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ГІСТОЛОГІЧНІ ТА МОРФОМЕТРИЧНІ ОСОБЛИВОСТІ МУКОЦИЛІАРНОЇ СИСТЕМИ СЛИЗОВОЇ ОБОЛОНКИ НИЖНЬОЇ ТА ЗАДНЬОЇ СТІНОК ЛОБОВОЇ ПАЗУХИ ЛЮДИНИ У НОРМІ

Метою даної роботи було вивчення особливостей мукоциліарної системи слизової оболонки лобової пазухи людини у нормі. Групний матеріал, отриманий від осіб обох статей віком від 26 до 75 років, які померли від причин, не пов'язаних з патологією приносних пазух, дослідження виконане відповідно до міжнародних рекомендацій з біологічних досліджень. Досліджувані залози являють собою складні альвеолярно-трубчасті залози, які складаються із системи вивідних проток з ацинусами. Результати нашого дослідження показали певну різницю у будові ацинусів залоз нижньої стінки, де вони були поділені на 2 типи. Перший тип характеризується утворенням ацинусів кубоподібними клітинами, а другий – пірамідними. За секреторною продукцією залози нижньої стінки визначаються як білково-слизові, а залози задньої стінки – змішані. Морфометрія показала, що значення середньої товщини (мкм) підслизового шару задньої стінки з обох боків були на 74% нижчими порівняно з показником для нижньої стінки. Достовірної різниці зовнішнього діаметра (мкм) ацинусів залоз вказаних стінок з обох сторін не виявлено.

Ключові слова: людина, лобова пазуха, слизова оболонка, залози.

Introduction. The late and recent data show that inflammation of the mucous membrane of the nasal vestibule, nasal cavity and paranasal sinuses, in particular the frontal sinus (FS), affects approximately 12% of the population (Kwah, & Peters, 2019). It is also known that acute and chronic paranasal sinusitis can cause complications in the adjacent structures, namely, the skull cavity and the orbit (meningitis, epidural abscess, venous sinus thrombosis, orbital abscess, etc). Despite numerous achievements of contemporary medicine, the above complications carry the risk of death and significant morbidity (Ziegler et al., 2018).

Numerous recent and later publications have been devoted to the study of the mucociliary system of the mucous membrane of the FS, though quite a large number of unsolved problems exist in contemporary rhinology and morphology to date (Dovbnia, 2017; Negus, 1957; Pronina et al., 2016). Therefore, in our opinion, the study of histotopographic and morphometric peculiarities of

the glands of the mucous membrane of the frontal sinuses is of great practical importance for modern morphology and clinical medicine.

The aim of investigation was to determine and compare the histological structure of the glands of the mucous membrane of the inferior and posterior walls of the normal human frontal sinus with the obtained morphometric indicators.

Material and methods. After obtaining the mucous membranes of the FS, their fragments were fixed in phosphate buffered 2.5% glutaraldehyde solution and embedded in the Epon-812 epoxy resin (Bilokon et al., 2014; Hryn et al., 2016; Kostylenko et al., 2007).

To obtain the semi-thin sections, the «Selmi» UMTP-7 (Sumy PA) ultramicrotome was used. Evaluation of the quality of the obtained sections was carried out using a stereoscopic microscope. For high-quality attachment of histological sections to the surface of the slide glass, the latter together with sections were kept in a thermostat for 24 hours at a temperature of 45–50°C. The sections were stained with 0,1% toluidine blue solution and 1% methylene blue according to J.A. Lynn, or polychrome method of staining histological preparations was used (Lynn, 1965; Shepitko et al., 2012; Yakushko et al., 2013).

The obtained preparations were studied on the «Konus» light microscope, equipped with the Sigeta DCM-900 9.0 MP digital microphoto attachment and the Biorex 3 (serial number 5604) software, adapted for the above studies.

To obtain morphometric parameters, the MOV-16 ocular-micrometer was used (Avtandilov, 1990). Morphometric method was used to determine metric parameters, namely, the outer diameters of the acini of the glands of the mucous membrane of the human FS. Statistical processing of the resulting data was carried out on a personal computer using the Statistica 13 and Microsoft Excel 2010 software packages (Tanavalee et al., 2016).

Results and discussion. The study of the frontal sinuses (FS) has raised our interest since the incidence of sinusitis (frontitis) and other pathological processes of the sinuses, and frontal sinuses in particular, is increasing yearly. For our study, the topographical and anatomical and functional peculiarities of this area, its relationship with other paranasal sinuses and structures of the skull are of great importance (Ziegler et al., 2018).

The inflammatory process that develops in FS has its own features, since the specified sinus is a closed cavity with a small amount of oxygen. This ensures favorable growth of anaerobic microorganisms, destruction of immunoglobulins and production of proteolytic enzymes with inhibition of microflora, adapted in the upper respiratory tract (Athanasopoulos et al., 2008).

In a small amount of oxygen, that is, hypoxia, the mucous membrane switches to anaerobic glycolysis with the accumulation of underoxidized metabolic products, which creates an acidic environment. This, in turn, leads to further disruption of mucociliary transport with disruption of metabolism in the mucous membrane, caused by the pathological process (Layko et al., 2013). Metabolic acidosis leads to inhibition of lysozyme action, i.e., immunological homeostasis disorder and increased inflammatory alterations of the mucous membrane (Dostbil et al., 2011). It also leads to such irreversible changes, as an increase in the number of goblet cells, squamous metaplasia of the respiratory epithelium with atrophic changes in the epithelium in conditions of plastic failure of the regenerative process with pronounced sclerotic changes in the lamina propria (Kirtsreesakul et al., 2009; Snidvongs et al., 2014).

The studies report that the morphological changes of the mucous membranes of the FS do not always correspond to the clinical manifestations of inflammatory processes of this sinus (Ramadan, 2009; Šuchaň et al., 2014).

The main feature of a normal or pathologically changed mucous membrane is its thickness. Studies have been conducted on 56 healthy people, in which, macroscopic study revealed no pathological changes, whilst histological study showed fibrous thickening of the subepithelial layer in 37 of them. These thickenings indicate the existing inflammatory process, or past inflammation (Sun et al., 2010).

The functional load on different walls of the FS is quite different. The mucous membrane of the inferior wall of the human frontal sinus tends to be more essential; it is connected to the nasal cavity through the middle nasal passage by the spine. It has been reported that the average thickness

of the submucous layer of the mucous membrane of the human FS has significant differences in the thickness of the submucous layer on different walls, and this made it possible to divide the values into two groups. It was shown that the thickness of the submucous layer on the anterior and inferior walls, for which the greatest values of the thickness of the mucous membrane were established, was by 3–4 times higher compared to the values of the posterior wall and septum of the frontal sinus (Serbin et al., 2019). Interesting data were obtained about the ratio of the thickness of the mucous membrane to the submucous layer. Notably, it was 1:3 for the anterior and inferior walls, and 1:2 for the posterior wall and septum (Serbin et al., 2019).

Additionally, the maximum concentration of glands in the mucous membranes of the frontal sinuses has been identified on the inferior wall, where they are located in two layers: superficial and deep. Glands with long and short excretory ducts were identified. The ductal part was represented by intralobular, interlobular and common excretory ducts (Довбня та ін., 2015).

The findings of the study of the mucous membrane of the inferior wall of the human FS have established that its submucous layer was formed by loose fibrous connective tissue with a pronounced network of microvessels, in which 2 types of complex branched glands were found, consisting of the acini and the system of excretory ducts.

In the Type I glands, the acini were formed by the cuboidal cells. Numerous secretory granules were found in the cytoplasm. The orbicular nuclei contained mainly decondensed chromatin and small grains of condensed chromatin, which were diffusely located in the karyoplasm. The nucleolus, mostly one, was eccentric. The optically dense secretory products and leucocytic cells, namely, lymphocytes, macrophages and plasma cells, were found in the lumens (Fig. 1A).

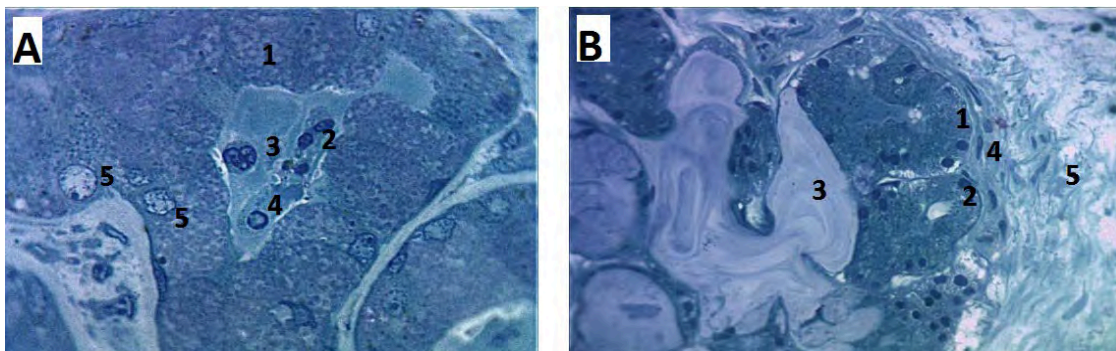


Fig. 1.

A: Acini of the Type I glands of the inferior wall of the human frontal sinus. Semi-thin section.

Methylene blue stain: Oc. lens: 10×magnification; Obj. lens: 100× magnification;

B: Type II glands in the mucous membrane of the inferior wall of the human frontal sinus. Semi-thin section. Polychrome stain: Oc. lens: 10×magnification; Obj. lens: 100× magnification.

A: 1 – glandulocyte; 2 – lymphocyte; 3 – plasma cell; 4 – macrophage; 5 – nucleus of glandulocyte;

B: 1 – acinus; 2 – nucleus of epitheliocyte; 3 – secretory products in the lumen of the duct; 4 – nuclei of fibroblasts; 5 – loose connective tissue.

A significant number of macrophages and plasma cells were detected around the acini of the glands.

The acini of the Type II glands contained pyramidal cells, the cytoplasm of which was densely filled with secretory granules. Optically dense nuclei were located in the basal parts of the cells. Basophilic layered secretory products were detected in the excretory ducts, which were a direct continuation of the acini of the glands (Fig. 1B).

Microvessels around the glands of both types were represented by the capillary-type vessels. The loose connective tissue around the glands was mainly represented by a fibrillar component (collagen and elastic fibers) with sporadic fibroblasts.

The findings of the study of the glands of the mucous membrane of the posterior wall have established that they were localized in the submucous layer, where collagen fibers and few cellular

elements, namely, fibroblasts, fibrocytes, the cells of hematogenous origin, prevailed. The glands of the mucous membrane were complex, branched and consisted of the acini and excretory ducts.

The cells of the acini were formed by the cylindrical cells with basophilic cytoplasm. Numerous fine secretory granules were found in the apical segments, which, when stained with toluidine blue, showed the α -reaction, indicating the predominance of proteins in their composition. Nuclei of a regular rounded shape with decondensed chromatin, indicating their functional activity, and one eccentric nucleolus were found in the central parts of epitheliocytes. On the semi-thin sections, the basal parts of the cells had a fine honeycomb appearance, due to the enlargement of the cisterns of the granular endoplasmic reticulum.

Sporadic cambial cells were characterized by optically dark homogeneous cytoplasm and small, irregularly shaped nuclei. Optically inhomogeneous basophilic contents were found in the lumens of the acini. Plasma cells with enlarged cisterns of the granular endoplasmic reticulum and characteristic arrangement of condensed chromatin in the nuclei were found very close to the basal membrane of individual acini. Nearby, mast cells with centric nuclei were detected in the secretory granules, indicating the apocrine type of heparin secretion into the surrounding connective tissue. Fibroblasts and collagen fibers, circularly oriented around the basement membrane, formed a capsule around the acini (*Fig. 2A*).

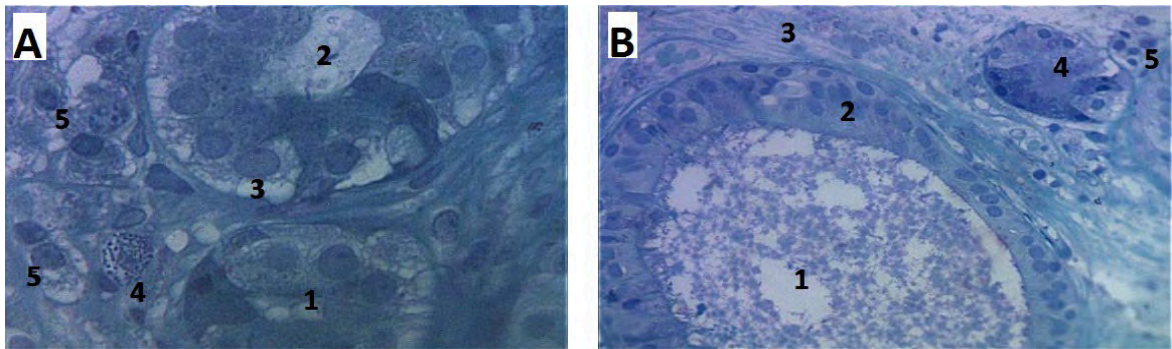


Fig. 2.

A: Acini of the glands of the posterior wall of human frontal sinus. Semi-thin section. Methylene blue stain: Oc. lens: 10×magnification; Obj. lens: 100× magnification;

B: Excretory ducts of the serous glands of the posterior wall of human frontal sinus. Semi-thin section. Toluidine blue stain: Oc. lens: 10×magnification; Obj. lens: 40× magnification.

A: 1 – acinus; 2 – lumen; 3 – nucleus of ductal epitheliocyte; 4 – plasma cell;

B: 1 – the acini; 2 – lumen of the duct; 3 – loose connective tissue of the submucous layer; 4 – secretory products in the lumen of the duct; 5 – microvessels.

The excretory ducts of the glands collect the secretory products from the acini. In the mucous membrane of the posterior wall of the FS, they were long, strongly branched and had a wide lumen near the surface. Their wall was formed by one or two layers of epitheliocytes. Fibrillar-granular contents of heterogeneous optical density were found in the lumens of the ducts.

Cylindrical cells with microvilli on the apical surface and basophilic cytoplasm were conjoined with the lumen of the duct. Small optically clear secretory granules were found in the supranuclear part. The orbicular nuclei were centric and contained mainly decondensed chromatin and one eccentric nucleolus.

Cuboidal cells were found near the basement membrane and had weakly basophilic homogeneous cytoplasm. The long axis of their ovoid nuclei was directed parallel to the basement membrane. Two nucleoli were detected in the karyoplasm.

Outside the basement membrane, bundles of collagen fibers and sporadic fibroblasts with spindle-shaped nuclei formed a rather dense capsule, which separated the ducts from the surrounding connective tissue. Microvessels were found behind it (*Fig. 2B*).

The morphometric study of the thickness of the structural elements of the mucous membrane of the inferior wall of the human FS revealed no statistically significant difference between the

average thickness of the submucous layer on the left and the right, accounting for $423,67 \pm 21,33 \mu\text{m}$ and $426,45 \pm 16,77 \mu\text{m}$, respectively. The mean values of the outer diameter of the acini of the glands of the inferior wall of the human FS were $30,42 \pm 2,36 \mu\text{m}$ on the left and $31,01 \pm 1,34 \mu\text{m}$ on the right. No significant differences in the dimensions of the acini on both sides were found (Table 1).

Table 1

The ratio of the morphometric parameters of the thickness of the submucous layer and the outer diameter of the acini of the glands of the inferior and posterior walls of the normal human frontal sinus, μm

Parameter	Inferior wall		Posterior wall	
	on the left (n=10)	on the right (n=10)	on the left (n=10)	on the right (n=10)
Thickness of the submucous layer	423.67 ± 21.33	426.45 ± 16.77	111.17 ± 9.77 *	115.47 ± 6.48 *
The outer diameter of the acini of the glands	30.42 ± 2.36	31.01 ± 1.34	29.77 ± 2.07	30.17 ± 2.25
Note: $\infty - p < 0.05$ compared to the values of the opposite side.			Note: * - $p < 0.05$ compared to the values of the inferior wall.	

The findings of the study of the posterior wall showed no statistically significant difference between the average thickness of the submucous layer on the left and the right, accounting for $111,17 \pm 9,77 \mu\text{m}$ and $115,47 \pm 6,48 \mu\text{m}$, respectively. The resulting morphometric data were by 74% lower compared to the value of the inferior wall, respectively. The findings of the morphometric study of the outer diameter of the acini of the glands of the posterior wall of the human FS, revealed that the mean values were $29,77 \pm 2,07 \mu\text{m}$ on the left and $30,17 \pm 2,25 \mu\text{m}$ on the right. We did not find any significant differences between the parameters of the dimensions of the acini of the glands of the inferior wall of the FS (Table 1).

Conclusions. The findings of our study have established that the complex branched alveolar-tubular glands were located in the mucous membrane of the inferior and posterior walls of the FS. These glands consisted of a system of excretory ducts with the acini. The glands of the inferior wall were divided into 2 types: the acini of the glands of the first and second type were formed by the cuboidal cells and pyramidal cells, respectively. The cells of the acini of the glands of the posterior wall were formed by the cylindrical cells. According to the nature of the secretory products, the glands of the inferior wall were proteinaceous and mucous, and those of the anterior wall were mixed.

The resulting metric data showed that the values of the average thickness (μm) of the submucous layer of the posterior wall (PW) on both sides were by 74% lower compared to the value of the inferior wall (IW) (PW: on the left – $423,67 \pm 21,33$; right – $31,01 \pm 1,34$ / IW: left – $111,17 \pm 9,77$; right – $115,47 \pm 6,48$). No significant difference in the outer diameter (μm) of the acini of the glands of the abovementioned walls on both sides has been found (PW: left – $30,42 \pm 2,36$; right – $426,45 \pm 16,77$ / IW: left – $29,77 \pm 2,07$; right – $30,17 \pm 2,25$).

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HISTOLOGICAL AND MORPHOMETRIC FEATURES OF THE MUCOCILIARY SYSTEM OF THE MUCOUS MEMBRANE OF THE INFERIOR AND POSTERIOR WALLS OF THE FRONTAL SINUS IN A NORM

The present paper was aimed at the study of the peculiarities of the mucociliary system of the mucous membrane normal human frontal sinus (FS).

Object and methods. Cadaveric material obtained from people of both sexes aged 26 to 75 years who died of causes not related to pathology of the paranasal sinuses has been studied in accordance with international guidelines for biological research.

Results. The investigated glands are complex alveolar-tubular glands, which consist of a system of excretory ducts with the acini. The findings of our study showed a certain difference between the structure of the acini of the glands on the inferior wall, where they were divided into 2 types. The first type is characterized by the formation of the acini by the cuboidal cells, and the second type by the pyramidal cells. By the secretory products, the glands of the inferior wall are defined as proteinaceous and mucous, and the glands of the posterior wall are mixed. The morphometry showed that the values of the average thickness (μm) of the submucous layer of the posterior wall (PW) on both sides were by 74% lower compared to the parameter for the inferior wall (IW). No significant difference in the outer diameter (μm) of the acini of the glands of the specified walls on both sides was found.

Keywords: human, frontal sinus, mucous membrane, glands.

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