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Evaluation of the Possibility of E-Cadherin, P-Catenin and Cytokeratins Expression in the Diagnostics and Prognosis of the Mammary Gland Accessory Lobe Diseases

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Abstract **The aim** of our study was to develop new criteria for the differential diagnosis of variants of intraepithelial neoplasia of the mammary gland accessory lobe based on the use of a comprehensive morphological method of investigation using an optimal panel of markers to improve the quality of diagnosis and assessment of invasive cancer risk. **Background.** Along with breast cancer, the number of cases of the mammary gland accessory lobe cancer has increased in recent years. This trend requires careful screening among women from the high-risk group, which include, first of all, patients with precancerous lesions of the breast. The method of immunohistochemistry in recent years has become widely used by researchers in the study of intraepithelial neoplasia of the breast and its lobe with the aim of early diagnosis and prognosis. **Material and methods.** The study was based on the results of a retrospective examination of 182 patients with mammary gland accessory lobe cancer. According to the morphological examination results, the patients were divided into 4 groups. The intensity of reactions localized in the cytoplasm and on cell membranes was evaluated by a semi-quantitative method on a point scale from O to 3 using the Leica Q550 image analyzer. **Results.** In 31 (50.0%) cases of dysplasia the process was located in the right breast, in 27 (43.6%) women - in the left breast, and in 4 (6.4%) cases the lesion was bilateral. There were no significant differences in the location of the pathological process in invasive ductal carcinoma. At invasive lobular carcinoma in situ in 12 (42.9%) cases the pathological process was located on the left, and in 16 (57.1%) cases – on the right. **Conclusion.** Thus, the expression of cytokeratins 5/6 and 14 types is significantly more often observed in dysplasia (p= 0.0005), which allows them to be used for additional differential diagnosis of dysplasia and highly differentiated carcinomas in situ. Determination of the levels of cytokeratins 5/6, H, cyclin D1, E-cadherin and 3-catenin is the most important tool for the differential diagnosis of various types of breast neoplasia and its additional lobes as an independent factor in assessing tumor invasiveness.

Keywords Breast cancer, Mammary gland accessory lobe cancer, Immunohistochemistry, Dysplasia, Invasion

1. Introduction

To date, breast cancer (BC) continues to occupy the first place in the frequency of occurrence among malignant neoplasms in women and is one of the main causes of mortality from cancer. Along with breast cancer, the number of cases of the mammary gland accessory lobe cancer has increased for recent years. The incidence of cancer in the mammary gland accessory lobe makes up 0.2–0.3% of its total frequency [1]. According to most experts, the prognosis of mammary gland accessory lobe cancer (MGALC) is determined by its early diagnosis. This trend requires careful screening among women from the high-risk group, which include, first of all, patients with precancerous lesions of the breast [2-5]. When studying the latest researches on breast diseases, we found that not so much work has been done that gives clear recommendations for diagnosis and treatment, as well as for the prediction of MGALC, which is why this study seems to be relevant and interesting both in terms of clinical and practical oncology [1,6].

In recent years, issues of verification and determination of the prognostic value of the so-called pre-invasive breast lesions and its accessory lobe have been intensively developed, among which there are severe dysplasia, in situ breast cancer (carcinoma in situ - CIS), cancer with the onset of invasion [7]. These diseases differ significantly from each other in the course and risk of subsequent development of invasive cancer. E-cadherin, P-catenin and high-molecular-weight cytokeratins are considered the most informative markers that allow a differential diagnosis between ductal and lobular neoplasia of the breast [8-9]. In this regard, the expression of these proteins was studied by us with the aim of confirming the morphological diagnosis and determining their diagnostic specificity.

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First of all, E-cadherin, a transmembrane protein, which is an adhesion protein, belongs to the markers of differential diagnosis. Loss of membrane expression of E-cadherin is considered a hallmark of lobular neoplasia, while most ductal carcinomas and dysplastic lesions, as well as normal ductal epithelium, are highly protein-expressing. However, the occasional loss of E-cadherin expression in ductal carcinoma cells, as well as aberrant protein expression in lobular neoplasia, can lead to diagnostic errors [10]. In such cases, a number of researchers use additional markers, such as high-molecular cytokeratin, specific for lobular neoplasia cells, and alpha, beta and gamma-catenins which are specific for ductal cells.

The method of immunohistochemistry has become widely used by researchers in the study of intraepithelial neoplasia of the breast and its lobe for early diagnosis and prognosis in recent years [2,11]. The immunohistochemical method (IHC) allows not only to determine the histogenetic features of precancerous lesions of the mammary gland and its additional lobe, the expression of hormonal receptors, various oncoproteins, proliferative activity, but also to identify the presence of minimal invasion, often significantly changing the prognosis of the disease. In this regard, the IHC method has become widely used by researchers in the study of intraepithelial neoplasia of the mammary gland and its lobe. However, in most published studies using this method, a limited number of markers does not allow to fully clarify the biological profile of the studied diseases. There is no data on the prognostic significance of these types of neoplasia. A comprehensive diagnostic algorithm combining morphological and immunohistochemical criteria has not been determined [12]. All of the above mentioned testifies to the high relevance of developing criteria issue for the verification of pre-invasive breast and its accessory lobe diseases for oncology and pathological anatomy and determines the need for additional research in this direction.

The aim of our study was to develop new criteria for the differential diagnosis of intraepithelial neoplasia variants of the mammary gland accessory lobe based on the use of a comprehensive morphological method of investigation using an optimal panel of markers to improve the quality of diagnosis and assessment of invasive cancer risk.

2. Material and Methods

Our study was based on the results of a retrospective examination of 182 patients with mammary gland accessory lobe cancer treated at the Republican Specialized Scientific and Practical Medical Center of Oncology and Radiology from 2010 to 2020. According to the morphological examination results, the patients were divided into 4 groups. The 1st group included 62 (34.1%) patients with severe dysplasia, which was defined below as "dysplasia". The 2nd group consisted of 71 (39.0%) patients with ductal carcinoma in situ, hereinafter referred to as " invasive ductal carcinoma (IDC)". The 3rd group included 28 (15.4%) women with lobular carcinoma in situ, hereinafter referred to as "invasive lobular carcinoma (ILC)". The 4th group included 21 patients (11.5%) with carcinoma in situ with the onset of invasion, this group was designated as "microinvasive carcinoma ". It is usually referred to as DCIS with microinvasion (Tab.1).

Table 1. Distribution of patients by groups

Groups	Number	%
Dysplasia	62	43.1
Invasive ductal carcinoma in situ	71	39.0
Invasive lobular carcinoma in situ	28	15.4
Microinvasive carcinoma	21	11.5
Total	**182**	**100**

The material for the study by immunohistochemistry was fixed with 10% neutral formalin for 24 hours, poured into paraffin, prepared sections with a thickness of 4 microns, which were applied to highly adhesive glasses and dried at a temperature of 37°C for 18 hours. Restoration of antigenic activity was carried out in a 2100 Retrieval mini-autoclave (Pick Cell) at 121°C for 20 min followed by cooling for 90 min. The recovery was carried out in a citrate buffer pH 6.0 or in a tris-EDTA buffer pH 9.0. Protease processing was used for some antibodies. The system "EnVision" ("Dako") was used as a detection system, diaminobenzidine and AES were used as a chromogen. To obtain results suitable for quantitative processing, the reactions were carried out using an automatic immunohistostainer "Autostainer Dako".

The intensity of reactions localized in the cytoplasm and on cell membranes was evaluated by a semi-quantitative method on a point scale from O to 3 using the Leica Q550 image analyzer: 0 - no reaction, 1 - weak reaction, 2 - moderate reaction, 3 - strong reaction. The results of reactions with antigens having nuclear localization were evaluated by counting the number of colored nuclei per 100 nuclei in 3 fields of view. The results obtained were expressed as a percentage. Data processing was carried out on a personal computer in the Statistics program (Statsoft, Inc). Comparative analysis of parametric features was carried out using the Student's criterion, nonparametric quantitative features were carried out using the Mann-Whitney criterion. Chi-squared and Fischer criteria were used to compare qualitative features. A correlation analysis was performed using Spearman's rank correlation coefficient to determine the presence and quantitative characteristics of the severity of the relationship between individual structural and functional indicators.

3. Results

In 31 (50.0%) cases of dysplasia the process was located in the right breast, in 27 (43.6%) women - in the left breast, and in 4 (6.4%) cases the lesion was bilateral. There were no significant differences in the location of the pathological process in invasive ductal carcinoma: in 35 (49.3%) patients

the lesion was found in the left mammary gland and in 36 (50.7%) cases - in the right one. A similar pattern was observed at microinvasive carcinoma in situ: in 10 (47.6%) cases the lesion was located on the left, in 10 (47.6%) - on the right, and in 1 (4.8%) case the lesion was bilateral. At invasive lobular carcinoma in situ in 12 (42.9%) cases the pathological process was located on the left, and in 16 (57.1%) cases – on the right (Tab.2).

Table 2. Distribution of patients by localization

Groups	Right lobe		Left lobe		Both lobes	
	n	%	n	%	n	%
Dysplasia (n=62)	31	50.0	27	43,6	4	6.4
Invasive ductal carcinoma in situ (n=71)	36	50.7	35	49.3	-	-
Invasive lobular carcinoma in situ (n=28)	16	57.1	12	42.9	-	-
Microinvasive carcinoma (n=21)	10	47.6	10	47.6	1	4.8
Total (n=182)	**93**	**51.1**	**84**	**46.2**	**5**	**2.7**

The expression of E-cadherin was detected in all dysplasia samples (100%), in 68 (95.7%) invasive ductal carcinoma samples, and in 19 (90.4%) microinvasive carcinoma samples in our study. In most cases of invasive ductal carcinoma in situ, the expression level was estimated as 3+ (66 (93%) samples) and only in 2 cases was estimated as 2+. Figure 1 presents a characteristic picture of full membrane staining with antibodies to E-cadherin in typical ductal carcinoma. Three cases in which the expression of E-cadherin was absent were initially characterized as highly differentiated ductal carcinoma in situ of a solid structure according to morphological criteria, however, immunohistochemical studies allowed them to be classified as invasive lobular carcinoma in situ.

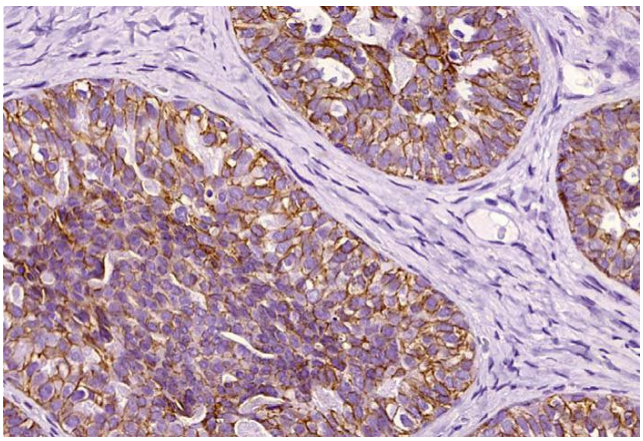

Figure 1. Positive expression of E-cadherin in ductal carcinoma in situ. x400

A complete absence of E-cadherin expression was determined in all cases of classical intralobular carcinoma of the first type (19 (68%) cases). 4 (14.2%) cases of full-membrane cell staining were identified among 9 cases qualified by morphological examination as intralobular cancer with severe cellular atypia (type 2). In three cases, the intensity of staining was estimated as 3+, in one case - as 2+. The case identified as having mixed morphological features also showed pronounced expression of E-cadherin, regarded as 3+. In general, staining for E-cadherin was absent in 23 (82%) cases of morphologically diagnosed lobular carcinoma (Fig.2).

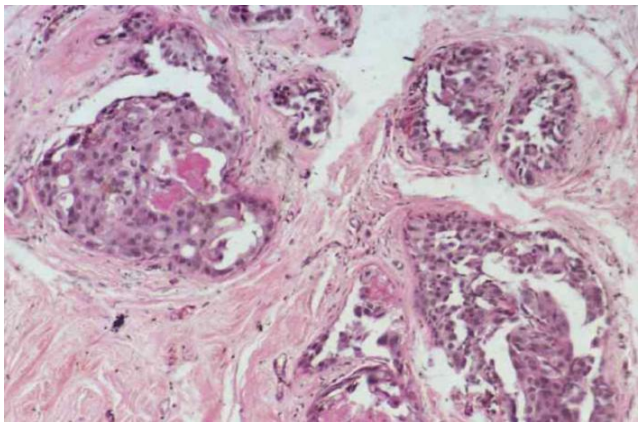

Figure 2. Expression of E-cadherin in the studied samples. x400

The same trend was noted when determining the expression of P-catenin in various types of precancerous lesions of the mammary gland. Significantly more often, P-catenin was determined in dysplasia and Invasive ductal carcinoma (in 98%) and 95.7%> cases), as well as in microinvasive carcinoma in 89% of cases. (Fig.3).

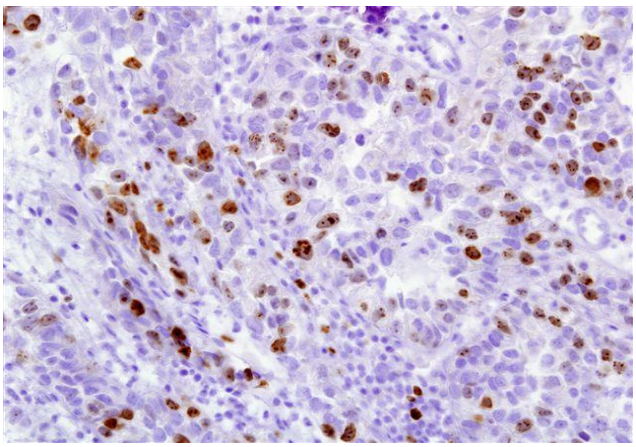

Figure 3. Expression of P-catenin in the studied samples. x400

Positive staining was obtained in 2 of 3 E-cadherin negative intraductal carcinomas, confirming the ductal nature of these lesions. In one case, the expression of P-catenin was also not determined. Thus, this case was qualified as intralobular carcinoma. In 2 from 4 cases of lobular carcinoma in situ with aberrant expression of E-cadherin, expression of P-catenin was detected, which made it possible to classify these tumors as intraductal, despite the primary morphological diagnosis. In 2 cases staining for P-catenin was negative. When evaluating the

results obtained, it was noted that positive staining was detected in most cases of classic lobular carcinoma in situ (16 cases from 19 (84%)), and its intensity varied from 2+ to 3+.

There was no expression of E-cadherin and 3-catenin in one of these four cases, which further confirmed the lobular nature of the tumor. In general, the expression of high-molecular cytokeratin was detected in lobular carcinoma in situ significantly more often (p=0.0006). In microinvasive cancer, the expression of high-molecular cytokeratin was found only in 1 (4.7%) case with the histological structure of lobular cancer. However, the staining was uneven and was estimated as +1 and +2 points in different areas of the tumor (Fig.4).

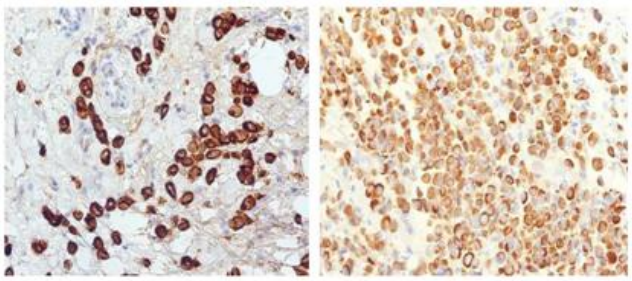

Figure 4. Positive cytoplasmic expression in tumor cells of the mammary gland accessory lobe of total cytokeratins (left) and high-molecular cytokeratins (right). x200

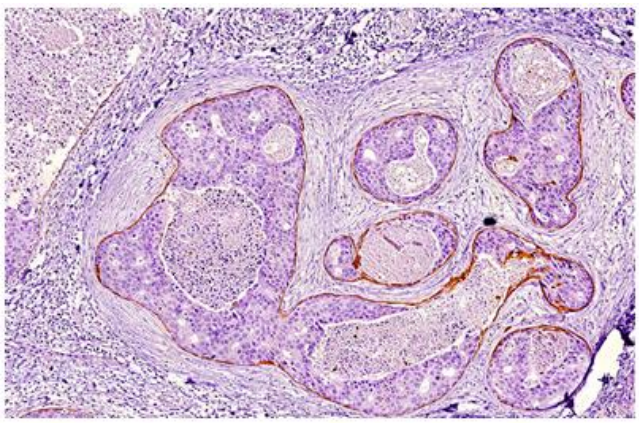

Figure 5. Expression of cytokeratin types 5/6 by myoepithelial cells in case of intraductal carcinoma in situ. x200

In addition, an immunohistochemical reaction with antibodies to cytokeratin types 5/6 and 14 was evaluated, which was performed not only for differential diagnosis between ductal hyperplasia and cancer in situ, but also for detection of basal cell differentiation. It was found that the expression of cytokeratins of types 5/6 and 14 is significantly more often detected in dysplasia (38 (56%) cases), and scattered protein expression of varying degrees of severity was characteristic. In the study of carcinomas in situ, the following data were obtained: positive expression of cytokeratin types 5/6 was determined in 3 cases of intraductal and 1 case of intralobular cancer. Expression of cytokeratin type 14 - additionally in 1 case of ductal carcinoma in situ (except for 3 cases in which the expression of cytokeratin 5/6 and cytokeratin 14 was detected simultaneously) and in 1 case of intralobular carcinoma (except for one case where the expression of CK 5/6 and CK14 was simultaneous). Expression of cytokeratins 5/6 and 14 was found in 4 cases of ductal and 2 cases of lobular carcinoma in situ, which was 5.6% and 7.1%, respectively. In other cases, these proteins were expressed only by myoepithelial cells (Fig.5).

4. Conclusions

Thus, the expression of cytokeratins 5/6 and 14 types is significantly more often observed in dysplasia (p= 0.0005), which allows them to be used for additional differential diagnosis of dysplasia and highly differentiated carcinomas in situ.

E-cadherin proved to be highly specific as a marker characteristic of intraductal carcinoma; its expression was determined significantly more often in this type of tumor (p= 0.0005).

Obviously, these markers cannot be used for differential diagnosis between lobular and ductal carcinomas in situ, since there are no significant differences in their expression in these types of neoplasia.

However, they allow us to further characterize the lesion: in all positive cases, there was also no expression of receptors for estrogen and progesterone, which allowed them to be attributed to the basal cell type of cancer in situ.

Histological examination of precancerous lesions of the mammary gland should be supplemented with an assessment of the expression of immunohistochemical markers.

Determination of the levels of cytokeratins 5/6, H, cyclin D1, E-cadherin and P-catenin is the most important tool for the differential diagnosis of various types of breast neoplasia and its accessory lobes as an independent factor in assessing tumor invasiveness.

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The article is published for the first time and is part of a scientific work.

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