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STRUCTURAL ORGANIZATION OF THE CEREBELLUM OF 17-18 WEEK HUMAN FETUSES DURING INTRAUTERINE DEVELOPMENT

Abstract. *The study established micrometrical parameters of the cerebellar hemispheres and the brain worm in 17-18-week human fetuses, as well as structural organization, morphometric parameters of the cerebellum and morphology of the radial glia.*

Key words: *cerebellar hemispheres, the brain worm, morphometric parameters, fetal development, radial glia.*

Introduction. The study is conducted within the frame of the scientific-research work on the topic: "Detection of regularities of the organ- and histogenesis and topography of the internal thoracic and abdominal organs, the structures of the central nervous system in human fetuses (macroscopic, histological, immunohistochemical, and US-examination). Comparison of the findings obtained with the similar ones of fetuses with congenital developmental defects", State Registration № 0113U005070.

The functional system is evaluated as a unit of anatomical-physiological integration in every certain case combining various nervous systems and dynamics of nerve processes into one adjustment reaction. For example, the fetus is characterized by acceleration and selective development of those structures of the central nervous system and their functions essential for a newborn to perform various kinds of vital activity [4].

The cerebellum plays an important role in adaptation and setup of motor programs to make movements accurate by means of trial-and-error method (for example, learn to play baseball and other games requiring body movements). Although the cerebellum is more often considered from the point of view of its control over movements, it is also involved into certain cognitive functions, such as language. These functions of the cerebellum are not still studied so

well that they can be described in details. The recent years has been marked by an increasing interest of researchers to investigation of the central nervous system (CNS) of the fetus, and it is not accidentally, as now morbidity and mortality rates due to congenital cerebral defects occupies one of the leading positions among all other developmental defects in childhood. To our mind, one of the main reasons of such situation is untimely detection and difficulty in accurate differential diagnostics of a number of nosological forms of congenital cerebral defects of the fetus [1].

Therefore, in many countries of the world timely diagnostics (especially prenatal one), prevention and prognosis of this pathology are of a great priority. Within the span of human life the cerebellar structure undergoes qualitative and quantitative changes having a certain interest not only from the point of theoretical and practical aspects of medicine. They are important factors to understand age neuromorphology and changes in pathological conditions [1].

Vimentin has been found to be detected in embryogenesis in the cells of radial glia (neural stem cells) which are precursors of neuron, glioblasts, expressed during differentiation of neuroblasts [2].

In the germinal layer the cells are differentiated into neuroblasts and glioblasts and migrate into a reverse direction. The movement of cells through the layer of Purkinje cells is directed by radial

(Bergmann's) glia [8].

Due to this fact investigation of age and individual changeability of the cerebellar structure in prenatal human ontogenesis is of a considerable importance both for practical activity and theoretical design, because in spite of all the studies conducted rather many gaps remain unsolved.

Objective: to determine macrometric parameters of the cerebellar hemispheres and brain worm, as well as cytoarchitectonics and morphometric parameters of the cerebellar structures of human fetuses of 17-18 weeks of the intrauterine development.

Materials and methods.

Anatomical-histological, immunohistochemical and morphometric examinations of the cerebellar hemispheres and brain worm of 15 human fetuses with gestation period (GP) of 17-18 weeks have been conducted. The fetuses were obtained during late abortion at the Regional Pathological-Anatomical Bureau in Vinnytsia. Congenital defects of the CNS were not found. Parietal-coccygeal length (PCL) was $-136,0 \pm 6,7$ mm, mass $-230,0 \pm 10,1$ g (Fig.1). Sizes of the head:

transverse $-53,0 \pm 2,7$ mm, longitudinal $-57,0 \pm 2,9$ mm, height $-56,0 \pm 2,9$ mm. Sizes of the frontal sinciput: longitudinal $-22,0 \pm 1,0$ mm, transverse $-33,0 \pm 1,5$ mm. Sizes of the parietal sinciput: longitudinal $-16,0 \pm 0,8$ mm, transverse $-14,0 \pm 0,8$ mm. The material obtained was fixed in 10% neutral formaldehyde solution, then the cerebellum was filled in paraffin and celloidin blocks. After series of cerebellar sections 10-12 mcm thick were made, the specimens were stained with hematoxylin and eosin, toluidine blue and by Van Gieson's stain. During immunohistochemical examination diagnostic monoclonal antibodies were used manufactured by "DacoCytomation": vimentin, Ki-67 and synaptophysin.

To conduct morphometric examination the microscopes SIGETA and MBC-10 were used. Photofixation and morphometry of the sections obtained was made by means of the camera ETREK Ucmos and computer program ToupViem (computed histometry).

Macrometric parameters of the cerebellar hemispheres and brain worm were detected by means of our own methods developed [3].

The digital data obtained were statistically processed by means of the standard program package "Statistica 6.0" by the firm Statsoft.

Results and discussion. In the process of the study we have obtained the following macrometric parameters of the cerebellar hemispheres and brain worm: transverse size of the cerebellum $-18,0 \pm 0,7$ mm, left hemisphere: longitudinal size $-11,0 \pm 0,7$ mm, height $-7,0 \pm 0,4$ mm; transverse size $-7,0 \pm 0,4$ mm; right hemisphere: longitudinal size $-11,0 \pm 0,7$ mm; height $-7,0 \pm 0,4$ mm; transverse size $-7,0 \pm 0,4$ mm. Transverse size of the brain worm $-4,0 \pm 0,2$ mm; longitudinal size of the brain worm $-7,0 \pm 0,4$ mm; height of the brain worm $-6,0 \pm 0,5$ mm; cerebellum mass $-700,0 \pm 35,7$ mg. Transverse fissures are found on the surfaces of the cerebellar hemispheres and brain worms forming the layers of the cerebellum. A deep horizontal fissure is also found. It begins in the point where the middle peduncle enters the cerebellum (Fig. 1). The left and right cerebellar hemispheres and the brain worm are clearly visualized. Liu F. (2011) found that the primary cerebellar fissure of the human fetus is detected on the 14th week. Since

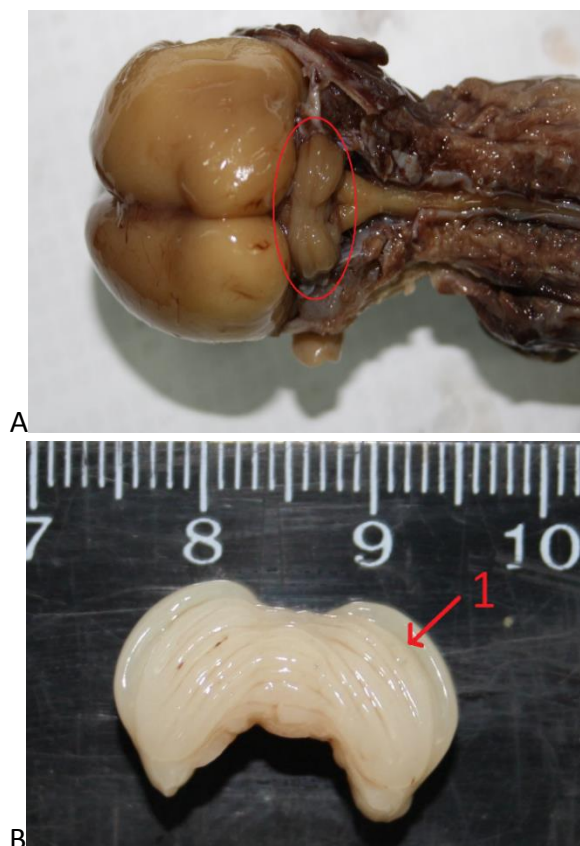


Fig. 1. Human fetus of 17-18 weeks of intrauterine development. PCL $-136,0$ mm. A-general view. B-cerebellum (upper surface): 1-horizontal fissure.

the 16th week the secondary fissure and dentate nucleus can be identified [6].

Yamaguchi K. (1997) states that after the 22nd week of the intrauterine development primary curves or fissures are found on the lateral surface of the cerebellar hemispheres. At the same time, curves are formed on all the surfaces since the 28-29th weeks [9].

Rakic P. (2004) characterized the layers of the cerebellum in the period from the 7th to 40th week of gestation. Till the 10th week proliferation of cells was limited by the ventricular zone. External granular layer is isolated separately in the 10-11th week, and Purkinje cells appear till the 13th week. In the 20-21st week they cross intermediate layer (future molecular layer). The 5th layer appears since the 32nd week. During examination of the histocytoarchitectonics of the cerebellar hemispheres and brain worm in this gestation term the following layers are clearly visualized: ventricular, intermediate and cerebellar cortex. Rakic P. (2004) differentiates the similar cerebellar layers [8].

Rakic P. (2004) describes that the cerebellum is formed at the expense of overgrowth of the dorsal-lateral wall of the neural tube in the area of the hindbrain. In the first weeks of the human development neuroblast migration of the matrix zone results in anlage of the nuclei and Purkinje cells. In the 9-11th weeks matrix stem cells are isolated from the ependymal layer and migrate (primary migration) on the surface of the cerebellum germ. They form external germinal layer there (till the 21st week of development its thickness is 6-9 cellular layers).

The study detected that histocytoarchitectonics of the cerebellar hemispheres in this gestation term is clearly visualized. On the horizontal section of the cerebellum the dentate nucleus is of a thin concave tape shape turned laterally and dorsally by its concave part. In the medial direction the outlines of the dentate nucleus are not closed. This place is termed the gate of the dentate nucleus. The three layers have been found: ventricular zone, intermediate zone, cortical zone, which in its turn is divided into the internal granular, intermediate, external granular layers (Fig. 2).

General thickness of all the cerebellar layers in the right and left hemispheres varies. Thus,

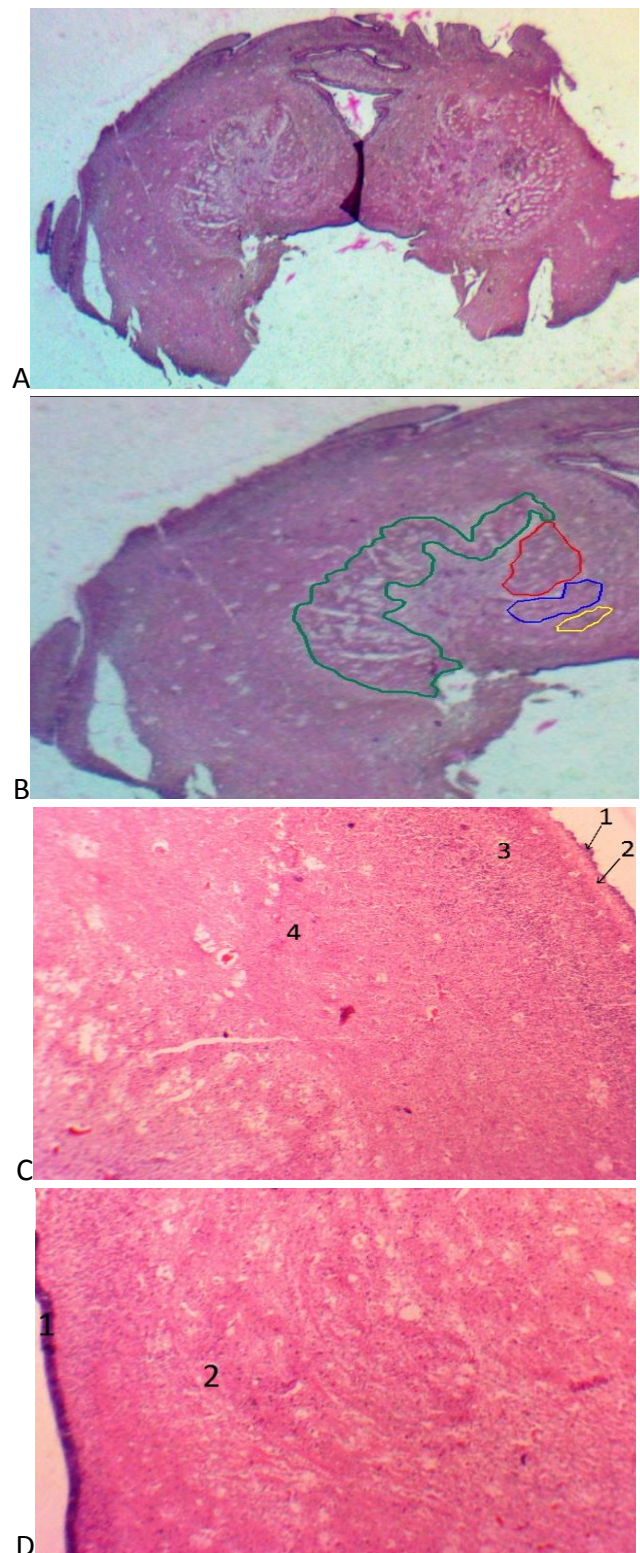


Fig. 2. Cerebellum of the human fetus 17-18 week of gestation. A – horizontal section of the cerebellum.

Hematoxylin-eosin; x6. B – nuclei of the right cerebellar hemisphere: dentate nucleus (green), crustate nucleus (red), spherical nucleus (blue), topical nucleus (yellow). Hematoxylin-eosin; x6. C – the right hemisphere: 1-external granular zone, 2-molecular zone, 3-internal granular zone, 4-intermediate zone. Hematoxylin-eosin; x40. D – the right hemisphere: 1-ventricular zone, 2-intermediate zone. Hematoxylin-eosin; x40.

general thickness of all the layers of the right hemisphere is $4189,7 \pm 234,6$ mcm, general thickness of the gray matter of the right cerebellar hemisphere – $447,3 \pm 21,9$ mcm, external granular – $28,3 \pm 1,3$ mcm, molecular – $56,0 \pm 2,7$ mcm, internal granular – $363,0 \pm 19,2$ mcm, intermediate zone – $3711,0 \pm 144,7$ mcm, ventricular zone – $31,4 \pm 1,8$ mcm. General thickness of all the layers of the left cerebellar hemisphere is $4056,2 \pm 229,4$ mcm, general thickness of the gray matter of the left cerebellar hemisphere – $441,1 \pm 20,8$ mcm, external granular – $27,2 \pm 1,2$ mcm, molecular – $55,4 \pm 2,6$ mcm, internal granular – $358,5 \pm 18,7$ mcm, intermediate zone – $3584,0 \pm 193,5$ mcm, ventricular zone – $31,1 \pm 1,8$ mcm. General square of the right dentate nucleus was $0,25 \pm 0,01$ mm², general square of the left dentate nucleus was – $0,26 \pm 0,01$ mm². General square of the right crustate nucleus was $0,05 \pm 0,002$ mm², the left one – $0,05 \pm 0,002$ mm². General square of the right spherical nucleus was $0,03 \pm 0,001$ mm², the left one – $0,03 \pm 0,001$ mm². General square of the topical right nucleus was $7875,3 \pm 417,4$ mcm², the left one – $8291,8 \pm 406,3$ mcm².

The highest density of the neutral stem cells (NSC) was detected in the ventricular zone of all the structures of both hemispheres and it was $18,3 \pm 8,4$ cells per $0,01$ mm². In the external granular layer (neurons and glial cells) – $14,0 \pm 0,5$ cells per $0,01$ mm². In the intermediate zone – $6,3 \pm 0,2$ cells per $0,01$ mm². In the internal granular layer – $9,0 \pm 0,3$ cells per $0,01$ mm². The least density of cells was visualized in the molecular zone – $3,7 \pm 0,1$ cells per $0,01$ mm².

Therefore, to our mind, investigation of the cellular growth in the cerebellum of human embryos and fetuses is of a great importance as it can be used for evaluation of the cortical growth and appearance of the cerebellar nuclei in the white matter of the cerebellum, non-invasive studies and improve the analysis of embryonic disorders of the cerebellum.

During the use of proliferation protein Ki-67 more intensive proliferation of cells in the ventricular zone of the cerebellum and less intensive in the white matter were found (Fig. 3). Acton A. (2012) describes that in the studies performed by means of immunocytochemical marker of the proliferative protein Ki-67 in the

cerebellum of the human fetus in the term of gestation of 17-21 weeks more intensive proliferation of cells was found in the ventricular zone and external granular layer [5].

During the use of radial glia marker we have found that fibers of the radial glia begin from the ventricular zone, penetrate all the zones of the cerebellum in the radial direction and finish in the external granular layer. Vimentin expression in the fibers of radial glia was found to be relatively moderate in the external granular and intermediate zones, and relatively strong in the ventricular and internal granular zones. An average length of the short radial glia fibers was $179,5 \pm 8,4$ mcm, and the long ones – $284,3 \pm 13,1$ mcm (Fig. 3).

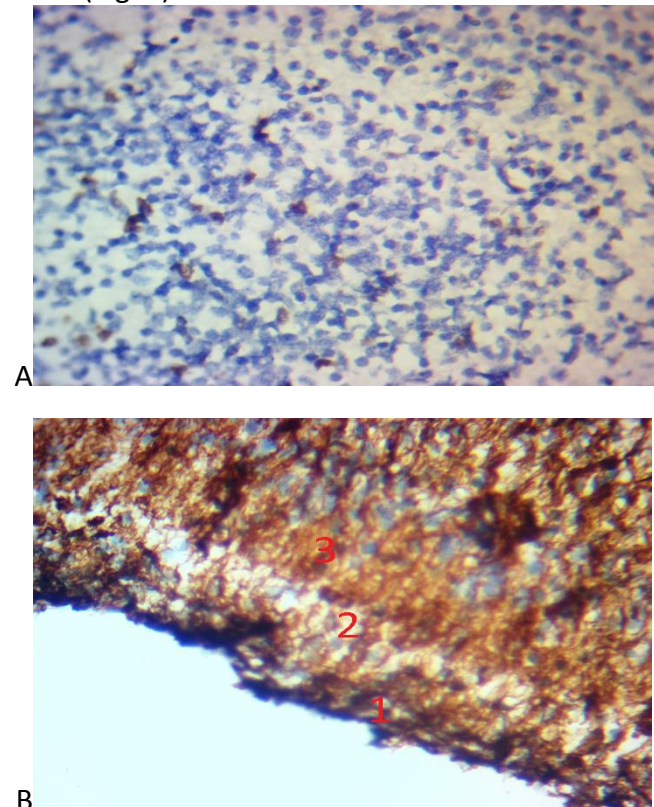


Fig. 3. A – proliferation of glioblasts in the cerebellar hemispheres (brown) Ki-67; $\times 400$. B – cerebellar hemisphere. 1-external granular zone, 2-molecular zone, 3-intermediate zone. Vimentin; $\times 400$

While examining expression of synaptophysin the expression of the cells was found in all the layers of the cerebellum at this age (Fig. 4). Our studies correspond to the investigations conducted by Nag T.C. (2001). The author indicates that expression of synaptophysin cells was found in all the zones of the cerebellum since the 16th week of the intrauterine development [7].

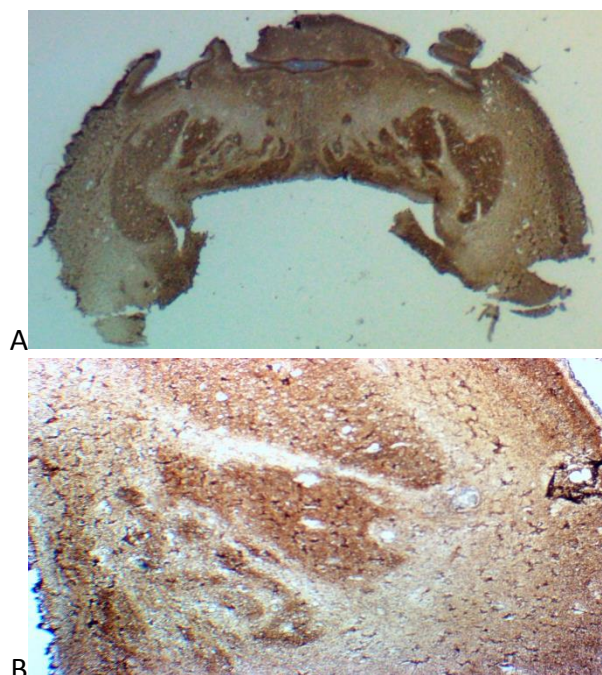


Fig. 4. A – cerebellar hemisphere. Synaptophysin; $\times 6$.
B - Synaptophysin; $\times 100$.

Therefore, in the process of investigation we have detected macrometric parameters of the cerebellar hemispheres, peculiarities of cytoarchitectonics and morphometric parameters of the cerebellar hemisphere structures of the human fetuses in the 17-18th weeks of intrauterine development.

Conclusions.

1. In the 17-18th weeks on the upper and lower surfaces of the cerebellar hemispheres and brain worm transverse fissures are found passing through the hemispheres and worm forming the layers of the cerebellum. A deep horizontal fissure is formed.

2. Three layers are clearly visualized in the cerebellar hemispheres: ventricular layer, intermediate layer and cortical layer. The highest density of the neural stem cells was found in the ventricular zone – $18,3 \pm 8,4$ cells per $0,01 \text{mm}^2$. The least density of cells was found in the molecular zone – $3,7 \pm 0,1$ cells per $0,01 \text{mm}^2$. The highest thickness was found in the intermediate zone – $3711,0 \pm 144,7 \text{mcm}$, the least thickness was found in the external granular layer $28,3 \pm 1,3 \text{mcm}$.

3. The biggest proliferation of cells was found in the ventricular cerebellar zone, the least intensive proliferation was detected in the intermediate zone. Expression of synaptophysin was found in all the layers of the cerebellum.

4. The fibers of the radial glia begin from the

ventricular zone and finish in the external granular layer. Vimentin expression in the fibers of radial glia was found to be relatively moderate in the external granular and intermediate zones, and relatively strong in the ventricular and subventricular zones. An average length of the short radial glia fibers was $179,5 \pm 8,4 \text{mcm}$, and the long ones – $284,3 \pm 13,1 \text{mcm}$.

Prospects of further studies. Further studies suggest detection of regularities of the location of the white and gray matter of the human cerebellum in the prenatal period using immunohistochemical methods.

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