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Investigating the expression of cytokeratin 5/6 in benign and malignant breast lesions

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Abstract

Introduction: Breast cancer (BC) is the most common cancer in women, which is the second most common malignancy in terms of mortality and prevalence after lung cancer. BC is a group of very diverse diseases that can be detected at a molecular, histopathological and clinical level.
Objectives: The aim of the present study was to evaluate the expression of CK5/6 and its relationship to some of the factors affecting prognosis, such as tumor grade, lymph node involvement.
Patients and Methods: In this cross-sectional study with descriptive and analytical aspects, 40 malignant and 20 benign tumors were collected in 2020 from the archives of the pathology department of two educational hospitals in the city of Ahvaz. The demographic and clinical characteristics of each specimen, including age, patient, lymphovascular and perineural invasion, lymph node involvement, tumor grade and tumor type were extracted from the patients' record. Then the expression of CK5/6 intensity staining was examined by immunohistochemistry.
Results: All benign breast lesions had positive expression for CK5/6 and staining intensity between six and nine. In the malignant group, 33 samples showed negative expression of CK5/6 and only seven samples (17.5%) showed positive expression with a low-staining index. A positive expression with a low-staining index. All seven positive specimens were invasive ductal carcinoma (IDC) lesions with staining index 2, 4, and 6. Additionally, none of the ductal carcinoma in situ (DCIS) specimens which immunostained were positive for CK5/6. In the present study, all IDCs with weak expression of CK5/6 were grade III. No statistically significant relationship was observed between perineural and lymphovascular invasion and lymph node involvement with the intensity of CK5/6 expression.
Conclusion: Our study showed that cytokeratin immunohistochemical intensity is able to distinguish benign lesions from malignant IDC and DCIS lesions and accordingly in determining of tumor grade after weak staining in high-grade IDC, which may be due to squamous metaplasia in these tumors; however, more extensive research with a larger sample size are required to assess its effect in lymphovascular and perineural invasion and also lymph node involvement.

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Introduction

Breast cancer (BC) is the most common cancer in women, which is the second most common cancer in terms of mortality and prevalence after lung cancer (1). Various factors are involved in BC prognosis and survival, including grading and staging (2). In addition to the factors mentioned, the role of age, the status of estrogen and progesterone receptors, Erb2 (human epidermal growth factor receptor 2) are well detected as the prognostic factors (3).

Breast cancer is a diverse disease that can be detected at clinical, histopathological and molecular levels (4). Its heterogeneity at the molecular level is characterized by recurrent changes in the frequency and size of genomic abnormalities and also changes in gene expression. This heterogeneity means that BC categorization could include classification and histopathologic grade of the tumor. Additionally, immunohistochemical parameters can provide more data to choose better therapy options (5,6). Milk ducts are composed of three types of epithelial cells, including stem cells, myoepithelial/basal cells and duct cells. Invasive ductal carcinoma (IDC) is the most common form of BC originating in epithelial cells. The classification of these cells provides information from tumor markers that enable early detection and prognosis of BC

Key point

Breast cancer is the most common cancer in women. In this cross-sectional study the expression of CK5/6 intensity staining was examined by immunohistochemistry. Cytokeratin immunohistochemical intensity can distinguish benign lesions from malignant invasive ductal carcinoma (IDC) and ductal carcinoma in situ (DCIS) lesions.

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(7). Cytokeratins are tumor markers that are essential in determining the cancer prognosis. Moreover, cytokeratins as intermediate filaments, are mainly found in epithelial cells and are considered the key component of skeletal system that help the nucleus stabilization and maintenance of cells' morphology (8). Cytokeratins have 20 subtypes, the expression of which depends on the type of epithelial cell and the degree of differentiation. Different profiles of cytokeratins are expressed in cancerous tissues (carcinomas) caused by epithelium (9). Recent studies have focused on the relationship between cytokeratins, malignancy type and the prognosis and also grade of tumors. Cytokeratins 5 and 6 (CK5/6) are neutral type II peptides from the cytokeratin's family that are expressed in both keratinized/nonkeratinized squamous epithelium of the prostate, breast, and salivary glands and accordingly they are sensitive markers for scaly differentiation (10). Moreover, this peptide is expressed in both malignant and benign tumors of the myoepithelium, squamous mucosa and epithelium. Cytokeratins 5 and 6 expression has been used to differentially diagnose basal-like BC from other triple-negative breast cancers (TNBCs), while CK5/6 expression in TNBC is associated with poor prognosis, high-grade differentiation and lymph node metastasis (11). Cytokeratins 5 and 6 expression is associated with the epithelial-mesenchymal transition state process, which plays an important role in metastasis (12).

Objectives

Considering that most studies on the expression of CK5/6 expression in BC have been conducted in triple negative BC and few studies have been conducted on the relationship between CK5/6 expression and clinical features in other BCs (13). The aim of the present study was to evaluate the expression of CK5/6 and its relation to some of the factors affecting prognosis such as tumor stage, lymph node involvement and grade of malignancy.

Materials and Methods

Patients, samples collection and immunohistochemical assay

In this cross-sectional study with descriptive and analytical aspects, 40 malignant and 20 benign (non-cancerous) tumors were collected in 2020 from the archives of the pathology department of Imam Khomeini and Golestan hospitals in the city of Ahvaz. Inclusion criteria were all patients with benign and malignant breast tumors, adequate tumor mass, having no necrosis/bleeding, presence of pathologic lymph node sections and full medical records. The demographic and clinical characteristics of each specimen, including age, patient, depth of tumor, lymphovascular and perineural invasion, involvement of lymph node, and tumor grade and type were extracted from the patient record. To examine the expression of CK5/6, a labeled antibody immunohistochemical technique (D5/16B4 polyclonal antibody, catalog #180267, Thermo Fisher Scientific, USA) was conducted based on the protocols mentioned in previous studies.

Prostate gland tissue was considered as the positive control tissue. To assess the expression of CK5/6, immunohistochemical staining was scored for the staining pattern (cytoplasmic or membranous) and the proportion and intensity of staining of the tumor cells using a 0-3 scale (14).

Statistical analysis

Frequency, percent and descriptive statistics (e.g. mean ± SD) for quantitative variables were employed for qualitative variables. In the analytical part, the data were analyzed using chi-square tests. Significance level $P<0.05$ and all analysis were performed using SPSS software version 25.

Results

Demographic and clinicopathological characteristics

The mean age of the patients in the cancer group and in the benign group was 50.8 ± 12.1 years and 45.8 ± 10.90 years, respectively. Regarding the type of histological diagnosis in the two groups, IDC (IDC) accounts for 33 (82.5%) cases in the cancer group and in the benign group 14 (70%), 5 (25%) and one (5%) cases were fibroadenoma, fibrocystic or benign phyllodes tumors respectively. In the cancer group, most tumors were grade II (52.5%). Regarding the age distribution of the cancer group, most cases 16 (40%) were between 41 and 50 years old. The mean tumor size in the cancer patient group was 2.5-1.6 cm. In addition, perineural, lymphovascular and lymph node involvement was reported in 14 (35%), 15 (75%), and 25 (62.5%) of cases, respectively. Demographic and clinicopathological information for the cancer group listed in Table 1.

CK5/6 staining intensity and the relationship with clinicopathological factors

In the cancer group, only seven samples (17.5%) were positive for the CK5/6 marker, while in the benign group, all 20 samples were positive and this difference was statistically significant ($P<0.001$). The staining intensity of CK5/6 in the cancer group was reported between 2 and 6; however, in the benign group the staining intensity was higher and varied between 6 and 9 (Figure 1). Based on the results of the present study, a statistically significant relationship was observed between the cancer and the benign group in terms of CK5/6 staining intensity expression ($P<0.001$). The results of the present study showed that among the clinicopathological factors, a significant relationship was observed only between the tumor grade and the intensity of CK5/6 staining, therefore the staining intensity increases with the higher tumor grade, but there is no significant relationship between other factors such as lymphovascular and perineural invasion and also lymph node involvement with expression of CK5/6 staining intensity. According to the analysis of the present study, all seven samples positive for the CK5/6 marker were related

Table 1. Demographic and clinicopathological characteristics in cancer group

Characteristics		
Histological, No. (%)	IDC	33 (82.5)
	ductal carcinoma in situ	7 (17.5)
Tumor grade, No. (%)	Grade I (well-differentiated)	11 (27.5)
	Grade II (moderately-differentiated)	21 (52.5)
	Grade III (poorly-differentiated)	8 (20)
Age distribution (y), No. (%)	≤ 40	8 (20)
	≥ 60	9 (22.5)
	41-50	16 (40)
	51-60	7 (17.5.5)
Lymphovascular Invasion, No. (%)	Yes	16 (40)
	No	24 (60)
Perineural invasion, No. (%)	Yes	14 (35)
	No	16 (65)
Lymph node involvement, No. (%)	Yes	25 (62.5)
	No	15 (37.5)
Age (y), Mean ± SD		50.8 ± 12.1
Tumor size (cm), Mean ± SD		2.5 ± 1.6

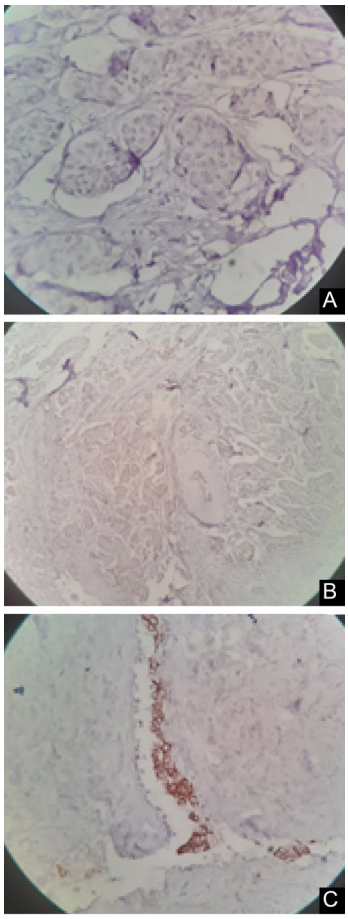

Figure 1. Representative of CK5/6 immunostaining in in malignant and benign breast samples: From the above left: (A) negative CK5/6 in IDC; (B) weakly positive CK5/6 in high grade IDC; (C) positive CK5/6 in fibroadenoma.

to grade III tumor. Of seven positive samples, four samples showed a weak staining intensity of 2, two samples showed a moderate staining intensity of 4, and one sample showed a strong staining intensity of 6. Likewise, of seven positive samples, four samples showed a weak staining intensity of 2, while in two samples and one sample reported moderate staining intensity of 4 and strong staining intensity of 6, respectively (Table 2).

Discussion

This study was conducted to evaluate the immunohistochemistry of CK5/6 on 60 breast biopsy samples, including 20 benign lesions and 40 malignant lesions. All benign breast samples had a positive expression of CK5/6 and the staining intensity of the benign samples varied between 6 and 9, which was consistent with other investigations. The highest staining intensity in the present study was index 9, observed in fibrocystic cases. In cases with fibroadenoma, the intensity of the staining index was mostly 6 and 7. Even in benign phyllodes, few cellular areas were observed that showed cytoplasmic staining with a staining index of 6. Similar to the present study, in the study of Bhalla et al, all benign lesions except for the lactating adenoma were immunopositive for CK5/6 expression and the staining index varied between 6 and 9 (11). In the present study, in the malignant group, 33 samples showed negative expression of CK5/6 and only seven samples (17.5%) showed positive expression with a low-staining index. All seven positive specimens were IDC lesions with staining index 2, 4 and 6. Furthermore, none of the DCIS specimens immunostained positive for CK5/6 in this study. With staining index 2, 4 and 6. Besides, none of the DCIS specimens immunostained positive for CK5/6. The results of our study are in line with other investigations in this area. In a previous study, six out of 25 samples demonstrated positive immunoreactivity for CK5/6, all of which were IDC lesions with a staining index varying between 2 and 6 (11). In the study by Ivković-Kapicl et al, of 117 analyzed tumor specimens, 22% were immunohistochemically positive for CK5/6 and the immunoreactivities were directly associated with triple-negative phenotype and higher histological grade (15). In another study, Ding et al showed that in 38 cases of benign breast lesions, the positive rate of CK5/6 expression was 100%. In five cases of atypical ductal hyperplasia, there were few positive cells in the ducts. They showed, in 19 cases of CDIS, no tumor cells expressed CK5/6. In 19 cases of IDC, almost no CK5/6 was detectable (16). The study by Otterbach et al showed that only three out of 39 invasive BCs were positive for CK 5/6 expression (17). Based on previous studies, CK 5/6 expression may be useful to distinguish usual ductal hyperplasia from the atypical ductal hyperplasia (ADH)/DCIS spectrum. Based on the present study and the other studies, CK5/6 expression may help to distinguish benign fibrocystic lesions and fibroadenomas from malignant DCIS lesions. Due to the

Table 2. Correlation between CK5/6 staining intensity and clinicopathological factors

Variable		CK5/6 staining intensity index				*P* value
		0	2	4	6	
Tumor grade	Grade I (well-differentiated)	11 (75%)	0 (0%)	0 (0%)	0 (0%)	0.000
	Grade II (moderately-differentiated)	21 (100%)	0 (0%)	0 (0%)	0 (0%)	
	Grade III (poorly-differentiated)	1 (12.5%)	4 (50%)	2 (25%)	1 (12.5%)	
	Total	33 (82.5%)	4 (10%)	2 (5%)	1 (2.5%)	
Lymphovascular Invasion	Yes	12 (75%)	2 (12.5%)	1 (6.3%)	1 (6.3%)	0.587
	No	21 (87.5%)	2 (8.3%)	1 (4.2%)	0 (0%)	
	Total	33 (82.5%)	4 (10%)	2 (5%)	1 (2.5%)	
Perineural invasion	Yes	10 (71.4%)	1 (7.1%)	2 (14.3%)	1 (7.1%)	0.108
	No	23 (88.5%)	3 (11.5%)	0 (0%)	0 (0%)	
	Total	33 (82.5%)	4 (10%)	2 (14.3%)	1 (7.1%)	
Lymph node involvement	Yes	21 (84%)	2 (8%)	1 (4%)	1 (4%)	0.797
	No	12 (80%)	2 (13.3%)	1 (6.7%)	0 (0%)	
	Total	33 (82.5%)	4 (10%)	2 (5%)	1 (2.5%)	

higher staining intensity in benign lesions compared to malignant lesions, the CK5/6 staining intensity index can be used to distinguish benign from malignant lesions (18). It is known that CK5/6 expression is generally positive in most of the benign ductal lesions and that it is additionally expressed in myoepithelial cells and glandular epithelium. Nevertheless, in DCIS nearly all multiplying cells were CK5/6-negative (19). In a previous study, Böcker et al showed that all 23 cases of benign breast lesions and 5% of 25 cases of malignant breast lesions showed positive expression of CK5/6. Since, this difference in expression in different studies may be caused by different patient groups or differences in sample size (20). No medullary BC cases were included in the present study, however, in the study by Tot et al, positive expression of CK5/6 was shown in 25% of typical cancers, 43% of atypical carcinomas and 20% of medullary metastases (21). In the present study, all IDCs were low-level expression of CK5/6 grade III, consistent with other investigations. In one investigation, all grade III malignancies had weakly positive CK5/6 immunoreactivity (11). Abd El-Rehim et al, showed a significant positive correlation between grade III carcinoma and CK5/6 positivity. Additionally, they found that invasive grade III carcinoma with extensive central necrosis has an aggressive behavior and basal phenotype (22). Similarly, in the study by Al-Maghraby et al, CK5/6-positive IDCs were mostly grade III. The positivity of CK5/6 in poorly-differentiated (grade III) carcinoma is due to the fact that high-grade IDCs, which correspond to basal BCs, share the same expression pattern as breast basal/myoepithelial cells (23).

In contrast to the present study and above studies, van de Rijn et al observed that the expression of basal-type cytokeratins in breast carcinoma is a tumor grade- independent prognostic factor (24). In our study, no statistically significant association between lymphovascular and perineural invasion and also lymph node involvement with the intensity of CK5/6 expression was detected. Likewise, Abd El-Rehim et al showed a positive correlation between CK5/6 expression with local and regional recurrence and also distant metastases was seen (22). Van de Rijn et al, also demonstrated that CK 5/6 expression in BC was associated with a significantly shorter survival; however, it had no predictive value in patients with lymph node metastasis (24). In general, further studies with a larger sample size are needed to determine definitive results, particularly regarding the relationship between lymphovascular and perineural invasion and also lymph node involvement with the intensity of CK5/6 expression.

Conclusion

Based on the results of the present study, the immunohistochemical intensity of cytokeratin can distinguish benign lesions from malignant IDC and DCIS lesions and also in determining tumor grade after weak staining in high-grade (III) IDC, which may be due to squamous metaplasia, in these tumors. However, further studies with larger sample sizes are needed to determine its role in lymphovascular and perineural invasion and lymph node involvement.

Limitations of the study

One of our limitations was the small number of study patients. It is suggested that more people be investigated in the next studies.

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Authors' contribution

Conceptualization: Shahram Bagheri.
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Funding acquisition: Shahram Bagheri.
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Methodology: SML, Shahram Bagheri.
Resources: Seyed Abbas Rezaei Naserabad, Parvin Kheradmand.
Supervision: Shahram Bagheri.
Validation: Seyed Abbas Rezaei Naserabad.
Visualization: Shahram Bagheri.
Writing—original draft: Seyed Abbas Rezaei Naserabad, Shahram Bagheri.
Writing—review and editing: Shahram Bagheri, Parvin Kheradmand.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical issues

The research followed the tenets of the Declaration of Helsinki. The Ethics Committee of Golestan Hospital, Ahvaz Jundishapur University of Medical Sciences approved this study. (IR.AJUMS.HGOLESTAN.REC.1401.038). Accordingly, written informed consent was taken from all participants before any intervention. This study was extracted from MSc thesis of Seyed Abbas Rezaei Naserabad at this university (Thesis: CRC-0104). Ethical issues (including plagiarism, data fabrication, double publication) have been completely Observed by the authors.

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