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CHARACTERISTICS OF THE EXPRESSION OF MONOCLONAL ANTIBODIES DURING THE PERIOD OF THE MARGINAL AND THORACIC NUCLEI FORMATION

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Embryology is one of the most relevant and popular areas in medicine. Without exaggeration, it is one of the most important branches of medicine and biology. Thanks to embryology, we can better understand the development processes of organisms, the causes of birth defects, and the possibilities of treating infertility. Immunohistochemical methods have significantly improved the understanding of the mechanisms that control the differentiation of neural stem cells into neurons and glial cells, as well as the processes of targeted migration. The aim of the study - to characterize the expression of monoclonal antibodies during the development of marginal and thoracic nuclei. 134 preparations of human embryos and fetuses aged from 6-7 to 39-40 weeks of intrauterine development were used, which developed in the womb without the influence of harmful factors, both internal and external environment. The research was conducted with the mothers' written consent and in compliance with all ethical and deontological norms. To perform immunohistochemical methods, monoclonal antibodies from the company "DacoCytomation" (Denmark): vimentin, synaptophysin, Ki-67, CDX2, S-100 were used. As a result of the study, it became clear that the expression of monoclonal antibodies is completely dependent on the processes inherent in each of the periods of intrauterine development. Before the end of the period of intrauterine development, the expression of Ki-67 is weak, and vimentin and CDX-2 are completely absent. Instead, the expression of S-100 and synaptophysin in the studied nuclei is alternately relatively strong.

Key words: *central nervous system, spinal cord, gray matter, intrauterine development, neuron.*

Connection of the publication with planned research works.

It is part of the research work of the National Pirogov Memorial Medical University, Vinnytsya, which was carried out as part of the research works of the Human Anatomy Department: "Establishment of morphological changes in the formations of the human central nervous system during the prenatal period of ontogenesis (macroscopic, histological, morphometric and immunohistochemical research)", state registration number 0118U001043.

Introduction.

The central nervous system controls and coordinates the activity of all body systems, ensuring the integration of sensory information and the corresponding motor reactions of the human body. This is the dominant structure that ensures the harmonious functioning of the human body as a single, integrated system [1].

The study of histogenesis and organogenesis is a key point in understanding the mechanisms of birth defects. Therefore, it is important for students, lecturers, scientists and for the practicing medical community in general to understand the stages of embryogenesis and histogenesis, including the mechanisms of the occurrence of intrauterine malformations of the structures of the central nervous system [2].

The spinal cord is a significantly important component of the nervous system that provides transmission and processing of information, regulation of movements, and integration of sensory and motor signals. It plays a leading role in harmonious intrauterine development, is necessary for the coordinated work of the entire organism and maintenance of motor activity [3].

It is out of question that the spinal cord provides the processing of sensory information, its transport to the brain and the integration of sensory signals to ensure adequate reactions and coordination of movements. Its role in the integration of sensory and motor information is the basis for the normal functioning of the nervous system and ensuring adaptive responses to changes in the environment [4].

Immunohistochemistry is a powerful tool both in scientific research and in clinical diagnostics. Its use allows obtaining detailed information about the expression and localization of proteins, which is key to understanding the pathogenesis and mechanisms of disease development [5].

Despite a number of studies and the relevance of the problem of formation of the structures of the central nervous system. Most of the publications are outdated, and the data obtained should be updated [6].

Why does the study of neurogenesis not lose its relevance? Because the immunohistochemical method expands our possibilities in the research field. Thus, we have the opportunity to work in a new informative direction. Changes in technologies and equipment, increasing opportunities and reliable results of scientific research contribute to a deeper understanding of the processes of embryogenesis, which has a great impact on both fundamental science and practical medicine [7].

The aim of the study.

To characterize the expression of monoclonal antibodies during the development of marginal and thoracic nuclei.

Object and research methods.

For this study, 134 preparations of human embryos and fetuses aged from 6-7 to 39-40 weeks of intrauterine development were used, which developed in the womb without the influence of harmful factors, both internal and external environment. When conducting the research, we performed everything according to "Observance of ethical and legislative norms and requirements in the performance of scientific morphological studies", in addition, it was confirmed by the Commission on Biomedical Ethics of the National Pirogov Memorial Medical University, Vinnytsya, Ukraine, an expert opinion

was issued (minutes of the meeting of the Committee of Bioethics of the M.I. Pirogov VNMU No. 10 dated December 6, 2018). According to the 2017 agreement between National Pirogov Memorial Medical University, Vinnytsya and VPAB (Vinnytsya Pathological-Anatomical Bureau), agreed on all points of joint scientific and practical activities with the further formation of protocols of pathological examination in accordance with the form No. 013-2/o approved by the order of the Ministry of Health of Ukraine dated 14.08.2004 No. 417. The local bioethics committee confirmed the legality of all performed procedures in accordance with international norms and conventions.

Immunohistochemical methods were based on the use of monoclonal antibodies from DacoCytomation (Denmark): vimentin, synaptophysin, Ki-67, CDX2, S-100. Vimentin and CDX2 were used to study the morphology of radial glia; synaptophysin – a marker of nerve fibers, synaptic connections and myelination processes; S-100 is a marker of astrocytic glia. Ki-67 is a nuclear non-histone protein expressed in proliferating cells and is an important link in the chain of cell proliferation mechanisms.

Research results and their discussion.

In the period of 6-7 weeks of intrauterine development, proliferation processes are actively taking place in the dorsal neuroepithelium, therefore, the results of the study on the Ki-67 protein expression in different parts of the spinal cord at this gestational period are different. The greatest proliferative activity of neural stem cells was observed in the dorsal part of the neuroepithelium, within the future posterior horns of the cervical segments. We also note that proliferation in the mantle layer of the posterior horns occurs only in gliocytes, while neuroblasts continue to differentiate **(fig. 1)**. A strong expression of CDX-2 was found as well. After studying the expression of synaptophysin, it became clear that it is weakly expressed in the area of the dorsal neuroepithelium.

At 8-9 weeks, high proliferative activity remained, due to which Ki-67 expression is relatively most pronounced in the cervical segments. As for vimentin, its most pronounced expression is observed precisely at the border of the posterior horns and posterior cords. As for the expression of CDX-2, the same trend as in the gestation period of 6-7 weeks is maintained. The expression of S-100 is characteristic of the basal membrane of the neuroepithelial layer of the posterior horns.

In the period of 11-12 weeks of gestation, Ki-67 expression is most pronounced in the cervical and thoracic segments. As for vimentin and S-100, the expression characteristics are the same as at 8-9 weeks of gestation. It should be noted that synaptophysin showed a strong expression in the thoracic nucleus, and moderate expression in the peripheral nucleus.

At 14-15 weeks, Ki-67 expression indicators, in comparison with the previous weeks of gestation, decrease and are weak. However, the relative highest activity is observed in the posterior horns of the cervical and lumbar segments and was 13%. As for the expression of vimentin in this area of the posterior horns, it is moderate, while the expression of the protein S-100 is strong, and CDX-2 is absent

The expression of synaptophysin corresponds to the gestation period of 11-12 weeks **(fig. 2)**.

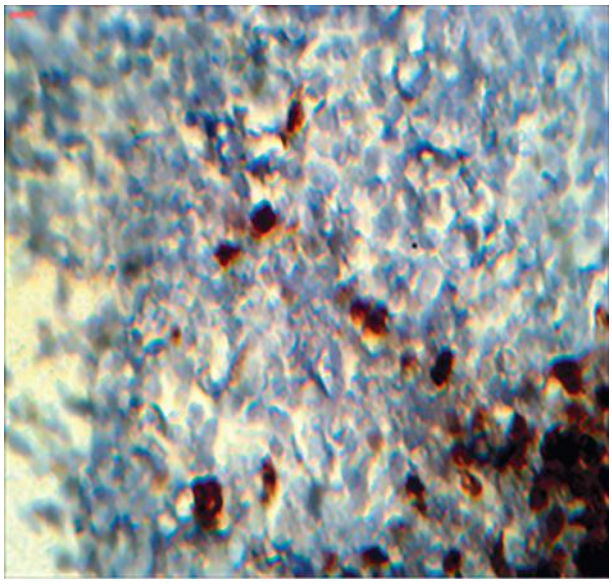

Figure 1 – Intensity of proliferation in dorsal neuroepithelium in cervical segments (6-7 weeks of intrauterine development). Ki-67. Magnification: ×100.

At 20-21 weeks of gestation, Ki-67 expression in general decreases compared to previous periods of gestation and amounts to 11%. If we give a description of the spinal cord, it is relatively most pronounced in the cervical and lumbar segments. As for vimentin and CDX-2, there is no expression in the region of the thoracic and marginal nuclei at this stage of gestation. The trend with S-100 expression remains the same as at 14-15 weeks of gestation. Synaptophysin expression is characterized by a decrease in the thoracic nucleus and an increase in the peripheral nucleus **(fig. 3)**.

At 29-30 weeks, Ki-67 expression is weak in the area of the posterior horns of the spinal cord segments. The expression of vimentin and CDX-2 in the thoracic and marginal nuclei is absent, while the expression of the S-100 protein is strong in the middle part of the posterior horns of the spinal cord segments, but less pronounced in the area of the marginal nucleus. As for the expression of synaptophysin, it will be relatively moderate in

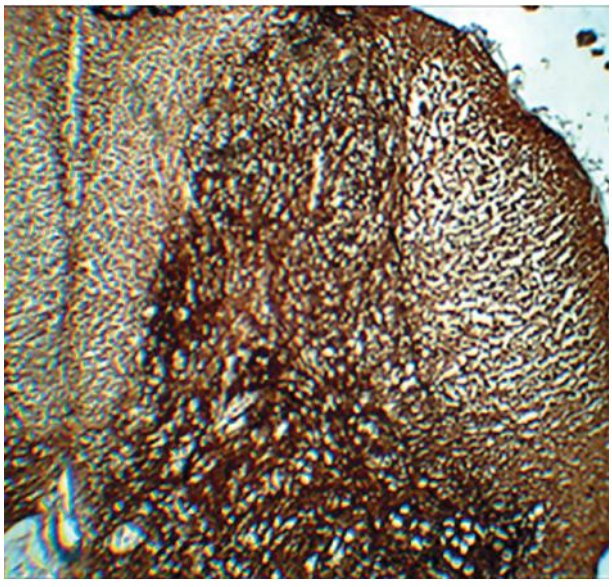

Figure 2 – Posterior horns of the spinal cord at the level of the cervical thickening (17-18 week of gestation). Synaptophysin. Magnification: ×40.

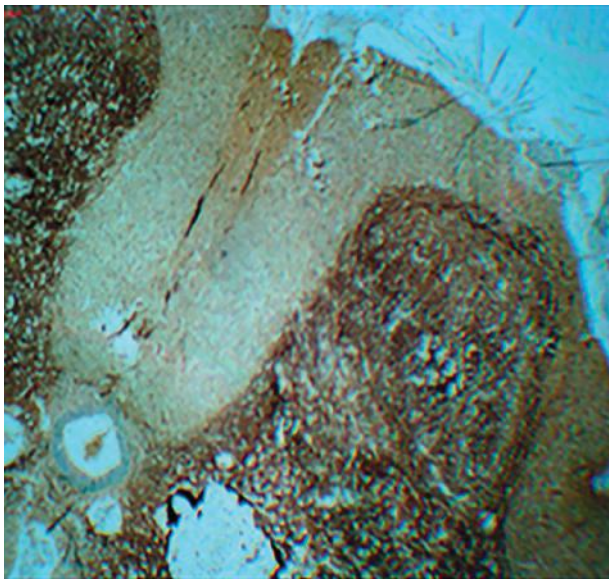

Figure 3 – Expression of synaptophysin in the thoracic and marginal nuclei (20-21 weeks of gestation). Synaptophysin. Magnification: ×40.

the area of the thoracic nucleus, and within the border it will increase and reach maximum values at 39-40 weeks.

Conclusions.

The most active proliferation occurred at 5-6 weeks of gestation, strong expression of Ki-67 was detected in 92% of cells. Later, a gradual decrease in Ki-67 expression was observed. As for the expression of vimentin and CDX-2, the highest indicators were established at the end of the embryonic period, from the 14th to the 15th week onwards, the expression in the area of the thoracic and marginal nucleus is completely absent. A relatively strong expression of synaptophysin in the thoracic nucleus was noted at 12-13 weeks, and at 39-40 weeks in the peripheral. Instead, a strong expression of S-100 was detected starting from the 14-15th week of gestation in the marginal nucleus, and at 29-30 weeks in the thoracic nucleus.

Prospects for further research.

Neuroembryology and neuroanatomy are clearly promising directions and the obtained results are implemented both in the educational process and will be no less interesting for familiarization by practicing doctors.

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ХАРАКТЕРИСТИКА ЕКСПРЕСІЇ МОНОКЛОНАЛЬНИХ АНТИТІЛ В ПЕРІОД ФОРМУВАННЯ КРАЙОВОГО ТА ГРУДНОГО ЯДЕР

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Резюме. *Вступ.* Не зважаючи на низку проведених досліджень, вивчення внутрішньоутробного розвитку спинного мозку не втрачає своєї актуальності. Це одна із структур центральної нервової системи, що забезпечує взаємозв'язок структур людського організму із головним мозком, а також забезпечує передачу усіх видів чутливості та рухових імпульсів. Крім того з'явилася можливість порівняти нещодавно отримані дані із даними десятирічної давнини та виконати у подальшому глибокий аналіз. Вивчення ембріогенезу дозволяє ідентифікувати причини та механізми виникнення вроджених вад спинного мозку, що є важливим для розробки методів їхньої діагностики, лікування та профілактичних заходів. Однозначно, отримані результати стануть в нагоді не лише в навчальних закладах, але й будуть становити наукову цінність для практикуючих лікарів у діагностичній сфері. Імуногістохімічні методи посідають важливу роль, є важливими для прогресу в медицині та біології, оскільки дозволяють дослідникам точно і специфічно вивчати зміни в тканинах.

Об'єкт і методи дослідження. Досліджено 134 препаратів ембріонів та плодів людини віком від 6-7 до 39-40 тижнів внутрішньоутробного розвитку, використовуючи імуногістохімічний метод. Для дослідження було обрано моноклональних антитіл фірми «DacoCytomation» (Denmark): віментин, синаптофізин, Кі-67, CDX2, S-100.

Результати. 5-6 тиждень гестації характеризується активними процесами проліферації, саме тому спостерігалась сильна експресія Кі-67. Надалі, поступово процеси проліферації згасають, що прямопропорційно спостерігається і на експресії Кі-67. Щодо S-100, то в ембріональний період експресія слабко виражена і починає наростати та відзначається, як сильна з 14-15 тижня гестації. Щодо віментину та CDX-2, то для них характерна сильна експресія в кінці ембріонального періоду.

Висновки. На основі результатів імуногістохімічних методів виявлено, що до закінчення періоду внутрішньоутробного розвитку експресія Кі-67 є слабкою, а віментину та CDX-2 взагалі відсутня. Натомість експресія S-100 та синаптофізину в досліджуваних ядрах почергово відносно сильна.

Ключові слова: центральна нервова система, спинний мозок, сіра речовина, внутрішньоутробний розвиток, нейрон.

CHARACTERISTICS OF THE EXPRESSION OF MONOCLONAL ANTIBODIES DURING THE PERIOD OF THE MARGINAL AND THORACIC NUCLEI FORMATION

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Abstract. *Introduction.* Despite the number of conducted studies, the study of intrauterine development of the spinal cord does not lose its relevance. It is one of the structures of the central nervous system, which ensures the interconnection of the structures of the human body with the brain. Also, it provides the transmission of all types of sensitivity and motor impulses. In addition, there was an opportunity to compare recently obtained data with data from ten years ago and perform further in-depth analysis. The study of embryogenesis makes it possible to identify the causes and mechanisms of birth defects of the spinal cord, which is important for the development of their diagnosis, treatment and preventive measures methods. Immunohistochemical methods play the main role, and are important for progress in medicine and biology, as they allow researchers to accurately and specifically study changes in tissues.

Object and research methods. 134 preparations of human embryos and fetuses aged from 6-7 to 39-40 weeks of intrauterine development were studied using the immunohistochemical method. Monoclonal antibodies from the company "DacoCytomation" (Denmark) were chosen for the study: vimentin, synaptophysin, Ki-67, CDX2, S-100.

The results. The 5-6th week of gestation is characterized by active proliferation processes, which is why a strong expression of Ki-67 was observed. In the future, the proliferation processes gradually fade away, which is directly proportional to the expression of Ki-67. As for S-100, in the embryonic period the expression is weakly expressed and begins to increase and is noted as strong from the 14-15th week of gestation. As for vimentin and CDX-2, they are characterized by strong expression at the end of the embryonic period.

Conclusions. Based on the results of immunohistochemical methods, it was found that before the end of the period of intrauterine development, the expression of Ki-67 is weak, and vimentin and CDX-2 are completely absent. Instead, the expression of S-100 and synaptophysin in the studied nuclei is alternately relatively strong.

Key words: central nervous system, spinal cord, gray matter, intrauterine development, neuron.

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Conflict of interest:
The Authors declare no conflict of interest.

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ANATOMICAL AND FUNCTIONAL CHARACTERISTICS OF COMPLEX FLAPS OF THE SUBCLAVIAN ARTERY BASIN IN RECONSTRUCTIVE SURGERY OF THE HEAD AND NECK

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The article presents the anatomical variations of the blood vessels supplying the muscles that are clinically important for the safe performance of surgical procedures such as skin-muscle flap reconstruction. Because much of the discussion revolves around the arterial anatomy of muscles, venous drainage is often overlooked. However, a possible cause of flap loss may be impaired venous drainage. Purpose: to consider the anatomical variants of branching of the vessels of the subclavian artery and to investigate the peculiarities of the blood supply of the skin part of the skin-muscle flap of the sternocleidomastoid muscle in the experiment. It was established that the anatomical