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HISTOGENESIS OF SOLID TYPE DUCT CANCER OF A WOMAN'S BREAST GLAND

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In Ukraine, the development of ductal cancer of the mammary gland continues to be a growing problem for the health of women, not only of the elderly but also of young age, and the cause of death in 18–20 % of cases. Non-invasive forms of cancer, which account for 5–8 % of cases, are associated with the proliferative process in the alveolar part of the gland. The structural and functional organization of the epithelium in the terminal duct of the gland on the autopsy material of women of reproductive age who died from infectious diseases was studied, as well as the solid type of ductal cancer of the gland was retrospectively identified, according to the WHO classification (2003); histological, histochemical, immunohistochemical methods were applied (markers p63, bcl-2, α -sma, VEGF from DakoCytomation) with karyometric analysis. The paper presents a new theoretical justification and solution to the topical issue of histogenesis of solid breast cancer based on a comparison with the epithelium of the terminal part of the duct – the acinus-button. The histogenesis of cancer with an extreme degree of cataplasia associated with the terminal part of the duct is presented.

Key words: histogenesis, solid cancer, mammary gland, immunohistochemistry, karyometry.

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ГІСТОГЕНЕЗ СОЛІДНОГО ТИПУ ПРОТОКОВОГО РАКУ МОЛОЧНОЇ ЗАЛОЗИ ЖІНКИ

В Україні розвиток протокового раку молочної залози продовжує бути зростаючою проблемою здоров'я жінок не тільки похилого а й молодого віку, та причиною смерті у 18–20 % випадків. Неінвазійні форми раку, що складають 5–8 % випадків, пов'язані із проліферативним процесом в альвеолярній частині залози. Вивчалась структурно-функціональна організація епітелію термінальної протоки залози на аутопсійном матеріалі жінок репродуктивного віку, померлих від інфекційних захворювань, а також ретроспективно ідентифікований солідний тип протокового раку залози, згідно класифікації ВООЗ (2003); застосовані гістологічний, гістохімічний, імунногістохімічний методи (маркери p63, bcl-2, α -sma, VEGF фірми DakoCytomation) з кариометричним аналізом. В роботі приведено нове теоретичне обґрунтування і рішення актуального питання гістогенезу солідного раку молочної залози на підставі порівняння з епітелієм термінальної частини протоки – ацинуса-бруньки. Представлено гістогенез раку з крайнім ступенем катаплазії, що пов'язаний із термінальним відділом протоки.

Ключові слова: гістогенез, солідний рак, молочна залоза, імунногістохімія, кариометрія.

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Wartime causes stress disorders in a woman's body, causing hormonal imbalance, disrupts the regulation of the reproductive organs' function, in particular the mammary glands, and provokes the development of oncopathology – breast cancer. The challenge of time requires improvement of pathomorphological diagnostics and treatment tactics. Finding out the tissue origin (histogenesis) of malignant tumors is important not only for the pathomorphological diagnosis of the tumor, but also, which is especially important, for the justified treatment of the patient. The reason for this is the fact that tumors of different tissue origin in the body are not equally sensitive to radiation therapy and to chemical means of treatment – medicines [5, 11]. As it is known, the histogenesis of a tumor is determined by a pathomorphologist as follows: by studying various stages of the ontogenetic development of the the organ's tissue, studying its genotype and comparing it with the morphological structure of neoplasia. If the tumor is built of highly differentiated cells, its histogenesis is relatively easy to establish, this is facilitated by the partial preservation of the morphological and functional similarity of its cells to certain derived cell elements of the organ. However, there are still discussions about the tissue origin of the smooth muscle cells of the mammary duct, whose morpho-functional variability correlates with the degree of their differentiation, susceptibility to dysplastic and metaplastic damage, which complicates their verification [5, 13]. With regard to tumors with a low degree of differentiation, the establishment of histogenesis based on phenotypic features loses its significance and requires additional immunohistochemical verification. At the same time, the ultrastructural level of malignant tumors components' study indicates that, in general, they do not have specific signs and are characterized by nuclear and cellular polymorphism, the heteromorphic structure of the host cell, which depends on the degree of its differentiation, the peculiarities of unstable gene and chromosomal apparatus for restoring the structure DNA and other anomalies [7, 8, 9, 10].

Partial specific signs of differentiation of tumor elements can also be observed, which indicates the preservation, despite malignancy, of some of the specific properties of mammary gland cells acquired during phylogenesis and ontogenesis [4, 5, 10, 12, 13]. This thesis leaves hope for the search for alternative ways of establishing the histogenesis of a solid type of breast cancer (breast cancer). As you know, tumors arise in the tissue of the organ, where during regeneration the most intense cell reproduction takes place – the proliferative growth centres (germinal zones). It is here that the least of differentiated elements are found – cambial, stem, semi-stem cells, blast forms, precursor cells. With them, as well as with disturbances in the molecular pathways of regulating cell proliferation and differentiation [7, 8], the development of cellular metaplasia, dysplasia with subsequent transformation, under certain conditions, into a malignant tumor is associated. Previous studies have established the localization of areas of proliferative activity in the terminal ducts of the woman's mammary gland in reproductive age. According to the theory of tumor progression, individual properties of malignant tumors can vary significantly, manifest independently of each other and create different combinations of signs. The trophism of neoplastically transformed cells parasitizing the body, the influence of the stroma on tumor progression and spread in the body are of great importance in this [14]. The independent progression of various signs of tumors is regulated at the genetic level, which is also inherent in a malignant tumor of the mammary gland [3, 4, 6].

But the debate continues on the issue of the histogenesis of a solid type of the MG malignant neoplasia, which differs in its morphology and functional behavior from other types of cancer in the affected organ. The subject of the study was the histogenesis of a solid type of breast cancer, based on the morphological features of the terminal duct of the gland (acinus-button) in a woman of reproductive age.

The purpose of the study was to find out histochemical, molecular features and the degree of cataplasia of a solid type of breast cancer in comparison with the epithelium of the terminal duct – acinus-button for the development of diagnostic methods and differentiated treatment in the clinic.

Materials and methods. The collection of material was carried out from the database of the archive and current histological research in the KP “Poltava Regional Pathological and Anatomical Bureau of the POR” for the years 2020–2024, taking into account the law of Ukraine on burial and funeral affairs, and the agreement on scientific and practical cooperation between the PDMU and the KP “Poltava Regional Pathological and Anatomical Bureau of the POR”. A morphofunctional study of elements in the solid type of breast cancer of the terminal duct was carried out on archival material of surgically removed mammary glands with a confirmed diagnosis of cancer in women aged 27 to 60 years (21 cases with 294 microsections of tumor tissue samples) and current autopsy material of intact breast MG (3 observations and 24 microsections of MG tissue of women deceased from infectious diseases under the age of 30). Standard fixation of the material: 10 % solution of cold neutral formalin, embedding in paraffin for further immunohistochemical (IHC) research performed at a certified laboratory in Dnipro. Sections (thickness 4–5 µm), staining with hematoxylin and eosin (g-e) for inspection microscopy; histochemical combined staining according to the Bergman method: (Shif-iodine acid) SHIA reaction + Alcian blue BSHAB (Bergman Shif-iodine acid alcian blue), picrofuchsin according to Van Gieson (VG), in some cases Nile blue staining was performed. Microscopy takes into account the degree of basophilia of the cell nucleus, the presence of SHIA-positive inclusions [1, 2]; in the case of immunohistochemical examination (IHC), standard tissue sections were deparaffinized and applied to glass slides. To restore the disturbed antigenic structure, due to fixation in formalin, thermal induction of the epitope (antigen site) was performed by heating the slides in a citrate buffer with a pH of 6.7 in an autoclave to a temperature of +1210C for 8 minutes; incubation of micropreparations' sections with primary antibodies took place in humid chambers at a temperature of +23–250C for 30 minutes.

In the future, the following ready-made monoclonal antibodies from Dako Cytomation were used: to the oncoprotein p63 (clone JAY), a marker of cambial cells, to the apoptoregulatory protein bcl-2 (clone 124), to smooth muscle actin a-sma (clone 1A4), VEGF-a – vascular endothelial growth factor marker (VGI clone). Polymorphic dextrin molecules containing secondary antibodies, immunological to primary antibodies, and multiple peroxidase molecules – horseradish root enzymes that give the object a brown color – are used in the visualization system. To differentiate tissue structures, the sections were additionally stained with Mayer's hematoxylin for 3 minutes. Dehydration and inclusion of tissue sections in balsam was carried out according to standard principles. The qualitative reaction of the antigen binding to the antibody (AG+AT) was visually observed under a light microscope and the location of the immune complex (AG+AT) was identified by the intensity of tissue staining: -, +, ++, +++, i.e. negative, low, moderate, markedly positive (semi-qualitative-quantitative gradation). The expression for each marker was evaluated individually in at least 10 fields of view of an Olympus BX 41 light microscope with x20x40x100 objectives. Photographing of microsections was carried out with a digital camera “C3040-АДУР” connected to this microscope. Together with this, cell

karyometry was carried out, which is the most informative method of morphometry (per 100 standard enlarged photo-objects) with the calculation of the nuclei volume (V) according to the formula for a rotating ovoid, where the axis of rotation coincides with the large diameter of the cell nucleus. Further logarithmization of the nucleus took place according to the formula:

$$\text{LgV}=\text{Lg}(P\times\pi/6)\times d^2D,$$

where LgV of the cell nucleus is equal to: Lg of P multiplied by the number π and divided by six, then multiplied by d squared and the value D of the cell; the obtained data are determined in μm^3 . P is constant multiplicity of increase, $\pi - 3.14$; d – small core diameter, D – large core diameter.

Computer processing of core measurements was carried out using the licensed software “Microsoft office Exel, 2013”, core classes acquired values as a percentage (%) of the maximum class. The set of results consisted of a karyocovariogram, which more objectively compares the distribution of the maximum and other modal classes of the cell population, which is related to the level of structural organization and differentiation. The reliability and generalization of the research results was carried out with the maximum reduction of random errors, in compliance with legal and legislative norms and requirements for scientific morphological research (V.D. Mishanov et al., 2007).

Results of the study and their discussion. As it is shown by our previous studies of an intact MG of a young woman in reproductive age, the terminal (proximal) areas of the duct of the gland, are formed by the acinus button at rest (without milk secretion), located within the lobule and surrounded by loose connective tissue (Fig. 1).

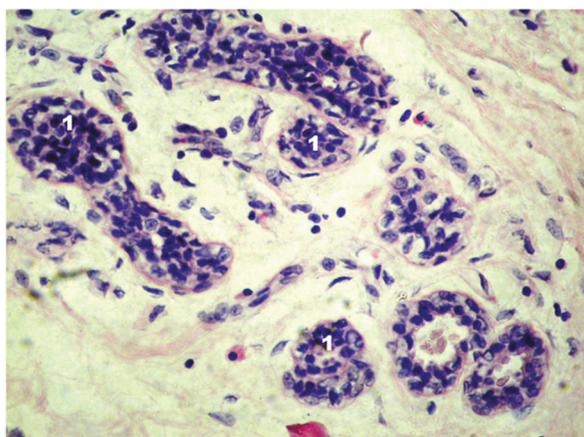


Fig. 1. Terminal ducts of the mammary gland of a woman with acini-buttons. Staining with hematoxylin and eosin. Magnification x400: 1 – acinus button at rest.

The morphological feature of the button is an almost absent, in histological sections, free opening filled with two types of cells: a small number – with basophilic nuclei surrounded by a narrow rim of eosinophilic cytoplasm, and, on the outside – a more numerous groups of cells with angular cytoplasm with appendages and basophilic core. There are capillary-type vessels in the loose connective tissue around the duct. The above provides favorable conditions for mammary gland trophism, which can be mediated by angular cells resembling smooth muscle cells. B+SHIA+AS staining of the terminal duct reveals inclusions around the nucleus of cells, and in their cytoplasm, which have a crimson-red color, corresponding to complex carbohydrates. Logarithmization of the

nuclei of the cell population of ductal buttons revealed the existence of two nuclear classes. Among them, there are those with a nuclear lgV of 0.7 and identified as myoepithelium, as well as those with an lgV of 0.55 and a much lower quantitative percentage are classified as cambial cells. The IHC characteristic of the latter is the pronounced (+++) expression of the p63 marker by cell nuclei, which confirms the hypothesis of cambial elements. Sometimes among them there are those with doubled nuclei and moderate (++) expression of p63 (being in anaphase of the mitotic cycle). The bcl-2 marker revealed local moderate (++) expression in the cytoplasm of these cells. In addition, in single cells of the duct button, the expression of the marker is diffuse in the cytoplasm. A population of cells of the duct is of an angular shape with a nuclear lgV of 0.7 according to IHC features has a moderate (++) expression of p63. The a-sma marker for smooth muscle actin has a pronounced expression (+++) both in the cytoplasm and in the processes of these cells. The latter cover the proliferating cell population in the center of the button of the proximal duct with their linear extensions of the cytoplasm and penetrate between it. Retrospective analysis of the morphological structure of the solid type of DMGC (duct mammary gland cancer). In the examination staining with hematoxylin and eosin, the latter is represented by atypical polymorphic epithelium, sometimes with signs of proliferation, which does not form special structures, filling the opening of the duct with an increase in its volume. At the same time, cell nuclei are polymorphic, basophilic and surrounded by deformed karyolemma. In figures of cell mitosis, there is an uneven distribution of chromatin between the division poles. The nuclei of cells are surrounded by eosinophilic cytoplasm of uneven intensity with admixtures of basophilic substance of a lamellar appearance. In some cellular elements, the cytoplasm has a bubbly appearance with clearing around the nucleus. In the basal sections of the cancerous duct, atypical epithelium with a small basophilic nucleus surrounded by a narrow rim of cytoplasm, as well as deformed, angular-shaped cells with cytoplasm that have rod processes of different

lengths are found. In a polymorphic-looking atypical population of cells of the solid type of DMGC, the mathematical calculation of the decimal lgV of the cell nucleus determined four stable nuclear classes with the lgV of the nucleus 0.95, 1.25, 1.4, 1.6. At the same time, each nuclear class of cancerous tumor cells is characterized by its structural and functional state. IHC analysis with a line of markers p63, a-sma, and bcl-2 and VEGF revealed the following features of these neoplasia cells. Thus, atypical cells with lgV core 1.6 have absent expression of p63 (-) light-basophilic color when stained with Mayer's hematoxylin. This atypical epithelium also has a negative reaction (-) to a-sma in its cytoplasm. A feature of the ICG reaction with bcl-2 was its diverse expression in terms of expression and localization in atypical cells. In some places, there is an almost complete lack of reaction of neoplastic cells with bcl-2. The GC feature of the reaction of the above-mentioned nuclear class with combined histochemical staining according to B+III+AC is the detected SHIA-positive blocks that are diffusely located in the cytoplasm of cells. This GC reaction is observed in cells of the nuclear class that have a negative expression of bcl-2. However, VEGF expression is absent in the neoplasia. Among other populations of cellular elements of this malignant neoplasia, a class with lgV core 1.6 was found. It corresponds to cells with large nuclei, a tortuous course of the karyolem and the content of granular chromatin located in their marginal part. The wide rim of the cytoplasm of cells, when examined with hematoxylin and eosin staining, is optically almost empty. Histochemical staining with Nile blue revealed blue vacuoles in the cytoplasm. Finally, the nuclear class, which makes 25 % (relative to the maximum) with lgV 1.4. It corresponds to cells with a dark basophilic deformed nucleus and barely basophilic cytoplasm. There is no connection between neoplasia cells and the basal membrane of the duct, heteropolarity is noted. These cells have a negative (-) expression of p63, and a constant, pyknotic expression of bcl-2. However, among them there are those that have a pyknotic nucleus. The lining of a cancerous duct of a solid type of DMGC is also formed by cells, the nuclear class of which is small in number and is not subjected to mathematical logarithmization.

However, according to IHC properties, those with a small dark-basophilic nucleus expressing p63 (+++) and surrounded by a narrow rim of cytoplasm are determined. (Fig. 2). At the same time, these cells have intense (+++) dot expression of bcl-2. Finally, IHC examination of angular cells with processional cytoplasm located along the wall of the cancerous duct revealed pronounced (+++) nuclear expression of p63. This expression of the marker had its continuation among the polymorphic population of neoplasia, dividing it into separate nodes.

A clear intraductal separation of the nodes of neoplasia with the formation of a gap is revealed, which has a connection with the connective tissue structure of the stroma of the MG, which contains a nearby vessel with the same expression of a-sma (Fig. 3). IHC bcl-2 has a high activity (+++) punctate reaction, covering almost all visible cellular elements, and continuing in processes of angular smooth muscle cells SMCs. The apparent basement membrane of the cancerous terminal duct-bud has alcian-positive staining with HC staining according to BSHAB. It is blue in color and intermittent in nature.

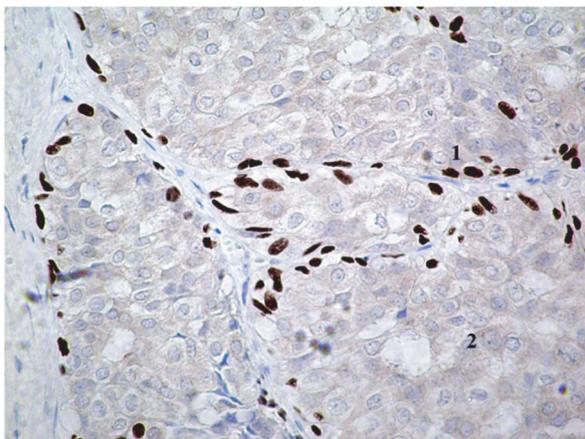


Fig. 2 Expression of p63 in the basal parts of the cancerous terminal duct with a solid type of breast cancer. IHC reaction with additional staining with Mayer's hematoxylin. Magnification x400. 1 – basally located cambial cells expressing p63; 2 – solid fields of the epithelium with no expression of the marker.

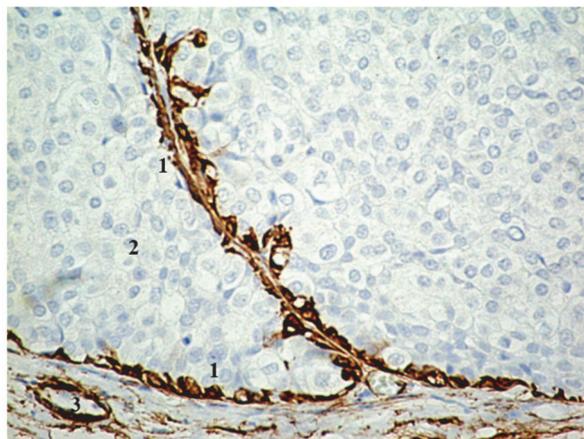


Fig. 3 Expression of α -sma on the periphery of intraductal nodules of a solid type of breast cancer. IGH reaction with additional staining with Mayer's hematoxylin. Magnification x400. 1 – basally located cells expressing a-sma; 2 – solid fields of atypical epithelium with absent a-sma expression; 3 – expression of a-sma by smooth muscle cells of the arteriole.

A comprehensive study of the histogenetic origin of solid breast cancer reveals the degree of differentiation, proliferative and apoptotic activity of the tumor in the patient at the time of the study. Clinicians-oncologists rely on these indices to select specific treatment to reduce the impact on the body.

Thus, Nel Janske et al. [1], propose the use of liposomes as a “Trojan horse” – a bioavailable dosage form that can contain antibodies, proteins, and functional peptides. At the same time, monoclonal antibodies have a selective effect on atypical cambial cells, cells of the parenchyma of a breast tumor, thanks to specific transmembrane, intracellular receptors and enzymes. But for this, pathohistological establishment of the tumor tissue’s histogenesis and its proliferative activity, apoptosis, etc. is important.

The myoepithelial component of the tumor, which separates its solid fields from the surrounding stroma, is also discussed in the scientific work of Maria Foschini et al. [5] and Anvar Shams [10], especially its barrier function that inhibits tumor invasion. Attention is focused on possible changes in the myoepithelium – from the degree of differentiation to hyperplastic growth, metaplastic and neoplastic transformations. In our work, based on the use of p63 and α -sma markers, it was established that the differentiation state of the myoepithelium is of cambial eodermal origin, but according to the content of smooth muscle actin in the cytoplasm, it is of mesenchymal origin, i.e. chimeric cells. Moreover, in the work of Anvar Shams [13], attention is focused on the need to stimulate the differentiation of the myoepithelium as a restraining factor of tumor invasion. Support of the extreme degree of cataplasia of the tumor by the bcl-2 protein restrains the apoptosis of cancerous cells, which supports the viability of the tumor regardless of the control by the “universal guardian of the cellular genome” – the p53 gene. The apoptotic role of the latter is highlighted in the work of Mihalcea [9].

Conclusions

1. Processes confirmed by immunohistochemical markers take place in the resting terminal duct of the MG: a) proliferation of the cambial epithelium (p63) in the proximal part of the button duct; b) hormone-directed apoptosis (bcl-2) of acinous button cells; c) smooth muscle cells (α -sma) in the composition of the acinar button functionally affect the trophism and differentiation of the cambial epithelium of the terminal duct.

2. A histogenetically solid type of ductal breast cancer originates from the cambial epithelium of the proximal part of the terminal duct – the acinus-button.

3. According to IHC properties, the extreme type of cataplasia is characteristic of solid cancer: with the expression of p63 and α -sma in the epithelium of the basal sections of the duct and the absence of these markers, in contrast to the expression of the antiapoptotic protein bcl-2, which indicates avoidance of apoptosis.

4. Ways of survival of atypical cells in a solid type of cancer of the terminal ducts of the MG in conditions of hypoxia are the capture of macro-organism glucose and its polymerization into glycogen (SHIA-positive reaction), as well as precursors of lipids (acetal lipids) and neutral fat (positive Nile blue).

Prospects for further research. The prospect of using the obtained results is to improve the molecular level of pathomorphological diagnosis of malignant neoplasia and differentiated therapy, taking into account the maturity and apoptotic activity of the tumor.

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CRANIOTOPOGRAPHIC CHARACTERISTICS OF RECTUS SINUS OF THE DURA MATER OF THE BRAIN IN PEOPLE OF MATURE AGE IN HEALTH

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For our investigation, we collected cadaveric material from 40 people of different ages and gender. We have prepared corrosive preparations of the rectus sinus of the dura mater of the brain. Our research has a variety of research methods for morphological research: topographic-anatomical preparation on the macroscopic and microscopic levels, cranial-morphometric examination of the rectus sinus, hardening of acrylic plastic for the preparation of corrosive preparations for the rectus sinus of the dura mater of the brain, injection method. The morphometric indicators we collected from the rectus sinus were subject to variation-statistical analysis. We also use the method of computer-graphic analysis. The data from our research can be used in planning surgical interventions on the structures of the dura mater of the brain – first for everything on the rectus sinuses of the dura mater of the brain.

Key words: human, mature age, dura mater of the brain, rectus sinus, craniotopographic method.

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КРАНІОТОПОГРАФІЧНА ХАРАКТЕРИСТИКА ПРЯМОЇ ПАЗУХИ ТВЕРДОЇ ОБОЛОНКИ ГОЛОВНОГО МОЗКУ У ЛЮДЕЙ ЗРІЛОГО ВІКУ У НОРМІ

Для нашого дослідження був використаний трупний матеріал від 40 людей різного віку та статі. Нами були виготовлені корозійні препарати прямого синуса твердої оболони головного мозку. У нашому дослідженні були використані різноманітні методи дослідження для морфологічних досліджень: топографо-анатомічне препарування на макроскопічному та мікроскопічному рівнях, краніо-морфометричне дослідження прямої пазухи, застосування акрилової пластмаси для виготовлення корозійних препаратів прямого синуса твердої оболони головного мозку, метод ін'єкції. Отримані нами морфометричні показники щодо прямої пазухи піддавалися варіаційно-статистичному аналізу. Також нами був застосований метод комп'ютерно-графічного аналізу. Дані нашого дослідження можливо використати при плануванні оперативних втручань на структурах твердої оболони головного мозку – перш за все на прямих пазухах твердої оболони головного мозку.

Ключові слова: людина, зрілий вік, тверда оболонка головного мозку, пряма пазуха, краніотопографічний метод.

The study is a part of the research project "Morphofunctional study of human internal organs and laboratory animals in various aspects of experimental medicine", state registration No. 0121U108258.

Vascularization of the human brain was and is the subject of close attention and the object of careful study by specialists in theoretical and practical medicine. However, the need for further study of the sinuses of the dura mater is due to the anatomical and functional importance of this part of the venous system of the brain [7–9].

Despite the fact that recently a considerable number of works devoted to the sinuses of the dura mater of the human brain have been published, there are quite a few literary sources devoted to the anatomical and topographic features of the direct sinus of the dura mater of the brain [3, 8, 9].

Rectus sinus is the second venous collector that provides blood outflow from the brain and is one of the complex formations of the dura mater of the brain and performs the transport function of blood outflow, while connecting the vena magna Galeni and the confluens sinuum. Quite a lot of information has been devoted to these issues in recent years [2–4, 6, 11–13, 15].