Morphological features of the pons in human fetuses 14-15 weeks of intrauterine development
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Knowledge of human embryonic development is essential to improve our understanding of human fetal anatomy and to help understand the etiology of congenital malformations [3, 13, 29].

In the conditions of a difficult demographic situation in Ukraine, the problem of saving newborns is becoming more and more urgent. Environmental pollution, non-observance of a healthy lifestyle, unbalanced nutrition leads to the development of birth defects, sometimes to disability and death of newborns [5, 17, 18, 23].

Timely detection of a birth defect allows you to make a decision to terminate a pregnancy or prepare for the birth of a sick child. Early prenatal diagnosis allows adequate monitoring of the course of pregnancy, childbirth and the neonatal period. Modern methods of analysis make it possible to investigate placental insufficiency - the cause of fetal development delay, intrauterine hypotrophy and hypoxia, and ultimately - the possibility of intrauterine death.
of the fetus [22, 28].

Most often, congenital malformations of the brain were diagnosed before the 22nd week of intrauterine development, which were the result of a violation of the main processes of brain development - the formation of the neural tube, the division of its cranial division into paired formations, migration and differentiation of nerve cell elements [4, 11].

According to some studies [7, 21, 27], at the 7th week of pregnancy, the development of the pons as a derivative of the metencephalon is observed, at the 12th week of intrauterine development, the differentiation of the basilar and tegmental parts of the pons occurs, at the 14th week, migrating neurons are identified in the basilar part of the pons, which form pons nuclei among the white matter, at the 18th week of gestation in the pons cover, the nuclei of the abducutor and facial nerves are identified, at 28 weeks, formed cells are observed in the nucleus of the abducens nerve, at 32 weeks of intrauterine development, multipolar cell complexes that form the nuclei of the abducutor and facial nerves increase, at 36 weeks, the size of neurons increases, they have clearly defined nuclei and nucleoli.

In the 2nd trimester of pregnancy, the organ of hearing begins to develop in human fetuses. R. Lim and A. Brichta [15] described the development of the human hearing organ with intussusception of the ear sac at 4 weeks of gestation, the growth of the semicircular canals from 5 weeks and the formation of the auricle from 10 weeks of gestation. A number of authors proved that from the 12th week, the vestibular node of the vestibulocochlear nerve was gradually filled with glial cells, and in the cochlear nerve, at the 22nd week, the peripheral processes began to be myelinated, which suggests that the maturation of peripheral glial cells in the hearing organ of the human fetus begins its development from 9 to 22 weeks of gestation [10, 16, 19].

Embryonic development of the pons, according to literary sources of foreign and domestic scientists, was studied on animals, which could not always be extrapolated to humans [14]. Currently, the structures of the posterior cranial fossa and brain stem are of great interest to researchers, because a large number of nuclei are located in the pons area, which play an important role in ensuring vital functions [1].

The aim of the scientific work is to establish the morphological features of the pons of human fetuses at 14-15 weeks of gestation, the size and area of the nuclei of cranial nerves and neurons located in the pons area.

Materials and methods

The work was performed as part of the SRW of the Department of Human Anatomy, National Pirogov Memorial Medical University, Vinnytsya “Establishment of patterns of organ and histogenesis and topography of internal organs of the thoracic and abdominal cavities, as well as structures of the central nervous system of human fetuses (macroscopic, histological, immunohistochemical and ultrasound examination)".

The study was approved at the meeting of the Biomedical Ethics Committee of the National Pirogov Memorial Medical University, Vinnytsya (Excerpt from the minutes of the meeting of the Bioethics Committee of the National Pirogov Memorial Medical University, Vinnytsya No. 10 dated November 23, 2017), in compliance with the basic provisions of GCP (1996), Convention on the Protection of Human Rights and Dignity in Connection with the Application of Advances in Biology and Medicine (1996). The research materials do not contradict the basic ethical norms of the Declaration of Helsinki on the ethical principles of conducting scientific and medical research involving human subjects, adopted by the 59th General Assembly of the World Medical Association in 2008.

Anatomical and histological research was performed on 6 human fetuses aged 14-15 weeks of gestation. The average fetuses weight was 93.30±4.66 g, parieto-occipital length - 118.0±5.9 mm.

The cadaver material for the study was obtained as a result of late abortions according to medical indicators from the maternity hospitals of the city of Vinnytsya, Ukraine.

The weight of the fetus and the weight of the pons were determined using electronic scales. According to the method of Arvandilov G. G. [2], with the help of a ShC-125 caliper, the dimensions of the head were determined, the height of the pons (sulcus bulbopontinus from below and the legs of the midbrain from above), the width of the pons (the most prominent point from one angulus pontocerebellaris to the other), the thickness of the pons (the most prominent point from the posterior surface to the anterior surface of the pons). The obtained pons preparations from human fetuses were fixed in a 10% solution of neutral formalin according to our own method. Subsequently, serial sections of the pons were made from paraffin and celloidin blocks with a thickness of 8-9 microns. Preparations were stained with hematoxylin-eosin, toluidine blue modified by Nissl. Microscopic research was carried out using the MBS-9 microscope and the Euromex iScope series and the Euromex Microscope camera B. V. DC. 1359 F100 using the following magnifications: x1, x4, x10, x40, x100, x400. Computer histometry (Toup View) was used for morphometric research.

Statistical digital data were processed on a personal computer using Microsoft Excel 2016 and "Statistica 6.1" software (license number BXXR901E246122FA).

Results

In human fetuses at 14-15 weeks of gestation, the following head sizes are established: circumference - 110.6±5.5 mm, height - 44.60±2.23 mm, transverse size - 30.91±1.54 mm, longitudinal size - 38.58±1.93 mm. The size of the front fontanelle: longitudinal - 22.00±1.10 mm, transverse - 19.17±0.96 mm. The size of the posterior fontanelle: longitudinal - 10.71±0.53 mm, transverse - 11.10±0.55 mm.

Macrometric dimensions of the pons: height - 6.75±0.337
mm, thickness - 6.354±0.318 mm, width - 9.152±0.458 mm. The weight of the pons was 4.757±0.238 g.

On the histological preparation of the pons, the roof, the base, and the nucleus of the trapezoidal body are clearly defined at the boundary between them by the gestation period of 14-15 weeks.

The boundaries of the nucleus of the trigeminal nerve in human fetuses at 14-15 weeks of fetal development are unclear. The nucleus has an oval, somewhat elongated shape, with an area of 0.024±0.002 mm². Neurons of the nucleus are single, most of them are represented by neuroblasts (Fig. 1).

The nucleus of the abducens nerve is located near the cavity of the fourth ventricle on the posterior surface of the pons. The boundaries of the nucleus are unclear. The nucleus is elongated and has a rounded shape. The core area is 0.183±0.009 mm². Neurons are unformed, almost identical, spherical or oval in shape. The area of neurons is 35.29±1.76 μm². Nerve cells with a large round nucleus, in which a dense basophilic nucleolus and a homogeneous eosinophilic cytoplasm are defined. The vestibular nuclei of the vestibulocochlear nerve are located in the posterior-lateral area of the pons preparation. The nucleus has no clear boundary, it is just beginning to form, the area of the nucleus is 0.053±0.002 mm². Neurons are at the stage of differentiation, their number is small, but they already have processes. The average value of the neuron area is 140.1±7.0 μm². The area of the cell nucleus is on average 60.1±3.01 μm². The nucleoleus and chromatia are clearly visualized in the nuclei.

The cochlear nuclei of the vestibulocochlear nerve are located above the vestibular nuclei in the lateral part of the pons preparation. The nucleus has an irregular oval shape, the boundaries of the nucleus are not clear. Its area is 0.014±0.001 mm² (Fig. 2). Neurons are immature, rounded in shape, and do not have appendages. The sizes of the nuclear cells are almost the same, represented by poorly differentiated nerve cells of a spherical shape with a nucleus in which a dense basophilic nucleolus and a homogeneous eosinophilic cytoplasm are noted. The area of the neuron is 113.8±5.7 μm². The area of the cell nucleus is 36.01±1.80 μm² on average.

Discussion

According to Hamano S. I. et al. [6], as well as Hossain M. I. et al. [8], who studied the development of the nuclei of the trigeminal nerve, at the 12th week of gestation, the nuclei have not yet been determined. In our study of human fetuses of 14-15 weeks of gestation, vague, slightly elongated, oval-shaped borders of the trigeminal nerve nucleus with single neurons were found.

Neurons of the main nucleus of the trigeminal nerve in terms of cell location, number of Nissl bodies, and neuron morphology approached adult neurons at approximately 33 weeks of gestation [24]. In their work, Lavezzi A. M., Mehboob R. and Matturri L. [12] pointed out that the structures of the human trigeminal nucleus are still poorly studied. But the trigeminal nerve has a functional significance in the early phases of the development of the central nervous system and in the regulation of autonomic functions.

K. Yamaguchi and K. Honma [30] indicated that the nucleus of the abducens nerve was identified at 20 weeks. Core neurons were clearly distinguished from glial cells by their clear nuclei. The cells of the nuclei of neurons had different shapes and sizes, larger neurons were located in the center of the nucleus. At the same time, Padmini M. P. and Rao B. N. [21] proved that the nucleus of the abductor nerve was identified at the 18th week of gestation in the covering of the pons. According to our research, the
boundaries of the nucleus of the abducens nerve at 14-15 weeks of gestation are unclear, the neurons are not formed, and are almost the same in size.

A morphometric study by Rao B. N. and Padmini M. P. [25] showed that primitive migrating cells of the nucleus of the facial nerve settle down at 10 weeks of fetal development, and rounded neuroblasts form at 12-18 weeks. Our results confirm the results of this study that from 12 to 18 weeks of gestation, the nuclei of the facial nerve are formed by rounded neuroblasts.

S. Jang et al. [9] studied the development of the vestibular nuclei of the vestibulocochlear nerve in human fetuses of different gestational periods. Morphometric analysis of the study showed that vestibular nuclei neurons differed from glia only after 16 weeks of gestation, cytoarchitectonically divided into medial and lateral at 21 weeks. When examining human fetuses at the stage of 14-15 weeks, we established that the vestibular nuclei of the vestibulocochlear nerve are just beginning to form, the nucleus without a clear boundary, the nucleolus and chromatin are clearly visualized in the nuclei.

S. Saini et al. [26] in their study indicated that the cochlear nuclei can be identified at 10 weeks of fetal development, and dorsal and ventral cochlear nuclei can be identified at 16 weeks. A sudden jump in the growth of the total area of nuclei, the number of neurons and astrocytosis occurs at week 18, and at week 22, an increase in proliferation and apoptosis is observed. In his scientific work, Mishra S. et al. [20] concluded that the morphological and functional maturation of the cochlear nuclei of the vestibulocochlear nerve in humans occurs simultaneously in the middle of pregnancy, which is a critical period of development, and lasts until the 37th week of gestation. Data from the scientific work of Saini S. et al. [26] also confirm the results of our study that at 14-15 weeks of intrauterine development in human fetuses, irregular oval-shaped cochlear nuclei of the vestibulocochlear nerve with immature neurons are determined.

Thus, in human fetuses of 14-15 weeks of intrauterine development, differences in the sizes and areas of cranial nerve nuclei and neurons that form nuclei in the pons area were found.

In the future, further studies plan to establish patterns of expression of immunohistochemical markers in the prenatal period of human ontogenesis.

Conclusions

1. In human fetuses at 14-15 weeks of intrauterine development, we found the nuclei of the trigeminal, abducens and vestibulocochlear nerves. The nucleus of the facial nerve at the stage of 14-15 weeks of gestation was represented by single polygonal nerve cells with eosinophilic cytoplasm.

2. The nucleus of the abductor nerve had the largest area, the cochlear nuclei of the vestibulocochlear nerve had the smallest area.

3. The largest area of neurons was in the vestibular and cochlear nuclei of the vestibulocochlear nerve, the smallest - in the nuclei of the abducens and facial nerves.

4. At 14-15 weeks of intrauterine development of human fetuses, the area of the cell nucleus could be determined only in the nuclei of the vestibulocochlear nerve, while the cell nuclei of the trigeminal, facial, and abducens nerves were represented by spherical nerve cells with a nucleus in which a basophilic nucleus and a homogeneous eosinophilic cytoplasm.

References


з еозинофільною цитоплазмою. Найбільшу площу мало ядро відвідного нерву, найменшу - завиткове ядро присінково-завиткового нерву. Найбільша площа нейронів була у ядрах присінково-завиткового нерву, найменша - у ядрах відвідного та пицевого нервів. На 14-15 тиждів внутрішньоутробного розвитку плодів людини площу ядра клітини можна було визначити лише у ядрах присінково-завиткового нерву, тоді як ядра клітин у трійчастого, пицевого та відвідного нервів були представлені нервовими клітинами кулястої форми з ядром, в якому відмічалося базофільне ядерце та гомогенна еозинофільна цитоплазма. Таким чином, у плодів людини 14-15 тижнів внутрішньоутробного розвитку були виявлені відмінності у розмірах та площах ядер черепних нервів і нейронів, які формують ядра в ділянці моста.

Ключові слова: плоди людини, гестаційний вік, ембріональний розвиток, міст, ядра моста, нейрони ядер моста.

Author’s contribution
Lopatkina O. P. - research, methodology and writing of the original draft, data visualization.
Tykholaz V. O. - review writing and editing, formal analysis and validation, resources.
Shkolnikov V. S. - supervision, project administration.
Zalevskyi L. L. - software, conceptualization.