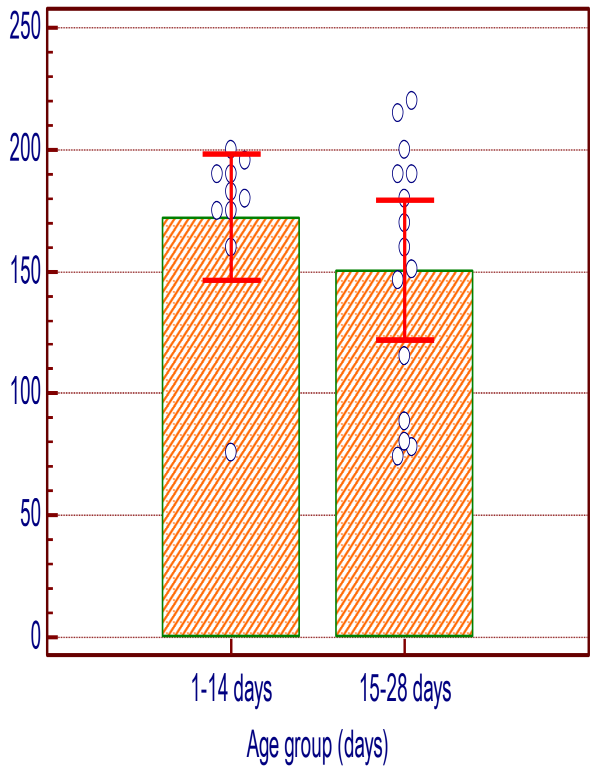

Figure 1. histograph illustrating the mean difference (its 95% interval confidence AP length in, between the 2 age groups

Table 2. The difference in mean thickness of 4 brain lobes the 2 age groups

	Age group (days)		
	1-14 days	**15-28 days**	**P**
Frontal lobe thickness (mm)-mean			<0.001
Range	(2.42 to 3.72)	(3.11 to 4.28)	
Mean	3.05	3.67	
SD	0.39	0.33	
SE	0.125	0.086	
N	10	15	
Effect of older age compared to younger age			
Difference in mean		0.62	
Cohen's d		1.75	
Occipital lobe thickness (mm)-mean			0.01
Range	(2.54 to 3.46)	(2.91 to 4.5)	
Mean	3.09	3.59	
SD	0.35	0.48	
SE	0.11	0.123	
N	10	15	
Effect of older age compared to younger age			
Difference in mean		0.5	

Cohen's d		1.15	
Temporal lobe thickness (mm)-mean			0.48[NS]
Range	(2.12 to 4.16)	(2.77 to 4.94)	
Mean	3.73	3.55	
SD	0.61	0.59	
SE	0.193	0.151	
N	10	15	
Effect of older age compared to younger age			
Difference in mean		-0.18	
Cohen's d		-0.3	
Parietal lobe thickness (mm)-mean			0.39[NS]
Range	(3.25 to 4.54)	(3.08 to 4.1)	
Mean	3.66	3.53	
SD	0.38	0.35	
SE	0.119	0.091	
N	10	15	
Effect of older age compared to younger age			
Difference in mean		-0.13	
Cohen's d		-0.36	

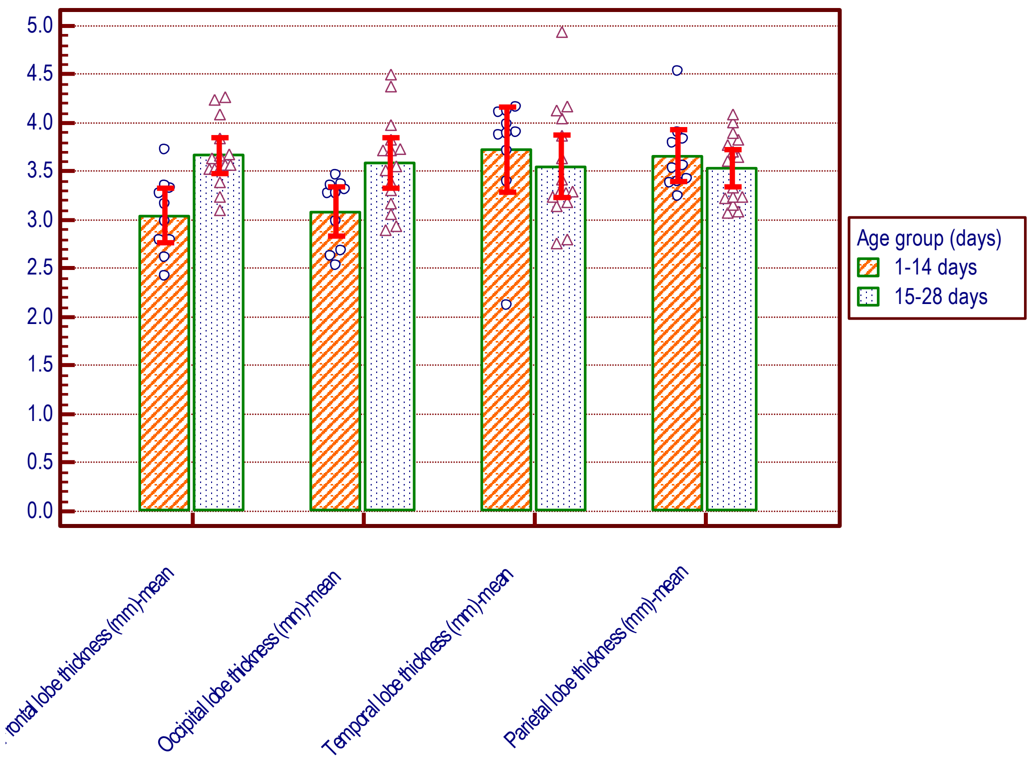

Figure 2. Diagram with in error bars showing the variation in mean (with its 95% confident interval) thickness of 4 brain lobes the 2 age groups

Table 3. The mean thickness of 4 different brain lobes among subjects in the first age group (1-14 days).

	Frontal lobe thickness (mm)-mean	Occipital lobe thickness (mm)-mean	Temporal lobe thickness (mm)-mean	Parietal lobe thickness (mm)-mean	P (repeated measure)
1-14 days					0.023
Range	(2.42 to 3.72)	(2.54 to 3.46)	(2.12 to 4.16)	(3.25 to 4.54)	
Mean	3.05	3.09	3.73	3.66	
SD	0.39	0.35	0.61	0.38	
SE	0.125	0.11	0.193	0.119	
N	10	10	10	10	

P value for paired comparisons between:

Mean Frontal lobe thickness (mm) x Mean Occipital lobe thickness (mm) = 0.68[NS]

Mean Frontal lobe thickness (mm) x Mean Temporal lobe thickness (mm) = 0.004

Mean Frontal lobe thickness (mm) x Mean Parietal lobe thickness (mm) = 0.002

Mean Occipital lobe thickness (mm) x Mean Temporal lobe thickness (mm) = 0.021

Mean Occipital lobe thickness (mm) x Mean Parietal lobe thickness (mm) = 0.006

Mean Temporal lobe thickness (mm) x Mean Parietal lobe thickness (mm) = 0.72[NS]

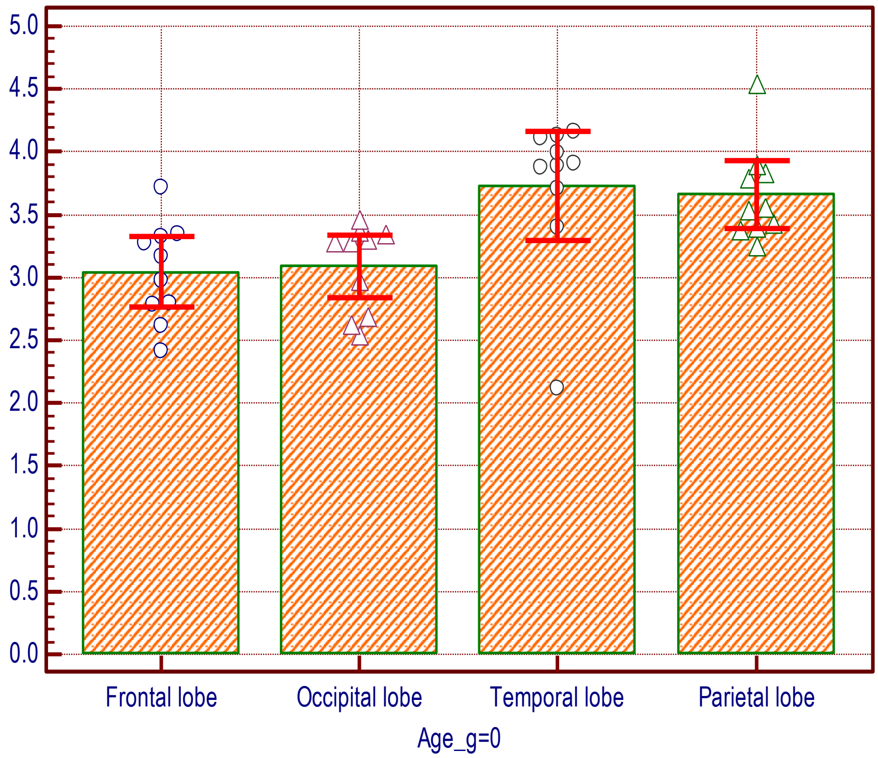

Figure 3. Dot with S -error bars show the variation difference in mean (with its 95% confidence interval) thickness of 4 brain lobes among subjects 1-14 days old.

Table 4. The mean thickness of 4 different brain lobes among subjects in the second age group (15-28 days).

	Frontal lobe thickness (mm)-mean	Occipital lobe thickness (mm)-mean	Temporal lobe thickness (mm)-mean	Parietal lobe thickness (mm)-mean	P (repeated measure)
15-28 days					**0.66[NS]**
Range	(3.11 to 4.28)	(2.91 to 4.5)	(2.77 to 4.94)	(3.08 to 4.1)	
Mean	3.67	3.59	3.55	3.53	
SD	0.33	0.48	0.59	0.35	
SE	0.086	0.123	0.151	0.091	
N	15	15	15	15	

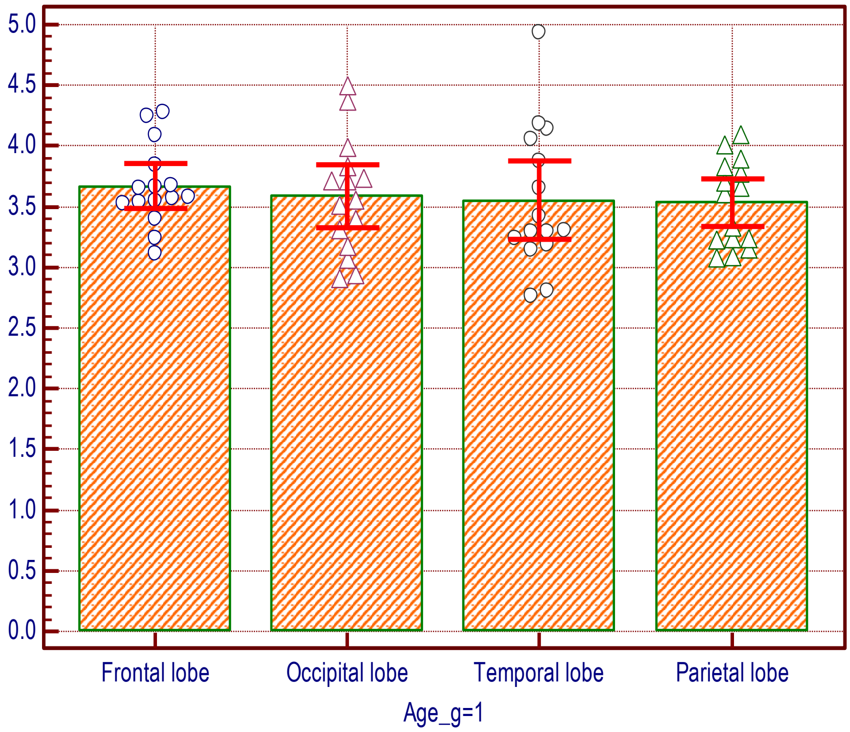

Figure 4. Dot diagram with error bars showing the difference in mean (with its 95% confidence interval) thickness of 4 brain lobes among subjects 15-28 days old.

Table 5. standard deviation (variability) of 3 repeated parameters or measurements for each brain lobe thicknessing between the two age groups.

	Age group (days)		
	1-14 days	**15-28 days**	
Frontal lobe thickness (mm)-SD of 3 repeated measurements			0.27[NS]
Range	(0.23 to 1.46)	(0.16 to 1.21)	
Mean	0.77	0.61	
SD	0.4	0.3	
SE	0.126	0.076	
N	10	15	
Effect of older age compared to younger age			

Difference in mean		-0.16	
Cohen's d		-0.47	
Occipital lobe thickness (mm)-SD of 3 repeated measurements			0.59[NS]
Range	(0.19 to 1.26)	(0.18 to 1.33)	
Mean	0.73	0.65	
SD	0.39	0.34	
SE	0.125	0.087	
N	10	15	
Effect of older age compared to younger age			
Difference in mean		-0.08	
Cohen's d		-0.22	
Temporal lobe thickness (mm)-SD of 3 repeated measurements			0.002
Range	(0.49 to 1.59)	(0.05 to 1.36)	
Mean	1.14	0.59	
SD	0.38	0.37	
SE	0.121	0.096	
N	10	15	
Effect of older age compared to younger age			
Difference in mean		-0.55	
Cohen's d		-1.47	
Parietal lobe thickness (mm)-SD of 3 repeated measurements			0.05
Range	(0.35 to 1.56)	(0.01 to 1.18)	
Mean	0.8	0.5	
SD	0.4	0.32	
SE	0.125	0.082	
N	10	15	
Effect of older age compared to younger age			
Difference in mean		-0.3	
Cohen's d		-0.85	

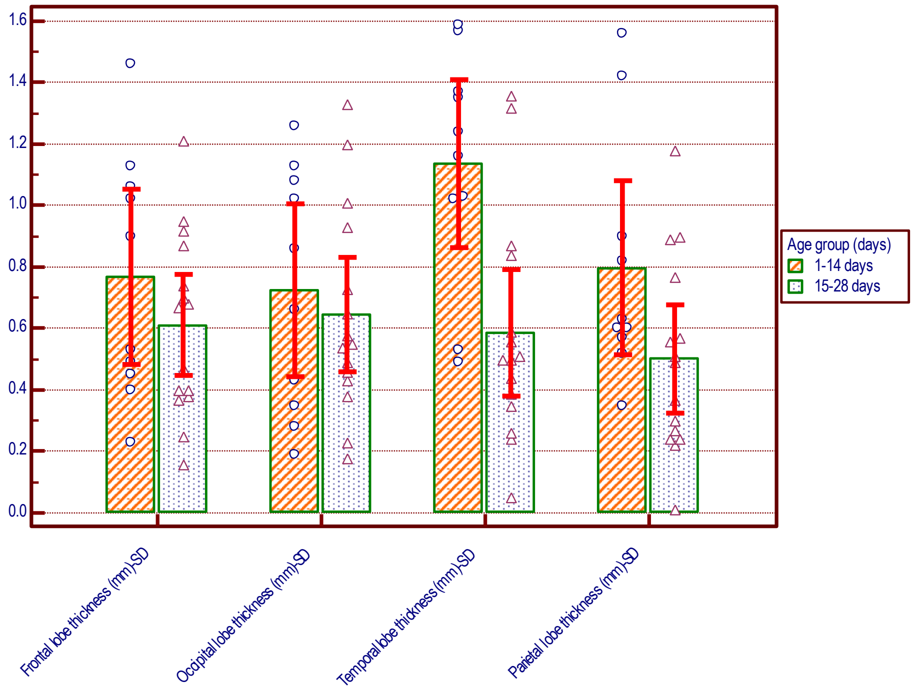

Figure 5. Dot diagram with error bars showing the difference in mean (with its 95% confidence interval) variability (standard deviation) of 3 repeated measurements for each brain lobe thickness between the 2 age groups.

Table 6. Simple linear regression with age in days as the explanatory (predictor) variable and mean Frontal lobe thickness (mm) as the dependent (outcome) variable.

	Regression Coeffedent	P
(Constant)	2.98	<0.001
Age (days)	0.03	<0.001

P (Model) <0.001

R^2=0.419

Table 7. Simple linear regression with age in days as the explanatory (predictor) variable and mean Occipital lobe thickness (mm) as the dependent (outcome) variable.

	Regression Coefficient	P
(Constant)	3.0	<0.001
Age (days)	0.02	0.005

P (Model) = 0.005

R^2=0.292

Table 8. Simple linear regression with age in days as the explanatory (predictor) variable and mean Temporal lobe thickness (mm)- as the (outcome) variable

	Regresed Coefficient	P
(Constant)	3.78	<0.001
Age (days)	-0.01	0.37[NS]

P (Model) = 0.378[NS]

R^2=0.034

Table 9. Simple linear regression with age in days as the explanatory (predictor) variable and mean Parietal lobe thickness (mm)- as the dependent (outcome) variable

	Regression Coefficient	**P**
(Constant)	3.70	<0.001
Age (days)	-0.01	0.28[NS]

P (Model) = 0.28[NS]

R^2=0.05

Discussion

The human brain grows the most dramatically during the pre and post-natal development (Alexander-Bloch et al., 2018).

In this study a deep convolutions neuronal network – based approach, we put in our mind that changes during pre-natal are followed up by post-natal changes and this is evident including segmentation, gyri and sulci formation (Ball et al., 2012).

This study focusing to be measure the thickness of brain cortex at different lobes and in comparison with two age groups (1- 14 day 1st group and 15- 28 day of 2nd group), taking the consideration of measuring from the pial membrane to the bordres of white matter, so we propose a new electronic digital caliber was utilized.

The present resultation referred that mean length of gray matter of frontal lobe at 1-14 day post natally was 3.05 mm and 3.67 mm at 15- 28 day, this reflect significant variation at P<0.5 (Ball et al., 2013) mentioned that the mean length of brain cortex 0.5 – 0.6 mm thickness at early stage of life, this reflect that result was a way from the data recorded in our study., otherwise the mean thickness of brain cortex of parietal lobe at 14 day was 3.66 mm and at 28 day next was 3.55 mm, this indicate slow development and even regression of cerebral cortex at 28 day and this agree with (Glasser & Van Essen, 2011) who recorded regression for this lobe thickness after few weeks of development.

The mean thickness of temporal lobe at 14 day post natally was recorded 3.75 mm and 3.55 mm at 28 day, this result confirm the data recorded at parietal lobe, that mild regression of thickness stay in this defiant time or stag (Mak Fan et al., 2012).

The thickness of occipital lobe at 14 day was 3.09 mm and the progress to 3.59 mm at 28 day, tis result was significant in comparison in between groups. This reflect the capacity of this lobe to be grow faster than in parietal and temporal lobe, so this result was in agreement with (Nagy et al., 2011).

The histomorphometry indicated that gray matter was increase in whole cases during the advancing of age after 28 day post- natally.

The whole gyri in whole lobes indicated the present six layers of neuronal pyramidal cells and glial supporting cells stored by molecular layer sub pial membrane and terminating in the polymorphic layer adjacent to the white matter which was in agreement with most previous investigators such as (Fan et al., 2018; Mingyang et al., 2022).

Conclusion

Twenty-five neonatal brain of humans were studied of its morphometry. the samples were distributed into two groups, group one were of 2 weeks, and the other was 15-28 day.

Measurements were applied using electron caliber, the present study reverted that the mean thickness of frontal lobe (3.67mm) of older age in comparison to younger age (3.05 mm). the whole lobes of brain indicated the presence of six layers of neonatal and glial cells of whole groups.

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