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Study of Molecular Subtypes and Clinic-PathoLogical Features of Breast Cancer in Iraq

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Abstract: ***Breast cancer is the most common cancer in women worldwide constituting 25.1% of all new cancer cases. In 2012, worldwide breast cancer deaths were approximately 521,907 as reported by GLOBOCAN data base. The histopathological classification essentially plays a role in identifying the various histologic variants of breast carcinoma; namely, tubular, medullary, mucinous/colloid carcinomas, and others. A new therapeutically relevant molecular classification has been developed, based on gene expression profiling using complementary DNA microarrays. This observational retrospective study was carried out in Al-Amal national hospital for cancer treatment / Baghdad during the period from January 2012 to December 2016. A total of 250 women were selected with excluding male patients, recurrent cases. The following archival information were obtained from each patient using KHUH's laboratory information system: age at diagnosis, tumor size, histopathological subtype, presence or absence of carcinoma in situ, lymph node status and immunohistochemical profile of the hormonal receptors ER and PR, and immunohistochemical profile of HER2 in the invasive malignant cells. The tumor size measurement was obtained on ultrasound reports of the breast prior to biopsy. The number of lymph nodes identified and the number of lymph nodes positive for metastasis were determined. The immunohistochemical antibodies used for estrogen, progesterone, and HER2 are anti-estrogen receptor antibody (SP1), anti-progesterone receptor antibody (1E2), and anti-HER-2 (4B5) rabbit monoclonal primary antibody. The diagnosis, SBR grading, and hormonal receptor and HER2 status assessment were carried out and verified independently by at least 2 qualified histopathologists. Results of this study showed that breast cancer classification by immunohistochemistry revealed that in our community luminal A tumors were the most common subtype, followed by the rest tumors. Breast cancer subtypes exhibited particular characteristics. Luminal A tumors were associated with an increased frequency of ductal carcinomas. The HER2-positive and triple negative tumors were associated with an increased frequency of a large tumor size and poorly differentiated carcinomas and are thereby more aggressive. In addition, triple negative tumors least frequently showed a component of carcinoma in situ. It can be concluded from our study that there is no correlation between lymph node status and molecular subtypes.***

Keywords: Breast cancer, Molecular subtypes, Clinic-pathological feature

1. Introduction

Breast cancer is a cancer that develops from breast tissue [1]. Signs of breast cancer may include a lump in the breast, a change in breast shape, dimpling of the skin, fluid coming from the nipple, a newly-inverted nipple, or a red or scaly patch of skin [2].

In those with distant spread of the disease, there may be bone pain, swollen lymph nodes, shortness of breath or yellow skin [3].

In this classification, breast carcinomas are divided into five major molecular groups: Luminal A, Luminal B, human and HER2 is used to classify these tumors as it is easier, more cost-effective and yields similar molecular subtypes [5, 6]. Molecular subtyping by immunohistochemistry is now regarded as the cornerstone for the detection of tumor sensitivity to hormonal therapy and subsequent Trastuzumab therapy [6, 7]. Racial differences in the distribution of breast cancer are well documented [8, 9]. While luminal A is the most prevalent subtype in most regions [8], it is worth noting that the frequency of triple negative tumors is high among certain communities, such as African American [10], epidermal growth factor receptor 2 (HER2), basal, and normal-like [4]. In clinical practice, the immunohistochemical status of estrogen receptor (ER), progesterone receptor (PR), [11].

In 2010, Tamimi et al [12] analyzed 5 immunohistochemistry markers (ER, PR, HER2, epidermal growth factor receptor [EGFR], and Chromosome [CK] 5/6) in 231 breast cancer cases located in the Eastern Province of Iraq. Their results showed that in our population, there is a higher prevalence of HER2-positive and lower prevalence of luminal type tumors compared with western populations.

They also reported that approximately 40% of their cases tested negative for all mentioned immunohistochemical markers and fell under the category of unclassified. These statistics highlight the difference between the Iraqi and Western societies' pattern of distribution [13, 14]. There is a need for further studies to elaborate and define the nature of breast cancer in Iraq.

In this study, we aimed to assess the prevalence of breast cancer subtypes in the central region of Iraq and their associated clinicopathological features, hoping that such an endeavor will increase our understanding of breast cancer and improve its management.

2. Patients and Methods

This is an observational retrospective study based on data retrieved from Al-Amal national Hospital for cancer Treatment Baghdad laboratory archival information system). We utilized KKUH's histopathology laboratory reports to identify all patients diagnosed with primary invasive breast cancer in the past 5 years, between January 2012 and December 2016.

We excluded male patients, recurrence cases, and non-Arab patients. A total of (250) cases were selected for this study.

Using KKUH's laboratory archival information system, we obtained the following parameters for each patient: age at diagnosis, tumor size, histopathological subtype, Scarff-Bloom-Richardson (SBR) grade, presence or absence of carcinoma in-situ component, lymph node status, immunohistochemical profile of the hormonal receptors ER and PR and immunohistochemical profile of HER2 in the invasive malignant cells.

The tumor size measurement was obtained on ultrasound reports of the breast prior to biopsy. If no ultrasound reports were found, then reports from other radiological modalities such as MRI, computed tomography, or mammogram were used. The status of lymph node metastasis was determined using radiological modalities, trucut biopsy of axillary lymph nodes or evaluation of lymph nodes obtained at mastectomy, including sentinel lymph nodes.

The number of lymph nodes were identified and the number of lymph nodes positive for metastasis were determined. Patients with a positive lymph node status in whom the lymph nodes have not been quantified (in cases of positive trucut axillary lymph node biopsies) were labelled as "undetermined".

The immunohistochemical antibodies used for estrogen, progesterone, and HER2 are anti-estrogen receptor antibody (SP1), anti-progesterone receptor antibody (1E2), and anti-HER-2 (4B5) rabbit monoclonal primary antibody. The machine used for immunohistochemistry staining is Bench Mark XT. The HER2 was scored from 0 to 3+ in which: score 0 or 1 are negative; 2+ is equivocal; and 3+ is positive. A 3+ score is for an intense full circumferential cytoplasmic membrane staining in more than 10% of invasive malignant cells. Specimens showing equivocal HER2 staining were sent for fluorescent in situ hybridization (FISH) from Targos Molecular Pathology GmbH, Kassel, Germany, and their results were documented.

The diagnosis, SBR grading, and hormonal receptor and HER2 status assessment were carried out and verified independently by at least 2 qualified histopathologists.

3. Statistical Analysis

The Statistical Package for Social Sciences software version 21.0 (IBM Corp, Armonk, NY, USA) was used for statistical analysis. Descriptive statistics, frequency, and percentages of categorical variables were reported. Chi square test was used for categorical variables. The results were considered statistically significant if the p-value was <0.05.

4. Results

A total of (250) breast cancer cases were included: Most cases, 84.8% (n=212) were ductal, 10.0% (n=25) were lobular, and the other remaining cases 15(5.2%) were of other subtypes including medullary, tubular, mucinous, metaplastic, adenoid cystic, and encysted papillary carcinoma. The average tumor size at diagnosis was 3.50±2.05 cm. More than half of our patients had a tumor size between 2-5 cm (n=138, 55.2%), while only a third (n=75, 30.0%) exhibited a tumor size <2 cm and the rest of 37 cases 14.85 were more than 5 cm. More than half of the cases (n=150, 60.0%) presented with lymph node metastases as shown in table (1) and (2).

Table 1: Distribution of clinico-pathological characteristics according to hormonal and molecular subtypes in (250) women with invasive breast cancer

Characteristics	Luminal A	Luminal B	HER-2 Positive	Triple negative	Total	p- value
Total	150(60.0)	35(14.0)	30(12.0)	35(14.0)	250(100)	
Age(years)						
≤50	71(58.7)	23(47.9)	21(55.3)	25(58.1)	140(56.0)	0.633
>50	50(41.3)	25(52.1)	17(44.7)	18(41.9)	110(44.0)	
Tumor size (cm)						
≤2	52(37.4)	11(30.6)	3(10.0)	9(20.0)	75(30.0)	
>2-≤5	70(50.3)	20(55.5)	20(66.6)	28(62.2)	138(55.2)	0.058
>5	17(12.3)	5(13.9)	7(23.4)	8(17.8)	37(14.8)	
Lymph nodes metastasis						
Negative	53(39.2)	14(40.0)	12(33.3)	21(47.7)	100(40.0)	
positive						
1-3	27(20.0)	8(22.8)	7(19.4)	8(18.2)	50(20.0)	0.951
≥4	17(12.6)	5(14.4)	5(14.0)	3(6.8)	30(12.0)	
Undermined	38(28.2)	8(22.8)	12(33.3)	12(27.3)	70(28.0)	
Data are presented as number and percentage (%)						

Results in table (1) and (2) showed that the ER immune stain was positive in 72.0% and the PR in 65.0%. Human epidermal growth factor receptor 2 immune stain was positive in 20.0% and equivocal in 21.0% of the cases. The most prevalent subtype was luminal A (n=150, 60.0%) followed by, in descending order of frequency, triple negative (n=35 14.0%), luminal B (n=35, 14.0%), and HER2-positive (n=30, 12.0%). The distribution of clinical and pathological characteristics among the various molecular subtypes is ill. Human epidermal growth factor receptor 2-positive and triple negative tumors occurred in higher frequency (55.3-58.1%) in patients who were younger than 50 years of age compared with luminal tumors. However, this was not statistically significant (p=0.633). Human epidermal growth factor receptor 2-positive (n1) had a tumor mass size of >2 cm in 90.0%, and triple negative tumors (n2) in 80% of patients; in which most ranged between 2 cm and 5 cm (n1= 20, 66.6% and n2=28, 62.2%) and the remaining were >5 cm (n1= 7, 23.4% and n2=8, 17.8%) (p=0.058).

In addition, these subtypes had aggressive microscopic features with approximately two-thirds of them showing poorly differentiated carcinomas. In addition, triple negative tumors least frequently displayed an in situ component (27.0%, p=0.032).

Table (2): Distribution of histopathological characteristics according to hormonal and molecular subtypes in (250) women with invasive breast cancer

Characteristics	Luminal A	Luminal B	HER-2 Positive	Triple negative	Total	p- value
Total	150(60.0)	35(14.0)	30(12.0)	35(14.0)	250(100)	
Histology						
Ductal	110(78.0)	32(91.4)	30(96.8)	40(93.0)	212(84.8)	0.018
Lobular	22(15.6)	2(5.7)	1 (3.2)	0	25(10.0)	
Others	9(6.4)	1(2.9)	0	3(7.0)	13(5.2)	
Tumor grade						
Grade I	30(19.6)	5(11.4)	0	0	35(14.0)	0.0001
Grade II	80(52.3)	17(38.6)	13(43.3)	7(30.4)	117(46.8)	
Grade III	43(28.1)	22(50.0)	17(52.7)	16(69.6)	98(39.2)	
Carcinoma in situ						
Present	80(51.2)	19(57.6)	10(41.7)	10(27.0)	119(47.6)	0.032
Absent	76(48.8)	14(42.4)	14(58.3)	27(73.0)	131(52.4)	
Data are presented as number and percentage (%)						

Table 3: Distribution of molecular subtypes of breast carcinomas immunohistochemistry in various regional and western countries

Variables	Mehdi et al [16]	Yang et al [22]	Cheng et al [25]	Vallejos et al [33]	Fourati et al [17]	Carey et al 19		Present study
						African Americans	Non- African Americans	
Setting	Oman	Poland	China	Peru	Tunis	Carolina, USA		IRAQ
Number of patients	542	804	628	1198	966	196	300	250
Years	2006-2010	2000-2003	2007-2010	2000-2002	2007-2009	1993-1996		2012-2016
Luminal A	34.7%	69.0%	46.5%	49.3%	50.7%	47.4%	54.0%	60.0%
Luminal B	15.9%	6.0%	17.0%	13.2%	13.4%	12.7%	17.3%	14.0%
HER2/NEU	24.1%	8.0%	15.0%	16.2%	13.4%	8.2%	5.6%	12.0%
Triple negative	25.3%	18.0%	21.5%	21.3%	22.5%	31.6%	23.0%	14.0%
HER2-hyman epidermal growth factor receptor 2								

5. Discussion

We studied the distribution of the molecular subtypes of breast cancer in a tertiary hospital setting and evaluated the differences in clinicopathological features between these subtypes. The average age of diagnosis in our study was 47.5 years, which is equivalent to that reported by the Iraqi Cancer Incidence Report [15], and a previous study conducted in Amal national Hospital for cancer Treatment Baghdad [2001-2010] [16].

Most of our cases (56.0%) occurred in women <50 years, which is similar to the Omani study [17]. In contrast, in the United States, 65.1% of cases occurred in women older than 55 years according to the Surveillance, Epidemiology and End Results (SEER) Cancer Statistics Review in the period from 2001 to 2005 [18] . While only 30.0% of our patients presented with a tumor size <2 cm, in countries like the United States and Poland, the percentage of patients presenting with a tumor size ≤2 cm is considerably higher, being 58.4% and 51.9%, respectively [9,19]. This signifies late diagnosis in our community and it may be due to multiple factors including inadequate information in the community, pertaining to breast cancer and the presence of a non-comprehensive screening program.

The distribution of molecular subtypes in our study was mostly consistent with the findings in other published studies from various regional and western countries (table 3). Regarding the distribution of the molecular classification, luminal A was the most frequently encountered subtype, as recognized in most studies. Like our findings, about quarter of the cases (25.3%) in Mehdi et al's [17] study were triple negative, with luminal tumors comprising only 34.7%.

Although the frequency of the molecular subtypes differ from one population to another, most have a similar order of distribution with triple negative carcinomas being the second most prevalent subtype.

Human epidermal growth factor receptor 2-positive and triple negative tumors were associated with a greater frequency of poorly differentiated carcinomas [13, 20]. Compared to luminal A, these subtypes are associated with an increased frequency of a larger tumor size [21, 22], and a younger age group [21, 23].

In our present study, we found no association between the different molecular subtypes and lymph node status. While multiple studies failed to detect such an association [21, 24], several studies identified a high frequency of lymph node metastasis with HER2-positive tumors and a low frequency with basal-like tumors [25, 26].

In contrast to half of luminal A tumors (51.2%), only 27.0% of triple negative tumors (p=0.032) displayed an in-situ component. Zaha et al [27] demonstrated that 45 cases of their luminal tumors (n=124) showed an in-situ component.

The role of mammography to detect the different molecular subtypes has been suggested in one study [28], which concluded that HER2-positive tumors and triple negative tumors were less likely to be detected by mammography.

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