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Original article

Association between common risk factors and molecular subtypes in breast cancer patients

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ABSTRACT

Background: Breast cancer is the most commonly diagnosed cancer in women worldwide and characterized its by molecular and clinical heterogeneity. Gene expression profiling studies have classified breast cancers into five subtypes: luminal A, luminal B, HER-2 overexpressing, basal-like, and normal breast-like. Although clinical differences between subtypes have been well described in the literature, etiologic heterogeneity have not been fully studied. The aim of this study was to assess the associations between several hormonal and nonhormonal risk factors and molecular subtypes of breast cancer.

Methods: This cross-sectional study consisted of 1884 invasive breast cancer cases. Variables studied included family history, age at first full-term pregnancy, number of children, duration of lactation, menstruation history, menopausal status, blood type, smoking, obesity, oral contraceptive use, hormone replacement therapy and in vitro fertilization. The odds ratios (OR) and 95% confidence intervals (CI) were estimated using multivariate logistic regression analysis.

Results: Thousand two-hundred and forty nine patients had luminal A, 234 had luminal B, 169 had HER-2 overexpressing and 232 had triple negative breast cancer. The age of ≥40 years was found to be a risk factor for luminal A (OR 1.41 95% CI 1.15–1.74; $p = 0.001$) and HER-2 overexpressing subtype (OR: 1.51, 95% CI: 1.01–2.25; $p = 0.04$). Women who were nulliparous (OR 1.48, 95% CI 1.03–2.13; $p = 0.03$) or who had their first full-term pregnancy at age 30 years or older (OR 1.25 95% CI 0.83–1.88; $p = 0.04$) were at increased risk of luminal breast cancer, whereas women with more than two children had a decreased risk (OR 0.68, 95% CI 0.47–0.97; $p = 0.03$). Breast-feeding was also a protective factor for luminal subtype (OR 0.74, 95% CI 0.53–1.04; $p = 0.04$) when compared to non-luminal breast cancer. We found increased risks for postmenopausal women with HER-2 overexpressing (OR 2.20, 95% CI 0.93–5.17; $p = 0.04$) and luminal A (OR 1.87, 95% CI 0.93–3.90, $p = 0.02$) breast cancers, who used hormone replacement therapy for 5 years or more. Overweight and obesity significantly increased the risk of triple negative subtype (OR 1.89 95% CI 1.06–3.37; $p = 0.04$ and OR 1.90 95% CI 1.00–3.61; $p = 0.03$), on the contrary, decreased the risk of luminal breast cancer (OR 0.63 95% CI 0.43–0.95; $p = 0.02$ and OR 0.50 95% CI 0.32–0.76; $p = 0.002$, respectively) in premenopausal women. There were no significant differences between risk of breast cancer subtypes and early menarche, late menopause, family history, postmenopausal obesity, oral contraseptive use, smoking, in vitro fertilization, blood groups and use of hands.

Conclusions: Reproductive and hormonal characteristics (breastfeeding, parity, age at first full-term birth, hormone replacement therapy) were associated with luminal subtype, compared to non-luminal breast cancer, as consistent with previous studies. Obesity and overweight increased the risk of triple negative subtype, particularly in premenopausal women. Older age and use of hormone replacement therapy were related to the risk of HER-2 overexpressing breast cancer. Our data suggest a significant heterogeneity in association of traditional breast cancer risk factors and tumor subtypes.

Introduction

Breast cancer is the most commonly diagnosed cancer in women worldwide, with more than one million new cases diagnosed per year, and the second leading cause of cancer mortality

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among women. According to the GLOBOCAN 2008, the crude as well as age-standardized incidence and mortality rates of breast cancer in Turkey per 100,000 were 10.6; 28.3 and 6.4; 12.4 (10,065 new cases and 4311 deaths), respectively.[1]

Age, family history, early menarche, late menopause, nulliparity, late age at first full-term pregnancy and use of hormone replacement therapy (HRT) are well-established risk factors for the development of breast cancer.[2] It has been suggested that risk factors which are associated with estrogen receptor (ER) and progesterone receptor (PR) positive breast tumors involve mechanisms related to endogenous hormone exposure, whereas the etiology of ER and PR negative breast cancers may be non-hormonal.[3–5]

Breast cancer is characterized by its molecular and clinical heterogeneity. Studies profiling gene expression have classified breast cancers into five distinct subtypes: luminal A (ER positive and/or PR positive, human epidermal growth factor receptor 2 (HER-2) negative), luminal B (ER positive and/or PR positive, HER-2 positive), HER-2 overexpressing (ER negative, PR negative, HER-2 positive), basal-like (ER negative, PR negative, HER-2 negative, cytokeratin 5/6 positive and/or epidermal growth factor receptor positive) and normal breast-like tumors (unclassified).[6,7] Basal-like and normal breast-like tumors both have a triple negative phenotype (ER negative, PR negative, HER-2 negative), although approximately 70% of triple-negative tumors are basal-like.[8]

Clinical differences between these breast cancer subtypes have been well described in the literature. Five-year breast cancer specific survival rates are 65–94% for luminal A, 83–92% for luminal B, 39–71% for HER-2 overexpressing tumors, 51–93% for basal-like tumors, and 44–91% for normal breast-like tumors.[6] However, data regarding differences in the associations between well-established breast cancer risk factors and molecular subtypes are limited.

Few studies have explored the associations between common breast cancer risk factors and the molecular subtypes of breast cancer.[5,9–11] Most epidemiological studies found some differences in risk factor profiles according to ERPR status, although specific findings have not been consistent across studies.[4,12,13] Thus, the majority of these studies had small sample size with less than optimal receptor data availability (usually <60% of cases) or limited numbers of cases with triple negative or HER-2 overexpressing breast cancers. A better understanding of the etiology of triple negative tumors, which account for 10–20% of breast cancers, is particularly important because they include most clinically aggressive tumors.[9,13]

The main aim of this study was to assess the associations between several hormonal and nonhormonal risk factors and molecular subtypes of breast cancer defined by ER, PR and HER-2 status.

Materials and methods

This retrospective cross-sectional study consisted of 2005 women diagnosed with breast cancer between 1983 and 2011 who have been followed up in Department of Medical Oncology at Hacettepe University, Institute of Oncology.

Cases with missing data of ER ($n = 45$), PR ($n = 45$) and HER-2 ($n = 78$) were excluded as we were unable to classify these cases as luminal, HER-2 overexpressing, or triple negative. Women diagnosed with breast carsinoma in situ that were neither ductal ($n = 43$) nor lobular ($n = 10$) were also excluded. After exclusion of cases with missing data of ER, PR, HER-2 status and cases of carcinoma-in-situ, 1884 cases of invasive breast cancer were eligible for analysis.

Medical doctors conducted a face-to-face interview with each patient at the time of the diagnosis. Patients were asked detailed information on family history of breast and/or ovarian cancer, age at first full-term pregnancy, number of biological children, duration of lactation, menstruation history and menopausal status. Additional informations were collected on age at breast cancer diagnosis, blood type, smoking and use of hands. Use of oral contraceptives, HRT and in vitro fertilization were also inquired.

Before statistical analysis, risk factors were classified as follows: age at diagnosis (<40, ≥40 years), family history (no, yes), age at first full-term pregnancy (nulliparous, <30, ≥30 years), number of children (0, 1, ≥2 children), breastfeeding (no, yes) and age at menarche (<12, ≥12 years), menopausal status (pre-, peri, postmenopause), age at menopause (<55, ≥55 years). Early menarche was defined as first menstruation occurring before the age of 12 years and late menopause was defined as menopause at age of 55 years or more.[2] Data on HRT and oral contraseptive use classified as (no, <2 years, ≥2 to 5 years and ≥5 years), in vitro fertilization (no, yes) and smoking (no, yes). Body weight and height recorded accurately at the time of admission. Body mass index (BMI) was calculated as BMI = weight (kg)/height^2 (m^2), and classified into three categories: (i) normal body weight (BMI: 18.5–24.9 kg/m^2); (ii) overweight (BMI: 25–29.9 kg/m^2) and (iii) obese (BMI ≥30.0 kg/m^2), using criteria of World Health Organization.[14]

Data on ER and PR status and HER-2 expression were obtained from medical record review. ER and PR status were assessed by immunohistochemistry (IHC). The nuclear staining in more than 5% of tumor cells were considered as positive. Expression of HER-2 was also determined immunohistochemically. HER-2 positivity (a score of 3+) was defined as strong complete membrane staining in more than 10% of tumor cells; scores of 0 and 1 were considered negative, and fluorescence in situ hybridization was done for all 2+ tumors. Finally, tumor subtypes were classified as luminal A (ER positive and/or PR positive/HER-2 negative), luminal B (ER positive and/or PR positive/HER-2 positive), HER-2 overexpressing (ER negative/PR negative/HER-2 positive) and triple negative (ER negative/PR negative/HER-2 negative).[6]

Statistical analysis

Differences between subtypes with regard to demographic reproductive and characteristics and common breast cancer risk factors were examined using one-way ANOVA for continuous variables and Pearson chi-square tests for the categorical variables.

We analyzed three major groups (luminal, HER-2 overexpressing and triple negative breast cancer). Multivariate logistic regression analysis was used to estimate odds ratios (OR) and 95% confidence intervals (95% CI) for the association between risk factors and each breast cancer subtype. One-sample Kolmogorov–Smirnov test was performed to check the normal distribution of variables. All variables were normally distributed ($p > 0.05$). We selected both HER-2 overexpressing and luminal subtypes as the referent while evaluating triple negative breast cancer (comparing triple negative and non-triple-negative patients), triple negative and luminal subtypes as the referent while evaluating HER-2 overexpressing breast cancer, and triple negative and HER-2 overexpressing subtypes as the referent while evaluating luminal breast cancer (comparing luminal and non-luminal cases). We also evaluated luminal tumours separately by luminal A and luminal B, using logistic regression analysis. When a significant risk factor was determined for luminal breast cancer, we re-analyzed for four groups (luminal A, luminal B, HER-2 overexpressing and triple negative breast cancer), comparing luminal A to non-luminal A and luminal B to non-luminal B. Molecular subtypes were considered as outcome variables and risk factors were considered as explanatory variables. The approach was comparable to performing a series of simple binary logistic regression models. We evaluated

confounding between each of the risk factors. Final multivariate analyses for hormone therapy use and BMI were adjusted for the type of menopause. *P* values to test for heterogeneity of effects between tumor subtypes were also obtained using logistic regression analyses.

All data was entered and analysed using Statistical Package for Social Sciences version 15.0 (SPSS, Inc., Chicago, IL, USA). Appropriate statistical analysis was carried out with a two-sided level of 0.05 and 95% CI.

Results

A total of 66.3% ($n = 1249$) of the patients had luminal A, 12.4% ($n = 234$) had luminal B, 9.0% ($n = 169$) had HER2 overexpressing and 12.3% ($n = 232$) had triple negative breast cancer. The odds ratios and 95% CIs of demographic and reproductive risk factors by tumour subtypes were given in Table 1.

Age. The mean age of the patients with luminal A subtype was 49.1 ± 11.7 years, luminal B was 46.2 ± 11.8 years, triple negative was 47.7 ± 12.1 years and HER-2 overexpressing breast cancer was 49.7 ± 10.9 years ($p = 0.002$). To investigate a possible association between young age at diagnosis and breast cancer subtype, we divided patients into two groups (<40 years and ≥40 years). The age of ≥40 years was found to be a risk factor for luminal A (OR 1.41 95% CI 1.15–1.74; $p = 0.001$) and HER-2 overexpressing subtype (OR: 1.51, 95% CI: 1.01–2.25; $p = 0.04$).

Family history. Four hundred and forty two (23.5%) patients had breast and/or ovarian cancer history in the family. Association with family history of breast cancer did not differ significantly across molecular subtypes, although the highest proportion of patients with family history was observed in triple negative group (27.3%; $p = 0.11$).

Parity, age at first full-term pregnancy and breast-feeding. 13.2% of the patients were nulliparous. When luminal subtype compared to non-luminal breast cancer, nulliparous women had an increased risk of luminal breast cancer (OR 1.48, 95% CI 1.03-2.13; $p = 0.03$), whereas patients with more than two children had a decreased risk (OR 0.68, 95% CI 0.47–0.97; $p = 0.03$). Women who had their first full-term pregnancy at age 30 years or older were also at increased risk of luminal breast cancer (OR 1.25 95% CI 0.83–1.88; $p = 0.04$). Overall, 85.1% of the women had breastfed their babies, with a mean duration of 12.9 ± 9.9 months. Breast-feeding was also noted to be a protective factor for luminal breast cancer (OR 0.74, 95% CI 0.53–1.04; $p = 0.04$). When we analyzed luminal tumours separately by luminal A and luminal B subtype; we observed similar and significant findings in reproductive factors for both luminal A and B breast cancer ($p < 0.05$).

Early menarche and late menopause. The mean menarche age was 13.32 ± 1.37 years (range, 9–21 years). A total of 27% of the patients had menarche before age 12 years. There was no difference in risk of developing breast cancer among subtypes ($p = 0.86$). Overall, 45.1% of the patients ($n = 845$) were postmenopausal and 23% ($n = 193$) of these were 55 years and over. There was no significant difference between risk of breast cancer subtypes and late menopause ($p = 0.39$).

Oral contraceptive use. Four hundred and sixteen (22.6%) patients had a history of oral contraceptive use with a mean duration of use of 26.3 months. 254 women had used for <2 years, 96 women had used for 2 to 5 years and 66 patients had used for 5 years or more. We observed no significant difference in oral contraseptive use among breast cancer subtypes.

Hormone replacement therapy (HRT). A total of 15.7% of the patients ($n = 288$) had a history of HRT use. The mean duration of therapy was 24.1 months. 9.9% of these had used for <2 years, 3.2% had used for 2–5 years and 2.6% had used for 5 years or more. Women who had used HRT ≥5 years were at an increased risk of luminal A breast cancer (OR 1.73 95% CI 0.92–3.26; $p = 0.02$). There were also elevated risks for HER-2 overexpressing and luminal B breast cancer with borderline p values (OR 2.02 95% CI 0.92–4.41; $p = 0.05$ and OR 1.47 95% CI 1.14–2.55; $p = 0.06$, respectively). When we stratified by menopausal status (peri- and postmenopausal women), there was no significant association between HRT use and risk of perimenopausal breast cancer. However, we found significant increased risks for women with HER-2 overexpressing (OR 2.20, 95% CI 0.93–5.17; $p = 0.04$) and luminal A (OR 1.87, 95% CI 0.93–3.90, $p = 0.02$) breast cancers, who used HRT for 5 years or more, in postmenopausal women. There was no significant impact of HRT use on triple negative breast cancer, regardless of menopausal status.

Smoking. A total of 17.0% of the patients were smokers. The percentages of smokers among subtypes were similar ($p = 0.93$). We observed no significant association between smoking and breast cancer subtypes.

Obesity. Body mass index was calculated for 1630 patients. A total of 32.9% of the patients were normal weight, 36.8% were overweight, 30.3% were obese. Obesity was associated with a significant increased risk of triple negative breast cancer (OR 1.58 95% CI 1.02–2.44; $p = 0.01$) and decreased risk of luminal subtype (OR 0.70 95% CI 0.52–0.94; $p = 0.02$). When we stratified by menopausal status, we found no association between obesity and tumour subtypes in postmenopausal women. However, there were strong associations in premenopasual women. Overweight and obesity significantly increased the risk of triple negative subtype (OR 1.89 95% CI 1.06–3.37; $p = 0.04$ and OR 1.90 95% CI 1.00–3.61; $p = 0.03$), on the contrary, decreased the risk of luminal breast cancer (OR 0.63 95% CI 0.43–0.95; $p = 0.02$ and OR 0.50 95% CI 0.32–0.76; $p = 0.002$, respectively).

In vitro fertilization. Sixty-six patients (3.6%) had a history of in vitro fertilization before diagnosis. There was no significant difference of in vitro fertilization history among breast cancer subtypes.

ABO blood groups. The blood groups were known for 1441 patients. The most frequent blood types were A (43.2%) and 0 (33.1%), respectively. We found no difference between ABO blood groups and molecular subtypes of breast cancer.

Use of hands. 85.1% of patients were using right hand, 4.1% were using left hand and 0.9% were using both. No significant association was found between breast cancer subtypes and use of hands.

Discussion

Demographic and reproductive factors varied significantly by tumour subtypes in the present study. Compared with other subtypes, triple negative and luminal B cases were more likely to be younger at diagnosis ($p = 0.002$). Kwan et al. found that patients with luminal B and triple negative disease were younger than patients with luminal A.[5] Many studies reported that young age was associated with triple negative (basal-like) breast cancer and older age was related to hormone receptor positive tumors. [4,6,9,11] The incidence of breast cancer increases with age, doubling about every 10 years until the menopause, when the rate of increase slows dramatically.[2] In this study, older age was found to be a risk factor for luminal A and HER-2 overexpressing subtype.

Nulliparous women had an increased risk of both luminal A and B breast cancers, while women with more than two children had a decreased risk, when compared to hormone receptor disease. Several studies suggested that reproductive factors which are related to endogenous estrogen and progesterone exposure, are strongly associated with the risk of hormone receptor positive breast cancer.[12,15,16] An epidemiologic meta-analysis of breast

Table 1
The odds ratios and 95% confidence intervals of demographic, reproductive and non-hormonal risk factors by breast cancer subtypes.[a]

Risk factor	Luminal breast cancer ($n=1483$)		Luminal A breast cancer ($n=1249$)		Luminal B breast cancer ($n=234$)		HER-2 overexpressing breast cancer ($n=169$)		Triple negative breast cancer ($n=232$)	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR	*P*	OR	*P*	OR	*P*
Age										
<40 years	1	0.77	1	0.001*	1	<0.001*	1	0.04*	1	0.16
≥40 years	0.96 (0.74–1.24)		1.41 (1.15–1.74)		0.56 (0.42–0.75)		1.51 (1.01–2.25)		0.80 (0.59–1.09)	
Family history										
No	1	0.88	1	0.60	1	0.27	1	0.07*	1	0.16
Yes	1.02 (0.78–1.32)		1.06 (0.85–1.32)		0.82 (0.59–1.169		0.68 (0.45–1.03)		1.24 (0.91–1.70)	
Blood type										
A	0.91 (0.61–1.35)	0.90	0.92 (0.66–1.28)	0.72	0.75 (0.46–1.23)	0.06*	1.27 (0.71–2.26)	0.93	0.96 (0.58–1.56)	0.85
B	0.94 (0.63–1.38)		1.04 (0.75–1.44)		0.78 (0.48–1.26)		1.14 (0.64–2.03)		0.99 (0.61–1.61)	
AB	0.98 (0.67–1.44)		0.88 (0.65–1.21)		1.19 (0.76–1.85)		1.09 (0.62–1.91)		0.95 (0.59–1.51)	
0	0.79 (0.46–1.35)		0.87 (0.55–1.38)		0.69 (0.33–1.43)		1.10 (0.49–2.46)		1.31 (0.69–2.48)	
Use of hand										
Right-handed	0.80 (0.40–1.61)	0.68	1.19 (0.70–2.04)	0.28	0.34 (0.15–0.79)	0.05*	0.95 (0.37–2.42)	0.97	1.52 (0.59–3.94)	0.52
Left-handed	0.93 (0.23–3.71)		1.11 (0.70–1.76)		0.75 (0.41–1.38)		1.01 (0.45–2.25)		1.73 (0.74–4.03)	
Age at menarche										
Menarche ≥12 years	1	0.56	1	0.40	1	0.89	1	0.41	1	0.98
Menarche <12 years	1.07 (0.83–1.39)		1.09 (0.88–1.36)		0.97 (0.71–1.34)		0.85 (0.57–1.25)		0.99 (0.72–1.36)	
Age at menopause										
Menopause <55 years	1	0.99	1	0.87	1	0.85	1	0.49	1	0.54
Menopause ≥55 years	0.99 (0.88–1.12)		0.97 (0.70–1.35)		0.95 (0.56–1.61)		0.94 (0.79–1.11)		1.04 (0.90–1.21)	
Age at first full-term pregnancy										
<30-years old	1	0.03*	1	0.04*	1	0.01*	1	0.36	1	0.19
≥30-years old	1.25 (0.83–1.88)		1.14 (1.08–1.30)		1.57 (1.08–2.30)		0.96 (0.55–1.69)		0.71 (0.41–1.22)	
Nulliparous	1.48 (1.03–2.13)		1.44 (1.05–1.91)		1.59 (1.01–2.46)		0.67 (0.39–1.15)		0.72 (0.46–1.12)	
Number of children										
0	1	0.03*	1	0.01*	1	0.03*	1	0.37	1	0.22
1	0.79 (0.50–1.26)		0.60 (0.36–1.08)		0.66 (0.39–1.11)		1.49 (0.76–2.89)		1.05 (0.58–1.89)	
≥2	0.68 (0.47–0.97)		0.59 (0.38–0.71)		0.66 (0.45–0.97)		1.46 (0.85–2.50)		1.37 (0.87–2.15)	
Breast-feeding										
No	1	0.04*	1	0.03*	1	0.04*	1	0.24	1	0.27
Yes	0.74 (0.53–1.04)		0.71 (0.56–0.98)		0.69 (0.48–0.96)		1.34 (0.81–2.21)		1.26 (0.83–1.92)	
Oral contraceptive use										
No	1	0.71	1	0.56	1	0.96	1	0.49	1	0.76
Yes (0–2 years)	0.89 (0.65–1.23)		1.01 (0.77–1.32)		1.04 (0.70–1.55)		1.21 (0.78–1.88)		1.01 (0.67–1.51)	
Yes (≥2–5 years)	0.92 (0.51–1.66)		1.24 (0.81–1.90)		1.00 (0.53–1.86)		0.68 (0.29–1.58)		0.91 (0.47–1.74)	
Yes (≥5 years)	1.26 (0.73–2.16)		1.33 (0.79–2.23)		0.83 (0.37–1.84)		0.66 (0.23–1.84)		1.41 (0.72–2.74)	
HRT use										
No	1	0.03*	1	0.02*	1	0.06*	1	0.05*	1	0.39
Yes (0–2 years)	1.04 (0.51–2.12)		1.51 (1.09–2.10)		1.04 (0.66–1.65)		0.50 (0.25–1.01)		0.87 (0.54–1.41)	
Yes (≥2–5 years)	1.06 (0.55–2.02)		1.17 (0.67–2.01)		1.14 (0.53–2.44)		0.94 (0.37–2.41)		0.95 (0.42–2.12)	
Yes (≥5 years)	1.45 (0.96–2.19)		1.73 (0.92–3.26)		1.47 (1.14–2.55)		2.02 (0.92–4.41)		0.30 (0.07–1.25)	
In vitro fertilization										
No	1	0.50	1	0.65	1	0.26	1	0.98	1	0.41
Yes	1.24 (0.65–2.34)		1.12 (0.67–1.86)		1.45 (0.75–2.83)		0.98 (0.42–2.32)		0.70 (0.30–1.64)	
Obesity[b]										
Normal weight	1	0.02*	1	0.64	1	0.55	1	0.54	1	0.01*
Overweight	0.81 (0.61–1.09)		0.90 (0.71–1.14)		1.11 (0.79–1.57)		1.08 (0.75–1.54)		1.37 (0.89–2.12)	
Obese	0.70 (0.52–0.94)		0.98 (0.76–1.26)		0.92 (0.63–1.33)		1.22 (0.84–1.77)		1.58 (1.02–2.44)	
Smoking										
No	1	0.98	1	0.18	1	0.54	1	0.88	1	0.88
Yes	1.03 (0.73–1.37)		1.19 (0.91–1.55)		0.88 (0.59–1.31)		1.03 (0.66–1.60)		0.97 (0.65–1.43)	

CI, confidence interval; OR, odds ratio; HRT, hormone replacement therapy.

[a] The ORs and 95%CIs were found by comparing triple negative to non-triple-negative; luminal to non-luminal and HER-2 overexpressing to non-HER-2 overexpressing cases.

[b] Normal weight (BMI: 18.5–24.9 kg/m^2); overweight (BMI: 25–29.9 kg/m^2); obese (BMI ≥30.0 kg/m^2).

cancer concluded that nulliparity and delayed childbearing were associated with increased risk of ER positive but not with ER negative breast cancer.[13] There were no significant associations between reproductive characteristics and risks of HER-2 overexpressing and triple negative breast cancers in our study, as consistent with previous studies. The Polish Breast Cancer Study (PBCS) found a strong reverse risk of parity for luminal A tumors and reported that increasing parity did not show protection against HER-2 overexpressing and triple negative subtypes.[9] The Carolina Breast Cancer Study (CBCS) also found no association between HER-2 overexpressing subtype and reproductive characteristics.[6]

Women with first full-term pregnancy at age ≥30 years also had significantly elevated risk of luminal breast cancer, when compared to hormone receptor negative cases. The Breast Cancer Association Consortium (BCAC) reported that, being parous was associated with a 16% decreased risk and each additional live birth was associated with an 11% decrease in risk, while each five-year increment in age at first birth was associated with a 7% increase in risk.[17] Previous studies showed that pregnancy has a dual effect on risk of breast cancer; it transiently increases the risk after child birth (short-term effect) by stimulating the malignant cell transformation but reduces the risk in later years (long-term effect) by inducing the differentiation of normal mammary stem cells.[18]

The effect of breastfeeding is controversial in breast cancer. In the present study, breast feeding was noted to be a protective factor for luminal A and B subtypes. No protective effect was observed for triple negative or HER-2 overexpressing breast cancers. Similar to our results, Phipps et al. reported that women who breastfed for at least six months experienced a lower risk of luminal disease.[11] On the other hand, Kwan et al. found no differential associations of breastfeeding among subtypes.[5] The mean duration of breast-feeding was 12.9 ± 9.9 months for our patients. A meta-analysis of breastfeeding and breast cancer risk, by the Collaborative Group on Hormonal Factors in Breast Cancer, showed 4.3% of relative risk reduction for every 12 months of breast-feeding. The protective effect of breastfeeding may be associated with the complete differentiation of breast cells and the shorter exposure to endogenous sex hormones, which are reduced during lactation-induced amenorrhea.[19]

The mammary gland undergoes regional proliferation, differentiation, and programmed cell death in response to the hormonal fluctuations of the menstrual cycle. Early age at menarche and late age at menopause are associated with high cumulative exposure to ovarian hormones.[20] This extended period is susceptible for breast cancer development as the undifferentiated breast tissue is exposed to mitogenic estrogen and progesterone.[21] In the study of BCAC, each one-year increase in age at menarche was found to be associated with a 4% (95% CI 2–5%) decrease in breast cancer risk.[17] Early menarche has been more strongly linked to ER and PR positive tumors,[3,6,12,16] however, we observed no significant difference among breast cancer subtypes and early menarche or late menopause, as consistent with the results of a previous meta-analysis of 10 studies.[13]

A total of 15.7% of our patients had a history of HRT use with a mean duration of 24.1 months. The Million Women Study reported that the risk of breast cancer for current users of combined formulations was duration dependent, ranging from a 45% relative increase for less than 1 year of use to a 131% relative increase for more than 10 years of use, with excess relative risk of 117% for 5–10 years use.[22] We observed that postmenopausal women who used HRT for more than 5 years had a significantly increased risk of luminal A breast cancer. Holli et al. reported a preponderance of oestrogen receptor positive tumour in HRT users.[23] Phipps et al. found that current use of estrogen plus progestin hormone therapy was associated only with risk of luminal breast cancer (OR 1.7, 95% CI 1.3–2.1) and women with triple negative breast cancer were more likely to have never used HRT.[10,11] In our study, triple negative disease was the only subtype which use of HRT showed no increase in risk.

In our study, 22.6% of the women had a history of oral contraceptive use. We found no significant difference between breast cancer subtypes and oral contraseptive use. Cotterchio et al. reported that use of oral contraceptives was not significantly associated with either hormone receptor positive or negative breast cancer risk.[12] Data regarding the effects of oral contraceptives on the risk of breast cancer is conflicting. Some studies have found no association between oral contraceptive use and breast cancer risk,[2,24] however, other studies have showed modest increase in risk among women who were currently using oral contraceptives, or who had stopped using them in the preceding 10 years.[25] We also found no significant difference among cases with history of in vitro fertilization.

Obesity and overweight were associated with a significant increased risk of triple negative breast cancer and decreased risk of luminal subtype in premenopausal women. As similar to our findings, Cotterchio et al. suggested that obesity was associated with an increased ERPR negative and decreased ERPR positive breast cancer risk in premenopausal women.[12] PBCS reported that increased BMI among premenopausal women reduced the risk of luminal A and B breast cancer, whereas no protective association was seen against basal-like or unclassified tumors.[9] Kwan et al. also observed that premenopausal triple-negative cases tended to have higher BMI, which was in agreement with the basal-like cases in the CBCS.[5,6] It has been suggested that among postmenopausal women, the conversion of androgens to estrogens in adipose tissue may increase breast cancer risk and may preferentially lead to ERPR positive breast cancer risk.[16] However, Cotterchio et al. found no significant difference among subtypes in postmenopausal women,[12] as consistent with our findings.

In obese premenopausal women, the hormonal milieu is different and obesity has been associated with low serum hormone-binding globulin, hyperandrogenism, hyperinsulinemia, increased insulin-like growth factor-I and high serum leptin levels suggesting a pathway not mediated by endogenous sex hormones.[26,27] Leptin exerts stimulatory effects on ER-negative breast cancer cell proliferation, invasion and angiogenesis, where estrogen action is not a factor, both directly and by way of induction of vascular endothelial growth factor (VEGF) and heparin-binding epidermal growth factor-like growth factor (HBEGF) and hepatocyte growth factor (HGF) expressions. The data suggests an interaction among obesity, adipokines, triple-negative tumors, and poor prognosis compared with other types of breast cancer.[28]

A total of 17.0% of the patients were smokers. The proportion of smokers were similar among subtypes ($p = 0.93$). We observed no significant association between smoking and breast cancer subtypes. There was not enough consistent evidence to determine whether smoking plays a causal role in breast cancer. However, an elevated risk of breast cancer in former smokers (9%) and current smokers (16%) was observed in a recent large prospective study of postmenopausal women.[29,30]

A total of 23.5% of our patients had breast and/or ovarian cancer history in the family. We found no significant difference in family history among breast cancer subtypes. However, family history was associated with decreased risk of HER-2 overexpressing breast cancer with a borderline p value ($p = 0.07$). Previous studies found no association between family history and HER-2 status[6,7] whereas others showed a lower percentage of HER-2/neu positivity in agreement with our data.[31,32] PBCS reported that family history of breast cancer did not differ significantly for subtypes, whereas the greatest increase in risk was found for basal-like tumors.[9] Although

no statistically significant difference was observed, the highest percentage of patients with TNBC were observed in patients with family history (27.3%; $p = 0.11$). Some studies found that tumors of patients with family history are more likely to be ER/PR negative, particularly in BRCA-related tumors, but most of the studies found no significant difference as consistent with our results.[31–33] In contrast to our findings, Phipps et al. observed that luminal cases were more likely to have a first-degree family history of breast cancer.[11]

This study does have some limitations. First, our study was not designed as a case-control study, which made it difficult to quantify the exact risk for the development of breast cancer subtypes. However, Beg et al reported that, case-case analyses among tumor subtypes are a useful exploratory tool to examine etiologic heterogeneity.[34] In addition, there are case-case studies evaluated differences among breast cancer subtypes, like our study, in the literature.[5,6] Second, we had no IHC data on cytokeratin 5/6 expression and epidermal growth factor receptor to further classify triple-negative tumors into basal-like and normal breast-like. We grouped together all triple-negative tumors, thus, previous findings did not indicate substantial differences in the epidemiologies of basal-like and normal breast-like triple-negative tumors.[6,8,9] Another limitation was that, we had no data on past or current use of oral contraceptives and HRT, although durations of use were recorded.

In summary, consistent with prior reports, reproductive and hormonal characteristics (breastfeeding, parity, age at first full-term pregnancy, HRT use) were associated with luminal breast cancer, compared to hormone receptor negative disease. Obesity and overweight increased the risk of triple negative subtype, particularly in premenopausal women. Older age and HRT were the only variables associated with the risk of HER-2 over-expressing breast cancer. Some of the modifiable factors were related to the development of a specific breast cancer subtype. Maintaining a healthy weight may reduce the number of poor prognostic triple negative tumors. Higher parity and longer breast-feeding could reduce the risk of luminal breast cancer. Potential risks and benefits should be considered before deciding to treat with HRT for decreasing the risk of HER-2 overexpressing breast cancer.

The application of the cellular systems biology approach to cancer diagnostics has prognostic and predictive value for patient stratification. In addition to the basic immunohistochemistry measurements, combination with clinical and pathological data the quantitative measurements of biomarkers (NF-κB, CD3ζ, β-catenin, NKG2D, STAT, P21, p53, etc.) may determine the risk factors of molecular subtypes, assess risk of recurrence, survival and predict responses to specific therapy options.[35] Our data suggested a significant heterogeneity in association of common breast cancer risk factors and tumor subtypes, however, our findings should be replicated in a population-based case–control study.

Conflict of interest statement

None declared.

References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008. Globocan 2008. *International Journal of Cancer* 2010;**127**:2893–917.
2. McPherson K, Steel CM, Dixon JM. ABC of breast diseases. Breast cancer-epidemiology, risk factors, and genetics. *British Medical Journal* 2000;**321**:624–8.
3. Huang WY, Newman B, Millikan RC, Schell MJ, Hulka BS, Moorman PG. Hormone-related factors and risk of breast cancer in relation to estrogen receptor and progesterone receptor status. *American Journal of Epidemiology* 2000;**151**:703–14.
4. Britton JA, Gammon MD, Schoenberg JB, Stanford JL, Coates RJ, Swanson CA, et al. Risk of breast cancer classified by joint estrogen receptor and progesterone receptor status among women 20–44 years of age. *American Journal of Epidemiology* 2002;**156**:507–16.
5. Kwan ML, Kushi LH, Weltzien E, Maring B, Kutner SE, Fulton RS, et al. Epidemiology of breast cancer subtypes in two prospective cohort studies of breast cancer survivors. *Breast Cancer Research* 2009;**11**:R31.
6. Carey LA, Perou CM, Livasy CA, Dressler LG, Cowan D, Conway K, et al. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. *JAMA* 2006 Jun 7;**295**:2492–502.
7. Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proceedings of the Natural Academy of Sciences* 2001;**98**:10,869–10,874.
8. Millikan RC, Newman B, Tse CK, Moorman PG, Conway K, Dressler LG, et al. Epidemiology of basal-like breast cancer. *Breast Cancer Research Treatment* 2008;**109**:123–39.
9. Yang XR, Sherman ME, Rimm DL, Lissowska J, Brinton LA, Peplonska B, et al. Differences in risk factors for breast cancer molecular subtypes in a population-based study. *Cancer Epidemiology, Biomarkers & Prevention* 2007;**16**:439–43.
10. Phipps AI, Malone KE, Porter PL, Daling JR, Li CI. Body size and risk of luminal, HER2-overexpressing, and triple-negative breast cancer in postmenopausal women. *Cancer Epidemiology, Biomarkers & Prevention* 2008;**17**:2078–86.
11. Phipps AI, Malone KE, Porter PL, Daling JR, Li CI. Reproductive and hormonal risk factors for postmenopausal luminal, HER-2-overexpressing, and triple-negative breast cancer. *Cancer* 2008;**113**:1521–6.
12. Cotterchio M, Kreiger N, Theis B, Sloan M, Bahl S. Hormonal factors and the risk of breast cancer according to estrogen- and progesterone-receptor subgroup. *Cancer Epidemiology, Biomarkers & Prevention* 2003;**12**:1053–60.
13. Ma H, Bernstein L, Pike MC, Ursin G. Reproductive factors and breast cancer risk according to joint estrogen and progesterone receptor status: a meta-analysis of epidemiological studies. *Breast Cancer Research* 2006;**8**:R43.
14. World Health Organization. *Obesity-preventing and managing the global epidemic: report of a WHO expert committee*. Geneva: World Health Organization; 1998.
15. Colditz GA, Rosner BA, Chen WY, Holmes MD, Hankinson SE. Risk factors for breast cancer according to estrogen and progesterone receptor status. *Journal of Natural Cancer Institute* 2004;**96**:218–28.
16. Althuis MD, Fergenbaum JH, Garcia-Closas M, Brinton LA, Madigan MP, Sherman ME. Etiology of hormone receptor-defined breast cancer: a systematic review of the literature. *Cancer Epidemiology, Biomarkers & Prevention* 2004;**13**:1558–68.
17. Milne RL, Gaudet MM, Spurdle AB, Fasching PA, Couch FJ, Benítez J, et al. Assessing interactions between the associations of common genetic susceptibility variants, reproductive history and body mass index with breast cancer risk in the breast cancer association consortium: a combined case-control study. *Breast Cancer Research* 2010;**12**:R110.
18. Lambe M, Hsieh C, Trichopoulos D, Ekbom A, Pavia M, Adami HO. Transient increase in the risk of breast cancer after giving birth. *New England Journal of Medicine* 1994;**331**:5–9.
19. Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and breastfeeding: collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries, including 50,302 women with breast cancer and 96973 women without the disease. *Lancet* 2002;**360**:187–95.
20. Iversen A, Thune I, McTiernan A, Emaus A, Finstad SE, Flote V, et al. Ovarian hormones and reproductive risk factors for breast cancer in premenopausal women: the Norwegian EBBA-I study. *Human Reproduction* 2011;**26**:1519–29.
21. Li CI, Malone KE, Daling JR, Potter JD, Bernstein L, Marchbanks PA, et al. Timing of menarche and first full-term birth in relation to breast cancer risk. *American Journal of Epidemiology* 2008;**167**:230–9.
22. Beral V, Million Women Study Collaborators. Breast cancer and hormone-replacement therapy in the Million Women Study. *Lancet* 2003;**362**:419–27.
23. Holli K, Isola J, Cuzick J. Low biologic aggressiveness in breast cancer in women using hormone replacement therapy. *Journal of Clinical Oncology* 1998;**16**:3115–20.
24. Marchbanks PA, McDonald JA, Wilson HG, Folger SG, Mandel MG, Daling JR, et al. Oral contraceptives and the risk of breast cancer. *New England Journal of Medicine* 2002;**346**:2025–32.
25. Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and hormonal contraceptives: further results. *Contraception* 1996;**54**:1S–106S.
26. Kroenke CH, Chen WY, Rosner B, Holmes MD. Weight, weight gain, and survival after breast cancer diagnosis. *Journal of Clinical Oncology* 2005;**23**:1370–8.
27. Probst-Hensch NM, Steiner JH, Schraml P, Varga Z, Zurrer-Hardi U, Storz M, et al. IGFBP2 and IGFBP3 protein expressions in human breast cancer: association with hormonal factors and obesity. *Clinical Cancer Research* 2010;**16**:1025–32.

28. Vona-Davis L, Rose DP. Adipokines as endocrine, paracrine, and autocrine factors in breast cancer risk and progression. *Endocrine-Related Cancer* 2007;**14**:189–206.
29. Hamajima N, Hirose K, Tajima K, Rohan T, Calle EE, Heath Jr CW, et al, Collaborative Group on Hormonal Factors in Breast Cancer. Alcohol, tobacco and breast cancer-collaborative reanalysis of individual data from 53 epidemiological studies, including 58,515 women with breast cancer and 95,067 women without the disease. *British Journal of Cancer* 2002;**87**:1234–45.
30. Luo J, Margolis KL, Wactawski-Wende J, Horn K, Messina C, Stefanick ML, et al. Association of active and passive smoking with risk of breast cancer among postmenopausal women: a prospective cohort study. *British Medical Journal* 2011;**342**:d1016.
31. Chappuis PO, Nethercot V, Foulkes WD. Clinico-pathological characteristics of BRCA-1 and BRCA-2 related breast cancer. *Seminars in Surgical Oncology* 2000;**18**: 287–95.
32. Lakhani SR, Van De Vijver MJ, Jacquemier J, Anderson TJ, Osin PP, McGuffog L, et al. The pathology of familial breast cancer: predictive value of immunohistochemical markers estrogen receptor, progesterone receptor, HER-2, and p53 in patients with mutations in BRCA1 and BRCA2. *Journal of Clinical Oncology* 2002;**20**:2310–8.
33. Russo A, Herd-Smith A, Gestri D, Bianchi S, Vezzosi V, Rosselli Del Turco M, et al. Does family history influence survival in breast cancer cases? *International Journal of Cancer* 2002;**99**:427–30.
34. Begg CB, Zhang ZF. Statistical analysis of molecular epidemiology studies employing case-series. *Cancer Epidemiology, Biomarkers & Prevention* 1994;**3**: 173–5.
35. Critchley-Thorne RJ, Miller SM, Taylor DL, Lingle WL. Applications of cellular systems biology in breast cancer patient stratification and diagnostics. *Combinatorial Chemistry & High Throughput Screening* 2009 Nov;**12**(9):860–9.