

DOI: 10.34921/amj.2023.4.019

**ALIN CİBLƏRİ ARAKƏSMƏSİNİN SELİKLİ QIŞASINDA OLAN VƏZİLƏRİN
İMMUNOHİSTOKİMYƏVİ XARAKTERİSTİKASI****O.M.Pronina, S.M.Bilash, M.M.Koptev, A.V.Piroq-Zakaznikova, B.S.Kononov,
Ya.O.Olinichenko, S.V.Donchenko, V.V.Oleksienko, O.V.Mamay**
Poltava Dövlət Tibb Universiteti, Poltava şəhəri, Ukrayna

Xülasə. Məqalədə alın payı cibləri arakəsmələrinin selikli qışasında olan vəzilərin immunohistokimyəvi xarakteristikasını öyrənmək məqsədilə aparılmış tədqiqat işi haqqında məlumat verilmişdir.

Tədqiqat 30 yaşla 87 yaş arasında vəfat etmiş 52 şəxsin meyitindən əldə edilmiş preparatlar üzərində aparılmışdır. Bu məqsədlə 52 nəfərin alın payı ciblərinin selikli qışasından hazırlanmış 120 preparatdan istifadə edilmişdir. Bu materiallardan yarımnazik preparatlar hazırlanılaraq, bilavasitə immunohistokimyəvi analiz üçün lazım gələn metodla işlənmişdir.

Tədqiqat göstərmişdir ki, alın arakəsməsi ciblərinin selikli qışasında olan vəzilərin stromasında lokalizasiya edən CD-3-müsbət hüceyrələr yerli qoruyucu bəyər funksiyasına malikdir. Bundan əlavə, preparatlarda çoxlu sayda periasinar yerləşmiş T-limfositlər müşahidə edilir. Arakəsmənin periasinar birləşdirici toxumasında CD-20⁺ hüceyrələrin sayı olduqca azdır.

Axacaqətrafi stromada B-limfositlər əhəmiyyətli dərəcədə çoxdur. Alın payı arakəsməsinin ciblərinin selikli qışasında olan vəzilərin periasinar stromasında plazmosit hüceyrələrin sayı da əhəmiyyətli dərəcədə çoxdur. Bu hüceyrələr bazal membranlarda zəncirşəkilli topalar təşkil edir və topaların hər birində 10-12 hüceyrə olur. Axacaqətrafi birləşdirici toxumada CD-138⁺ hüceyrələr periasinar sahədəkinə nisbətən azdır.

Açar sözlər: alın payı ciblərinin arakəsməsi, selikli qışa, immunhistokimyəvi analiz

Ключевые слова: перегородка лобной пазухи, слизистая оболочка, иммуногистохимический анализ

Key words: the frontal sinus septum, mucosa, immunohistochemical analysis

**IMMUNOHISTOCHEMICAL CHARACTERIZATION OF MUCOSAL GLANDS OF THE
HUMAN FRONTAL SINUS SEPTUM MUCOSA IN NORMAL STATE****O.M.Pronina, S.M.Bilash, M.M.Koptev, A.V.Pirog-Zakaznikova, B.S.Kononov,
Ya.O.Olinichenko, S.V.Donchenko, V.V.Oleksiienko, O.V.Mamai**
Poltava State Medical University, Poltava, Ukraine

The immunohistochemical characteristics of the glands of the mucous membrane of the septum of the human frontal sinus were studied in normal conditions.

The study was conducted on 120 preparations of the glands of the mucous membrane of the septum of the frontal sinus in 52 corpses of people of both sexes aged from 30 to 87 years who died from causes not related to the pathology of the paranasal sinuses. Our study used such methods as collecting material from corpses and the subsequent method of preparing the material for histochemical studies, serial semi-thin sections, and immunohistochemical analysis.

According to an immunohistochemical study, it was established that in the stroma of the septal glands, there are CD 3-positive cells, which are elements of the local protective barrier, as well as a large number of periacinar T-lymphocytes. The number of CD 20-positive cells is negligible in the periacinar connective tissue of the septum. The number of B lymphocytes in the periductal stroma was significant. Many plasma cells were identified in the periacinar stroma of both protein and mucous glands of the mucous membrane of the human frontal sinus. The cells formed chains of 10-12 cells along the basement membrane. The number of CD 138-positive cells in the periductal connective tissue was lower compared to the periacinar tissue.

Among the most common chronic human diseases, chronic diseases of the upper respiratory tract occupy a significant place [1-3]. It should be noted that chronic inflammatory processes, such as inflammation of the paranasal sinuses, nasal mucosa, and pharynx, are among the most common chronic diseases of the upper respiratory tract. They occupy a large share in quantitative values, both in absolute numbers and in their share in the structure of ORL-pathology in the face of an increasing trend of such pathologies against the backdrop of several years of the Covid-19 pandemic [4-5], the Russian-Ukrainian war [6], as well as multiple exo- and endogenous factors [7-8], including the state of the nervous system under stress and other psychopathologies [9-10].

Scientific studies of the frontal sinus still occupy a significant part in the work of anatomists, histologists, topographic-anatomists and other specialists and, naturally, otorhinolaryngologists, as specialists who directly deal with the pathologies of this sinus [11-13]. The frontal sinus has close contact with other paranasal sinuses, the orbit, the anterior cranial fossa, and in the case of purulent-inflammatory processes in this sinus, there is a high probability of pus spreading to adjacent areas, which complicates some surgical procedures. Studies of this sinus from different points have recently occupied a significant share in scientific articles.

From our point of view, little attention is paid to studies of the immunohistochemical characteristics of the glands of the mucous membrane of the frontal sinus and the septum, which is a significant drawback.

Immunohistochemical studies make it possible to analyze the state of the local immune system of the mucous membranes and the glandular apparatus itself. In our opinion, such studies will allow us to understand better and

analyze the causes of inflammatory processes in the frontal sinus, their diagnosis, and treatment. In the literature, more attention should be paid to the functional relationships between the state of the glandular apparatus of the mucous membrane of the septum of the human frontal sinus and the local immune system. Therefore, the relevance and feasibility of our research, in our opinion, are beyond doubt.

The purpose of our work is to study the immunohistochemical features of the glands of the mucous membrane of the septum of the human frontal sinus in normal conditions.

Research materials. *The object of the study* was the glands of the mucous membrane of the septum of the human frontal sinus.

The subject of the study was the immunohistochemical characteristics of the glands of the normal mucous membrane of the septum of the frontal sinus of the human nose.

The materials for the study were taken in the conditions of the Poltava Regional Pathological Bureau of the Health Department of the Poltava Regional Military Administration, as well as in the conditions of the forensic morgue of the Forensic Medical Examination Bureau of the Health Department of the Poltava Regional Military Administration. A total of 120 examples were taken. The glands of the mucous membrane of the septum of the frontal sinus were studied in 52 corpses of people of both sexes aged 30 to 87 years who died from causes unrelated to the pathology of the paranasal sinuses. The distribution of observation objects is presented in *table 1*.

Research methods. Our study was carried out using the technique of collecting material from corpses, followed by preparing the material for histochemical studies, serial semi-thin sections, and immunohistochemical analysis. The latter method is based on the specific interaction of poly- or monoclonal antibodies with tissue antigens of the glands of the mucous membrane of the septum of the human frontal sinus, which manifests itself due to a certain mark at the light-optical level.

Table 1. Distribution of the studied material by age groups and gender

Groups	Age	Number of objects		
		men	women	total
Early adulthood	20-40 years	5	5	10
Middle adulthood	40-60 years	12	5	17
Late adulthood	60 years and older	13	12	25
Total		30	22	52

In the conditions of our study, we were able to reduce the immunohistochemical study (IHC) to two stages using polymeric dextran molecules with secondary antibodies immunogenic to the primary ones and multiple peroxidase molecules. This IHC technique has significantly increased specificity and sensitivity by eliminating the biotin molecule from the system, possibly leading to falsely positive IHC results.

Our study used 4-6 μm thick sections mounted on SuperFrostPlus adhesive slides, which we deparaffinized. Because the material was fixed in formalin, which can reduce the immunogenicity of antigenic determinants, we carried out heat induction of epitope retrieval (HIER), thanks to which the antigenic properties of the tissues were restored. For this purpose, we used a citrate buffer with $\text{pH}=6.0$ heated in an autoclave to 121°C for 8 minutes under the symmetrical arrangement of glasses in the cuvette.

For our study, as primary antibodies, we used monoclonal antibodies to CD138, CD68 - a marker of macrophages, histiocytes (clone KP1, Dako), CD 20 - pan-B marker (clone L26, LabVision), CD3 - pan-T- marker (clone SP7, LabVision), as well as Ki-67 - a proliferation marker (clone SP6, LabVision). For each marker used, we conducted a control study to exclude false positive or false negative results. We used incubation of sections with primary antibodies in humid chambers at a temperature of $(23-25)^{\circ}\text{C}$. The titer was individually selected for each antibody, where a special antibody diluent solution (Dako) was used as a solvent.

The next stage of IHC consisted of visualization, for which the new generation UltraVisionLP (LabVision) system was chosen. Secondary antibodies enriched with chromium peroxidase molecules were applied to the sections and incubated in humidified chambers for 30 minutes with a mandatory post-incubation washing step of 10 minutes using a Tris-buffered solution. Identification of the reaction itself was carried out under the control of a microscope with the application of a chromogen (DAB (LabVision)), with manifestations in the form of dark brown staining of specific cellular structures, depending on the marker itself.

Differentiation of tissue structures was carried out by additional staining of sections with Mayer's hematoxylin for 1 to 3 minutes, followed by dehydration and imprisonment in balm.

Microscopy of the structures was carried out with an "OlympusBX 41" light optical microscope, which was combined with an "Olympus C 3040-A DUP" camera using $\times 10$, $\times 20$, $\times 40$, $\times 100$ lenses.

Research results and discussion

When studying paraffin sections of the mucous membrane of the nasal sinus septum, a large concentration of T-lymphocytes was determined in the terminal sections of the serous glands, localized in the periacinar connective tissue (*fig. 1*).

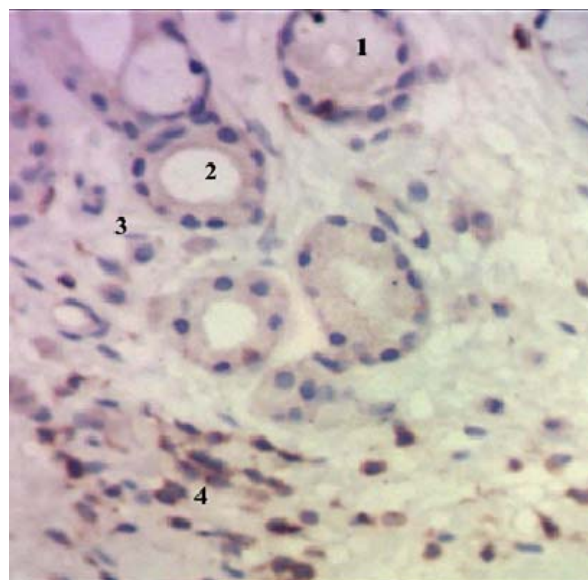


Figure 1. Reaction of monoclonal antibodies to CD 3 on intraepithelial and periacinar T-lymphocytes in the connective tissue of the serous glands of the mucous membrane of the septum of the human frontal sinus. Microphotography. Magnification: obh. 100, okh. 10: 1 – final section; 2 – excretory duct; 3 – periductal connective tissue; 4 – CD 3-positive cells.

Also, in this area, intraepithelial CD 3-positive cells were detected in the secretory epithelium of the terminal sections of the serous glands. They were detected close to the basement membrane between adjacent epithelial cells.

In the submucosa near the terminal mucosa of the septum of the human frontal sinus, CD 3-positive cells were not visualized (*fig. 2*).

Periductal single T-lymphocytes were detected, localized at a considerable distance from the basement membrane of the excretory ducts.

When conducting an immunohistochemical study in the stroma of the serous glands of the mucous membrane of the septum of the human frontal sinus, no B-lymphocytes were detected around the terminal sections.

Single CD 20-positive cells were localized perivascularly in the loose connective tissue between groups of acinar units, quite rarely - periductally (*fig. 3*).

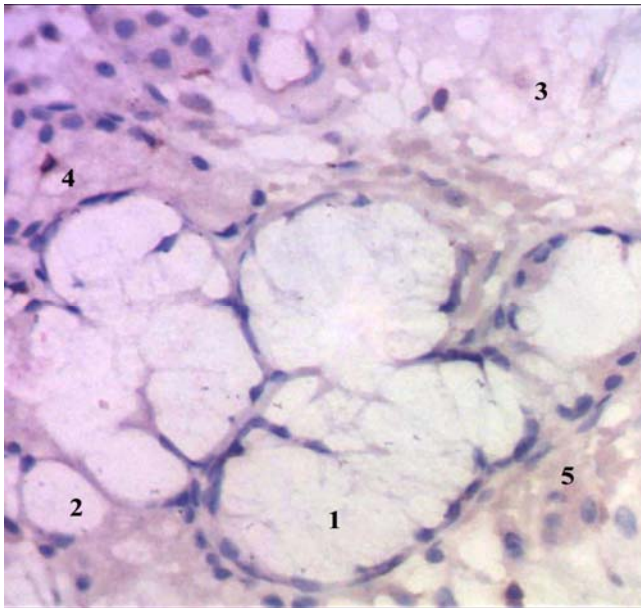


Figure 2. Reaction of monoclonal antibodies to CD 3 in the terminal sections and periacinar connective tissue of the mucous glands of the mucous membrane of the septum of the human frontal sinus. Microphotography. Magnification: obh. 100, okh. 10: 1 – mucous terminal section; 2 – venule; 3 – connective tissue of the mucous membrane; 4 – macrophage; 5 – collagen fibers.

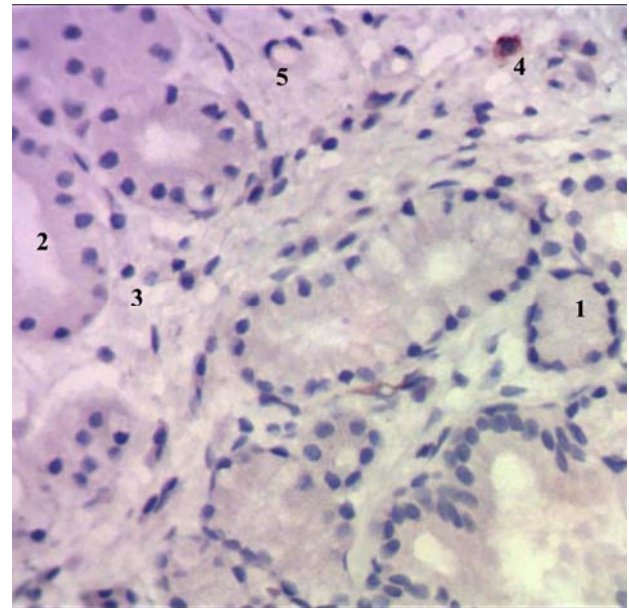


Figure 3. Reaction of monoclonal antibodies to CD 20 on B-lymphocytes in the connective tissue of the stroma of the serous glands of the mucous membrane of the septum of the human frontal sinus. Microphotography. Magnification: obh. 100, okh. 10: 1 – final section; 2 – excretory duct; 3 – periductal connective tissue; 4 – CD 20-positive cell; 5 – periductal CD 20-positive cell

Periacinar CD 20-positive cells in the mucous glands of the mucous membrane of the septum of the human frontal sinus were detected quite rarely (*fig. 4*).

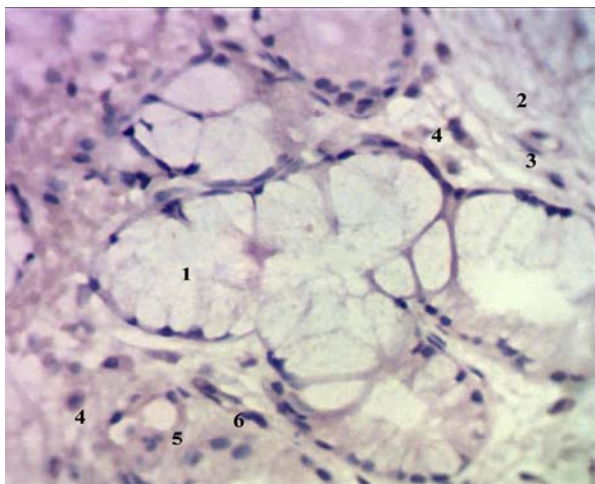


Figure 4. Reaction of monoclonal antibodies to CD 20 in the periacinar connective tissue of the mucous glands of the mucous membrane of the septum of the human frontal sinus. Microphotography. Magnification: obh. 100, okh. 10: 1 – mucous terminal section; 2 – collagen fibers; 3 – fibroblast body; 4 – CD 20-positive cells; 5 – venule; 6 – capillary.

In the connective tissue that surrounded the excretory ducts of the mucous glands of the mucous membrane of the septum of the human frontal sinus, B lymphocytes were not detected.

The number of CD 68-positive cells in the mucous membrane of the septum of the human frontal sinus was almost undetectable, both periacinar and periductal. The established phenomenon (a small number of antigen-presenting cells) indicates a slight antigenic load on the mucous membrane in this area.

A study of preparations of the human frontal sinus septum showed that the number of plasma cells around the terminal sections of the serous glands was significant in the mucous membrane. The marking intensity corresponded to 3 – “strong” when using a semi-quantitative assessment method.

CD 138-positive cells were localized predominantly in the connective tissue (“nodal interstitial compartments”) at the points of contact of the 3-4 terminal sections (*fig. 5*).

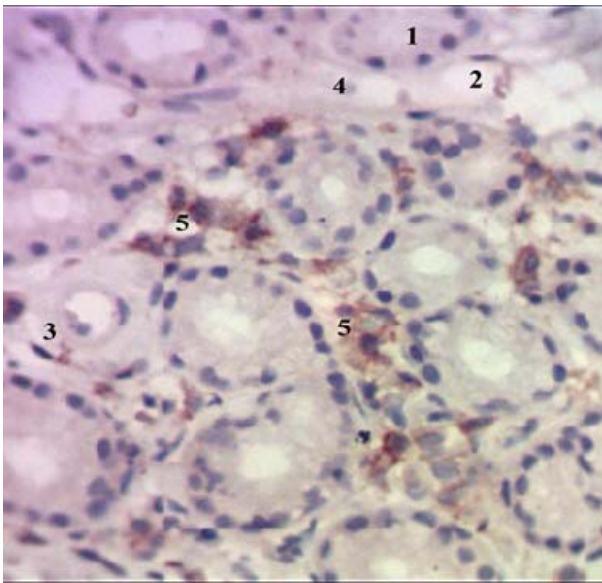


Figure 5. Reaction of monoclonal antibodies to CD 138 in the periacinar connective tissue of the serous glands of the mucous membrane of the septum of the human frontal sinus. Microphotography. Magnification: obh. 100, okh. 10: 1 - protein final section; 2 - venule; 3 - arteriole; 4 - periacinar connective tissue; 5 - CD 138-positive cells.

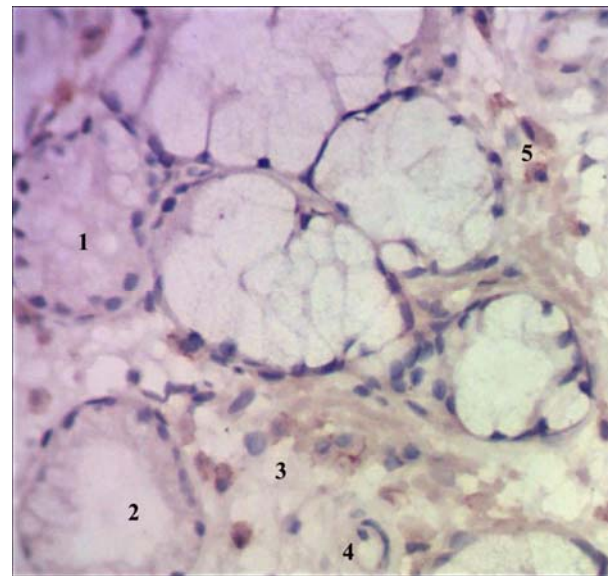


Figure. 6. Reaction of monoclonal antibodies to CD 138 on periacinar and periductal plasmacytes in the connective tissue of the mucous glands of the mucous membrane of the septum of the human frontal sinus. Microphotography. Magnification: obh. 100, okh. 10: 1 - final section; 2 - excretory duct; 3 - periductal connective tissue; 4 - venule; 5 - CD 138-positive cells.

CD 138-positive cells in the connective tissue of the mucous membrane of the septum of the human frontal sinus were single-around the terminal sections of the mucous glands.

The number of plasma cells in the periductal connective tissue stroma was significantly higher than in the periacinar stroma (*fig. 6*).

CD 138-positive cells were located in the stroma at a short distance from the basement membrane of the excretory ducts and formed groups of 2-3 cells.

In the mucous membrane of the septum of the human frontal sinus, Ki-67-positive cells were not detected in the secretory epithelium of the terminal sections of the serous glands.

As part of the epithelium of the excretory ducts, Ki-67-positive nuclei were found in the basal layer of pseudostratified epithelial cells.

In the periductal stroma of the glands, Ki-67-positive cells were detected, which were diffusely located between collagen fibers and fibroblast bodies (*fig. 7*).

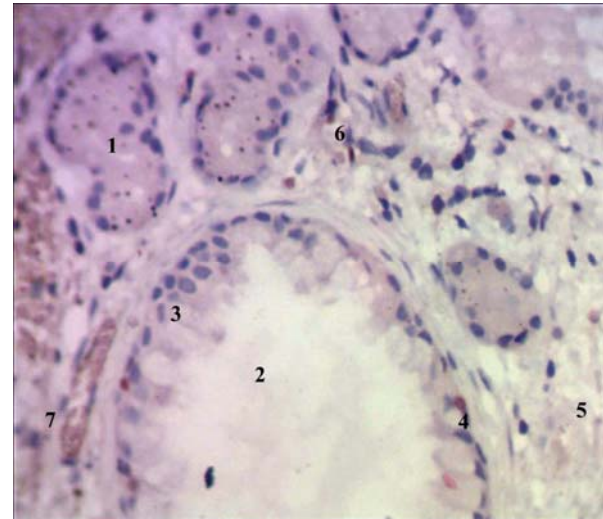


Figure. 7. Reaction of monoclonal antibodies to Ki-67 in the mucous membrane of the septum of the human frontal sinus. Microphotography. Magnification: obh. 100, okh. 10: 1 - final section; 2 - excretory duct; 3 - pseudostratified epithelium of the excretory duct of the serous gland; 4 - Ki-67-positive nuclei in the basal layer of the epithelium of the excretory duct of the serous gland; 5 - periductal connective tissue; 6 - Ki-67-positive cells; 7 - vein.

Conclusions. According to an immunohistochemical study, it was established that in the stroma of the septal glands, there are CD 3-positive cells, which are elements of the local protective barrier, as well as a large number of periacinar T-lymphocytes. The number of CD 20-positive cells is negligible in the periacinar connective tissue of the septum. The number of B lymphocytes in the periductal stroma was significant. Many plasma cells were identified in the periacinar

stroma of both protein and mucous glands of the mucous membrane of the human frontal sinus. The cells formed chains of 10-12 cells along the basement membrane.

The number of CD 138-positive cells in the periductal connective tissue was lower compared to the periacinar tissue. Thus, the data obtained in our study can become an essential element in analysing the causes of inflammatory processes in the frontal sinus, their diagnosis, and their treatment.

REFERENCES

1. Bousquet J, Pfaar O, Agache I, Bedbrook A, Akdis CA, Canonica GW, et al. ARIA-EAACI care pathways for allergen immunotherapy in respiratory allergy // *Clin Transl Allergy* 2021;11. <https://doi.org/10.1002/clin2.12014>.
2. Savchenko L, Mykytiuk M, Cinato M, Tronchere H, Kunduzova O, Kaidashev I. IL-26 in the induced sputum is associated with the level of systemic inflammation, lung functions and body weight in COPD patients // *Int J Chron Obstruct Pulmon Dis* 2018;Volume 13:2569–75. <https://doi.org/10.2147/COPD.S164833>.
3. Sousa-Pinto B, Sá-Sousa A, Vieira RJ, Amaral R, Klimek L, Czarlewski W, et al. Behavioural patterns in allergic rhinitis medication in Europe: A study using MASK-air® real world data // *Allergy* 2022;77:2699–711. <https://doi.org/10.1111/all.15275>.
4. Kaidashev I, Shlykova O, Izmailova O, Torubara O, Yushchenko Y, Tyshkovska T, et al. Host gene variability and SARS-CoV-2 infection: A review article. *Heliyon* 2021;7:e07863. <https://doi.org/10.1016/j.heliyon.2021.e07863>.
5. Boiko DI, Skrypnikov AM, Shkodina AD, Hasan MM, Ashraf GM, Rahman MH. Circadian rhythm disorder and anxiety as mental health complications in post-COVID-19 // *Environ Sci Pollut Res* 2022;29:28062–9. <https://doi.org/10.1007/s11356-021-18384-4>.
6. Haque U, Naeem A, Wang S, Espinoza J, Holovanova I, Gutor T, et al. The human toll and humanitarian crisis of the Russia-Ukraine war: the first 162 days // *BMJ Glob Heal* 2022;7:e009550. <https://doi.org/10.1136/bmjgh-2022-009550>.
7. Oliinichenko YO, Bilash SM, Pronina OM, Koptev MM, Oliinichenko MO, Bezeha O V., et al. The influence of the food additives complex on the remodeling processes of the small intestine structural components as an important medico-social problem of the public health current condition in Ukraine // *Bull Probl Biol Med* 2021;3:65. <https://doi.org/10.29254/2077-4214-2021-3-161-65-70>.
8. Harapko T V., Mateshuk-Vatseba LR. ELECTRONIC-MICROSCOPIC CHANGES OF THE SPLEEN UNDER THE ACTION OF SODIUM GLUTAMATE AND THEIR CORRECTION BY ORLISTAT. *Bull Probl Biol Med* 2020;3:217. <https://doi.org/10.29254/2077-4214-2020-3-157-217-221>.
9. Zhyvotovska L V., Skrypnikov AM, Sokil AA, Boiko DI. Affective disorders in long-term consequences of post-traumatic stress // *Bull Probl Biol Med* 2019;1:32. <https://doi.org/10.29254/2077-4214-2019-1-1-148-32-36>.
10. Shkodina A, Tarianyk K, Boiko D. Influence of sleep disturbances on cognitive decline in patients with parkinson's disease // *Ukr Sci Med Youth J* 2020;117:58–67. [https://doi.org/10.32345/USMYJ.3\(117\).2020.58-67](https://doi.org/10.32345/USMYJ.3(117).2020.58-67).
11. Koptev MM, Vynnyk NI, Kokovska O V, Pronina OM, Bilash SM. The use of immunohistochemical methods in the study of morphofunctional features of the human paranasal sinuses: literature review // *Wiad Lek* 2018;71:768–71.
12. Pronina OM, Koptev MM, Vynnyk NI, Proskurnya SA, Filenko BM. Current view on the structure and function of the frontal sinus: literature review // *Wiad Lek* 2018;71:1215–8.
13. Shkodina AD, Hrinko RM, Starchenko II. Modern conception as to the functional morphology of the olfactory system and its changes under the influence of some exogenous pollutants // *Med Ecol Probl* 2019;23:37–40. <https://doi.org/10.31718/mep.2019.23.3-4.09>.

ИММУНОГИСТОХИМИЧЕСКАЯ ХАРАКТЕРИСТИКА ЖЕЛЕЗ СЛИЗИСТОЙ ОБОЛОЧКИ ПЕРЕГОРОДКИ ЛОБНОЙ ПАЗУХИ ЧЕЛОВЕКА В НОРМЕ

Е.Н.Пронина, С.М.Билаш, М.Н.Коптев, А.В.Пирог-Заказникова, Б.С.Кононов,
Я.О.Олийниченко, С.В.Донченко, В.В.Олексиенко, О.В.Мамай

Полтавский государственный медицинский университет, г. Полтава, Украина

Резюме. Изучены иммуногистохимические характеристики желез слизистой оболочки перегородки лобной пазухи человека в норме.

Исследование проведено на 120 препаратах желез слизистой оболочки перегородки лобной пазухи у 52 трупов людей обоих полов в возрасте от 30 до 87 лет, которые умерли от причин, не связанных с патологией придаточных пазух носа. В нашем исследовании использовались такие методы как забора материала у трупов с последующим методом подготовки материала к гистохимическим исследованиям, методику серийных полутонких срезов и непосредственно самого иммуногистохимического анализа.

По данным иммуногистохимического исследования установлено, что в строме желез перегородки имеются CD 3-позитивные клетки, которые являются элементами местного защитного барьера, так же, как и большое количество периацинарных Т-лимфоцитов. Количество CD 20-положительных клеток незначительно в периацинарной соединительной ткани перегородки. Значительным было количество В-лимфоцитов в перипротоковой строме. Выявлено большое количество плазмочитов в периацинарной строме как белковых, так и слизистых желез слизистой оболочки лобной пазухи человека. Клетки формировали цепочки по 10-12 клеток вдоль базальной мембраны. Количество CD 138-положительных клеток в околопротоковой соединительной ткани было меньше, по сравнению с периацинарной.

Autor for correspondence:

Pronina Olena Mykolayivna, Poltava State Medical University, Department of anatomy with clinical anatomy and operative surgery

E-mail: vpbm@pdmu.edu.ua