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Assessment of Breast Cancer Immunohistochemical Properties with Demographics and Pathological Features; A Retrospective Study

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Abstract

Background: Breast cancer is considered the most common malignant disease in the female population. It is known as an emerging epidemy with a great burden on women's health, which can be associated with poor outcomes. Some factors including histological type, immunohistochemistry (IHC), tumor grade, and tumor size can have effects on breast cancer.
Objectives: This study aimed at assessing the effects of mentioned factors on IHC type of breast cancer.
Methods: This retrospective cross-sectional study was conducted on 142 patients, who were referred to one of the referral centers for breast cancer in Mashhad. Information including age, histological type, familial history, menopause status, tumor grade, tumor size, and IHC properties was collected from the patient's medical records. Allred score was used for reporting hormonal status. The data were analyzed by version 26 of SPSS software.
Results: The mean age of patient was 50.2 $\pm$ 12.7. The frequency of luminal A and luminal B type was calculated as 29.7 and 18.9%, respectively. In addition, triple-negative IHC type has a prevalence of 24.3% and HER2 had a prevalence of 27%. There were no significant differences between age (P = 0.34), familial history (P = 0.42), menopause (P = 0.36), histological type (invasive: P = 0.11, in situ: P = 0.45), and IHC properties. However, tumor diameter (P = 0.0001) and tumor grading (P = 0.002) had significant association with IHC properties.
Conclusions: Factors including tumor size and pathological grade can have effects on the gene expression properties of breast cancers. Luminal IHC type A is more common in breast cancer and is associated with better outcomes. However, age, histological type, familial history, and menopause status had no effects on the IHC properties of breast cancer.

Keywords: Breast Cancer, Immunohistochemical Study, Tumor Pathology

1. Background

Breast cancer is defined as the malignant proliferation of epithelial cells that cover the ducts or lobules of the breast (1, 2). It is among the top third most common malignant diseases in the majority of populations. It is considered the most common invasive malignancy among women, which can impose a great burden on the female population globally (3, 4). The incidence and prevalence of breast cancer have been rising during the last decades with the estimation of more than 1 million new cases diagnosed annually (5). However, the 5-year survival rate of patients with breast cancer has been improved to 83.4 to 98.4% in localized forms and 23.3% in metastatic breast cancer, which may contribute to the development of well-established screening programs (6).

Accordingly, several risk factors have been introduced that are associated with the emergence of breast cancer in the female population including age, hormonal factors, genetic predisposition, familial history, and nutritional factors (7-9). Immunohistochemically, this heterogeneous tumor group could be classified into 4 intrinsic subtypes including luminal A, luminal B, human epidermal growth factor receptor 2 (HER2/neu) positive, and basal or triple-negative, which are associated with different prognosis and treatment strategies (10). Modern studies surveyed that the steroid hormone receptor expression on tumor cells could determine the course of the disease. For instance, the luminal subtype of breast cancer is associated with better prognostic features and survival. However, triple-negative tumors and HER2-positive breast cancer could manifest as more aggressive invasive tumors with unfavorable outcomes (11-13).

At present, there is not much evidence for the assessment of immunohistochemistry (IHC) properties of breast cancer and its clinical and biological features especially in

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developing countries including Iran.

2. Objectives

This study aimed at investigating 4 IHC subtypes and comparing the association between pathological, demographics, and clinical behaviors of different IHC features of breast cancer.

3. Methods

3.1. Study Design

This retrospective cross-sectional study was conducted on female patients with breast cancer, who presented to Aria Hospital, Mashhad, Iran from 2012 to 2019. The patients who underwent surgery and had the pathological report in addition to the immunohistochemical properties in their medical records were included in the study. Furthermore, patients who had a history of neoadjuvant chemotherapy and patients who had pathologic species from other manners including fine-needle aspiration or core needle biopsy were excluded from the study. Convenience sampling method was used for patient selection and the study population was estimated to be at a minimum of 90 patients based on chi-squared (χ^2) test and power, which was assumed based on a similar study (14). Historically, the value of 0.05 has been used for type-I error (α). Accordingly, a type-I error (α) occurs when a null hypothesis is rejected and power is the probability of rejecting a false null hypothesis. Beta is the probability of a type-II error, which occurs when a false null hypothesis is not rejected. The sample size was calculated with the aid of PASS® software:

$$n = \delta_{\alpha,\beta}\left[\sum_{i=1}^{r}\sum_{j=1}^{c}\frac{(p_{ij}-p_{i.}\,p_{.j})^2}{p_{i.}p_{.j}}\right]^{-1}$$

Where,

$$X^2_{k-1}\left(X^2_{k-1}|\delta\right) = \beta$$

3.2. Data Collection

Patients' information including age, familial history of breast cancer, menopause status, histological type of breast cancer, immunohistochemical and hormonal properties of breast cancer, tumor grading, and size were collected from the medical records.

3.3. IHC Assessment

Patients with a history of breast cancer, who were confirmed with mammography and surgical pathology were assessed for immunohistochemistry properties. For the preparation of the species, a 0.4 millimeter of the species was extracted by a pathological needle puncture and separated with a thickness of one micrometer. The species were inserted on adhesion microscope slides and after wax deposition, the microscopic slides were hydrated by ethanol for 5 minutes. Antigen retrieval was performed with EDTA-TRIS solution (Sigma-Aldrich®, USA) at the temperature of 98° of centigrade for 20 minutes. After cooling down and washing with tris buffered saline (Sigma-Aldrich®, USA), species were inserted into hydrogen peroxide with 3% of concentration. For antibody incubation, species were washed 30 minutes with antibodies, and a post-primary block solution was used for 20 minutes. For the last step, after washing species with tris buffered saline (TBS) for the second time, microscopic slides were incubated with Novolink™ polymer for 20 minutes. Prepared slides were seen by a light microscope. The last guideline of the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) was used for reporting immunohistochemical results. All IHC samples were analyzed and commented on by 2 board-certified breast cancer pathologists.

3.4. Hormonal Receptor Assessment

Allred score was used for reporting the hormone receptors (15). PharmxDX kit (Agilent®, Dako, USA) was used for estrogen and progesterone hormonal assessment. Percentage score (PS) and intensity score (IS), which are two indicators of Allred score and suggest the proportion of colored cells, were used for hormonal assessment.

3.5. Statistical Analysis

Before starting the statistical analysis, the Kolmogorov-Smirnov test was used for evaluating the normality of data. Considering the lack of normality in data, the Mann-Whitney test and Kruskal-Wallis test were used for comparing the quantitative variables. Furthermore, the chi-square test and exact fisher test were used for analyzing qualitative data. A P-value below 0.05 is considered significant.

4. Results

4.1. Demographics

A total of 142 female patients were included in the final analysis. The mean age of the patient was 50.2 $\pm$ 12.7 (range: 20 - 75). The results of demographics and pathological features of patients are demonstrated in Table 1.

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Table 1. Study Variables

Variables	No. (%)
Age range	
< 40	24 (16.2)
40 - 50	49 (34.2)
> 50	69 (49.6)
Family history	
Positive	78 (54.9)
Negative	64 (45.1)
Menopause	
Positive	76 (53.5)
Negative	66 (46.5)
Immunohistochemical properties	
Luminal A	57 (29.7)
Luminal B	28 (18.9)
Triple-negative	27 (24.3)
Overexpression of HER2/NEU	30 (27)
Invasive histologic type	
Invasive ductal carcinoma	97 (87.3)
Invasive lobular carcinoma	8 (7.2)
Invasive medullary carcinoma	2 (1.8)
Invasive tubular carcinoma	4 (3.6)
In situ histologic type	
Ductal carcinoma in situ	28 (90.4)
Lobular carcinoma in situ	3 (9.6)
Tumor grade	
Grade 1	4 (3.1)
Grade 2	76 (53.6)
Grade 3	62 (43.3)
Tumor diameter	
T_1	17 (11.9)
T_2	94 (66.2)
T_3	21 (14.8)
T_4	10 (7.1)

4.2. IHC Properties

In the assessment of IHC properties, luminal A molecular type had the most frequency (29.7%). The disturbances of IHC type of breast cancers and study variables are demonstrated in Table 2. As seen in Table 2, variables including age (P = 0.34), familial history (P = 0.42), menopause (P = 0.36), and histological type (invasive: P = 0.11, in situ: P = 0.45) did not have any relationship with IHC properties of breast cancer. However, tumor diameter (P < 0.001) and tumor grading (P = 0.001) are significantly related to the IHC properties.

Accordingly, the Kruskal-Wallis test showed a significant difference between IHC type and tumor grade. According to the result, tumor grade was higher in both triple-negative and HER2/NEU IHC subtypes. Also, in terms of tumor diameter, the results showed a significant difference between IHC type and tumor diameter. Tumor diameter was higher in both triple-negative and HER2/NEU. However, both tumor grade and diameter were accordingly lower in luminal A IHC type (Table 2). Invasive ductal carcinoma is the most common type of invasive cancer. In addition, the most common type of IHC property observed in invasive ductal carcinomas was the overexpression of HER2/NEU. In addition, luminal A type was more prevalent among other types of breast cancer. However, this difference was not statistically significant (P = 0.119).

The relationship between medical information (age, histological type, familial history, menopause status, tumor grade, tumor diameter) and IHC type was studied by stepwise multinomial logistic regression by using medical information as independent factors and IHC as a dependent factor. Then, only significant independent factors were retained in the final regression model (Table 3). Therefore, the only variable, tumor diameter, was associated with IHC type in the presence of the other factors (P = 0.001).

5. Discussion

In this study, it was demonstrated that tumor size and tumor grading are two main factors affecting the IHC properties of breast cancer cells. Greater breast tumors and high-grade cancers are associated with unfavorable IHC properties such as the lack of hormonal receptors (triple-negative) or the lack of estrogen and progesterone receptors.

Breast cancer is the most common malignant neoplasm among women, which has a 5-year survival rate between 27 and 90% based on time of diagnosis, metastasis, epidemiology, and cancer subtype (16-18). In the study of Abedi et al., the 5-year survival rate of patients with breast cancer was 69.5% (19). In addition, Baghestani et al. concluded a 95% rate of 1-year survival, followed by a 79% 5-year survival, and a 50% 10-year survival rate among Iranian women diagnosed with breast cancer (20). Furthermore, several factors can affect the patient's survival including age, IHC type, histological type, and familial history (21). That said, molecular prognostic factors and hormonal factors assessed by IHC are at the center of attention nowadays (22).

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Table 2. The Disturbances of Immunohistochemistry Type of Breast Cancers and Study Variables [a]

Variables	Immunohistochemical Type				P-Value
	Triple-Negative	Luminal A	Luminal B	HER2/NEU	
Age range					0.34 [b]
< 40	2 (8.2)	8 (33.4)	8 (33.4)	6 (25)	
40 - 50	12 (24.5)	20 (40.8)	8 (16.3)	9 (18.4)	
> 50	13 (18.8)	29 (42)	10 (14.5)	17 (24.7)	
Family history					0.42 [c]
Positive	20 (25.6)	24 (30.7)	12 (15.4)	22 (28.3)	
Negative	13 (20.3)	21 (32.8)	12 (18.7)	18 (28.2)	
Menopause					0.36 [c]
Positive	19 (25)	23 (30.2)	13 (17.1)	21 (27.7)	
Negative	14 (21.2)	20 (30.3)	13 (19.7)	19 (28.8)	
Invasive histologic type					0.11 [b]
Invasive ductal carcinoma	26 (26.8)	25 (25.8)	17 (17.5)	29 (29.9)	
Invasive lobular carcinoma	1 (12.5)	4 (50)	3 (37.5)	0 (0)	
Invasive medullary carcinoma	0 (0)	2 (100)	0 (0)	0 (0)	
Invasive tubular carcinoma	0 (0)	2 (50)	1 (25)	1 (25)	
In situ histologic type					0.45 [b]
Ductal carcinoma in situ	0 (0)	21 (75)	7 (25)	0 (0)	
Lobular carcinoma in situ	0 (0)	3 (100)	0 (0)	0 (0)	
Tumor grade	2.69 ± 0.471	2.16 ± 0.554	2.12 ± 0.485	2.52 ± 0.509	0.001 [d]
Tumor diameter	3.63 ± 0.926	2.81 ± 0.480	3.07 ± 0.539	3.53 ± 0.681	< 0.001 [d]

[a] Values are expressed as No. (%) or mean ± SD.
[b] Exact fisher test.
[c] Chi-Square test.
[d] Kruskal-Wallis test.

IHC is used to distinguish surface proteins and antigens in different cells (23). In the IHC study, various specific markers are used to identify tumor subtypes and tissue origin, which are essential in the differentiation of primary tumors from the metastatic origin (24). Furthermore, IHC evaluations classify breast cancer cells based on their hormonal receptors (25). Estrogen receptors (ER), progesterone receptors (PR), human epidermal growth factor receptor-2 (HER2), and Ki-67 are 4 common biomarkers for the IHC study of breast cancer (26, 27). Based on hormonal receptors, IHC properties of breast cancers are classified as triple-negative tumors (negative for ER, PR, and HER2), luminal A (positive for ER, positive/negative for PR, negative for HER2, and < 14% of Ki-67), luminal B (positive for ER, positive/negative for PR, negative for HER2, and ≥ 14% of Ki-67), and HER2 positive (negative for ER, negative for PR, positive for HER2, and ≥ 14% of Ki-67) (28).

In the current study, luminal A type was the most commonly diagnosed IHC type of breast cancer, which was in association with previous studies (28, 29). There was no significant association between IHC properties and histological type of breast cancer. However, most invasive and in situ carcinomas were luminal A-type. Gupta et al. demonstrated no significant differences between the type of breast cancer and IHC properties (30). In a study conducted by Rao et al., ductal carcinoma in situ was mostly HER2 positive, but this association was not statistically significant (31). In contrast, few studies observed contraindicatory results. In the study of Jalava et al., lobular carcinomas were more associated with higher expression of ER and PR (32). Moreover, Holloway et al. demonstrated that triple-negative IHC type is frequently associated with ductal carcinoma (33). It seems that further studies are needed to evaluate the association between histologic types of breast cancers and their IHC properties.

Tumor grade is considered one of the important factors affecting the prognosis and the survival of patients with breast cancer (34). In our study, IHC type had a signifi-

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Table 3. Multinomial Logistic Regression by Using Medical Information as Independent Factors and IHC as A Dependent Factor

IHC Type (Dependent Variable) [a] and Independent Variable	Coefficient (B)	Std. Error	Wald	df	P-Value	Exp (B)
Luminal A						
Intercept	-19.711	7203.325	0.000	1	0.998	
Tumor diameter = T_1	21.097	7203.325	0.000	1	0.998	1452860761
Tumor diameter = T_2	20.067	7203.325	0.000	1	0.998	518878843
Tumor diameter = T_3	18.324	7203.325	0.000	1	0.998	90803797.6
Tumor diameter = T_4	0 [b]	-	-	0	-	-
Luminal B						
Intercept	-20.096	0.707	807.712	1	0.000	
Tumor diameter = T_1	20.096	1.581	161.542	1	0.000	534139968
Tumor diameter = T_2	19.942	0.809	607.372	1	0.000	457834258
Tumor diameter = T_3	20.096	0	-	1	-	534139968
Tumor diameter = T_4	0 [b]	-	-	0	-	-
HER2/NEU						
Intercept	-0.847	0.69	1.508	1	0.220	
Tumor diameter = T_1	-17.202	8306.049	0.000	1	0.998	3.38E-08
Tumor diameter = T_2	0.981	0.781	1.577	1	0.209	2.667
Tumor diameter = T_3	1.764	0.909	3.765	1	0.052	5.833
Tumor diameter = T_4	0 [b]	-	-	0	-	-

[a] The reference category: Triple-negative.
[b] This parameter is set to zero because it is redundant.

cant association with tumor grading. In the current study, grade 1 and grade 2 of breast cancers had luminal A as the highest IHC type. That said, the majority of high-grade tumor IHC types were diagnosed as triple-negative. In line with our study, Chand et al. stated that luminal A and luminal B IHC type had a significant correlation with age, tumor size, and tumor grade (22). Ayadi et al. suggested that the overexpression of HER2 is mostly associated with higher-grade breast cancer (35). Setyawati et al. concluded that low-grade breast cancers are related to luminal A IHC type. In addition, they concluded that high-grade cancers are mainly associated with luminal B and triple-negative IHC properties (36). All previous results were in line with the current study (37).

Tumor size is another important factor for breast cancer survival (38). Similar to previous studies, tumor size seems to affect the breast cancer IHC type (33, 36). Our study demonstrated that the majority of breast tumors with a mean size of 1-50mm were in the luminal group. Furthermore, tumor sizes of more than 50mm were associated with HER2 overexpression. Accordingly, a higher tumor diameter is associated with poor IHC properties.

This study has several limitations. Firstly, the present study is a retrospective cross-sectional study and not a prospective trial, which may increase the risk of potential selection and reporting bias. In addition, it can be argued that the present study may assess a few patients (n = 142). Therefore, we encourage physicians to evaluate the long-term oncological outcomes of patients with breast cancer with a larger sample population in a prospective design.

5.1. Conclusions

Factors including tumor size and pathological grade can affect the gene expression properties of breast cancers. Luminal IHC type A is more common in breast cancer and is associated with better outcomes. Age, histological type, familial history, and menopause status did not affect the IHC properties of breast cancer; although, further studies are needed to confirm these results.

Footnotes

Authors' Contribution: Study concept and design, K. P., and M. S.; Analysis and interpretation of data, R. S., and M. S.; Drafting of the manuscript, V. A. and R. S; Critical revision of the manuscript for important intellectual content, V. A. and K. P.; Statistical analysis, R. S.

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Conflict of Interests: The authors declare no conflict of interest.

Data Reproducibility: All data generated or analyzed during this study are included in this published article.

Ethical Approval: The purpose of this research was completely explained to the patients and they were assured that their information will be kept confidential by the researchers. The present study was approved by the medical ethics committee of the academy. The permission was obtained from the Medical Ethics Committee of the Medical Faculty of Islamic Azad University of Mashhad (registration no: IR.IAU.MSHD.REC.1396.37).

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