RESEARCH ARTICLE

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Relationships between Reproductive Risk Factors for Breast Cancer and Tumor Molecular Subtypes

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Abstract

Background: Due to wide clinical differences in the various pathological types of breast cancer and also close associations between disease prognosis and molecular subtypes, relationships of the latter with traditional risk factors have been suggested. Hence, the present study aimed to assess any associations. **Methods:** This bi-center cross-sectional study was performed on 800 consecutive women with known breast cancer referred to two Comprehensive Cancer Centers in Tehran between 2006 and 2016. Baseline information related to reproductive risk profiles as well as pathological tumor diagnosis and molecular subtypes determined using immunohistochemical analysis by immune-staining for ER, PR, and HER2 molecules were collected by reviewing hospital records. **Results:** Of 800 samples included for immunohistochemical analysis, 314 (39.3%) were diagnosed as of Luminal A subtype, 107 (13.4%) as Luminal B subtype, 153 (19.1%) as HER-2 over-expressing, and 226 (28.3%) as triple negative. Among all reproductive risk factors initially assessed, young age was associated with HER-2 over-expression, greater tumor size and a history of abortion with the luminal B subtype, lower age at pregnancy with the luminal A subtype, and lower gravidity and a shorter duration of breastfeeding with the triple negative subtype. **Conclusion:** Each molecular subtype of breast cancer in our population may be associated with specific reproductive risk factors.

Keywords: Reproductive- breast cancer- tumor marker- HER2- tumor molecular subtypes

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Introduction

Recent comprehensive researches on pathological and molecular patterns of breast cancer using advanced microarray technology and genetic detection tools have resulted in identifying new classification for this cancer (Colombo et al., 2011; Vuong et al., 2014). In this stratification, the cancer subtypes vary according to their gene expression signatures leading different clinical outcome (Cardoso, 2003). The main definitive subgroups defined for breast cancer includes luminal subtypes A and B, the HER2-subtype, and basal-like subtype (Roulot et al., 2016). Based on positive or negative reception for hormones including estrogen or progesterone, these subtypes have classified as luminal A (positive for estrogen and progesterone receptors, but negative for HER-2/neu), luminal B (positive for estrogen and progesterone receptors, but manifested by the pattern of HER-2/neu+), non-luminal HER-2/neu+ (negative for both estrogen and progesterone receptors but with the pattern of HER-2/neu+) and triple negative pattern which is negative for all pointed receptors (Lim et al., 2013; Skibinski and Kuperwasser, 2015). The interesting discover on these molecular pattern is the close relationship between these subtypes and both clinical prognosis and response to treatment approaches. Furthermore, this variation can help us to find out the exact etiology of breast cancer and its invasion. In fact, the heterogeneity of breast tumors molecular subtype may reflect its-related etiological pathways (Moriya et al., 2016). For instance, because both luminal subtypes A and B are positive for both estrogen and progesterone hormones, the main etiologies of these subtypes may be related to exposing these two hormones (Masood, 2016). More interestingly, due to close association between breast cancer subtypes and disease prognosis, the classic risk factors for this cancer leading poor disease prognosis may be linked to the molecular subtype of cancer (Akinyemiju et al., 2015).In fact, by assessing risk factors in relation to more homogenous subtypes we can identify novel risk profiles with combining both classic and molecular risk factors with the aim of better predicting disease prognosis and survival. The present study aimed to assess the association of

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reproductive risk factors for breast cancer (including age, age at first pregnancy, history of abortion, duration of breastfeeding, and history of contraceptive use) and also tumor-related diameters with tumor molecular subtypes.

Materials and Methods

This bi-center cross-sectional study was performed on women with known breast cancer who referred to two great referral hospitals as the Comprehensive Cancer Centers in Tehran between 2006 and 2016. In total, 800 cases with breast cancer were included into the study. All women reporting incident diagnoses of cancer were asked for permission to review their medical records to confirm the diagnosis and to classify cancers as in situ or invasive, by histologic type, size, and presence or absence of metastases. By reviewing the recorded files, the baseline information including classic risk factors for breast cancer age, size of tumor, age at first pregnancy, frequency of pregnancy, history of abortion, duration of breastfeeding, history of using oral contraceptives, and number of lymph nodes was collected. The information on molecular breast cancer subtypes were also extracted from the hospital recorded files that previously identified using immunohistochemical analysis by immune-staining for ER, PR, and HER2 molecules. In this regard, the subtypes were defined as luminal A (ER+ or PR+ and HER-2/neu−), luminal B (ER+ or PR+, HER-2/neu+), non-luminal HER-2/neu+ or HER-2 over expression (ER−, PR−, HER-2/neu+), and triple negative (ER−, PR−, and HER-2/ neu−). The study endpoint was to examine the relation between the clinical and tumor-related risk factors and various molecular subtypes of breast cancer.

For statistical analysis, results were presented as mean ± standard deviation (SD) for quantitative variables and were summarized by frequency (percentage) for categorical variables. Continuous variables were compared using ANOVA test or Kruskal-Wallis H test whenever the data did not appear to have normal distribution or when the assumption of equal variances was violated across the study groups. Categorical variables were, on the other hand, compared using chi-square test. P values of ≤ 0.05 were considered statistically significant. For the statistical analysis, the statistical software SPSS version 23.0 for windows (IBM, Armonk, New York) was used.

Results

The mean age of patients was 51.12 ± 11.72 years. Of 800 samples included for immunohistochemical analysis, 314 (39.3%) were positive for Luminal A subtype, 107 (13.4%) for Luminal B subtype, 153 (19.1%) for HER-2 over expression, and 226 (28.3%) for triple negative subtype. Regarding pathological types, the most common feature was IDC in 76.0% followed by ILC in 13.3%, in situ in 4.8%, mixed in 4.0% and other types in 2.0%. Overall, 14.2% had history of oral contraceptive use. As shown in (Table 1), those patients with HER-2 over expression pattern was significantly younger than other subgroups ($p = 0.006$). The mean size of tumor was more in those with luminal B subtypes ($p < 0.001$). Also, the mean age at first pregnancy was the lowest in luminal A subtype and the highest in triple negative subtype ($p< 0.001$). Also, the lowest and the highest number of gravity was revealed in triple negative and luminal A subtypes respectively with a significant difference across the subtypes ($p = 0.001$). The history of abortion was significantly found more in luminal B subtypes as compared to other molecular

Table 1.The Association between Class Risk Factors for Breast Cancer and Molecular Subtypes of the Tumor

Item	Luminal A	Luminal B	HER-2 over expression	Total physical activity
Age, year	52.32 ± 10.86	52.33 ± 12.23	48.85 ± 11.46	13.0
Size of tumor	2.55 ± 1.39	3.34 ± 4.10	2.46 ± 1.18	12.2
Number of positive node	2.55 ± 4.71	2.29 ± 4.08	2.28 ± 4.52	11.2
Age at first pregnancy, year	20.24 ± 6.18	21.19 ± 6.59	21.58 ± 5.28	14.5
Number of gravid	3.21 ± 1.96	3.12 ± 1.79	2.85 ± 1.43	14.7
Number of previous abortion	0.35 ± 0.83	0.92 ± 1.14	0.58 ± 0.82	11.8
Duration of breastfeeding				
Months	17.88 ± 8.19	16.11 ± 6.99	17.84 ± 7.08	13.1

Table 2. Association between Pathological Diagnosis and Molecular Subtypes of the Tumor

Item	Luminal A	Luminal B	HER-2 over expression	Total physical activity
IDC	322 (72.5)	91 (77.1)	151 (74.4)	0.001
ILC	87 (19.6)	17 (14.4)	26 (12.8)	12.9
In Situ	13 (2.9)	4 (3.4)	14 (6.9)	12.5
Mixed	15 (3.4)	4 (3.4)	11 (5.4)	10.5
Others	7 (1.6)	2 (1.7)	1 (0.5)	16.2
IDC	322 (72.5)	91 (77.1)	151 (74.4)	15
ILC	87 (19.6)	17 (14.4)	26 (12.8)	10
In Situ	13 (2.9)	4 (3.4)	14 (6.9)	12.7

Table 3. The Association between Class Risk Factors for Breast Cancer and Pathological Diagnosis of the Tumor

Item	IDC	ILC	In Situ	0.001 Total physical activity
Age, year	50.71 ± 11.34	52.90 ± 12.25	52.95 ± 11.35	12.9
Size of tumor	2.63 ± 1.51	2.72 ± 1.21	2.69 ± 1.20	12.5
Number of positive node	2.46 ± 4.42	2.75 ± 4.05	2.03 ± 4.06	10.5
Age at first pregnancy, year	21.70 ± 5.56	19.92 ± 5.91	19.58 ± 5.73	16.2
Number of gravid	2.78 ± 1.66	3.33 ± 2.29	2.89 ± 1.84	15.0
Number of previous abortion	0.66 ± 0.88	0.56 ± 0.84	1.00 ± 1.52	10.0
Duration of breastfeeding, Months	16.18 ± 7.25	17.90 ± 7.03	15.87 ± 8.28	12.7

Table 4. The Association between Class Risk Factors for Breast Cancer and Pathological Diagnosis of the Tumor

Type	OCP use (+)	OCP use (-)	Type	Total physical activity
IDC	540 (76.8%)	67 (69.1%)	IDC	-
ILC	89 (12.7%)	17 (17.5%)	ILC	12.9
In Situ	36 (5.1%)	6 (6.2%)	In Situ	12.5
Mixed	23 (3.3%)	7 (7.2%)	Mixed	10.5
Others	15 (2.1%)	0 (0.0%)	Others	16.2
Type	OCP use (+)	OCP use (-)	Type	15
IDC	540 (76.8%)	67 (69.1%)	IDC	10
ILC	89 (12.7%)	17 (17.5%)	ILC	12.7
In Situ	36 (5.1%)	6 (6.2%)	In Situ	0.001

subtypes ($p < 0.001$). Moreover, the duration of breastfeeding was significantly shorter in those who were triple negative subtypes. However, the number of positive lymph nodes was not associated with the molecular subtypes of the tumor. Considering various pathological types (Table 2), the prominent molecular subtypes was triple negative in IDC diagnosis, in ILC was luminal A subtypes, and in mixed and in situ diagnoses was HER-2 over expression indicating a significant difference in the molecular subtypes of tumor and pathological diagnosis ($p < 0.001$). No association was revealed between the use of oral contraceptive and the molecular subtype of breast cancer (15.7% in luminal A, 18.0% in luminal B, 16.3% in HER-2 over expression and 12.5% in triple negative subtypes, $p = 0.665$). Among all classic risk factors, only the age at first pregnancy was adversely associated with the presence of in situ diagnosis (Table 3). No association was found between the pathological type of tumor and history of oral contraceptive use (Table 4).

Discussion

In the present study of over 800 breast cancer subjects, some significant differences were revealed between some traditional risk factors for cancer and some specific molecular subtypes of the tumor. Among all reproductive risk factors initially assessed, young age was associated with HER-2 over expression pattern, more tumor size with luminal B subtype, lower age at first pregnancy with luminal A subtype, lower number of parity with triple negative pattern, the history of abortion with luminal B subtype, and shorter duration of breastfeeding with triple negative subtypes. In fact, each molecular subtype was specifically associated with specific reproductive risk factor. In other words, some hormonal risk factors were associated with hormone receptor negative subtypes. For instance, HER-2 over expression was more found in lower ages; and triple negative pattern was identified in those women with lower parity as well as in those with shorter breastfeeding. A number of previous studies have examined the association between breast cancer risk factors and tumor molecular subtypes. As shown by Turkoz et al., (2013) on Turkish women, the age of ≥40 years was found to be a risk factor for both luminal A and HER-2 overexpressing subtypes. Women who were nulliparous or who had their first full-term pregnancy at age 30 years or older were at increased risk of luminal breast cancer. Also, breastfeeding was a protective factor for luminal subtype. However they found no significant differences between the risk of breast cancer subtypes and early menarche, late menopause, family history, postmenopausal obesity, oral contraceptive use, smoking, in vitro fertilization, and blood groups. In another studyl in USA (Gaudet et al., 2011), triple negative tumors were associated with breastfeeding duration, increased body size was more strongly associated with both luminal B and triple negative tumors, a history of benign breast disease was associated with increased risk of luminal A tumors and also a family history of breast cancer was a risk factor for luminal A tumors. In a study by Islam et al., (2012) on Japanese women with breast cancer, a significant association was observed between early age at menarche and risk of luminal disease, however, no significant differences in association with parity, age at first live birth, breastfeeding history, age at menopause,

or synthetic hormonal use were seen across molecular subtypes of breast cancer. The Polish Breast Cancer Study also found that most established breast cancer risk factors such as age at menarche was associated with luminal A tumors (Yang et al., 2007). They also showed that having a family history of breast cancer was a risk factor for almost all subtypes although the magnitude of the effect was greatest for HER2-type breast cancers. Also, in a large study on 1,424 breast cancer cases in the USA, it was revealed that increasing parity was associated with reduced risk of luminal A tumors. Summing the studies in different geographical regions leads to the fact that various reproductive risk factors may be associated with specific molecular subtypes of the tumors and more interestingly these associations are considerably different among different patients' populations, emphasizing strong effect of gene variants on these associations. In fact, with respect to the etiological pathways for each pathological types of breast cancer, each correlative reproductive risk profile may act as a major ring of etiological chain for each pathological type of tumor in special population and thus this heterogeneity across different populations should be deeply examined in each population.

One of the main goals of the present study was to find out whether those molecular subtypes of breast cancer with poorer outcome and survival (such as triple negative subtype) are accompanied with poorer manifestations, and better molecular subtype was associated with better reproductive factors. In regards to that, we could show that the subtype of triple negative is associated with shorter duration of breastfeeding, higher age at first pregnancy and lower parity, also the luminal A subtype with best prognosis between other subtypes was related with good reproductive factors like younger age at first pregnancy and more parity but we could not find a relation between duration of feeding and this subtype.

It has been well demonstrated that the risk for developing triple negative subtype of tumor varies widely with pattern of breastfeeding and parity. Concerning that, it has been shown that the subtype of triple negative is manifested more in younger ages, and in those with shorter duration of breastfeeding (Millikan et al., 2008), however some other studies could not demonstrate these associations (Yang et al., 2007, Phipps et al., 2008).

In conclusion, in total, breastfeeding and parity have been well identified as potential protective factors against breast cancer and thus the lack of these factors can lead to poorer tumor-related outcome and shorter patients' survival (Morris, 2009). More parity and younger age in first pregnancy were related with luminal A subtype. In other words, shorter breastfeeding and primiparity are negative risk factors leading to an increased likelihood of breast cancer especially molecular subtypes with poorer outcome such as triple negative type in our study.

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