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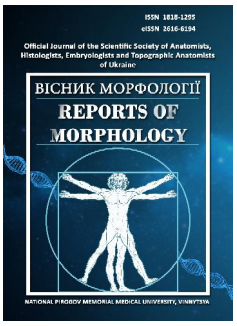

Immunohistochemical characteristics of the gray matter of the human spinal cord in the late prenatal period

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The study is dedicated to the relevant problem of studying the patterns of age-related (prenatal) restructuring in the brain and spinal cord and provides opportunities for predicting and correcting the occurrence of congenital defects. The aim of the research was to establish the nature of immunohistochemical marker expression in the gray matter structures of the human spinal cord during the late prenatal period. The material for the study consisted of spinal cord preparations from 27 human fetuses at gestational age 35-40 weeks. The following methods were used during the research: anatomical, general histological, special histological, immunohistochemical, morphometric, and statistical analysis of the obtained data. It was found that at 35-36 weeks of the gestational period, the proliferation of neural stem cells (NSCs) occurs more intensively in the ventral neuroepithelium of spinal cord segments compared to the dorsal neuroepithelium. In the ventral neuroepithelium, there are 5-6 mitotic or post-mitotic NSCs, while in the dorsal part, there are only 2-3 cells. In fetuses at 39-40 weeks, the proliferative activity of neural stem cells in the dorsal neuroepithelium is higher in cervical and lumbar segments, where Ki-67 expression is detected in 6 % of cells (reactive in 7-8 cells), and in thoracic and sacral segments, it is 4 % (reactive in 3-4 cells). In contrast to the dorsal neuroepithelium, in the ventral part of the neuroepithelium of the segments, the proliferative activity of neural stem cells is slightly less intense. In cervical and lumbar segments, Ki-67 expression occurred in 4 % of cells (reactive in 3-4 cells), and in thoracic and sacral segments, it was 2 % (reactive in 1-2 cells). At 35-36 weeks of gestation, high vimentin expression was observed around the neuroepithelium, at the base of the posterior horns, and along the posterior median septum. Vimentin expression in the mantle layer was relatively weak and persisted along blood vessels and in the area of spinal cord root formation. Before birth, relatively weak vimentin expression was detected in the remnants of radial glia surrounding the neuroepithelial layer. Vimentin expression was absent in the neuroepithelium proper, but focal vimentin expression was observed around blood vessels. The absence of vimentin expression in the neuroepithelium indicates the disappearance of radial cells. At 35-40 weeks of the gestational period, relatively strong synaptophysin expression was observed in the mantle layer of spinal cord segments, indicating the intensity of neuronal connectivity establishment and myelination of nerve fibers. These processes continue after birth. Synaptophysin expression was absent in the neuroepithelium proper.

Keywords: *brain, spinal cord, central nervous system, radial glia, neural stem cells, prenatal period, immunohistochemistry, neuron.*

Introduction

The stages of emergence, genetic mechanisms of development, and processes of differentiation of neural stem cells in the gray matter of the brain and spinal cord of human embryos and fetuses have always been a priority in scientific research [6]. Such studies contain a large amount of scientific data and facts, but to this day, the results of such work have conflicting aspects that require further clarification [3].

As a result of the emergence of modern research techniques, such as immunohistochemical or immunofluorescent analysis, as well as computer morphometry etc., new opportunities arise for thorough

studies of the structures of the human central nervous system [1].

It is known that neural stem cells and progenitor cells are the source of neuroblasts and glioblasts, which may exhibit different morphological and cytochemical properties at different stages of ontogenesis and in different structures of the central nervous system [11]. Neural stem cells arise in the neuroepithelium of the spinal cord. The coexistence of neuronal and glial cell precursors in the ventricular zone of the neural tube at very early stages has been confirmed using cell markers such as neuron-specific enolase and glial fibrillary acidic protein [4]. In contrast, the available literature lacks results regarding the application of immunohistochemical markers such as vimentin, S-100, CDX-2, and synaptophysin in the study of structures in the human fetal spinal cord.

The aforementioned techniques have allowed us to establish that neuroblasts, originating from the neuroepithelium, subsequently migrate in a targeted manner through amoeboid movements in various directions towards their sites of further differentiation along the radial glial fibers [5]. There is a viewpoint that these "guiding" glial cells disappear after the maturation of neurons. Cessation of further neuronal migration in the adult brain is attributed to the disappearance of glial radial processes [17]. However, the authors have not described the sequential morphology of radial glia during prenatal human ontogenesis, which requires further investigation and clarification. As the neuronal cytoplasm differentiates, the growth and differentiation of its processes occur, and intercellular connections, including the formation of synaptic structures, are established [7].

The active study of neural stem cells over the past twenty years has been associated with the creation of opportunities for tissue and organ regeneration in damaged or aging organisms, thus improving quality of life and extending lifespan [9, 16].

Thus, the significance of the research results on the morphology of the development of the brain and spinal cord in clinical practice is exempt from any doubt.

The aim of the study is to determine the expression pattern of immunohistochemical markers in the structures of the gray matter of the human spinal cord during the late prenatal period.

Materials and methods

The material for the study consisted of spinal cord specimens from 27 human fetuses at a gestational age of 35-40 weeks. These specimens were obtained during medical abortions or from relatively healthy women due to stillbirths. The fetuses had perished due to causes unrelated to anomalies in the development of the brain or spinal cord.

During the examination, it was found that the materials used in this study comply with the fundamental bioethical norms of the Helsinki Declaration adopted by the General Assembly of the World Medical Association, the Convention on Human Rights and Biomedicine of the Council of Europe (1977), relevant provisions of the WHO, the International Council of Medical Scientific Societies, the International Code of Medical Ethics (1983), the Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes of the Council of Europe of 18.03.1986, Directive 86/609/EEC of 24.11.1986, and Order No. 281 of the Ministry of Health of Ukraine of 01.11.2000. The bioethical examination has determined that this research may be presented for the publication of the obtained results.

This work is a part of the planned scientific research of the Department of Human Anatomy at Vinnytsia National Medical University named after M. I. Pirogov, titled "Establishment of Morphological Changes in the Formations of the Human Central Nervous System during the Prenatal Ontogenesis Period (Macroscopic, Histological, Morphometric, Immunohistochemical Study)", with the state registration number 0118U001043.

The spinal cord histological specimens were stained with silver nitrate (Bilshovsky impregnation), hematoxylin-eosin, and the Nissl staining method was applied.

Immunohistochemical techniques (monoclonal antibodies) were used as follows: Ki 67 as a marker of neural cell proliferation, vimentin as a marker of radial glial fibers and radial cells [10]; S-100 as a marker of glial cells, and synaptophysin as a marker of synaptic vesicles [8]. A semi-quantitative scale was used to assess protein expression intensity, with the following scoring: 0 - an absence of positive cells, weak - 30 % positive cells, moderate - 31-60 % positive cells, strong - 60 % or more stained cells [2].

Morphometry of the spinal cord formations was performed using a light microscope, with the following magnifications: x4, x10, x40, x100, and x200. Cytohistometry was carried out using PhotoM 1.21 software (computer histometry).

Statistical analysis of the quantitative data obtained from the results was performed on a personal computer using the standard software package "Statistica 6.1" by StatSoft (owned by the Scientific and Research Center of National Pirogov Memorial Medical University, Vinnytsia, license number BXXR901E246022FA). The correctness of the feature distribution was assessed for each variation series, including the calculation of mean values and standard deviations for each feature. The differences between independent variables were considered significant at $p<0.05$.

Results

In fetuses at 35-36 weeks of gestation, the proliferation of neural stem cells (NSCs) occurs more prominently in the ventral neuroepithelium of the spinal cord segments compared to the dorsal neuroepithelium. It has been found that the ventral neuroepithelium contains 5-6 mitotic or postmitotic NSCs (Fig. 1). While the dorsal region of the neuroepithelium has only 2-3 such cells.

NSCs migration from the neuroepithelium after mitosis occurs along the remnants of radial glial fibers. The radial direction is maintained only at a certain distance around

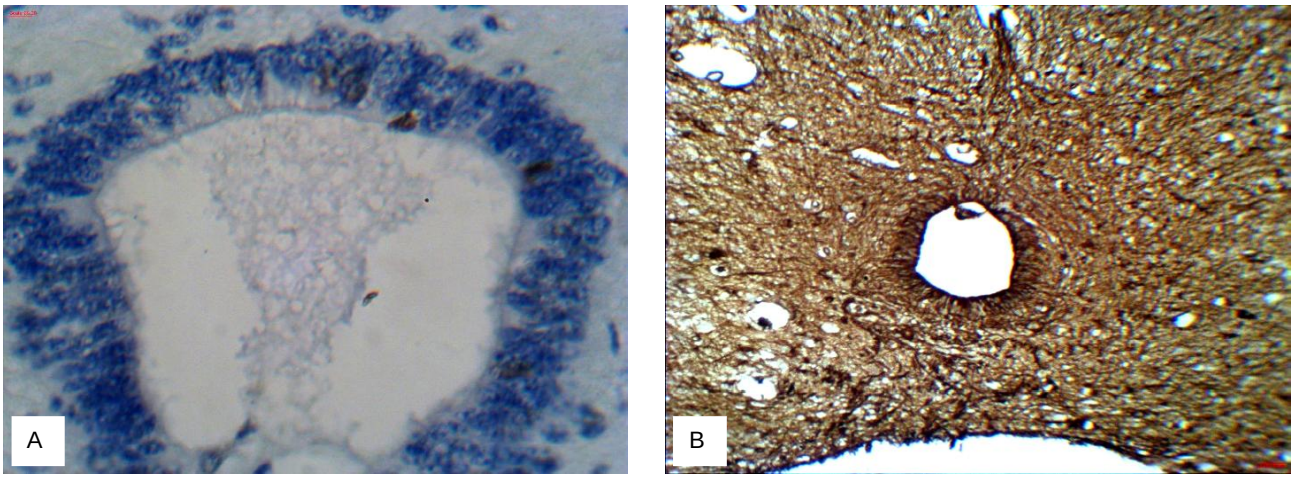

Fig. 1. Horizontal section of the human fetal spinal cord at 35-36 weeks. A - expression of Ki-67 in the neuroepithelium. Ki-67. x400. B - remnants of radial glia. Vimentin; x100.

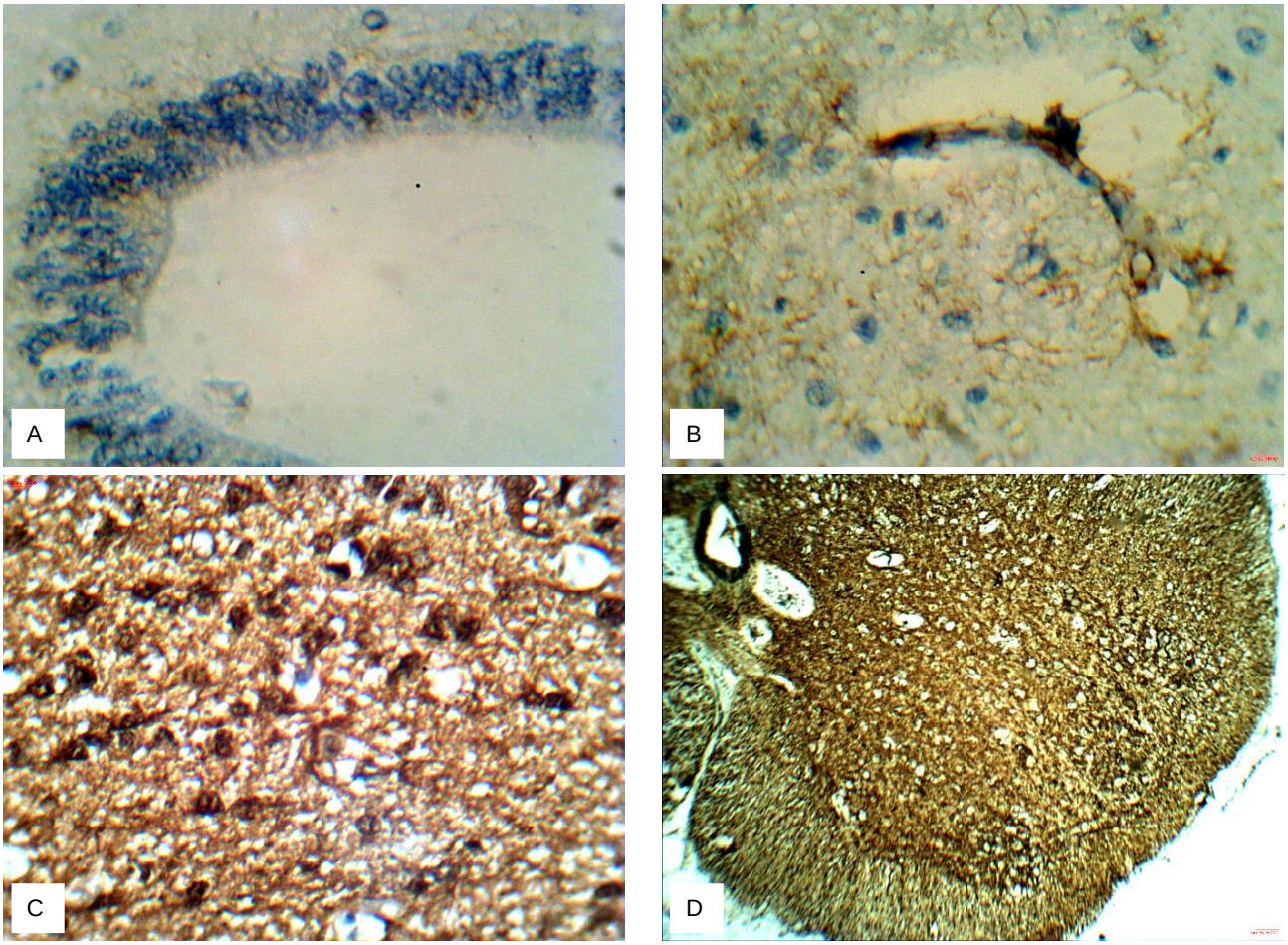

Fig. 2. Horizontal section of the human fetal spinal cord at 39-40 weeks. A - Ki-67 expression in the neuroepithelium. Ki-67. x400. B - remnants of radial glial fibers near blood vessels. Vimentin; x100. C - S-100 expression in the mantle layer glial cells of the segments. S-100. x400. D - a relatively strong expression of synaptophysin in the gray matter of the segments. Synaptophysin. x40.

the neuroepithelium (see Fig. 1). Additionally, the radial glial fibers change direction and extend in a fan-like pattern into the boundaries of the dorsal horns. Short fibers are located within the neuroepithelium, starting from the basal membrane and comprising its thickness. Long radial glial fibers extend from the basal membrane of the dorsal neuroepithelium and run along the posterior median septum. Strong vimentin expression was observed in the

neuroepithelium and along the posterior median septum (see Fig. 1). In the mantle layer, vimentin expression was relatively weak and persisted along the blood vessels and at the site of spinal cord root formation. Within the marginal zone, there was weak vimentin expression, indicating the absence of radial glia.

During the investigation of S-100 expression in glioblasts and gliocytes in spinal cord segments, it was found that strong expression occurred in the neuroepithelial layer (reacted in 100 % of cells). Additionally, strong S-100 expression was observed in the gray matter of the anterior horns (reacted in 90 % of gliocytes). We also observed a similar pattern of strong S-100 expression in glial cells of the gray matter in the posterior horns (reacted in 92 % of cells).

Relatively strong expression of synaptophysin was observed in the segments of the spinal cord, particularly in the mantle layer, indicating the intensity of neuronal connectivity processes. Additionally, synaptophysin expression in the marginal layer was moderate, while no expression of synaptophysin was detected in the neuroepithelium itself.

In fetuses at 39-40 weeks, the proliferative activity of neural stem cells in the dorsal neuroepithelium is relatively highest in the cervical and lumbar segments, where Ki 67 expression was observed in 6 % of cells (7-8 cells reacted), and in the thoracic and sacral segments, where it was 4 % (3-4 cells reacted) (Fig. 2). In contrast to the dorsal neuroepithelium, the ventral neuroepithelium of the segments showed the slightly lower intensity of proliferative activity of neural stem cells. Specifically, in the cervical and lumbar segments, Ki-67 expression occurred in 4 % of cells (3-4 cells reacted), and in the thoracic and sacral segments, it was 2 % (1-2 cells reacted) (see Fig. 2).

During this age period, a slowdown in the migration processes of NSCs is observed, both in the gray matter of the anterior regions and in the gray matter of the posterior horns, where their differentiation into neurons and glial cells occurs. In the mantle layer of the spinal cord segments, mitoses were only observed in glial cells. It should be noted that the intensity of glial cell mitoses was relatively higher in the posterior horns compared to the anterior horns, which may be associated with the relatively higher cell density in this area of the segments. In qualitative terms, Ki-67 expression in the posterior horns was weak, with 8 % of cells reacting, while in the anterior horns, it was 3 %. No mitoses of neuroblasts were detected in the mantle layer.

In fetuses at 39-40 weeks, vimentin expression was relatively weak in the remnants of radial glia surrounding the neuroepithelial layer. No vimentin expression was observed in the neuroepithelium itself, while focal vimentin expression was detected around blood vessels (see Fig. 2). Therefore, the absence of vimentin expression in the neuroepithelial layer indicates the absence of radial cells among the epithelial cells of the neuroepithelium.

S-100 expression in the segments of the spinal cord is relatively strong at the base of the anterior and posterior horns. There is a relatively higher density of glial cells in the posterior horns compared to the anterior horns. Specifically, S-100 expression occurred in 98 % of glial cells in the posterior horns and in 77 % of glial cells in the anterior horns (see Fig. 2).

Discussion

In their study, A. Ruiz-Sauri et al. [12] indicate that during the first half of the prenatal period, the intensity of mitosis in neural stem cells occurs more prominently in the ventral neuroepithelium than in the dorsal neuroepithelium. However, this pattern changes to the opposite before birth.

In our study, overall, evaluating the expression of Ki-67 in the neuroepithelium of the segments during this age period using a semi-quantitative scale, it was weak in both the ventral and dorsal regions. In terms of percentage, the cell expression accounted for 6 % in the ventral region and 3 % in the dorsal region.

It should be noted that further differentiation of neuroblasts takes place in the mantle layer itself. We did not observe neuroblast proliferation. In the gray matter of the anterior horns, the expression of Ki-67 in glial cells was weak, as only 10 % of the cells showed expression. The proliferation of satellite glial cells in the anterior horns was also low, with only 1-2 mitotic cells observed. Relative cell density was maintained in the gray matter of the posterior horns, despite a relatively low intensity of mitosis in glial cells within the posterior horns. The expression of Ki-67 in the mantle layer of the posterior horns was also weak, accounting for 17 % of the cells. The degree of Ki-67 expression in the marginal layer was quantitatively weak, where mitoses were observed in 6 % of the cells.

The results of our study coincide with the findings of V. S. Shkolnikov et al. [14], who state that strong expression in the fibers of the radial glia in the spinal cord segments occurs until the 17-18th week. Subsequently, a gradual involution of the radial glia takes place, and by birth, the expression of vimentin is observed only in its remnants within the neuroglial complexes and along blood vessels.

During the investigation of CDX-2 expression in the fibers of the radial glia in the spinal cord segments, it was found that for this age period, the expression of this protein is absent. The results of the study by V. S. Shkolnikov and S. V. Vernygorodskyi [13] provide data indicating that CDX-2 expression in the fibers of the spinal cord radial glia occurs only during the embryonic period.

Evidence that in the human fetal spinal cord at 39-40 weeks, the neuroepithelium is composed of ependymocytes and the absence of radial glial cells is the relatively strong expression of S-100 in the neuroepithelium. It should be noted that some neurons also exhibited S-100 expression. In their study, S. Skarlatou et al. [15] support the idea that S-100 is not expressed in neurons before birth, which is consistent with our findings at 39-40 weeks. In the neuroepithelial layer, radial glial cells are absent.

The relatively strong expression of synaptophysin in the gray matter of the spinal cord segments at 39-40 weeks, especially within the neuro-glia complexes, indicates the ongoing processes of establishing interneuronal connections and myelination of nerve fibers that continue until birth. However, synaptophysin expression is absent in the neuroepithelium proper.

The prospect of further research lies in studying the peculiarities of immunohistochemical marker expression in the formations of the spinal cord in mature individuals and comparing the obtained results with similar ones in fetuses.

Conclusion

1. At 35-36 weeks, the proliferation of neural stem cells is more intensive in the ventral neuroepithelium compared to the dorsal neuroepithelium. However, at 39-40 weeks, the proliferation of neural stem cells in the dorsal neuroepithelium becomes relatively higher than in the ventral neuroepithelium.

2. The vimentin expression indicates that at 35-36 weeks, radial glia is present around the neuroepithelium and in the posterior horns. However, by the time of birth, radial glia is only retained near the blood vessels of the gray matter segments.

3. Until birth, there is a relatively strong expression of S-100 in the glial cells of both the anterior and posterior horns.

4. The relatively strong expression of synaptophysin in the gray matter of the spinal cord segments, especially within the neuroglial complexes, indicates the ongoing processes of establishing interneuronal connections and myelination of nerve fibers.

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ІМУНОГІСТОХІМІЧНА ХАРАКТЕРИСТИКА СІРОЇ РЕЧОВИНИ СПИННОГО МОЗКУ ЛЮДИНИ У ПІЗНЬОМУ ПРЕНАТАЛЬНОМУ ПЕРІОДІ

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Дослідження присвячене актуальній проблемі вивчення закономірностей вікової (пренатальної) перебудови утворів головного та спинного мозку та надає можливості щодо прогнозування та корекції виникнення вроджених вад. Метою дослідження було встановлення характеру експресії імуногістохімічних маркерів у структурах сірої речовини спинного мозку людини у пізньому пренатальному періоді. Матеріалом для дослідження слугували препарати спинного мозку 27 плодів людини гестаційним терміном 35-40 тижнів. Під час проведення дослідження використані наступні методи: анатомічні, загальні гістологічні, спеціальні гістологічні, імуногістохімічні, морфометричні та статистичний аналіз отриманих даних. Встановлено, що у 35-36 тижнів гестаційного періоду проліферація нейральних стовбурових клітин (НСК) більш інтенсивно відбувається у вентральному нейроепітелії сегментів спинного мозку, ніж у дорзальному. У вентральному нейроепітелії налічується 5-6 мітотичних, або постмітотичних НСК, в той же час у дорзальній частині - 2-3 клітини. У плодів 39-40 тижнів проліферативна активність нейральних стовбурових клітин у дорзальному нейроепітелії є вищою у шийних і поперекових сегментах, де експресія Кі-67 відмічена в 6 % клітин (прореагувало 7-8 клітин), у грудних та крижових сегментах - 4 % (прореагувало 3-4 клітин). На відміну від дорзального нейроепітелію у вентральній частині нейроепітелію сегментів проліферативна активність нейральних стовбурових клітин дещо меншої інтенсивності. Так, у шийних та поперекових сегментах експресія Кі-67 відбулась у 4 % клітин (прореагувало 3-4 клітини) та у грудних і крижових - 2 % (прореагувало 1-2 клітини). У 35-36 тижнів гестації висока експресія віментину встановлена навколо нейроепітелію, в основі задніх рогів та вздовж задньої серединної перегородки. У мантійному шарі експресія віментину була відносно слабкою та зберігалась уздовж судин та у місці формування корінців спинного мозку. До народження у залишках радіальної глії навколо нейроепітеліального шару встановлена відносно слабка експресія віментину. У самому нейроепітелії експресія віментину була відсутньою, але навколо судин спостерігалась вогнищева експресія віментину. Відсутність експресії віментину у нейроепітелії свідчить про зникнення радіальних клітин. У 35-40 тижнів гестаційного періоду у сегментах спинного мозку встановлена відносно сильна експресія синаптофізину у мантійному шарі, що свідчить про інтенсивність процесів встановлення нейрональних зв'язків та мієлінізації нервових волокон. До народження дані процеси не закінчуються. Безпосередньо в нейроепітелії експресія синаптофізину відсутня.

Ключові слова: *головний мозок, спинний мозок, центральна нервова система, радіальна глія, нейральні стовбурові клітини, пренатальний період, імуногістохімія, нейрон.*