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## **MORPHOMETRIC PARAMETERS OF THE STRUCTURES OF THE MEDULLA OBLONGATA OF HUMAN FETUSES WITH SACROCOCYGEAL TERATOMA AT 17-18 WEEKS OF PRENATAL DEVELOPMENT**

**Abstract.** *The results of the study of morphometric parameters and structure of medulla oblongata in human fetuses at 17-18 weeks of prenatal development with sacrococcygeal teratoma are presented. The dimensions of nuclei of medulla oblongata, shapes and the degree of differentiation of neurons have been measured.*

**Keywords:** *teratoma, morphometric parameters, medulla oblongata, nuclei of medulla oblongata.*

**Introduction.** Teratomas are considered as tumors that develop from pluripotent cells and are represented by a wide range of tissues, which are not specific to the organ or body part [4].

The tumor is formed at the early stages of embryogenesis as a result of chromosomal abnormalities when the cells of the germ layers migrate to an area, which is atypical for normal development.

The most common teratomas in fetuses and newborns are the ones in the area of coccygeal and sacral bones. These tumors are 40% of all diagnosed tumors. Teratomas in the neck, in the ovaries or testes, in the brain, mediastinum and retroperitoneal space are less common. The incidence of sacrococcygeal teratomas (SCT) is 1 case per 40,000 births. The incidence of the tumors in girls is 4 times higher than the one in boys [14]. In 5-26% of cases teratomas are combined with other malformations. Thus, SCT growth and its immersion between the layers of cloacal membrane can lead to the formation of anorectal malformations in the form of urinary rectal fistulas and cause division of the scrotum and hypospadias. Front location of the tumor promotes the atresia of the anal canal and rectum [10]. In addition, teratomas are combined with the spinal dysraphia, agenesis of the sacral bone, meningocele [9]. Malformations of the spine were found in 80% of 45 patients with benign SCT. In isolated cases there are malformations of the heart in the form

of the interventricular septum defect and malformations of the digestive tube [6]. Structural organization of the white and gray matter of the segments of the spinal cord in fetuses with teratoma is described in detail in the works of V.S. Shkolnikov [3].

There are no data on the structure of the brain in fetuses with sacrococcygeal teratomas in the available scientific literature. Therefore, this problem requires more in-depth and detailed study of morphometric parameters of parts and structures of the brain in fetuses with this malformation.

**Objective:** to study morphometric parameters of medulla oblongata in human fetuses at 17-18 weeks of prenatal development, measuring the sizes and areas of nuclei of cranial nerves and neurons forming nuclei of medulla oblongata.

**Materials and methods.** The anatomical and histological study of the medulla oblongata of 2 female human fetuses with sacrococcygeal teratoma at 17-18 weeks of prenatal development has been performed. The crown-rump length was  $165,0 \pm 2,3$  mm, the weight was  $385,8 \pm 9,4$  g (Fig. 1).

Material for the study was obtained as a result of late abortions in the Vinnytsya Regional Pathoanatomical Office with the following fixing in 10% neutral formalin solution. From the celloidin and paraffin blocks serial horizontal sections of the medulla oblongata of 10-15 microns thick were performed. The samples



**A** **B**  
 Fig. 1. General view of human fetus with the teratoma of the sacrococcygeal spine. Gestational age (GA) – 17-18 weeks. A – side view. B – back view

were stained with hematoxylin-eosin, toluidine blue and by van Ghisoni, also silver impregnation was performed by Bilshovskiy.

Obtained samples were studied visually using microscopes Unico G380, MBS-9, the video capture was performed by camera Trek. For morphometric study computer histometry (ToupView) was used. Digital data were processed statistically.

Materials of the study do not contravene the fundamental bioethical norms of WMA Declaration of Helsinki adopted by the 59th World Medical Association in 2008.

**Results and discussion.** The primary olivary nuclei have the form of winding toothed plate (Fig. 2). The area of the right primary olivary nucleus in fetuses with teratoma is  $2,04 \pm 0,05 \text{ mm}^2$ , of the left –  $2,22 \pm 0,05 \text{ mm}^2$ . Area of the right medial accessory olivary nucleus is  $0,12 \pm 0,003 \text{ mm}^2$ , of the left one –  $0,14 \pm 0,003 \text{ mm}^2$ . The area of the dorsal right accessory olivary nucleus is  $0,14 \pm 0,003 \text{ mm}^2$ , and of the left one –  $0,16 \pm 0,003 \text{ mm}^2$ .

Neurons of the inferior olive are of oval or spherical shape with homogeneous eosinophilic cytoplasm and rounded basophilic nucleus (Fig. 3A). The average values of the areas and sizes of all the olivary neurons (main, medial and dorsal) are the same and are equal to  $47,8 \pm 1,7 \mu\text{m}^2$  and  $8,6 \pm 0,3 \times 7,8 \pm 0,2 \mu\text{m}$  accordingly. The area of the nucleus of the neuron is  $27,5 \pm 0,9 \mu\text{m}^2$  and its dimensions are  $6,2 \pm 0,2 \times 5,2 \pm 0,1 \mu\text{m}$ .

The process of differentiation of neurons

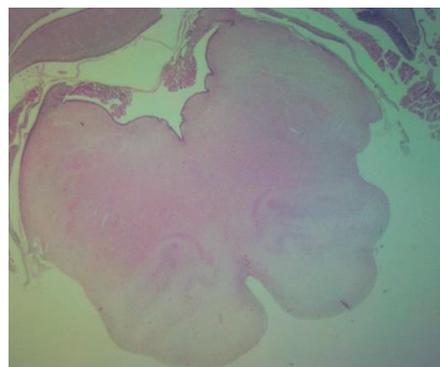


Fig. 2. The horizontal section of the medulla oblongata at the middle of the olive of the human fetus with teratoma. GA 17-18 weeks. Stained with hematoxylin-eosin. Magnification  $\times 10$

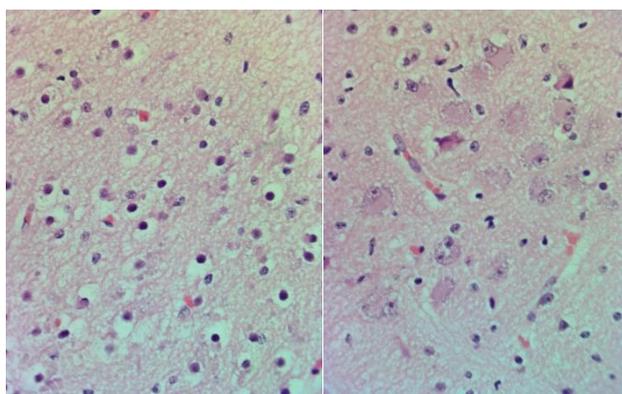
during the ontogenetic development from the early stages of the prenatal ontogenesis to the definitive age is accompanied by an increase in their size, formation of axon and mediators, development of interneuron communications and other structural and functional changes [1, 5].

Studies by L.D. Starlychanova [2] performed on the brains of 32 human fetuses and the ones by B. Narasinga Rao [12] performed on the brains of 25 fetuses of different gestational age, have found that the nucleus of inferior olive in fetuses of up to 20 weeks gestation are presented by poorly differentiated neurons of rounded and oval shapes, which is reaffirmed by our research.

Dual nucleus in fetuses with teratoma is located in a typical place of the medulla oblongata, which is more dorsally from the accessory dorsal olivary nucleus, or has an irregular oval shape. Its right area is  $0,06 \pm 0,002 \text{ mm}^2$ , the left one is  $0,04 \pm 0,001 \text{ mm}^2$ .

The nerve cells of the dual nucleus have irregular oval shape (Fig. 3B). The average area of a neuron is  $301,2 \pm 9,8 \mu\text{m}^2$ , its dimensions are  $20,2 \pm 0,6 \times 18,1 \pm 0,5 \mu\text{m}$ . In the neurons of the dual nucleus the nucleus with basophilic nucleolus and heterogeneous patches of chromatin is visualized. The area of the nucleus of a neuron is  $80,14 \pm 2,2 \mu\text{m}^2$  and its dimensions are  $10,0 \pm 0,3 \times 9,5 \pm 0,3 \mu\text{m}$ .

The nucleus of the hypoglossal nerve in fetuses with teratoma of 17-18 weeks of prenatal development is located in the medulla oblongata slightly posterior the midline at the bottom of the IV ventricle. It has no clear

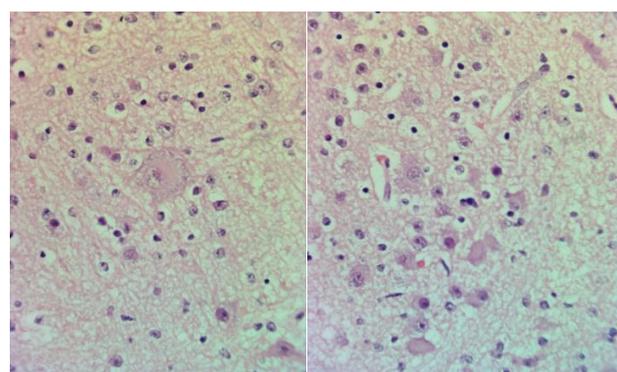


A.

B.

*Fig. 3. Neurons and glial cells in human fetus with teratoma. GA 17-18 weeks. A. Cells of the olivary nucleus. B. Cells of the dual nucleus. Stained with hematoxylin-eosin. Magnification×400*

contours and is presented by 2-6 neurons of various sizes (Fig. 4A). Thus, the area of a neuron is from  $205.2 \mu\text{m}^2$  to  $1021.4 \mu\text{m}^2$ , the area of a neuron nucleus is from  $30.2 \mu\text{m}^2$  to  $158.2 \mu\text{m}^2$ .



A.

B.

*Fig. 4. Neurons and glial cells in human fetus with teratoma. GA 17-18 weeks. A. Cells of the nucleus of the hypoglossal nerve. B. Cells of the dorsal nucleus of the vagus nerve. Stained with hematoxylin-eosin. Magnification×400*

The average area of a neuron is  $612,7 \pm 18,1 \mu\text{m}^2$ , its dimensions are  $30,72 \pm 0,9 \times 25,32 \pm 0,7 \mu\text{m}$ . The area of a neuron nucleus is  $85,4 \pm 1,6 \mu\text{m}^2$ , and its dimensions are  $10,1 \pm 0,2 \times 9,3 \pm 0,2 \mu\text{m}$ .

The main structural organization of the motor nuclei of the medulla oblongata is set in fetuses of 8-9 weeks of prenatal development. The cells with characteristics of mature neurons appear in the motor nuclei in 9-weeks fetuses, with the following gradual increase in their number and formation of an integral nucleus with differentiated nerve cells. The dual nucleus obtains its final organization and localization on

the 12<sup>th</sup> week, and the hypoglossal nerve nucleus – on the 13<sup>th</sup> week of gestation [7, 13]. Therefore, the arrangement of the motor nuclei in the medulla oblongata in fetuses of 17-18 weeks is the same to the one in adult and the motor nuclei is presented by differentiated neurons, which also confirmed by our research. Significantly different sizes of neurons of nuclei of hypoglossal nerve in fetuses of 17-18 weeks of prenatal development are not described in the available scientific literature. Also there are no data on this fact in fetuses with sacrococcygeal teratoma.

The dorsal nucleus of the vagus nerve is located near the bottom of IV ventricle of the caudal medulla oblongata some dorsally and laterally than the nucleus of the hypoglossal nerve, and in the medial medulla oblongata it is located more laterally than the nucleus of the hypoglossal nerve. The right and left dorsal nuclei of the vagus nerve in fetuses of 17-18 weeks teratoma have irregular oval shape and fuzzy contours, they consist of two additional nuclei (dorsal, ventral). The right area of the nucleus is  $0,11 \pm 0,003 \text{ mm}^2$ , and the left area is  $0,07 \pm 0,003 \text{ mm}^2$ . The dorsal nucleus of the vagus nerve is formed by the nerve cells that are irregular pear-shaped or oval-shaped (Fig. 4B). The average area of the neuron is  $282,3 \pm 8,4 \mu\text{m}^2$ , its dimensions are  $18,7 \pm 0,5 \times 16,4 \pm 0,4 \mu\text{m}$ . The average area of the nucleus of the neuron is  $64,01 \pm 1,8 \mu\text{m}^2$ , its dimensions are  $9,8 \pm 0,2 \times 8,5 \pm 0,2 \mu\text{m}$ .

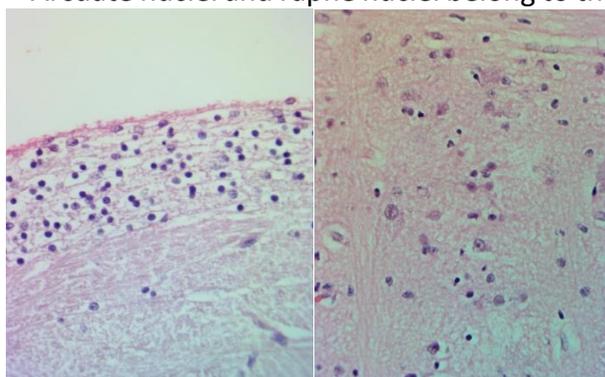
Structural changes in the localization of dorsal nucleus of the vagus nerve starts on the 13<sup>th</sup> week. In this gestational age the nucleus is represented by two accessory nuclei. Three accessory nuclei (caudal, dorsal, ventral) are clearly visible starting from 15<sup>th</sup> week of prenatal development. Major structural changes in the dorsal nucleus of the vagus nerve are completed to the 21<sup>th</sup> week, although the morphological differentiation of neurons lasts throughout all the period of fetal development [11, 8]. In our study, the dorsal nucleus of the vagus nerve is represented by two instead of three accessory nuclei, which contradicts the results of known scientific research.

Arcuate nucleus at the intersection of

pyramids is located in front of and more laterally to the pyramids, at the lower edge of the olive it is located in front of the pyramids, at the middle of the olive - in front of and more medially to the pyramids. It has a shape elongated plate. The right area of the nucleus is  $0,31 \pm 0,009 \text{ mm}^2$ , and the left one is  $0,29 \pm 0,008 \text{ mm}^2$ . The nucleus is represented by small spherical-shaped neurons (Fig. 5A). The average area of the neuron is  $21,2 \pm 0,6 \text{ }\mu\text{m}^2$ , and its dimensions are  $4,7 \pm 0,1 \times 5,2 \pm 0,1 \text{ }\mu\text{m}$ .

The raphe nuclei in fetuses with sacrococcygeal teratoma are located in the typical location of the medulla oblongata. The area of the nucleus raphes obscurus is  $0,4 \pm 0,01 \text{ mm}^2$ , the one of the nucleus raphe pallidus is  $0,1 \pm 0,003 \text{ mm}^2$ . These nuclei are represented by rounded-shaped neurons (Fig. 5B). The average area of the neuron is  $58,3 \pm 1,7 \text{ }\mu\text{m}^2$ , and its dimensions are  $9,2 \pm 0,2 \times 8,7 \pm 0,2 \text{ }\mu\text{m}$ .

Arcuate nuclei and raphe nuclei belong to the



A.

B.

**Fig. 5.** Neurons and glial cells in human fetus with teratoma. GA 17-18 weeks. A. Cells of the arcuate nucleus. B. Cells of the raphe nuclei. Stained with hematoxylin-eosin. Magnification  $\times 400$

caudal serotonergic system. Serotonergic neurons appear in 5-7-week embryo. On the 7<sup>th</sup> week the neurons form two discs on both sides of the midline with the following development of the nucleus raphes obscurus. Up to 20<sup>th</sup> week of pregnancy the nuclei of the caudal serotonergic system have their typical location in the medulla oblongata. In fetuses starting from the 20<sup>th</sup> week and in newborns the number of spherical neurons decreases and the content of spindle neurons increases, also the processes of their differentiation occur [15].

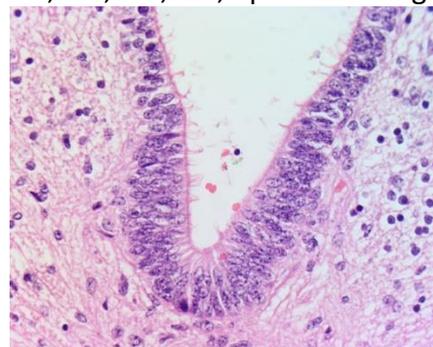
The nuclei of the caudal serotonergic system in our study in fetuses of 17-18 weeks of

prenatal development with sacrococcygeal teratoma are located in their typical places in the medulla oblongata and are presented by spherical undifferentiated nerve cells.

The solitary nucleus and spinal nucleus of the trigeminal nerve have not clear contours. These nucleus are presented by spherical and spindle single neurons. The average area of the neuron of the solitary nucleus is  $59,7 \pm 1,5 \text{ }\mu\text{m}^2$ , its dimensions are  $7,9 \pm 0,1 \times 7,3 \pm 0,1 \text{ }\mu\text{m}$ . The area of the nucleus of the neuron is  $21,1 \pm 0,6 \text{ }\mu\text{m}^2$ , its dimensions are  $5,1 \pm 0,1 \times 4,2 \pm 0,1 \text{ }\mu\text{m}$ . The average area of the neuron of the spinal nucleus is  $86,2 \pm 2,2 \text{ }\mu\text{m}^2$ , its dimensions are  $10,1 \pm 0,2 \times 8,7 \pm 0,2 \text{ }\mu\text{m}$ .

The solitary nucleus and spinal nucleus of the trigeminal nerve start to form on the 13<sup>th</sup> week of the prenatal development. Major structural changes and increase in the number of nerve cells in the nucleus of solitary tract occur from 21<sup>st</sup> to 25<sup>th</sup> weeks of gestation. [7] In our study the solitary nucleus has no clear contours and is presented by poorly differentiated neurons.

The neuroepithelial layer is formed by the neural stem cells (NSC) of spherical and ellipsoidal shape, which are located on the basal membrane (Fig. 6). The average area and dimensions of NSC of ellipsoidal shape are equal to  $41,5 \pm 1,6 \text{ }\mu\text{m}^2$  and  $10,7 \pm 0,4 \times 5,1 \pm 0,1 \text{ }\mu\text{m}$  accordingly. The average area and dimensions of NSC of spherical shape are equal to  $32,6 \pm 1,1 \text{ }\mu\text{m}^2$  and  $7,3 \pm 0,2 \times 6,7 \pm 0,3 \text{ }\mu\text{m}$  accordingly.



**Fig. 6.** NSC of the neuroepithelial layer in human fetus with teratoma. GA 17-18 weeks. Stained with hematoxylin-eosin. Magnification  $\times 400$

The density of NSC in the ventral neuroepithelium is considerably larger than the one in the lateral and dorsal parts. In addition, the highest frequency of ellipsoidal NSC is in the ventral part, and the highest frequency of

spherical NSC is in the lateral and dorsal parts. The thickness of the neuroepithelium in the ventral, lateral and dorsal parts is nearly the same and in average is equal to  $39,7 \pm 2,1 \mu\text{m}$ .

**Conclusions.** 1. In the samples of the medulla oblongata of human fetuses at 17-18 weeks of prenatal development with sacrococcygeal teratoma all the neural systems are clearly visualized and identified.

2. The group of motor neurons has the largest area. There are heterogeneous by size neurons in the neural systems that form the nucleus of hypoglossal nerve.

3. The dorsal nucleus of the vagus nerve is represented by two accessory nuclei which is not typical for this gestational age.

4. The neuron systems forming the inferior olive, sensitive nuclei of cranial nerves and nuclei of serotonergic system of medulla oblongata are presented by poorly differentiated neurons.

5. The highest density of neural stem cells is in the ventral part of the neuroepithelium. It was established that the ventral neural epithelium is mainly represented by ellipsoidal NSC, and the lateral and dorsal ones – by NSC spherical.

**Perspectives of further investigations.** In prospect it is planned to determine the topography of neurons and glia cells of the medulla oblongata in fetuses with teratoma by expression of immuno-histochemical markers and to compare the results with those in fetuses without malformations.

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