RESEARCH COMMUNICATION

Breast Cancer Molecular Subtypes and Associations with Clinicopathological Characteristics in Iranian Women, 2002-2011

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Abstract

Breast cancer is a heterogeneous disease that is affected by ethnicity of patients. According to hormone receptor status and gene expression profiling, breast cancers are classified into four molecular subtypes, each showing distinct clinical behavior. Lack of sufficient data on molecular subtypes of breast cancer in Iran, prompted us to investigate the prevalence and the clinicopathological features of each subtype among Iranian women. A total of 428 women diagnosed with breast cancer from 2002 to 2011 were included and categorized into four molecular subtypes using immunohistochemistry. Prevalence of each subtype and its association with patients' demographics and tumor characteristics, such as size, grade, lymph-node involvement and vascular invasion, were investigated using Chi-square, analysis of variance and multivariate logistic regression. Luminal A was the most common molecular subtype (63.8%) followed by Luminal B (8.4%), basal-like (15.9%) and HER-2 (11.9%). Basal-like and HER-2 subtypes were mostly of higher grades while luminal A tumors were more of grade 1 (P<0.001). Vascular invasion was more prevalent in HER-2 subtype, and HER-2 positive tumors were significantly associated with vascular invasion (P=0.013). Using muti-variate analysis, tumor size greater than 5 cm and vascular invasion were significant predictors of 3 or more nodal metastases. Breast cancer was most commonly diagnosed in women around 50 years of age and the majority of patients had lymph node metastasis at the time of diagnosis. This points to the necessity for devising an efficient screening program for breast cancer in Iran. Further, prospective surveys are suggested to evaluate prognosis of different subtypes in Iranian patients.

Keywords: Breast cancer - molecular subtypes - immunohistochemistry - clinicopathological characteristics - Iran

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Introduction

Breast cancer is the most common cancer in women worldwide (American cancer society, 2011) and in Iranian women (Iranian annual national cancer registration report, 2008). According to national cancer registry of Iran in 2007, the age-specific rate (ASR) for breast cancer incidence was 33.21 per 100,000. Despite the relatively low incidence of breast cancer in Iran, its cause-specific mortality is much higher in Iran compared to developed countries (Harirchi et al., 2011).

Breast cancer is a heterogeneous disease based on its clinical course, pathologic and therapeutic aspects, and prognosis (Huber et al., 2009; Zaha et al., 2010). Breast tumors with similar histology may express various clinicopathological features and differed in presentation and prognosis (Tamimi et al., 2010). Therefore, previous classifications focusing only on morphology could not fully capture the diversity of the disease (Zaha et al., 2010). To overcome this classification incompetency, other classification systems have been developed based on hormone receptor status and gene expression profiling of breast cancers (Pusztai et al., 2006). Recognition of each molecular subtype can improve the assessment of prognosis and can assist in advancement of molecular-targeted therapies, so that complications related to the current systemic therapies can be avoided (Ben Abdelkrim et al., 2010).

Nowadays, estrogen receptor (ER), progesterone receptor (PR) and HER-2 receptor status of breast tumors can be determined by using immunohistochemical (IHC) markers as surrogates for DNA-microarray in most pathology labs, and the breast cancer cases can be accordingly classified into at least four molecular subtypes including luminal A (ER and/or PR positive, HER-2 negative), luminal B (ER and/or PR positive, HER-2 positive), basal-like (ER negative, PR negative, HER-2 negative) and HER-2/neu (ER negative, PR negative, HER-2 positive) (Bhargava et al., 2009; Huo et al., 2009; Wiechmann et al., 2009; Zhao et al., 2009; Salhia et al.,

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2011). The HER-2/neu gene is a member of a gene family encoding growth factor receptors, including EGFR, HER-2, HER3 and HER4. Previous studies have shown that about 25 to 30% of invasive female breast cancers over express HER-2 (Slamon et al, 1987).

Based on geographical diversity in incidence and the mortality rates of breast cancer subtypes and because of correlations between clinical outcomes and race and ethnicity (Carey et al., 2006; Blackman & Masi, 2006), we carried out this study to find out the demographic distribution of breast cancer molecular subtypes among Iranian women. Further, we evaluated the association of each subtype with some of the clinicopathological features and prognostic parameters, including patient's age, histological type, tumor size, tumor grade, vascular and perineural invasions and lymph node involvement at the time of surgery.

Materials and Methods

Study Population

We included all female patients diagnosed with breast cancer at Atieh Hospital in Tehran, Iran, from 2002 to 2011. 428 patients with histologically confirmed primary breast cancer for whom ER, PR, HER-2 and Ki67 status were examined by IHC assays were included in the study. Patients with prior malignancy and those who had received neoadjuvant chemotherapy before surgery were excluded from the study.

Definitions and Laboratory Work

Histological grade was scored by the modified Scarf-Bloom-Richardson histological grading system. ER, PR and HER-2 status as well as Ki67 index were determined on the basis of IHC staining using monoclonal antibody (DAKO, Denmark). Hormone receptors (ER and PR) were considered positive if at least 10% of tumor cells nuclei were stained. Tumors were considered HER-2 positive if they were scored 3+ by IHC. Tumors with indeterminate IHC score (2+) were considered negative for HER-2 in the absence of fluorescent in situ hybridization (FISH) or CISH data. FISH and other types of in-situ hybridization are not routinely performed in our center and were not available for our patients. Nodal positivity was defined as the presence of any tumor cells in a lymph node and included micro and macrometastasis and isolated tumor cells.

Statistacal Analysis

Study data including patient's age at diagnosis, lymph node status, tumor size, histologic type, tumor grade and vascular and perineural invasions, in addition to ER, PR and HER-2 status and Ki67 index were extracted from pathology reports and analyzed using SPSS (version 16; SPSS, Chicago, IL, USA). The distribution of clinicopathological characteristics among the four subtypes were compared using Chi-square test for binary variables and analysis of variance (ANOVA) for continuous variables. Multivariate logistic regression analysis was used to determine whether subtype is an independent predictor of nodal involvement after being controlled for age, tumor size, and histological grade (luminal A was the reference group). All statistical tests were two sided, and a P value of less than 0.05 was considered significant.

Results

The mean age ±SD of the patients was 50±12 years at the time of surgery (range: 25-90). Pathology samples were obtained through mastectomy (56.9%), breast conserving surgery (23.7%), lumpectomy (14%) and other methods (5.3%).

The mean tumor size ±SD was 2.8±1.67 cm and 72.6% of the tumors were equal or less than 3 centimeters. There was not a significant correlation between tumor size and molecular subtype (P=0.052).

Mean number of nodes dissected at surgery was 14 nodes (range: 1-43). Among 343 patients with known lymph node status, 41.1% (n=141) had negative lymph nodes and for the rest of them median number of positive nodes was 3 (range: 1-43).

Regarding in-situ components of the tumors, the most frequent types observed were cribriform (in 35.5%) and solid (in 27.2%). Some tumors had more than one type of in-situ component and the type was missing in 51 patients. Morphological characteristics of tumors are presented in Table 1.The most common molecular subtype in our study was luminal A (63.8%). Other subtypes were Luminal B (8.4%), basal-like (15.9%) and HER-2 (11.9%). Table 2 summarizes clinicopathological features of tumors, additional demographic data and some of the significant differences. Tumors in the luminal A subtype were associated with more PR positivity than luminal B subtype (P=0.03). Luminal A subtype was associated with less Ki67 proliferation index compared to luminal B (P=0.004), basal-like and HER2 (P<0.001). Tumors in the basal-like subtype were associated with more Ki67 proliferation index compared to luminal A and luminal B subtypes (P<0.001). However, when Ki67 proliferation index ≥ 20% was considered positive, luminal B, basal-like and HER-2 tumors were all significantly (P<0.001)

Table 1. Morphological Characteristics in All Cases and by Molecular Subtype

		No.(%) within molecular Subtype				All cases
		LUMINAL		BASAL -CELL LIKE	HER-2	No.(%)
		A	B			
Nottingham's tubule formation	1	7 (9.1)	0	0	1 (7)	8 (7)
	2	25 (33)	4 (57)	2 (9)	2 (14)	36 (29)
	3	45 (58)	3 (43)	20 (91)	11 (79)	80 (65)
Nottingham's nuclear pleomorphism	1	14 (18)	0	0	0	14 (11)
	2	50 (66)	4 (57)	5 (23)	7 (50)	68 (55)
	3	12 (16)	3 (43)	17 (77)	7 (50)	41 (33)
Nottingham's mitotic figure	1	48 (64)	0	2 (9)	4 (29)	56 (46)
	2	21 (28)	6 (86)	10 (46)	8 (57)	47 (39)
	3	6 (8)	1 (14)	10 (46)	2 (14)	19 (16)
Tumor grade	1	75 (29)	0	4 (6)	0	81 (20)
	2	148 (57)	23 (70)	19 (30)	27 (54)	218 (53)
	3	38 (15)	10 (30)	40 (64)	23 (46)	113 (27)

Table 2. Tumor and Patient Characteristics by Subtype.

Characteristic		All cases N = 428	no. (% within molecular subtype) Luminal A n = 273	Luminal B n = 36	Basal-like n = 68	HER-2 n = 51	P value*
Age at surgery (Years):	≤35	52 (012)	27 (9.9)	8 (22.2)	11 (16.2)	5 (9.8)	0.02
	35-50	200 (046)	137 (50.2)	16 (44.4)	29 (42.6)	15 (29.4)	
	>50	183 (042)	109 (39.9)	12 (33.3)	28 (41.2)	31 (60.8)	
Tumor size (cm):	≤2	99 (28.5)	69 (31.4)	9 (32.1)	12 (21.4)	9 (20.9)	0.28
	2-5	219 (63.1)	137 (62.3)	17 (60.7)	38 (67.9)	27 (62.8)	
	>5	29 (08.4)	14 (6.4)	2 (7.1)	6 (10.7)	7 (16.3)	
Ve+ (perineural invasion)		70 (17.4)	53 (20.5)	6 (17.6)	5 (8.1)	6 (12.2)	0.09
Ve+ (vascular invasion)		207 (51.1)	128 (49.2)	18 (54.5)	27 (42.9)	34 (69.4)	0.03
Histologic type:	Ductal	361 (84.3)	218(79.9)	34 (94.4)	60 (88.2)	49 (96.1)	0.004
	Lobular	29 (06.8)	28 (10.3)	0	1 (1.5)	0	
	Others	38 (08.9)	27 (9.9)	2 (5.6)	7 (10.3)	2 (3.9)	
Number of positive lymph nodes:	0	141 (41.1)	88 (40)	10 (40)	27 (50.9)	16 (35.6)	0.36
	1-3	103 (30.0)	72 (32.7)	5 (20)	14 (26.4)	12 (26.7)	
	≥4	99 (28.9)	60 (27.3)	10 (40)	12 (22.6)	17 (37.8)	
Tumor grade:	Grade 1	79 (19.4)	75 (28.7)	0	4 (6.3)	0	<0.001
	Grade 2	217 (53.3)	148 (56.7)	23(69.7)	19(30.2)	27 (54.0)	
	Grade 3	111 (27.3)	38 (14.6)	10 (30.3)	40 (63.5)	23 (46.0)	
ER status:	Negative	121 (28.4)	2 (0.7)	0	68 (100)	51 (100)	<0.001
	Positive	307 (71.6)	271 (99.3)	36 (100)	0	0	
PR status:	Negative	169 (39.5)	38 (13.9)	12 (33.3)	68 (100)	51 (100)	<0.001
	Positive	259 (60.5)	235 (86.1)	24 (66.7)	0	0	
Ki67 proliferation index:	Mean index	26.6	20	30	45	37	<0.001
	Ki67 Negative	135 (39.7)	119 (54.1)	4 (13.3)	5 (10.0)	7 (17.5)	
	Ki67 Positive	205 (60.3)	101 (45.9)	26 (86.7)	45 (90)	33 (82.5)	<0.001

* 'Chi-square test was used for binary variables; for analyzing quantitative variables, analysis of variance was used when computational limits allowed, and Kruskal-Wallis was used if otherwise

Table 3. Multivariate Regression Analysis: Risk Factors for Having 3 Positive Lymph Nodes at Surgery

Variable		Adjusted OR (95% CI)	P value
Vascular invasion		41.68 (11.95-145.38)	<0.001
Tumor size(cm):	≤2	1	
	2 - 5	1.66(0.73-3.82)	0.23
	>5	22.51(5.05-100.40)	<0.001

OR: odds ratio; 95% CI: 95% confidence interval.

associated with positive Ki67 status and only luminal A was significantly Ki67 negative. Tumors in the luminal A group tend to be more of ductal and lobular rather than other histological types (P=0.004).

When evaluated for correlations with tumor grade, the following correlations were found (P<0.001): basal-like and HER-2 subtypes tend to be more frequently of grade 3 and less frequently of grade 1, luminal B subtype was associated with more tumor grade 2 and less grade 1, while luminal A tumors were mostly of grade 1 and less frequently of grade 3.Vascular invasion was more prevalent in HER2 subtype (P=0.032). This subtype was also associated with age groups of 36-50 and >50 years (P=0.021). Tumors of luminal A subtype were more prevalent in age group 36-50 years and luminal B was less frequently observed in patients ≤35 years (P=0.021).

The prevalence of HER-2 negative subtypes in our study was 79.7% (n=341) and 20.3% (n=87) of patients had HER-2 positive tumors. The HER-2 positive tumors were significantly associated with vascular invasion (P=0.013) and the HER-2 negatives were vascular invasion-free.

In multivariariate analysis, only tumor size greater than 5 cm and vascular invasion remained significant predictors of 3 or more nodal metastases (Table 3).

Discussion

Luminal A was the most prevalent subtype in our sample population, similar to most of the studies in western countries, China and Tunisia. The next prevalent subtype in our study was basal-like, followed by HER-2 and luminal B subtypes, respectively. These features are not distinct from breast cancer patients in western countries, where studies have shown that the prevalence of luminal A subtype varies from 47.9% (Munoz et al, 2009) to 75% (Nguyen et al., 2008), the luminal B subtype varies from 8 (Wiechmann et al., 2009) to 27.6% (Munoz et al., 2009), the Her-2 subtype ranges from 4 % (Nguyen et al., 2008) to 21.6% (Del Casar et al., 2009), and the basal-like subtype varies from 7.4 % (Spitale et al., 2009) to 21.2% (Ihemelandu et al., 2007). A series conducted in west Africa (Nigeria and Senegal) reported a much higher prevalence of triple-negative (ER−, PR−, Her-2−) tumors (55%) and a lower prevalence of luminal A and luminal B phenotypes (27 and 2% respectively) (Huo et al., 2009).

Like Saudi Arabian women (Tamimi et al., 2010), the majority of our patients were younger than 50 years old while in developed western countries, the median age of developing breast cancer is 60-65 years (Tamimi et al., 2010). In agreement with previous reports from Iran (Mousavi et al., 2007), more than half of our patients had lymph node metastasis at the time of diagnosis and their tumors were of grade 2 or more, whereas in more

developed countries, locally advanced disease is much less common (Zaha et al., 2010). This difference probably results from lack of effective screening programs and delayed diagnosis in Iran, which gives the tumor enough time to spread to adjacent tissues and lymph nodes before diagnosis.

Although there is much known about the strong role of ER in tumor responsiveness to endocrine therapy, the prognostic significance of PR presence in ER-positive tumors is still controversial (Ma et al., 2009). We found that the luminal A subtype was associated with more PR positivity than luminal B subtype (P=0.03). However, prospective controlled studies are highly recommended to determine the role of PR in prognosis and treatment outcome.

Ki67 is a nuclear marker of cell proliferation that its expression correlates proportionally to poorer clinical outcomes (Domagala et al., 1996; Trihia et al., 2003; Azambuja et al., 2007). In our study, luminal A subtype was associated with the least Ki67 proliferation index which is in agreement with previously published reports (Bhargava et al., 2009; Tamimi et al., 2010). The correlation of basal-like subtype and greater Ki67 proliferation indices has also been demonstrated by previous reports (Carey et al., 2006; Bhargava et al., 2009; Tamimi et al., 2010) and can be related to the poor clinical outcome of this tumor subtype.

Although we found that luminal A and HER2 tumors tend to be significantly of ductal and lobular histological types, in a similar study of Saudi Arabian women, there was no association between molecular classes and the histologic type of the cancer (Tamimi et al., 2010).

Like most of the similar studies (Sørlie et al., 2001; Casar et al., 2008; Abdelkrim et al., 2010) we found that basal-like and HER2 subtypes are more frequently of grade 3 and less frequently of grade 1. However, in the study of Miron et al., tumor differentiation grading did not correlate with the molecular subtype (Miron et al., 2008).

Although other studies have shown that tumors of HER-2 and basal-like subtypes were more likely to be larger in size than luminal tumors (Abdelkrim et al., 2010) and the triple-negative phenotype significantly correlates with tumor size (Tian et al., 2008), we were not able to find a significant correlation between tumor size and its molecular subtype. However, there was a near-significant correlation (P=0.052) between luminal A subtype and tumor size of 3 cm or less.

The prevalence of HER-2 positive subtypes in our study was almost similar to that observed in American women (22%) (Carey et al., 2006) and close to that observed in North Korean, indigenous African and Saudi Arabian women (around 17%) (Kim et al., 2006; Huo et al., 2009; Tamimi et al., 2010). The significant correlations we found between HER-2 positive tumors and vascular invasion is in agreement with previous studies that have shown HER2 over-expression is prognostically unfavorable (Slamon et al., 1987; Sørlie et al., 2001). Nevertheless, HER2 over-expression is associated with better responsiveness to specific types of chemotherapy (Keshgegian & Cnaan., 1995).

Unlike some of the previous reports on the association of basal-like and HER -2 subtypes with more than three lymph node metastases (Wiechmann et al., 2009; Abdelkrim et al., 2010), we could not find any significant correlations between molecular subtype of the tumor and lymph node metastasis neither in univariate nor in multivariate analysis. However, there are studies that state the subtype of the tumor is not associated or only weakly associated with its size and lymph node metastasis, suggesting that subtype is "intrinsic" and predetermined (Bhargava et al., 2009).

The risk factors we found in regression analysis for having 3 or more positive lymph nodes (tumor size and vascular invasion) can be explained by the malignant behavior of these tumors. In addition, progressive tumor growth and vascular dissemination is associated with more lymphatic dissemination. However, axillary lymph node involvement remains the most important prognostic factor in early-stage of breast cancer and there are reports on the association of basal subtype with a lower incidence of axillary nodal involvement compared to other subtypes (Crabb et al., 2008; Wiechmann et al., 2009). On the other hand, there has been reports on the correlation of the HER-2 subtype and a higher likelihood of four or more positive lymph nodes (Wiechmann et al., 2009). This information may be useful in planning the management options such as sentinel node biopsy, and locoregional radiation. (Wiechmann et al., 2009).

There are some potential limitations to this study. We have classified the tumors according to their ER, PR, and HER-2 status based on immunohistochemical surrogates, which is only an approximation of the underlying genotype-based breast cancer subtype. However, IHC profiles that use readily available clinical receptors are cost-effective and practical, and have been successfully used as surrogates for gene expression profiling by several studies (Brenton et al., 2005; Carey et al., 2006; Nguyen et al., 2008; Wiechmann et al., 2009). Compared to IHC, Genotyping is not always available, and is time-consuming and expensive (Ben Abdelkrim et al., 2010).The definition of luminal B in this study (ER and/or PR positive, Her-2 positive) does not identify all luminal B tumors, because only 30% to 50% of them are HER-2 positive, leading to misclassification of a subset of luminal B tumors into the luminal A subtype (Carey et al., 2006; Wiechmann et al., 2009). Further, some tumors might have been misclassified in our study because cases with the IHC score of 2+ for HER-2 were considered HER-2 negative in the absence of FISH data. Finally, triple-negative breast cancer (ER-negative, PR-negative, and Her-2-negative) is not quite the same as the basal-like subtype. Recent gene expression studies (Carey et al., 2006) have updated the immunohistochemical definition of basal-like subtype as "ER negative, PR negative, HER-2 negative, cytokeratin 5/6 positive, and/or HER-1 positive" and added another molecular subtype as unclassified (negative for all five markers), which is proven to be histologically less aggressive than basal-like tumors and more aggressive than luminal A tumors (Carey et al., 2006; Yang et al., 2007; Huo et al., 2009). However, the majority of triple-negative breast cancers carry the "basal-like" molecular profile on gene expression arrays (Anders & Carey, 2008)

and that is why we considered the triple negative subtype as basal-like.

In conclusion, luminal A was the most prevalent molecular subtype of breast cancer among our patients. Breast cancer was most commonly diagnosed in women around 50 years of age. The majority of patients had lymph node metastasis at the time of diagnosis. Therefore, it seems that a well-defined screening program is mandatory in Iran so that the tumors would be diagnosed in their early stages. Finally, our survey is a preliminary study of breast cancer molecular subtypes among Iranian women. Thus, further studies with larger sample sizes are recommended to accurately assess the situation in Iran. Authors also suggest prospective surveys to evaluate prognosis of different subtypes in Iranian patients which can assist in developing better therapies directed toward particular molecular subtypes.

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