ORIGINAL ARTICLE

MORPHOLOGICAL CHARACTERISTICS OF PLEOMORPHIC ADENOMAS OF SALIVARY GLANDS (ANALYSIS OF THE SURGICAL MATERIAL)

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ABSTRACT

The aim of the study is to reveal the morphological features of pleomorphic adenomas of the salivary glands during a comprehensive examination of the surgical material.

Materials and methods: Surgical material from 30 patients with pleomorphic adenomas of the salivary glands was studied. Microspecimens stained with hematoxylin and eosin were studied, using an Olympus BX-41 microscope (Japan) with subsequent processing with the Olympus DP-soft version 3.1 software, which was used to conduct a morphometric study. By morphometry in the tumor tissue, the specific volumes of the parenchyma and stroma, the thickness of the capsule located between the tumor tissue and the tissue of the salivary gland were determined; the absolute number of vessels in the field of view of the microscope was counted at × 100 magnification.

Results: Comprehensive morphological analysis of the surgical material of removed neoplasms of the salivary glands has showed that mesenchymal (15 cases, 50.0%) and mixed (10 cases, 33.3%) variants of pleomorphic adenomas are more common, and less often epithelial variants (5 cases, 16.7%). Pleomorphic adenoma is characterized by a different ratio of the epithelial (parenchymal) and mesenchymal (stromal) components forming this tumor, structural diversity and heterogeneity of the structure of these components, which do not have clear boundaries and are mixed with each other. A characteristic feature of pleomorphic adenoma is also the combination in each case of different types of epithelial cells and the structures that they form, as well as areas of various differentiation of the mesenchymal component. Mesenchymal and mixed variants of pleomorphic adenomas, in comparison with the epithelial variant, are more prone to progression and recurrence, as evidenced by our identified active processes of angiogenesis in tumor tissue, frequent tumor invasion of the capsule, thinning of the capsule or the absence of the capsule, less pronounced infiltration of the capsule by immune cells.

Conclusions: The morphological features of mesenchymal, mixed and epithelial variants of pleomorphic adenomas of the salivary glands revealed by the authors should be taken into account by clinicians during choosing the tactics for treating the patient, which will undoubtedly help to reduce the incidence of tumor malignization and its recurrence.

KEY WORDS: pleomorphic adenoma, salivary gland, morphology

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INTRODUCTION

Salivary gland tumors, according to the World Health Organization (WHO), represent 3 to 6% of all tumors of the head and neck region, with an annual incidence throughout the world ranging from 0.05 to 2 cases per 100,000 individuals [1]. Epidemiological data reveal different frequencies of salivary gland tumors in distinct ethic groups and geographic locations, which make it difficult to establish global estimates [2].

Pleomorphic adenoma ranks as the commonly occurring benign tumor and constitutes up to two-thirds of all salivary gland neoplasms [3]. Mostly, pleomorphic adenoma is located in the parotid glands (85%), minor salivary glands (10%) and the submandibular glands (5%). In the majority of cases, tumors originate in the superficial lobe. However, occasional cases may involve the deep lobe of the parotid gland and the parapharyngeal space. Minor salivary gland tumors are frequently encountered on the palate, followed by the lip, cheek, tongue and floor of the mouth [4].

The peak incidence of pleomorphic adenoma occurs at a mature age (30-50 years), with women prevailing among the cases (male: female ratio of about 1:1.4) [5]. Pleomorphic adenoma is a tumor with complex histoarchitectonics, characterized by slow growth, scant clinical symptoms and a tendency to recurrence [6]. It has been noted that 50% of recurrences of pleomorphic adenomas of the salivary gland are found in the first two years after surgery. By the end of the five-year follow-up, up to 80% of these neoplasms recur [7].

Clinicians often find it difficult to diagnose pleomorphic adenomas of the salivary glands. This leads to misdiagnosis and, as a result, the choice of the wrong tactics for treating the patient. Errors in diagnosis, according to various authors, range from 7 to 46% of cases. The greatest difficulties arise in the differential diagnosis of neoplastic and reactive-degenerative processes [8].

The most informative, accurate and valuable method of intravital diagnosis of pleomorphic adenoma is morphological examination of biopsy and surgical material. Despite numerous publications of domestic and foreign scientists, interest in the study of the morphological features of pleo-

morphic adenomas of the salivary glands has not weakened until now and remains relevant [9].

THE AIM

The aim of the study is to reveal the morphological features of pleomorphic adenomas of the salivary glands during a comprehensive examination of the surgical material.

MATERIALS AND METHODS

Surgical material from 30 patients with pleomorphic adenomas of the salivary glands was studied. The resulting material was fixed in a 10% solution of neutral buffered formalin, carried out according to the generally accepted method and embedded in paraffin. Serial sections 3-4 μm thick were made from paraffin blocks. Microspecimens stained with hematoxylin and eosin were studied, using an Olympus BX-41 microscope (Japan) with subsequent processing with the Olympus DP-soft version 3.1 software, which was used to conduct a morphometric study. By morphometry in the tumor tissue, the specific volumes (%) of the parenchyma and stroma, the thickness of the capsule (μm) located between the tumor tissue and the tissue of the salivary gland were determined; the absolute number of vessels in the field of view of the microscope was counted at × 100 magnification.

Statistical processing of the obtained digital data was carried out, using the Statistica 10.0 program. The means were compared, using the nonparametric Mann-Whitney U-test. Differences were considered significant at $p<0.05$.

RESULTS

Observational microscopy in all 30 cases revealed fragments of the salivary gland with adjacent tumor tissue, the structure of which corresponded to pleomorphic adenoma. The pleomorphic adenoma in all studied cases was characterized by structural diversity and heterogeneity, which was due, firstly, to the different ratio of the epithelial (parenchymal) and mesenchymal (stromal) components, forming this tumor, and secondly, to the complexity and multicomponent structure of the parenchyma itself and the stroma of the tumor.

The epithelial component in pleomorphic adenomas was represented by epithelial and myoepithelial cells. Epithelial cells were characterized by polymorphism, as they were of different size, shape and nuclear-cytoplasmic ratio. Epithelial cells in most fields of view were round, polygonal, or cubic. Epithelial cells were of the basaloid, spindle-cell, squamous, clear-cell, or plasmocytoid type in part of the visual fields. Less commonly, the epithelial component was represented by mucous, sebaceous, serous or multinucleated cells.

In all 30 cases, clusters of epithelial cells were in the form of nests or strands, which anastomosed with each other (fig. 1, 6). Epithelial cells in 22 cases formed solid, trabecular, cystic, glandular, ductal, or tubular structures (fig. 2, 3). In 4 cases, epidermoid differentiation with the formation of epithelial pearls was revealed in solid beds and ducts (fig. 4).

Myoepithelial cells were few in number, characterized by a focal location and were detected in 14 cases (fig. 3). These cells were located loosely or tightly adjoined to each other, were polygonal or spindle-shaped, sometimes resembling smooth muscle cells.

The mesenchymal (stromal) component of the pleomorphic adenoma in all cases was represented by connective tissue layers or fields with vessels of various sizes located between the fibers. In part of the visual fields, stromal hyalinosis was detected. Vessels in some of the visual fields looked dilated and full-blooded (fig. 5). Diapedetic hemorrhages were observed around some vessels (fig. 5). In all cases, the mesenchymal component of the tumor, in addition to connective tissue, was characterized by the presence of myxoid, chondroid and mucoid zones (fig. 1, 2, 4, 6, 7). In 2 cases, osteoid zones were found in the tumor stroma (fig. 5), and in 1 case – areas of lipomatosis (fig. 6).

It is interesting that in each case, a combination of, first, different types of epithelial cells and the structures that they formed, and, second, areas of different differentiation of the mesenchymal component, was revealed in a pleomorphic adenoma. A characteristic feature of all studied cases of pleomorphic adenomas was absence of clear boundaries between the epithelial and stromal components, mixed with each other.

In all studied 30 cases of pleomorphic adenomas, during morphometry, a different ratio of the specific volumes of the parenchymal and stromal components was noted. As a result, three variants of the tumor were identified. In 15 cases, a mesenchymal variant of pleomorphic adenoma was identified, characterized by a predominance ($p<0.05$) of the stromal component ((84.50±4.41)%) over the parenchymal component ((15.50±4.44)%). In 10 cases, a mixed or classic variant of a pleomorphic adenoma was determined, characterized by the absence ($p>0.05$) of differences between the specific volume of the parenchyma ((53.67±1.49)%) and stroma ((46.33±1.48)%). In 5 cases, an epithelial variant of the tumor was identified, in which the parenchymal component ((87.86±2.16)%) prevailed ($p<0.05$) over the stromal component ((12.14±2.18)%).

When calculating the absolute number of vessels, it was noted that the mesenchymal and mixed variants of pleomorphic adenomas were characterized by a large ($p<0.05$) content of vessels compared to the epithelial variant of the tumor. Thus, the absolute number of vessels in the mesenchymal, mixed and epithelial variants of pleomorphic adenomas was 12.83±1.02, 12.50±0.84, and 8.86±1.02, respectively.

In all cases of pleomorphic adenomas, the morphological features of the capsule located between the tumor tissue and the tissue of the intact salivary gland were assessed during the survey microscopy of the microspecimens and morphometric examination.

On survey microscopy, the capsule consisted of thickened connective tissue fibers with focal infiltration of immune cells, represented mainly by lymphocytes and macrophages (fig. 8). The latter, in part of the visual fields, penetrated the tumor tissue and the tissue of the salivary gland. The interesting thing is that the immune infiltration in the capsule was more pronounced in the epithelial variant of the pleomorphic

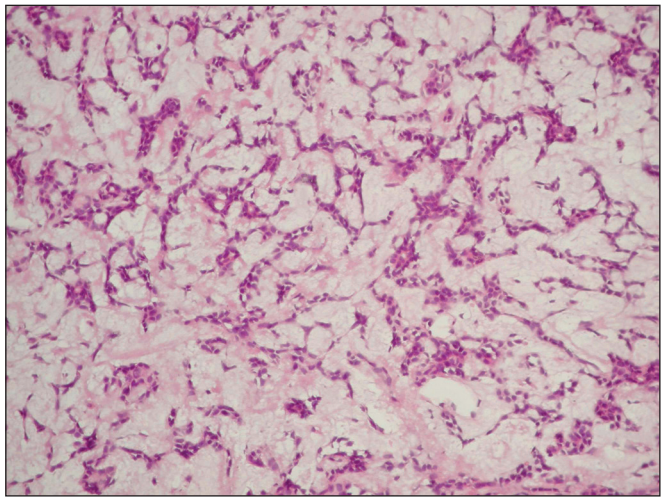

Fig 1. Epithelial cells of the tumor form nests and cords that anastomose with each other. The mesenchymal component of the tumor is represented by myxoid and mucoid zones. Stained with hematoxylin and eosin, × 200.

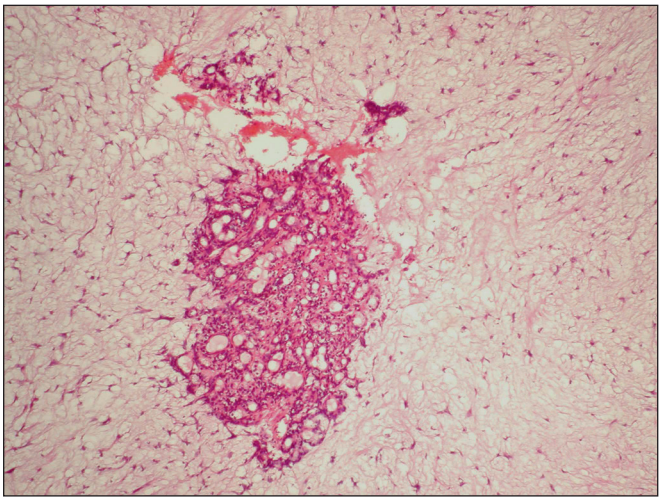

Fig. 2. Glandular, ductal and microcystic structures in the tumor parenchyma. The mesenchymal component of the tumor is represented by myxoid and mucoid zones. Stained with hematoxylin and eosin, × 100.

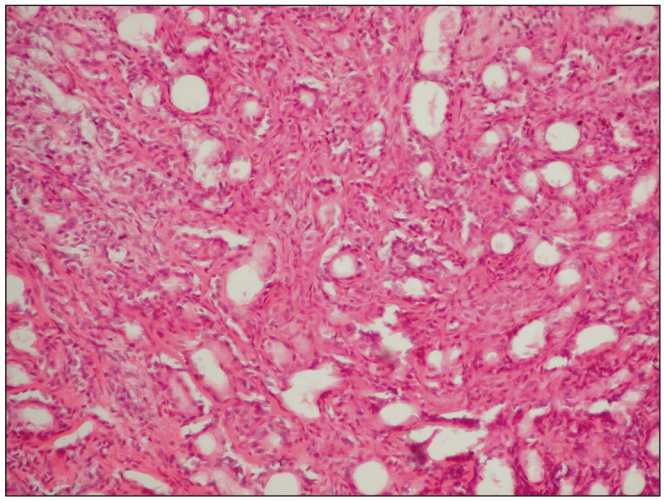

Fig. 3. Ductal and glandular structures in the epithelial component of the tumor. Few myoepithelial cells. Stained with hematoxylin and eosin, × 200.

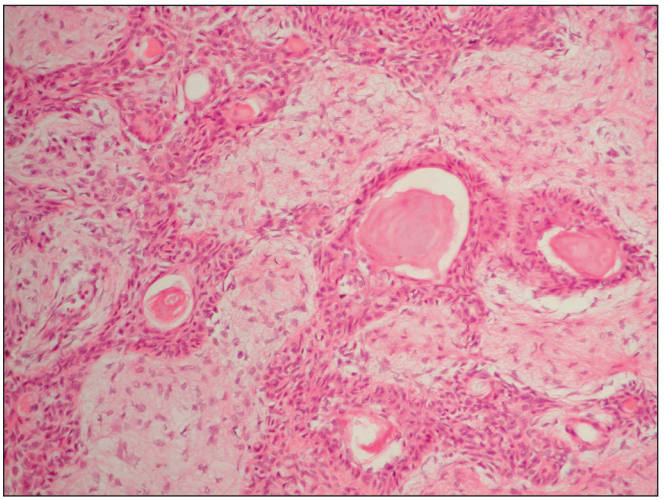

Fig. 4. Epidermoid differentiation in the epithelial component of the tumor with the formation of horny cysts. The stroma of the tumor is represented by connective tissue with myxoid and mucoid areas. Stained with hematoxylin and eosin, × 200.

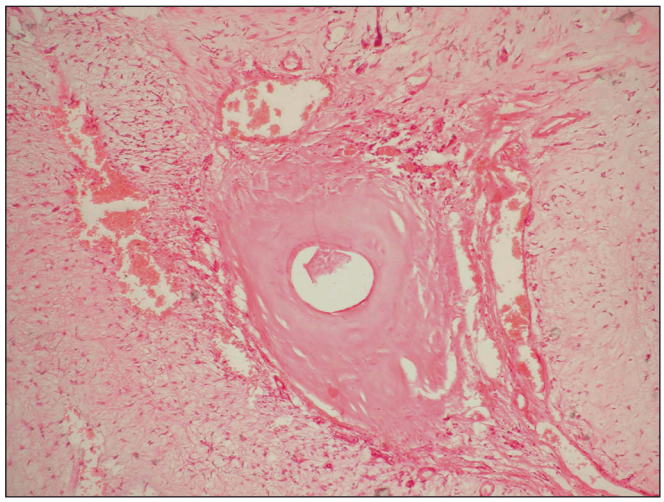

Fig. 5. Osteoid area in the tumor stroma. Hyperaemia of the stromal vessels with the formation of diapedetic hemorrhages. Stained with hematoxylin and eosin, × 100.

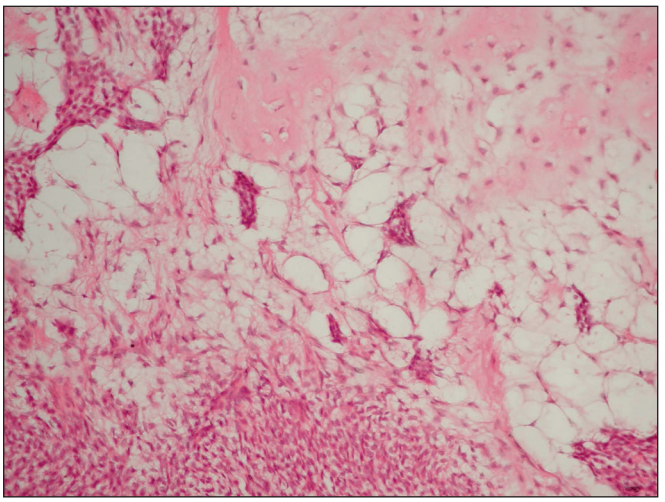

Fig. 6. Epithelial component of the tumor in the form of nests and intertwining strands. The mesenchymal component of the tumor is represented by myxoid, chondroid, mucoid zones and areas of lipomatosis. Stained with hematoxylin and eosin, × 200.

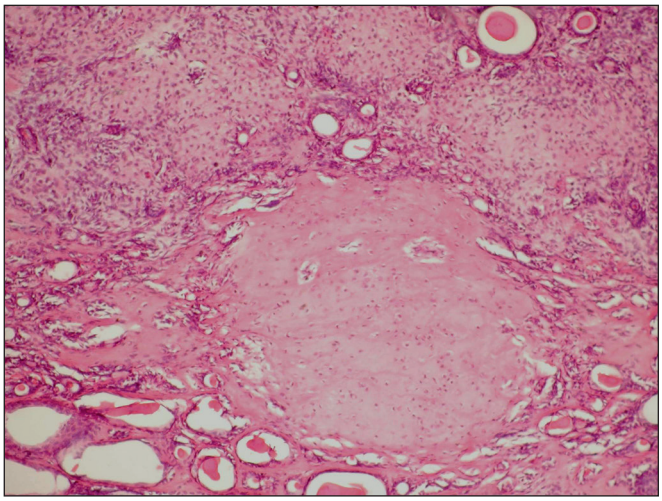

Fig. 7. Chondroid sites in the mesenchymal component of the tumor. Stained with hematoxylin and eosin, × 100.

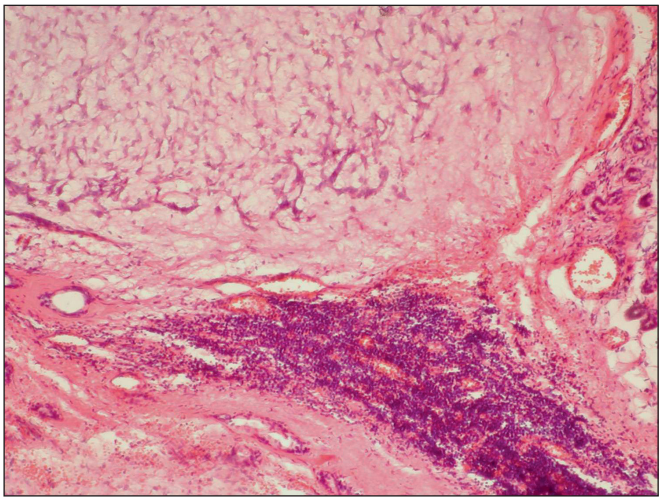

Fig. 8. An unevenly expressed connective tissue capsule separating the tumor tissue from the tissue of the salivary gland. Focal infiltration by immune cells of the capsule. Unevenly expressed neoangiomatosis. Stained with hematoxylin and eosin, × 100.

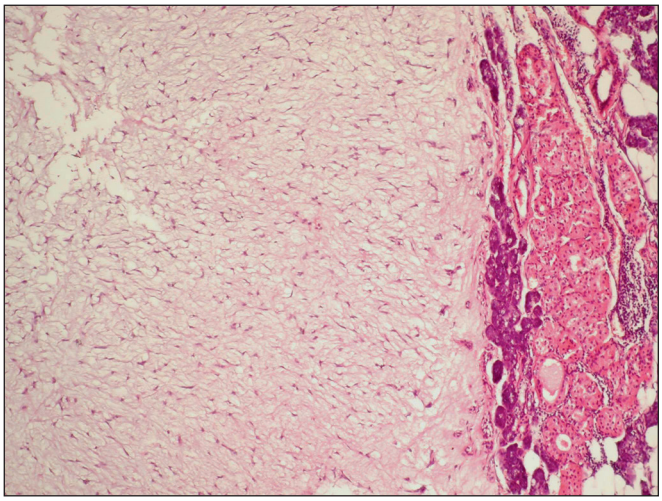

Fig. 9. Absence of a capsule between the tumor tissue and the tissue of the salivary gland. Stained with hematoxylin and eosin, × 100.

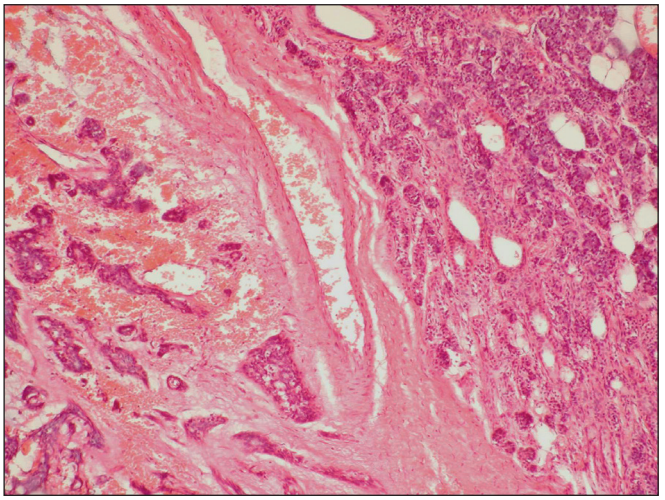

Fig. 10. Invasion of the tumor into the connective tissue capsule. Stained with hematoxylin and eosin, × 100.

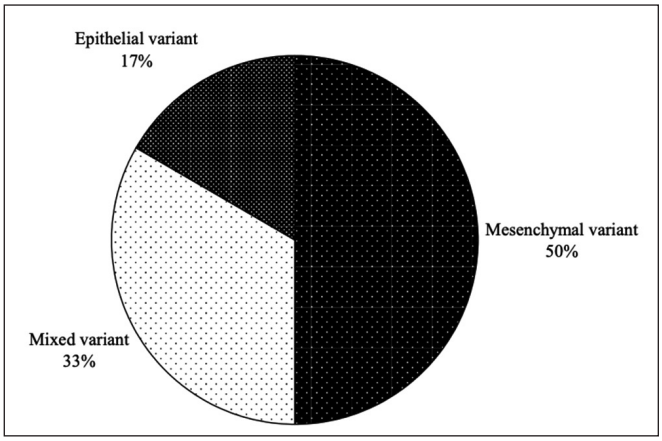

Fig. 11. Variants of pleomorphic adenomas among the studied cases.

adenoma, and less pronounced or not at all determined in the mesenchymal and mixed variants of the tumor.

In different variants of pleomorphic adenomas, an analysis of cases with an intact capsule, the absence of the capsule (fig. 9) and tumor invasion into the capsule (fig. 10) was carried out (table 1).

In cases where a capsule separating the tumor tissue from the tissue of the salivary gland was detected during the survey microscopy, its thickness was measured (table 1). It was noted that the mean value of the capsule thickness in the mesenchymal and mixed variants of the tumor was significantly ($p<0.05$) smaller than in the epithelial variant.

DISCUSSION

It is well known that the human salivary glands, performing protective-trophic, endocrine, excretory and regulatory functions, can be an arena for the development of tumor processes [8]. Pleomorphic adenoma is the most common benign tumor of the salivary glands [6]. Pleomorphic adenoma was first termed by Willis. In the earlier years, it was

Table 1. Morphological features of the capsule in different variants of pleomorphic adenomas

Pleomorphic adenoma variant	Capsule thickness (μm)	Intact capsule	Tumor invasion into the capsule	The capsule was not detected
		Number of cases,%		
Mesenchymal	6.33±1.32	3 cases, 20.0%	8 cases, 53.3%	4 cases, 26.7%
Mixed	6.95±1.22	2 cases, 20.0%	4 cases, 40.0%	4 cases, 40.0%
Epithelial	14.20±1.58	4 cases, 80.0%	1 case, 20.0%	–

also referred to as mixed tumor, enclavoma, branchioma, endothelioma, endochroma etc [3].

The histogenesis of pleomorphic adenomas of the salivary glands is a controversial issue, which explains the existence of various theories of the origin of the tumor today [10]. The source of pleomorphic adenomas of the salivary glands development can be epithelial cells lining the secretory sections and excretory ducts, as well as myoepithelial cells containing secretory elements in the cytoplasm. The development of this tumor from elements of the stroma is also possible [11]. An important role in the histogenesis of pleomorphic adenomas is assigned to the phenomenon of epithelial-mesenchymal transformation [12].

Pleomorphic adenoma, according to the literature [13] and our study, is characterized by a complex structure. In all 30 cases, it was characterized by a different ratio of its constituent epithelial (parenchymal) and mesenchymal (stromal) components, their structural diversity and heterogeneity, which did not have clear boundaries and were mixed with each other. A characteristic feature also for each case was a combination of various types of epithelial cells and the structures that they formed, as well as areas with different differentiation of the mesenchymal component.

Pleomorphic adenoma of the salivary glands is known to be of four variants. Traditional or classic version, which is characterized by the same content of epithelial and mesenchymal components. The mesenchymal variant of the tumor is characterized by the predominance of the mesenchymal component over the epithelial one. In the epithelial variant of the tumor, the epithelial component predominates over the mesenchymal one. The myoepithelial variant is characterized by a significant content of the myoepithelial component [14].

In our study, among 30 cases, mesenchymal, epithelial and mixed variants of the structure of pleomorphic adenomas were identified (fig. 11). A significant number of cases accounted for mesenchymal and mixed variants of the tumor structure.

Tumors, like normal tissues, need nutrients and oxygen to live, as well as the removal of metabolic products and carbon dioxide. These needs are met by neovascularization – the formation of a network of new blood vessels in the tumor [15].

Active processes of angiogenesis in a tumor, as is known, contribute to its malignancy, the progression of the process, and also increase the likelihood of recurrence [1]. Our survey microscopy, morphometric study with the calculation of the absolute number of vessels in the field of view of the microscope × 100 has showed that the mesenchymal and mixed variants of pleomorphic adenomas are characterized by the highest content of vessels, and the epithelial variant is characterized by the lowest content. The pronounced angiomatous component revealed by us, histogenetically associated with mesenchyme, in mesenchymal and mixed variants of pleomorphic adenomas of the salivary glands, may have an unfavorable prognostic value in terms of malignancy or recurrence of the tumor after its removal.

Benign tumors are known to be characterized by the presence of a well-defined connective tissue capsule. Numerous works have emphasized the barrier function of the capsule. Encapsulated tumors are less aggressive than non-encapsulated tumors [16].

In our study, in the case of mesenchymal and mixed variants of pleomorphic adenomas, in comparison with the epithelial variant of the tumor, more ($p<0.05$) cases with tumor invasion into the capsule and fewer ($p<0.05$) cases with an intact capsule were revealed (table 1). In the case of mesenchymal and mixed variants, cases with the absence of a connective tissue capsule were identified, however, in the epithelial variant of the tumor, such cases were not identified. During morphometry, it was noted that the connective tissue capsule was thinner ($p<0.05$) in mesenchymal and mixed variants of the tumor compared to the epithelial variant. Thus, the analysis of the survey microscopy and the performed morphometric study indicates that the mesenchymal and mixed variants of pleomorphic adenomas are more prone to recurrence compared to the epithelial variant.

Observation microscopy in the connective tissue capsule separating the tumor tissue and intact tissue of the salivary gland revealed focal immune infiltration, which was more pronounced in the epithelial variant of pleomorphic adenoma and less pronounced in the mesenchymal and mixed variants. This infiltration, from our point of view, plays a protective role [17], preventing the invasion of the tumor into the capsule, its progression and recurrence. Thus, the less pronounced immune infiltration in the capsule with mesenchymal and mixed variants of pleomorphic adenomas revealed by us indicates that these variants of the tumor are more prone to invasion into the capsule and penetration into the adjacent tissue of the salivary gland, as well as recurrence.

CONCLUSIONS

1. Comprehensive morphological analysis of the surgical material of removed neoplasms of the salivary glands has showed that mesenchymal (15 cases, 50.0%) and mixed (10 cases, 33.3%) variants of pleomorphic adenomas are more common, and less often epithelial variants (5 cases, 16.7%).

2. Pleomorphic adenoma is characterized by a different ratio of the epithelial (parenchymal) and mesenchymal (stromal) components forming this tumor, structural diversity and heterogeneity of the structure of these components, which do not have clear boundaries and are mixed with each other. A characteristic feature of pleomorphic adenoma is also the combination in each case of different types of epithelial cells and the structures that they form, as well as areas of various differentiation of the mesenchymal component.
3. Mesenchymal and mixed variants of pleomorphic adenomas, in comparison with the epithelial variant, are more prone to progression and recurrence, as evidenced by our identified active processes of angiogenesis in tumor tissue, frequent tumor invasion of the capsule, thinning of the capsule or the absence of the capsule, less pronounced infiltration of the capsule by immune cells.

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The Authors declare no conflict of interest.

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