

Expression of p53, Ki-67, bcl-2 Markers in Different Types of Neoplasia of the Accessory Lobe of the Mammary Gland

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Abstract The aim of the study was to determine the expression of markers of cell cycle regulation, proliferation and apoptosis (p53, Ki-67, bcl-2) in various types of neoplasia of the accessory lobe of the mammary gland. **Material and methods:** The study is based on the results of a retrospective examination of 182 patients who were treated for diseases of the accessory lobe of the mammary gland at the Russian National Medical Research and Medical Center and in the Namangan branch of the Russian National Medical Research and Medical Center from 2010 to 2022. The patients were divided into 4 groups according to the results of the morphological examination. **Results:** In general, a comparison of the expression profiles of markers p53, Ki-67, bcl-2 showed that microinvasive cancer of the accessory lobe was characterized by a combination of increased expression of p53 and Ki-67. The expression of p53 and Ki-67 was determined least often in patients with well-differentiated intraductal cancer, intralobular cancer and dysplasia of the accessory lobe of the mammary gland. At the same time, Bcl 2 was expressed significantly more often in these types of neoplasia, demonstrating opposite trends with p53 and Ki-67. **Conclusions:** 1. Histological examination of neoplasia of the accessory lobe of the mammary gland should be supplemented with immunohistochemical analysis, the results of which allow for differential diagnosis of various types of neoplasia in the accessory lobe of the mammary gland and make it possible to clarify the prognosis of the disease. 2. With an increase in the level of cataplasia of cellular elements of pre-invasive lesions of the accessory lobe of the mammary gland, the frequency of expression of Ki-67 and p53 ($p < 0.05$) significantly increases with a simultaneous decrease in the frequency of expression of receptors for estrogen and progesterone and bcl2 ($p < 0.05$), which makes it possible to select this optimal set of immunohistochemical markers into a panel used to assess the level of differentiation and malignant potential of neoplasia of the accessory lobe of the mammary gland. 3. A low degree of differentiation of tumor cells in the accessory lobe of the mammary gland in intraductal carcinoma in situ is associated with a decrease in the frequency of expression of bcl-2 ($p = 0.0005$), an increase in the frequency of expression of receptors for p53, Ki-67 ($p < 0.05$).

Keywords Cancer of the accessory lobe of the mammary gland, Immunohistochemistry

1. Introduction

Breast cancer is one of the most common oncological diseases, with over 2 million cases registered worldwide each year, 800,000 of which result in death within the first year. In Uzbekistan, 22,443 cases of breast cancer were registered in 2022, with an incidence rate of 120. The number of newly diagnosed cases per year is 4164, and the 5-year survival rate is 45.1, with a mortality rate of 5.2. In recent years, there has been progress in understanding the molecular biology of tumor histogenesis, leading to the development of modern methods for morphological, immunohistochemical, biological, cytogenetic, and molecular diagnostics [1,4]. The complexity of the mechanisms of breast cancer development complicates prevention and

treatment, but the prognosis at early stages of the disease is relatively favorable. However, the verification of the process at advanced stages, based on the presence of distant metastases, leads to high disability and mortality, reducing the effectiveness of all approaches in treatment strategies [3-5].

Molecular markers, particularly the mitotic index, Her2/neu, Ki-67, Bcl-2, and p53, are currently at the center of attention in domestic and foreign literature. The Bcl-2 apoptosis regulator is an intracellular protein that inhibits apoptosis in many cell systems, while the p53 protein is a tumor suppressor gene product expressed in all cells of the body. Its activation leads to the cessation of the cell cycle and DNA replication, inducing apoptosis under strong stress signals [6].

The increasing interest of clinicians and pathologists in precancerous lesions has led to the morphological and clinical classification of the disease and pathological processes, especially those preceding the development of cancer and other malignant tumors. Due to diagnostic

difficulties, non-invasive forms of breast cancer, such as ductal carcinoma in situ, are the primary targets. It remains unclear how often non-palpable DCIS, detected solely by mammography, progress to invasive cancer. [7-10]. Recent studies show that the prognosis of Ductal carcinoma in situ (DCIS) largely depends on its class. Therefore, screening is very useful for women with certain types of DCIS. For other types of DCIS, the question of whether the potential benefits of detection outweigh the side effects of screening (anxiety, unnecessary surgery, radiation therapy) remains. It is widely recognized that determining the prognosis of any tumor disease, including breast cancer, essentially requires the identification of markers that are in one way or another associated with the biology of the tumor, predict its natural history, and can forecast the choice of optimal treatment tactics and its effectiveness.

The aim of the study was to determine the expression of markers of cell cycle regulation, proliferation and apoptosis (p53, Ki-67, bcl-2) in various types of neoplasia of the accessory lobe of the mammary gland.

2. Materials and Methods

The study was based on the results of a retrospective examination of 182 patients who underwent treatment for breast adenosis at the Republican Scientific Practical Center for Oncology and Radiology (RSPCOR) and its Namangan branch from 2010 to 2022. The patients were divided into four groups based on the results of morphological examination. The first group comprised 62 patients (34.1%) with breast adenosis, the second group comprised 71 patients (39.0%) with in situ ductal carcinoma of the breast, the third group included 28 women (15.4%) with in situ lobular carcinoma in the breast, and the fourth group included 21 patients (11.5%) with in situ carcinoma and microinvasion in the breast. The average age of the patients was 56.4 years (20-75 years). 75 (41.2%) patients were in menopause, and 107 (58.8%) were in the reproductive period.

Exclusion criteria for the study were:

Age equal to or older than 80 years.

Combination of precancerous lesions and invasive breast cancer.

In the case of breast adenosis, 31 cases (50.0%) of the lesion were located in the right breast, 27 women (43.6%) in the left breast, and in 4 cases (6.4%) in both breasts. For in situ ductal carcinoma, significant differences in the location of the lesion were not observed, with 35 patients (49.3%) having the lesion in the left breast and 36 cases (50.7%) in the right. A similar pattern was observed for in situ lobular carcinoma, with the pathological process located on the left in 12 cases (42.9%) and on the right in 16 cases (57.1%).

For immunohistochemical analysis, 15 commercial sets of mono- and polyclonal antibodies were used, which were distributed according to their functional significance as follows: markers of proliferative activity - Ki-67; markers of apoptosis - Bcl-2; tumor growth suppressors - p53. The material was

fixed in 10% neutral formalin for 24 hours, then embedded in paraffin and cut into 4 μ m sections, which were placed on high-adhesion glass and dried at 37°C for 18 hours. Antigenic activity was restored in a mini-autoclave "2100 Retrieval" (Pick Cell) at 121°C for 20 minutes and then cooled for 90 minutes. The detection system used was "EnVision" (Dako), and the chromogen was diaminobenzidine and AES. The reactions were evaluated semi-quantitatively on a scale of 0 to 3 using the Leica Q550 image analyzer. The results were expressed as a percentage for antigens with nuclear localization (Ki67, p53, Bcl-2, estrogen and progesterone receptors) by counting the number of stained nuclei out of 100 nuclei in 3 fields of view. The results for estrogen and progesterone receptors were evaluated on an 8-point scale. Data processing was performed using the "Statistics" program (Statsoft, Inc). The Student's t-test was used for comparative analysis of parametric features, the Mann-Whitney test was used for non-parametric quantitative features, and the chi-square (χ^2) and Fisher's criteria were used for qualitative features. The Spearman rank correlation coefficient was used for correlation analysis. The expression of p53 was significantly lower ($p < 0.05$) in patients with low-differentiated intraductal carcinoma compared to those with ductal carcinoma in situ, microinvasive ductal carcinoma, and well-differentiated intraductal carcinoma. The highest expression of the marker was observed in patients with microinvasive ductal carcinoma, but the value of this indicator did not differ significantly ($p = 0.9024$) from that in the group of low-differentiated intraductal carcinoma.

The comparison of the expression of the apoptosis marker Bcl-2 showed that this marker is most commonly detected in patients with dysplasia, intraductal carcinoma, and well-differentiated intraductal carcinoma (85.5%, 89.3%, and 87.5% of cases). Patients with poorly differentiated intraductal carcinoma and microinvasion of the accessory lobe had significantly lower expression of this marker (39.4% and 39%), which was significantly different from the previous groups ($p = 0.0005$).

The evaluation of Ki-67 expression showed that it was most frequently encountered in patients with low-differentiated intraprotocolonic tumors of the accessory breast and in patients with microinvasion of the accessory breast, with values of 24.3% and 50% respectively. The levels of these values were significantly higher ($p < 0.05$) than the values of Ki-67 expression in patients with dysplasia, intraductal carcinoma, and high-differentiated prototypical carcinoma of the accessory breast of the breast (1.6%, 3.5%, 2.9%).

Overall, the comparison of expression profiles of the aforementioned markers showed that microinvasive carcinoma of the additional lobe was characterized by a combination of increased expression of p53 and Ki-67. The expression of p53 and Ki-67 was least frequently detected in patients with highly differentiated intraductal carcinoma, intralobular carcinoma, and adenosis of the additional lobe of the breast. At the same time, bcl-2 was expressed much more frequently in these types of neoplasia, demonstrating divergent trends with p53 and Ki-67.

Morphological characteristics and features of marker expression depending on the degree of differentiation of ductal carcinoma in situ of the accessory lobe of the mammary gland.

The comparison of morphological and immunohistochemical characteristics was conducted separately. The study found that cases of highly differentiated in situ ductal carcinoma of the accessory lobe of the breast accounted for 34 (48.9%) cases, while cases of poorly differentiated in situ ductal carcinoma accounted for 37 (52.1%). The study identified several features in patients with in situ ductal carcinoma, including the presence of necrosis only in poorly differentiated tumors and the prevalence of acine-like structures in tumor proliferation. The degree of polarization was highest in highly differentiated in situ ductal carcinoma, and the expression of estrogen receptors was positive in 95% of highly differentiated cases and 45% of poorly differentiated cases. The frequency of expression of progesterone receptors was similar, with 79% in highly differentiated cases and 27% in poorly differentiated cases, and was not dependent on the histological structure of tumor proliferation. Microcalcifications were found in 49% of the samples, with 16% being multiple, which was characteristic of poorly differentiated tumors.

The expression level of p53 was lowest in the subgroup with highly differentiated intraductal carcinoma of the additional lobe (14.7% of cases) and increased with decreasing tumor differentiation (40.5%). Meanwhile, the highest levels of this parameter were found in the cribriform and papillary structures of the additional lobe tumor. At the same time, the expression of the apoptosis marker bcl-2 was significantly associated with the degree of tumor differentiation (87.5% in highly differentiated intraductal carcinoma and 39.4% in low-differentiated carcinoma) and was associated with solid and papillary structures, respectively. The maximum level of expression of the proliferation activity marker Ki-67 was observed in the subgroup with a low degree of differentiation (24.3%) and decreased as the degree of differentiation increased (2.9%). The comparison of the expression levels of apoptosis and proliferation activity markers clearly indicates that as the level of cell element cataclysm increases, the level of proliferative activity sharply increases while the level of apoptosis decreases simultaneously. At the same time, a decrease in the degree of tumor differentiation is accompanied by an increase in the expression of the p53 protein, which is a poor prognostic factor, indicating an increase in the number of active atypical cells in tumor proliferates. The highest values of proliferation activity and differentiation disturbances were observed in papillary and cribriform structures.

3. Conclusions

Histological examination in neoplasms of the accessory lobe of the mammary gland should be supplemented with immunohistochemical analysis, the results of which allow for differential diagnosis of various types of neoplasms in the

accessory lobe of the mammary gland and provide an opportunity to refine the prognosis of the disease.

With an increase in the level of cell catalepsy of pre-invasive lesions of the accessory lobe of the mammary gland, the frequency of expression of Ki-67 and p53 significantly increases ($p < 0.05$) with a simultaneous decrease in the frequency of expression of estrogen and progesterone receptors and bcl-2 ($p < 0.05$), which allows to identify this optimal set of immunohistochemical markers in the panel used to assess the level of differentiation and malignant potential of neoplasms of the accessory lobe of the mammary gland.

Low degree of differentiation of tumor cells in the accessory lobe of the mammary gland in intraductal carcinoma in situ is associated with a decrease in the frequency of expression of bcl-2 ($p = 0.0005$), an increase in the frequency of expression of p53 and Ki-67 receptors ($p < 0.05$).

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