Molecular subtypes of breast carcinoma in Saudi Arabia

A retrospective study

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ABSTRACT

الأهداف : تحديد مدى انتشار الأنواع الجزيئية لسرطان الثدي في منطقة الرياض بالمملكة العربية السعودية، وكذلك تقييم العلاقة بين هذه الأنواع المختلفة والعمر عند التشخيص، وحجم الورم، والنوع التشريحي المرضي، ودرجة الورم، ووجود سرطانة لابدة، بالإضافة إلى حالة العقد اللمفاوية .

الطريقة : أجريت هذه الدراسة الرقابية الاسترجاعية خلال الفترة ما بين يناير2010م حتى ديسمبر 2014م، قمنا بتصنيف 359 حالة سرطان ثدي إلى أربعة أصناف جزيئية وذلك بالاعتماد على تقنية كيمياء الأنسجة المناعية للإستروجين ER وللبروجستيرون PR ولمستقبل عامل نمو البشرة البشري HER2. هذه الأنواع هي : نوع اللمعة (أ) (إيجابي ER و/أو PR وسلبي لـHER2) ، ونوع اللمعة (ب) (إيجابي ER و/أو PR وإيجابي لـHER2)، والنوع الإيجابي لمستقبل (HER2) (سلبي ER وPR وإيجابي لـHER2)، وأخيرًا النوع ثلاثي السلبية (سلبي ER وPR وHER2). قمنا بدراسة العلاقة بين هذه الأنواع الجزيئية والخصائص السريرية الأمراضية مستخدمين فحص مربع كاي .

النتائج : كان أكثر الأنواع تواترًا هو نوع اللمعة (أ) بنسبة (58.5%) متبوعًا ب وبتسلسل تنازلي حسب نسبة التواتر: ثلاثي السلبية (14.8%) ونوع اللمعة (ب) (14.5%) ثم الإيجابي لمستقبل (HER2) (12.3%) .

الخاتمة : كان الورم ذو اللمعة (أ) أكثر الأنواع مشاهدة في دراستنا بينما كان النوع الإيجابي لمستقبل (HER2) الأقل شيوعًا. وكانت غالبية سرطانات الثدي الفصيصية من نوع اللمعة (أ). يتميّز النوعان الإيجابي لمستقبل (HER2) وثلاثي السلبية بكثرة الأورام كبيرة الحجم والأورام ذات الدرجة النسيجية العالية. بالإضافة إلى ذلك، وبالمقارنة بالأورام اللمعية، تبين أن هذه الأنواع تحدث بتواتر عالٍ عند النساء الأقل من 50 عاماً. ومن الأمور الجديرة بالملاحظة هوَّ عدمُ شيوع وجود السرطانة اللابدة في الأورام ثلاثية السلبية، ولم توجد أي علاقة بين حالة العقد اللمفاوية والأنواع الجزيئية للأورام .

Objectives: To determine the distribution of various molecular subtypes of breast cancer in Saudi Arabia and to assess the association between these subtypes and age at diagnosis, tumor size, histopathological type, grade, presence of carcinoma in-situ, and lymph node status.

Methods: This observational retrospective study, between January 2010 and December 2014, was conducted at King Khalid University Hospital, Riyadh, Saudi Arabia. We classified 359 breast cancers into 4 molecular subtypes, using immunohistochemistry: luminal A (estrogen receptor [ER], or progesterone receptor [PR] positive and human epidermal growth factor receptor 2 [HER2] negative), luminal B (ER and/or PR positive and HER2 positive), HER2-positive (ER and PR negative and HER2 positive), and triple negative (ER, PR, and HER2 negative). We evaluated the relationship between these subtypes and clinicopathological features using Chi square test.

Results: The most prevalent subtype was luminal A (58.5%), followed in descending order of frequency by triple negative (14.8%), luminal B (14.5%), and HER2-positive (12.3%). The average age at diagnosis was 49.8 years, and average tumor size at diagnosis was 3.19 cm.

Conclusion: Luminal A tumor was the most common molecular subtype and HER2-positive was the least common. Most lobular carcinomas were luminal A tumors. Human epidermal growth factor receptor 2-positive and triple negative tumors had a higher histologic grade and a larger tumor size at diagnosis, and they were more common in women under 50 years. Carcinoma-in-situ was least common in triple negative tumors. We found no association between lymph node status and molecular subtypes.

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.[1] Although the incidence of breast cancer is lower in Saudi Arabia (age standardized rate per 100,000 is 29.6) than worldwide (age standardized rate per 100,000 is 43.1), it forms most cancer deaths in women.[1] The median age of diagnosis is 61 in the United States,[2] however, it occurs at a younger age group in Arab countries, including Saudi Arabia. The mean age of diagnosis of breast cancer in Saudi Arabia is 49 years,[3,4] and it is generally discovered at a later clinical stage.[5] Breast cancer is a heterogeneous entity with variable behavior and outcomes.[6,7] From a therapeutic point of view, the histopathological classification is of limited value as most breast carcinomas fall under the umbrella of ductal carcinomas not otherwise specified. The histopathological classification essentially plays a role in identifying the various histologic variants of breast carcinoma; namely, tubular, medullary, mucinous/colloid carcinomas, and others.[8] A new therapeutically relevant molecular classification has been developed, based on gene expression profiling using complementary DNA microarrays. In this classification, breast carcinomas are divided into 5 major molecular groups: Luminal A, Luminal B, human epidermal growth factor receptor 2 (HER2), basal, and normal-like.[7] In clinical practice, the immunohistochemical status of estrogen receptor (ER), progesterone receptor (PR), and HER2 is used to classify these tumors as it is easier, more cost-effective and yields similar molecular subtypes.[9,10] Molecular subtyping by immunohistochemistry is now regarded as the cornerstone for the detection of tumor sensitivity to hormonal therapy and subsequent Trastuzumab therapy.[10,11] Racial differences in the distribution of breast cancer are well documented.[12,13] While luminal A is the most prevalent subtype in most regions,[12] it is worth noting that the frequency of triple negative tumors is high among certain communities, such as African American,[14,15] Omani,[16] and Tunisian.[17] In 2010, Tamimi et al[18] 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 Saudi Arabia. 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.[18] These statistics highlight the difference between the Saudi and Western societies' pattern of distribution.[19,20] There is a need for further studies to elaborate and define the nature of breast cancer in Saudi Arabia. In this study, we aim to assess the prevalence of breast cancer subtypes in the central region of Saudi Arabia and their associated clinicopathological features. We hope that such an endeavor will increase our understanding of breast cancer and improve its management.

Methods. This is an observational retrospective study based on data retrieved from the King Khalid University Hospital's (KKUH) 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 2010 and December 2014. We excluded male patients, recurrence cases, and non-Arab patients. Non-Arab patients were excluded from this study as racial differences were noted in previous studies.[12,13] A total of 359 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. If no radiological size were available, tumor size at lumpectomy or mastectomy was employed. After size assessment, tumors were grouped in 3 categories: ≤2 cm, >2 but ≤5 cm, and >5 cm. Tumor grade evaluation was carried out according to the established Elston-Ellis Modification of the SBR system, which relies on the percentage of tubular differentiation, the presence of nuclear atypia/pleomorphism, and the number of

mitoses.[21] 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 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 BenchMark XT. Both antibodies and machine are manufactured by Ventana Inc., Tucson, Arizona, USA. The ER, PR, and HER2 were scored according to the guidelines of the College of American Pathologists.[21] Positive ER or PR requires ≥1% of invasive malignant cells that show nuclear staining/immunoreactivity. In addition, for ER and PR, another semi-quantitative scoring system called the Allred (Quick) scoring system was employed to assess the proportion of stained cells and the intensity of the nuclear staining.[21] 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. We classified the breast cancer into 4 molecular subtypes according to ER, PR, and HER2/neu status: luminal A (ER and/or PR positive and HER2/neu negative), luminal B (ER and/or PR positive and HER2/neu positive), HER2-positive (ER and PR negative and HER2/neu positive), and triple negative (ER, PR, and HER2/neu negative).[9,10]

Statistical analysis. The Statistical Package for Social Sciences software version 21.0 (IBMCorp, Armonk, NY, USA) was used for statistical analysis. Descriptive statistics, frequency, and percentages of categorical variables were reported. We studied the association between the molecular subtypes and age at diagnosis,

tumor size, histopathological subtype, grade, presence of foci of in situ carcinoma, and nodal status using Chi square test for categorical variables. We computed the odds ratio (OR) where appropriate and constructed 95% confidence interval (CI). The results were considered statistically significant if the p-value was <0.05.

Ethical considerations. There were minimal ethical implications and issues, as this is a retrospective study. Patient identity and confidentiality were protected by assigning each patient a serial number. Moreover, no one except for the investigating research team accessed the patients' records. We obtained the Institutional Review Board's approval as a consent form was not applicable to our study.

Results. A total of 359 breast cancer cases were included with an average patient's age at diagnosis of 49.8 years (standard deviation 12.28). Most cases, 85% (n=305) were ductal, 11.1% (n=40) were lobular, and the remaining cases were of other subtypes including medullary, tubular, mucinous, metaplastic, adenoid cystic, and encysted papillary carcinoma. Most cancers were moderately differentiated (n=171, 47.6%) followed by poorly differentiated (n=139, 38.7%). The average tumor size at diagnosis was 3.19 cm (SD 1.92). More than half of our patients had a tumor size between 2-5 cm (n=191, 53.2%), while only a third (n=119, 33.15%) exhibited a tumor size ≤2 cm (**Table 1**). Patients <50 years of age had greater odds of exhibiting a larger tumor size (p=0.036, OR=1.613, 95% CI, 1.030-2.526) than those in their sixth decade or more. Almost half of the cases (n=188, 59.5%) presented with lymph node metastases (**Table 1**).

The ER immunostain was positive in 70.8% and the PR in 63.8%. Human epidermal growth factor receptor 2 immunostain was positive in 18.7% and equivocal in 22.8% of the cases. The equivocal cases underwent FISH testing, and 34.2% of the equivocal cases were positive for HER2. The most prevalent subtype was luminal A (n=210, 58.5%) followed by, in descending order of frequency, triple negative (n=53, 14.8%), luminal B (n=52, 14.5%), and HER2-positive (n=44, 12.3%). The distribution of clinical and pathological characteristics among the various molecular subtypes is illustrated in **Tables 1 & 2**. Human epidermal growth factor receptor 2-positive and triple negative tumors occurred in higher frequency (66-70.5%) in patients who were younger than 50 years of age compared with luminal tumors. However, this was not statistically significant (p=0.124). Human epidermal growth factor

Table 1 - The distribution of clinico-pathological characteristics according to hormonal and molecular subtypes in 359 women with invasive breast cancer.

Characteristics	Luminal A	Luminal B	HER-2 positive	Triple negative	Total	*P*-value
Total	**210 (58.5)**	**52 (14.5)**	**44 (12.3)**	**53 (14.8)**	**359 (100)**	
Age (years)						0.124
≤50	114 (54.3)	28 (53.8)	31 (70.5)	35 (66.0)	**208 (57.9)**	
>50	96 (45.7)	24 (46.2)	13 (29.5)	18 (34.0)	**151 (42.1)**	
Tumor size (cm)						0.057
≤2	81 (40.1)	18 (34.6)	7 (17.5)	13 (25.0)	**119 (33.2)**	
>2 - ≤5	105 (52.0)	29 (55.8)	26 (65.0)	31 (59.6)	**191 (53.2)**	
>5	16 (7.9)	5 (9.6)	7 (17.5)	8 (15.4)	**36 (10.0)**	
Not determined					**13 (3.6)**	
Lymph nodes metastasis						0.656
Negative	78 (41.5)	17 (38.6)	13 (35.1)	20 (42.6)	**128 (40.5)**	
Positive						
1-3	42 (22.3)	12 (27.3)	5 (13.5)	12 (25.5)	**71 (22.5)**	
≥4	25 (13.3)	5 (11.4)	5 (13.5)	3 (6.4)	**38 (12.0)**	
Undetermined	43 (22.9)	10 (22.7)	14 (37.8)	12 (25.5)	**79 (25.0)**	

Data are presented as number and percentage (%)

Table 2 - The distribution of histopathological characteristics according to hormonal and molecular subtypes in 359 women with invasive breast cancer

Characteristics	Luminal A	Luminal B	HER-2 positive	Triple negative	Total	*P*-value
Total	**210 (58.5)**	**52 (14.5)**	**44 (12.3)**	**53 (14.8)**	**359 (100)**	
Histology						
Ductal	165 (78.6)	47 (90.4)	43 (97.7)	50 (94.3)	**305 (85.0)**	0.002
Lobular	35 (16.7)	4 (7.7)	1 (2.3)	0	**40 (11.1)**	
Others	10 (4.8)	1 (1.9)	0	3 (5.7)	**14 (3.9)**	
Tumor grade						
Grade I	46 (21.9)	3 (5.8)	0	0	**49 (13.6)**	0.0001
Grade II	116 (55.2)	27 (51.9)	16 (36.4)	12 (22.6)	**171 (47.6)**	
Grade III	48 (22.9)	22 (42.3)	28 (63.6)	41 (77.4)	**139 (38.7)**	
Carcinoma in situ						
Present	112 (53.3)	26 (50.0)	20 (45.5)	16 (30.2)	**174 (48.5)**	0.026
Absent	98 (46.7)	26 (50.0)	24 (54.5)	37 (69.8)	**185 (51.5)**	

Data are presented as number and percentage (%)

receptor 2-positive (n1) had a tumor mass size of >2 cm in 82.5%, and triple negative tumors (n2) in 75% of patients (*p*=0.018); in which most ranged between 2 cm and 5 cm (n1= 26, 65% and n2=31, 59.6%) and the remaining were >5 cm (n1= 7, 17.5% and n2=8, 15.4%) (*p*=0.057). 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 (30.2%, *p*=0.026). Lobular carcinomas occurred almost exclusively in the luminal A subtype (87.5%, *p*=0.002).

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 49.8 years which is equivalent to that reported by the Saudi Arabian Cancer Incidence Report,[3] and a previous study conducted in King Khalid University Hospital [2001-2010].[4] Most of our cases (57.9%) occurred in women ≤50 years, which is similar to the Omani study.[16] 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.[2] Notably, while only 33.15% of our patients presented with a tumor size ≤2 cm, in countries like

Table 3 - The distribution of molecular subtypes of breast carcinomas by 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		Saudi Arabia
Number of patients	452	804	628	1198	966	196	300	359
Years	2006-2010	2000-2003	2007-2010	2000-2002	2007-2009	1993-1996		2010-2014
Luminal A	34.7%	69.0%	46.5%	49.3%	50.7%	47.4%	54.0%	58.5%
Luminal B	15.9%	6.0%	17.0%	13.2%	13.4%	12.7%	17.3%	14.5%
HER2/NEU	24.1%	8.0%	15.0%	16.2%	13.4%	8.2%	5.6%	12.3%
Triple negative	25.3%	18.0%	21.5%	21.3%	22.5%	31.6%	23.0%	14.8%
HER2 - human epidermal growth factor receptor 2								

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.[13,22] 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 (**Table 3**). Unlike our findings, about half the cases (52.8%) in Tamimi et al's[18] study were triple negative, with luminal tumors comprising only 28.5%. 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 (**Table 3**).

Lobular carcinomas occurred almost exclusively in the luminal A group (87.5%, *p*=0.002) in our study, which was similar to the findings of Tamimi et al[18] and Yang et al.[22] However, only 55% of lobular carcinomas were luminal A in the Egyptian[23] and Norwegian studies.[220] Human epidermal growth factor receptor 2-positive and triple negative tumors were associated with a greater frequency of poorly differentiated carcinomas.[19,24] Compared to luminal A, these subtypes are associated with an increased frequency of a larger tumor size,[17,25] and a younger age group.[17,26] 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,[17,27] several studies identified a high frequency of lymph node metastasis

with HER2-positive tumors and a low frequency with basal-like tumors.[28,29] In contrast to half of luminal A tumors (53.3%), only 30.2% of triple negative tumors (*p*=0.026) displayed an in-situ component. Zaha et al[30] 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,[31] which concluded that HER2-positive tumors and triple negative tumors were less likely to be detected by mammography.

Study limitation. Our study was limited by the unavailability of Ki67, a cellular marker of proliferation that differentiates non-HER2 expressing luminal B from luminal A tumors.[10] We were also limited by the absence of cytokeratin 5/6, which helps in detecting Basal-like tumors, a subset of triple negative tumors.[29] In addition, molecular classification by immunohistochemistry, and by gene expression are not always identical with a discrepancy rate of 39%.[32]

In conclusion, breast cancer classification by immunohistochemistry revealed that in our community luminal A tumors were the most common subtype, followed by triple negative tumors. Breast cancer subtypes exhibited particular characteristics. Luminal A tumors were associated with an increased frequency of lobular 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. We found no correlation between lymph node status and molecular subtypes.

At diagnosis, most breast cancers in our study exceeded 2 cm in maximum dimension. The cause of this needs to be examined and addressed with some urgency.

We also recommend that the risk factors associated with the different molecular subtypes of breast carcinoma in Saudi Arabia are investigated. The impact of the various molecular subtypes of breast cancer on prognosis and survival should also be further investigated.

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