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Interaction of Estrogen and Progesterone Receptors, and Human Epithelial Receptor 2, with Breast cancer Risk factors: a Multi-center case-only study in Iran

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ABSTRACT

Background: The etiology of breast cancer and our understanding on the carcinogenicity of different risk factors is impotant for control programms. We investigated the expression of estrogen and progesterone receptors (ER, PR), and human epithelial receptor 2 (HER2) overexpression and its associations, with environmental risk factors among breast cancer patients.

Methods: We classified the patients into four groups including; triple negative (ER–/PR–/HER2–), HER2-overexpression (ER–/PR–/HER2+), luminal A (ER+ and/or PR+/HER2–) and luminal B (ER+ and/or PR+/HER2+). We used a case-only design and multinomial logistic regression analyses.

Results: In premenopausal patients, those with high BMI had lower prevalence of luminal B tumors compared to luminal A groups (OR= 0.42, 95% CI= 0.23 to 0.74). However, in the postmenopausal groups, prevalence of the luminal B tumors was less than luminal A tumors oral contraceptive pill (OCP) users (OR= 0.64, 95% CI= 0.42 to 0.98). In addition, among those who had an older age at menarche had a higher risk of ER2-overexpression tumors compared to luminal A tumors (OR= 2.82, 95% CI= 1.29-6.19).

Conclusion: Expression of HER2, ER, and PR, among breast cancer patients seems to be associated with OCP use, BMI, age at menarche and age at first pregnancy.

Keywords: Breast cancer, estrogen receptor, progesterone receptor, HER2, risk factors

Introduction

Breast cancer is the most common cancer and the leading cause of cancer deaths in women worldwide[1]. Although the incidence rate of breast cancer has decreased in the USA and in many other developed countries since the early 2000s[2], it has increased rapidly in many Asian countries[3]. For instance, the age standardized incidence rate (ASR) of breast cancer has increased by 50-100% in some Asian countries, including India and China, during the last two decades[4,5]. In Iran, breast cancer is the most common cancer among women with an ASR of 32.21 per 100 000 per year[3], and based on Globocan 2012, the annual number of new breast cancer cases will double in Iran by 2035, Given that the risk factor will not change[4].

Epidemiological and biological studies have shown that estrogen and progesterone play important roles in the development of breast cancer. Some established risk factors including; age at menarche and age at menopause, include hormonal mechanisms which are involved in the development of breast cancer[5]. Estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), are the tumor markers that have been widely studied in relation to the etiology, prognosis and treatment of breast cancer subtypes[6-8].

Epidemiologic studies have reported heterogeneity of breast cancer risk factors with hormone receptors and the expression of HER2[9,10]. Reproductive factors and BMI were shown to be associated with ER+ and PR+ breast cancer, compared with ER- and PR- tumors[6, 11-13]. In addition, the triple-negative tumors, defined as a lack of expression of estrogen, progesterone, and HER2, are characterized by a distinct etiology, aggressive histology, poor prognosis, and unresponsiveness to standard endocrine therapies, shorter survival, and BRCA1-related breast cancer[5,7,14].

Epidemiological studies in some Asian countries have suggested that there are different patterns of breast cancer subtypes according to ER, PR and HER2 status, compared to Western countries[2,15,16]. This contrasting pattern, as well as interaction between hormonal receptors and environmental risk factors has been suggested as reasons for the rapid increase of breast cancer incidence in these countries[2]. To date, only two studies have investigated the risk factors for breast cancer subtypes in Asia, with no statistical tests carried out on the interactions[15,17].

Although environmental risk factors for breast cancer have been studied in Iran[18-20], to the best of our knowledge, no study has so far evaluated the interaction between environmental risk factors and ER, PR and HER2 status. Therefore, we conducted a large multi-center study and investigated whether the association of established risk factors varies with ER, PR and HER2 expression.

Methods

Study subjects

We conducted a multicenter study which consisted of 3668 breast cancer patients from three large cities in Iran including; Tehran (627), Mashhad (1258), and Shiraz (1783), between 2000 and 2010. Eligible patients were women diagnosed with histopathologically confirmed invasive breast cancer. Patients were treated in three cancer center and signed an inform consent and allowed to conduct medical research on their data. Reproductive, demographic and anthropometric information collected using self-administrated questionnaire by each center at the time of admission. Clinical and pathological data was collected from medical records. Among these patients, a total of 2 706 patients had information on both their ER and PR status and 2 453 had information on their ER, PR and HER2 status. We

included only those patients who had information of both tumor markers and environmental risk factors in the analyses.

Outcome classification

Breast cancer patients were classified based on their ER, PR and HER2 status. Expression of these tumor markers was obtained from hospital records and they were measured by an immunohistochemistry (IHC) method. For HER2, scores 0 and 1+ were considered as negative, and score 3+ was considered to be positive. Cases with a 2+ score were considered as equivocal, and some of these cases were tested for gene amplification with fluorescence in situ hybridization (FISH). HER2 was considered positive if the IHC score was 3+ or 2+ with gene amplification identified by FISH.

According to the ER, PR and HER2 results, breast cancer patients were defined as luminal A (ER+ and/or PR+, and HER2–), luminal B (ER+ and/or PR+, and HER2+), triple negative (ER–, PR–, and HER2–) and HER2 overexpression (ER–, PR–, and HER2+). Similar to most other studies in this area (21, 22), luminal A was the most frequent subtype and then used as the reference group in this analysis. We also categorized breast cancer tumors by joint ER/PR status as; ER+/PR+, ER–/PR–, ER+/PR- and ER-/PR+. In the analyses, we only compared ER+/PR+ with ER–/PR– breast cancers, because these two subgroups had considerable differences from a biological viewpoint. In addition, we did not have sufficient power to analyze the other two groups (ER+/PR–, ER–/PR+).

Regional Research Ethics Committee of Tehran University of Medical Science, approved this study (No. 89-04-51-11833).

Risk factor classification

Information about established risk factors of breast cancer were collected as categorical and analyzed accordingly. Age at menarche was categorized as<12 years, 12-15 years and ≥15 years, family history of breast cancer as no vs. yes, age at first pregnancy as less than 25 years vs. more than 25 years, parity as nullipara vs. parous, duration of breastfeeding as never, 1-12 months, and more than 12 months and menopausal status as premenopausal vs. postmenopausal. Women with no history of the menstrual period during last 12 months and women with no hysterectomy or oopherectomy (not related to breast cancer treatment) were classified as postmenopausal. Oral contraceptive pill (OCP) use was categorized as never vs. ever users, BMI as less than 25 kg/m2, 25-30 kg/m2, and higher than 30 kg/m2. We did not collect information about hormone replacement therapy (HRT) that is an established risk factor of breast cancer, however, similar to many other Asian countries[23,24], less than one percent of Iranian women aged 50 years and older used HRT, often following surgical menopause[25].

Statistical analyses

Case-only odds ratios and corresponding 95% confidence intervals (CIs) were estimated using logistic regression modeling. To investigate the heterogeneity of the risk factors of interest among the four subtypes of breast cancer according to ER/ PR and HER2, we selected luminal A as the reference group and used multinomial logistic regression for the analyses. In addition, we studied the heterogeneity of breast cancer risk factors in ER+/PR+ versus ER-/PR- breast cancers, using binary logistic regression. All models were adjusted for potential confounders and the analyses were conducted separately for premenopausal and postmenopausal women. We also estimated the P-value for a trend test of the variables that had more than two strata. We used STATA (Ver. 11, Stata Corp LP, College Station, Texas, USA) for the statistical analyses.

Results

In total, we obtained data from 3,668 breast cancer patients from three hospitals. However, the ER and

PR status were available in 2,706 patients and the data on all hormone receptor status (ER, PR, HER2) were available in only 2,453 patients. The prevalence of ER+, PR+ and HER2+, were 66.4%, 59.2% and 39.5%, respectively. Menopausal status was available in 3,668 patients, in total 1,935 (52.75%) were premenopausal, and 1,733 (47.25%) patients had a postmenopausal breast cancer (Table 1). Luminal A had the highest prevalence compared to the other subtypes among both the premenopausal

Table 1: Distribution of environmental risk factors and expression of tumor markers by the menopausal status: Multi-center study in the Iranian women

Characteristic	Pre-menopausal	Postmenopausal	Total
	No. (%)	No. (%)	No. (%)
Age at menarche			
<12	57 (44.8)	70 (55.2)	127 (8.1)
12-15	686 (53.2)	604 (46.8)	1290 (81.6)
≥15	65 (39.9)	98 (60.1)	163 (10.3)
Family history			
No	1145 (53.3)	1003 (46.7)	2.148 (81.0)
Yes	260 (51.5)	245 (48.5)	505 (19.0)
Age at first pregnancy			
<25	789 (48.1)	850 (51.9)	1639 (78.0)
≥25	265 (28.2)	193 (41.8)	462 (22.0)
Parity			
Nulliparous	64 (56.6)	49 (43.4)	113 (5.0)
Parous	1087 (50.1)	1082 (49.9)	2.169 (95)
Breastfeeding			
Never	146 (59.8)	98 (40.2)	244 (12.5)
1-12	93 (51.4)	88 (48.6)	181 (9.3)
≥12	755 (49.5)	769 (50.5)	1524 (78.2)
Oral contraceptive use			
Never	627 (53.6)	542 (46.4)	1169 (49.5)
Ever	603 (50.5)	591 (49.5)	1194 (50.5)
BMI			
<25	520 (59)	362 (41)	882 (32.3)
25-29	543 (52.4)	494 (47.6)	1037 (38.0)
≥30	396 (48.9)	414 (51.1)	810 (29.7)
Expression of markers			
HER Status			
Negative	579 (61.6)	470 (55.3)	1535 (60.5)
Positive	361(38.4)	379 (44.6)	1002 (39.5)
ER Status			
Negative	359 (35.6)	291 (32.7)	912 (33.6)
Positive	647 (64.3)	597(67.2)	1805 (66.4)
PR Status			
Negative	400 (39.8)	368 (41.5)	1106 (40.8)
Positive	603 (60.1)	517 (58.4)	1607 (59.2)

Table 2: Association of environmental risk factors and hormone receptors (ER, PR) in premenopausal breast cancer patients: in case-case analyses[1]

Characteristics	ER+/PR+ (Control Group)	ER-/PR-		
	No. (%)	No. (%)	OR (crude)	OR[2] (Adjusted) (95% CI)
Age at menarche				
<12	30(6.80)	13(5.91)	0.86 (0.44-1.69)	1.02(0.46-2.26)
12-15	377(85.49)	189(85.91)	Ref	Ref
>15	34(7.71)	18(8.18)	1.05 (0.58-1.91)	1.13(0.56-2.28)
Test For Trend[3]				P=0.91
Family history				
No	492(84.25)	270(82.57)	Ref	Ref
Yes	92(15.75)	57(17.43)	1.12 (0.78-1.62)	0.65(0.34-1.26)
Age at first pregnancy				
<25	346(71.78)	187(74.50)	Ref	Ref
≥25	136(28.22)	64(25.50)	0.87 (0.61-1.23)	0.89(0.56-1.42)
Parity (child)				
Nuliparous	26(5.10)	17(6.07)	Ref	Ref
parous	484(94.90)	263(93.93)	0.83 (0.44-1.55)	3.00(0.42-21.39)
Breast feeding				
Never	59(12.91)	32(13.11)	Ref	Ref
1-12 months	54(11.82)	17(6.97)	0.58 (0.28-1.16)	0.49(0.12-1.97)
≥ 12	344(75.27)	195(79.92)	1.04 (0.65-1.66)	0.93(0.26-3.31)
Test For Trend				P=0.13
Oral contraceptive use				
Never	283(51.74)	143(49.14)	Ref	Ref
Ever	264(48.26)	148(50.86)	1.10 (0.83-1.47)	0.93(0.62-1.40)
BMI (kg/m2)				
<25	192(32.49)	116(35.69)	Ref	Ref
25-29	233(39.42)	125(38.46)	0.88 (0.64-1.21)	0.74 (0.46-1.18)
≥30	166(28.09)	84(25.85)	0.83 (0.59-1.13)	0.79 (0.47-1.30)
Test For Trend				P=0.37

1. In unconditional logistic regression, we calculated association between tumor subtypes and environmental risk factors of breast cancer (comparing case patient who were ER+/PR+ group)
2. For calculated odds ratios were adjusted all variables in the table
3. Test for trend

(54.0%) and postmenopausal (46.0%) patients. The prevalence of triple negative tumors was higher among the premenopausal women (62.2% vs. 37.8%; P=0.001), while HER2-overexpresion was more prevalent in the postmenopausal women (46.9 vs. 53.1; P=0.003). We found that the prevalence of late menarche (older than 15 years) was higher in the postmenopausal patients (60.1%) compared to the premenopausal group (39.9%) (p=0.002).

In the case-case analysis, we found no significant association between the studied risk factors and ER/PR status among the pre-menopausal cancers (Table 2). However, in the multivariate analysis, after adjustment for potential confounders, postmenopausal

patients with a menarche age older than 15 years, had an almost two-fold higher risk of developing ER-/PR- breast cancer (OR=1.99, 95% CI=1.04-3.79) compared to the women who reached menarche between 12-15 years (Table 3). Moreover, in both univariable and multivariable analyses, patients cancer (OR= 0.50, 95% CI=0.27-0.91) compared to those who gave birth before 25 years-of-age (Table 4). In the analyses based on ER/PR/HER2 status, we found that patients with a higher BMI were at who had had their first pregnancy after the age of 25 were at significantly lower risk of ER-/ER- breast

Table 3: Association of environmental risk factors and hormone receptors (ER, PR) in postmenopausal breast cancer: in case-case analyses[1]

Characteristics	ER+/PR+ (Control Group)	ER-/PR-		
	No. (%)	No. (%)	OR (crude)	OR[2] (Adjusted) (95% CI)
Age at menarche				
<12	34(9.26)	14(7.91)	0.88 (0.46-1.70)	0.74(0.34-1.62)
12-15	299(81.47)	139(78.53)	Ref	Ref
>15	34(9.26)	24(13.56)	1.51 (0.86-2.65)	1.99(1.04-3.79)
Test For Trend[3]				P=0.11
Family history				
No	390(80.75)	200(78.13)	Ref	Ref
Yes	93(19.25)	56(21.88)	1.17 (0.80-1.70)	1.16(0.67-2.03)
Age at first pregnancy				
<25	334(78.59)	191(86.04)	Ref	Ref
≥25	91(21.41)	31(13.96)	0.59 (0.38-0.92)	0.50(0.27-0.91)
Parity (child)				
Nuliparous	25(5.47)	13(5.35)	Ref	Ref
parous	432(94.53)	230(94.65)	1.02 (0.51-2.03)	1.15(0.20-6.38)
Breast feeding				
Never	46(11.00)	26(12.94)	Ref	Ref
1-12 months	45(10.77)	21(10.45)	0.82 (0.40-1.67)	0.45(0.12-1.64)
≥ 12	327(78.23)	154(76.62)	0.83 (0.49-1.39)	0.38(0.12-1.26)
Test For Trend				P=0.45
Oral contraceptive use				
Never	224(47.86)	113(47.28)	Ref	Ref
Ever	244(52.14)	126(52.72)	1.02 (0.74-1.39)	1.08(0.71-1.66)
BMI (kg/m2)				
<25	117(24.02)	74(28.24)	Ref	Ref
25-29	192(39.43)	103(39.31)	0.84 (0.58-1.23)	1.23(0.71-2.13)
≥30	178(36.55)	85(32.44)	0.75 (0.51-1.11)	0.97(0.55-1.72)
Test For Trend				P=0.30

1. In unconditional logistic regression, we calculated association between tumor subtypes and environmental risk factors of breast cancer (comparing case patient who were ER+/PR+ group)
2. For calculated odds ratios were adjusted all variables in the table
3. Test for trend

lower risk of developing luminal B than luminal A breast cancer (P-value for trend =0.003). In addition, the risk of developing luminal B was lower among the obese patients (BMI ≥30) compared to normal weight patients (BMI <25) (OR=0.42, 95% CI=0.23 to 0.74). Furthermore, the risk of HER2-over expression was lower in the obese patients (BMI ≥30) (OR= 0.49, 95% CI= 0.23-1.02) (Table 4). Among the postmenopausal patients, menarche later than 15 years was associated with a higher risk of HER-2overexpression (OR=%95 ,2.82 CI=-1.29 6.19) compared with those who reached menarche

Table 4: The association of environmental risk factors and tumor subtypes in premenopausal breast cancer: in case-case analyses [1]

Characteristics	Luminal A 393 (42.4)	Luminal B 245 (26.4)			Triple Negative 178 (19.2)			HER2-overexpresstion 110 (11.8)		
	No. (%)	No. (%)	OR (Crude)	OR[1] (95% CI)	No. (%)	OR (Crude)	OR (95% CI)	No. (%)	OR (Crude)	OR (95% CI)
Age at menarche										
<12	21 (42)	17 (34)	1.09 (0.56-2.14)	0.74(0.30-1.82)	7 (14.0)	0.79 (0.32-1.93)	1.14(0.42-3.12)	5 (10)	0.74 (0.27-2.04)	0.56(0.15-2.01)
12-15	254 (40.5)	187 (29.8)	Ref	Ref	106 (16.9)	Ref	Ref	81 (12.9)	Ref	Ref
>15	27 (44.3)	16 (26.2)	0.80 (0.42-1.53)	0.61(0.28-1.32)	11 (18)	0.97 (0.46-2.03)	1.04(0.45-2.41)	7 (11.5)	0.81 (0.34-1.93)	0.61(0.21-1.73)
Test For Trend[3]			P=0.62	P=0.17		P=0.82	P=0.85		P=0.53	P=0.25
Family history										
No	347 (43.3)	208 (25.9)	Ref	Ref	145 (18.1)	Ref	Ref	102 (12.7)	Ref	Ref
Yes	67 (41.4)	41 (25.3)	1.02 (0.66-1.56)	1.01(0.53-1.91)	40 (24.7)	1.42 (0.92-2.21)	0.95(0.43-2.08)	14 (8.6)	0.71 (0.38-1.31)	0.42(0.14-1.28)
Age at first pregnancy										
<25	253 (43.5)	153 (26.3)	Ref	Ref	111 (13.9)	Ref	Ref	64 (11)	Ref	Ref
≥25	88 (42.1)	61 (29.1)	1.14 (0.78-1.68)	1.09(0.65-1.80)	29 (19.1)	0.75 (0.46-1.20)	0.66(0.44-1.29)	31 (14.8)	1.39 (0.85-2.27)	1.59(0.84-2.98)

1. In unconditional logistic regression, we calculated association between tumor subtypes and environmental risk factors of breast cancer (comparing case patient who were luminal A group)
2. For calculated odds ratios were adjusted all variables in the table
3. Test for trend

Table 4:Continue...

	No. (%)	No. (%)	OR (Crude)	OR (95% CI)	No. (%)	OR (Crude)	OR (95% CI)	No. (%)	OR (Crude)	OR (95% CI)
Parity (child)										
Nulipa-rous	24 (52.2)	7 (15.2)	Ref	Ref	10 (21.7)	Ref	Ref	5 (10.9)	Ref	Ref
parous	341 (42.3)	215 (26.8)	2.16 (0.91-5.10)	1.93(0.25-14.65)	146 (18.2)	1.02 (0.47-2.20)	5.98(0.45-79.46)	99 (12.4)	1.39 (0.51-3.74)	2.25(0.10-47.36)
Breast feeding										
Never	47 (45.2)	27 (26)	Ref	Ref	19 (18.3)	Ref	Ref	11 (10.6)	Ref	Ref
1-12 months	33 (42.9)	27 (35.1)	1.42 (0.71-2.58)	1.10(0.21-5.61)	12 (15.6)	0.89 (0.38-2.10)	0.39(0.07-2.14)	5 (6.5)	0.64 (0.20-2.03)	0.71(0.06-8.33)
≥ 12	257 (42.7)	156 (25.9)	1.05 (0.63-1.76)	1.10(0.23-5.19)	110 (18.3)	1.05 (0.59-1.88)	0.46(0.09-2.22)	79 (13.1)	1.31 (0.65-2.65)	2.15(0.22-20.26)
Test For Trend			P=0.88	P=0.93		P=0.75	P=0.75		P=0.25	P=0.06
Oral con-traceptive use										
Never	186 (42.6)	115 (26.3)	Ref	Ref	77 (17.6)	Ref	Ref	59 (13.5)	Ref	Ref
Ever	199 (42.3)	131 (27.9)	1.06 (0.77-1.46)	0.92(0.59-1.44)	90 (19.1)	1.09 (0.75-1.57)	1.03(0.60-1.77)	50 (10.6)	0.79 (0.51-1.21)	0.67(0.37-1.19)
BMI (kg/m2)										
<25	118 (38.2)	86 (27.8)	Ref	Ref	64 (20.7)	Ref	Ref	41 (13.3)	Ref	Ref
25-29	166 (42)	112 (28.3)	0.92 (0.64-1.33)	0.66(0.40-1.09)	71 (18)	0.78 (0.52-1.19)	0.56(0.30-1.05)	46 (11.6)	0.79 (0.49-1.29)	0.65(0.34-1.25)
≥30	137 (50)	59 (21.5)	0.59 (0.39-0.88)	0.42(0.23-0.74)	50 (18.2)	0.67 (0.43-1.04)	0.65(0.34-1.25)	28 (10.2)	0.58 (0.34-1.00(	0.49(0.23-1.02)
Test For Trend			P=0.01	P=0.003		P=0.07	P=0.23		P=0.05	P=0.06

at 15–12 years. Any use of the OCP was also associated with a reduced risk of luminal B breast cancer compared with women who had never used this form of contraception (OR=0.64, 95% CI=0.42-0.98) (Table 5).

Discussion

We analyzed 2706 breast cancer patients from three major Iranian medical centers in eastern and southern cities, as well as Tehran, and found that reproductive and anthropometric factors varied by cancer subtypes in breast cancer. Of these cancer cases, luminal A was the most prevalent breast cancer subtype (44.3%) relative to luminal B (26.0%), along with HER2-overexpression (13.1%) and triple negative (16.6%). A study in Asia found 48% luminal A, 12% luminal B, 29% triple negative and 11% HER2-overexpression tumor cases[26]. In a large study conducted in the USA, the distribution of tumor subtypes were; 67% luminal A, 12% luminalB, 7% HER2-overexpression and 14% triple negative[22].

Table 5: The association of environmental risk factors and tumor subtypes in postmenopausal breast cancer: in case-case analyses[1]

Characteristics	Luminal A 393 (42.4)	Luminal B 245 (26.4)			Triple Negative 178 (19.2)			HER2-overexpresstion 110 (11.8)		
	No. (%)	No. (%)	OR[2] (Crude)	OR (95% CI)	No. (%)	OR (Crude)	OR (95% CI)	No. (%)	OR (Crude)	OR (95% CI)
Age at menarche										
<12	20 (37)	20 (37)	1.39 (0.72-2.68)	2.01(0.94-4.29)	5 (9.3)	0.86 (0.31-2.40)	1.01(0.31-3.29)	9 (16.7)	1.40 (0.61-3.22)	1.36(0.49-3.78)
12-15	222 (43)	159 (30.8)	Ref	Ref	64 (12.4)	Ref	Ref	71 (13.8)	Ref	Ref
>15	25 (32.9)	27 (35.5)	1.50 (0.84-2.69)	1.80(0.89-1.63)	9 (11.8)	1.24 (0.55-2.88)	1.76(0.67-4.58)	15 (19.7)	1.87 (0.93-3.75)	2.82(1.29-6.19)
Test For Trend[3]			P=0.11	P=0.03		P=0.68	P=0.29		P=0.06	P=0.009
Family history										
No	279 (42.7)	191 (29.2)	Ref	Ref	85 (13)	Ref	Ref	99 (15.1)	Ref	Ref
Yes	71 (42)	49 (29)	1.00 (0.67-1.55)	1.21(0.70-2.09)	24 (14.2)	1.10 (0.65-1.77)	1.12(0.52-2.38)	25 (14.8)	0.99 (0.59-1.65)	1.05(0.51-2.15)
Age at first pregnancy										
<25	255 (42.4)	170 (28.3)	Ref	Ref	81 (13.5)	Ref	Ref	95 (15.8)	Ref	Ref
≥25	63 (44.7)	50 (35.5)	1.19 (0.78-1.80)	1.06(0.62-1.80)	14 (9.9)	0.69 (0.37-1.31)	0.47(0.19-1.14)	14 (9.9)	0.59 (0.31-1.11)	0.62(0.29-1.31)
Parity										
Nulliparous	12 (31.6)	13 (34.2)	Ref	Ref	7 (18.4)	Ref	Ref	6 (15.8)	Ref	Ref
Parous	325 (43)	220 (29.1)	0.62 (0.27-1.29)	1.15(0.12-10.88)	96 (12.7)	0.50 (0.19-1.32)	0.42(0.04-4.08)	115 (15.2)	0.70 (0.25-1.92)	3.08(0.23-40.60)

1. In unconditional logistic regression, we calculated association between tumor subtypes and environmental risk factors of breast cancer (comparing case patient who were luminal A group)
2. For calculated odds ratios were adjusted all variables in the table
3. Test for trend

In our study, the overexpression of HER2 was comparable to the Asian study, but higher than in the USA study. The prevalence of HER2 positive has been found to be 18% to 25% in Western countries(27-29). In addition, the prevalence of HER2 positive patients was 29% in Malaysia, and 22% in China. However, the proportion of tumor subtypes observed was different in the ethnic groups in the Western and Asian studies[21,26].

Menopausal status has been associated with breast cancer subtypes in previous studies. Triple negative tumors were more prevalent among premenopausal patients compared to postmenopausal patients (p=0.001), while HER-2overexpression was

Table 5: Continue...

	No. (%)	No. (%)	OR (Crude)	OR (95% CI)	No. (%)	OR (Crude)	OR (95% CI)	No. (%)	OR (Crude)	OR (95% CI)
Breast feeding										
Never	27 (35.5)	23 (30.3)	Ref	Ref	13 (17.1)	Ref	Ref	13 (17.1)	Ref	Ref
1-12 months	26 (32.9)	33 (41.8)	1.48 (0.69-3.17)	2.58(0.53-12.39)	11 (13.9)	0.87 (0.33-2.31)	0.98(0.15-6.35)	9 (11.4)	0.71 (0.26-1.96)	0.44(0.09-2.15)
≥ 12	258 (45.3)	165 (28.9)	0.75 (0.41-1.35)	1.35(0.30-5.98)	62 (10.9)	0.49 (0.24-1.02)	0.48(0.08-2.77)	85 (14.9)	0.68 (0.33-1.38)	0.31(0.07-1.30)
Test For Trend			P=0.07	P=0.24		P=0.02	P=0.10		P=0.30	P=0.09
Oral contraceptive use										
Never	161 (42.3)	117 (30.7)	Ref	Ref	45 (11.8)	Ref	Ref	58 (15.2)	Ref	Ref
Ever	185 (43.4)	122 (28.6)	0.90 (0.65-1.26)	0.64(0.42-0.98)	55 (12.9)	1.06 (0.68-1.66)	0.88(0.48-1.60)	64 (15)	0.96 (0.63-1.45)	0.91(0.52-1.58)
BMI (kg/m2)										
<25	77 (38.3)	64 (31.8)	Ref	Ref	23 (11.4)	Ref	Ref	37 (18.4)	Ref	Ref
25-29	147 (43.4)	97 (28.6)	0.79 (0.52-1.20)	0.73(0.42-1.26)	49 (14.4)	1.11 (0.63-1.96)	1.67(0.72-3.86)	46 (13.6)	0.65 (0.38-1.08)	0.79(0.39-1.57)
≥30	129 (44.8)	82 (28.5)	0.76 (0.49-1.17)	0.74(0.43-1.29)	33 (11.5)	0.85 (0.46-1.56)	1.14(0.47-2.74)	44 (15.3)	0.70 (0.42-1.19)	0.67(0.33-1.38)
Test For Trend			P=0.24	P=0.33		P=0.53	P=0.94		P=0.65	P=0.29

more prevalent among postmenopausal patients (p=0.003). We found heterogeneity of association between the different risk factors and breast cancer subtypes in both the pre- and postmenopausal groups, and thus analyzed the data accordingly. Overall, we found more heterogeneity between the risk factors of breast cancer and tumor subtypes among postmenopausal patients, and significant associations were observed between ER-/PR- subtypes with age at menarche and age at first pregnancy in this group. In addition, in postmenopausal patients the age at menarche and the use of OCP had significant associations with HER2-overexpression and luminal B subtypes, respectively. In premenopausal women, we found an association between BMI with luminal B and HER2-overexpression subtypes. These results suggest a distinctive influence with these risk factors on the presentation of tumor markers when the patients developed breast cancer.

Strengths of our study include; appropriate study design, large sample size, and performing a multi-center study. Moreover, because we used a case-only design, we did not face any limitations in the selection of a control group, which is the main concern in a conventional case control study[30]. We analyzed all variables in both the premenopausal and postmenopausal groups, while other studies analyzed the combined data, usually because of small sample sizes. In addition, we analyzed the data according to hormone receptor statuses with different classifications of tumor subtypes. In our study, missing data were noticeably high for the hormone receptor

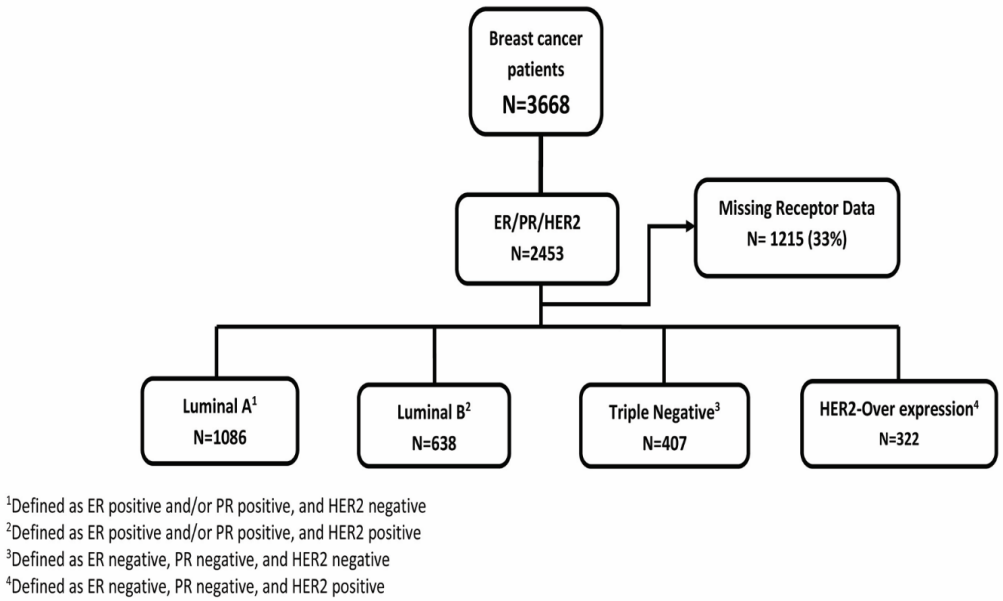

Fig 1: breast tumor subtypes defined by expression of estrogen receptor (ER), progesterone (PR), and epidermal growth factor receptor 2 (HER2) in a multi-center study

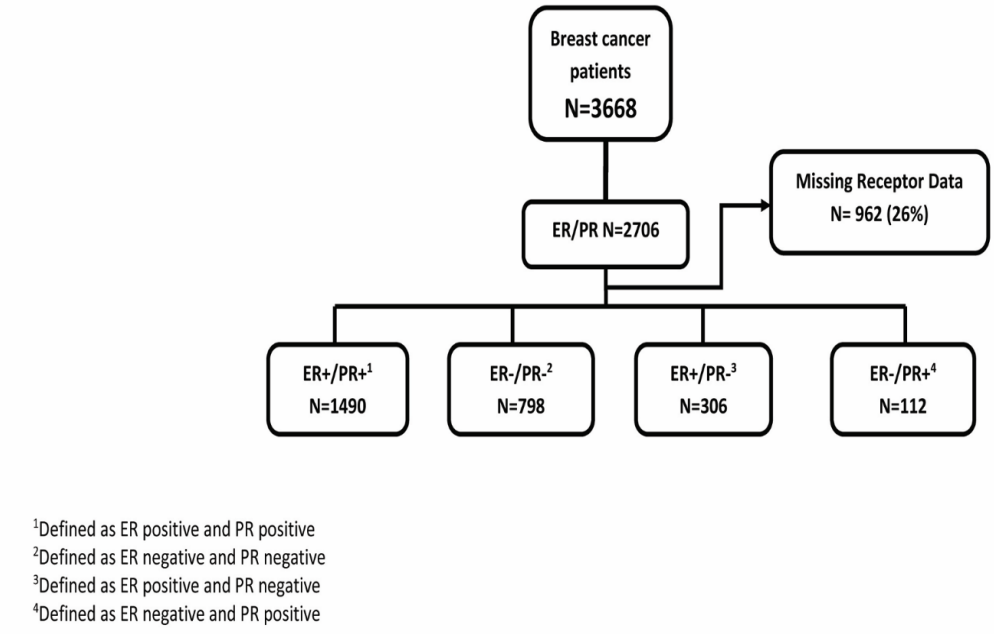

Fig 2: breast tumor subtypes defined by expression of estrogen (ER) and progesterone (PR) receptors in a multi-center study

status (**Figure 1, 2**), however, it was not related to the outcome or variables under study. Information bias may have influenced our findings because of non-differential misclassification of hormone receptor statuses. The IHC test may not be totally reliable and accurate in the service based laboratories[28, 31, 32], as the IHC showed a sensitivity range between 76-90% and specificity range between 83-85%[33].

Through comparison of ER-/PR- patients with the ER+/PR+ groups, we found no significant association between the studied risk factors in premenopausal patients. However, a pooled analysis of 23 studies, found that age at menarche, age at first pregnancy, parity and BMI varied according to the ER/PR statuses in all patients[22]. In another case-case analysis, the authors found a significant association between a family history of breast cancer and ovarian cancer in first degree relatives and ER+/PR+ tumors[10]. In our study, age at menarche and age at first pregnancy varied according to ER/PR status in postmenopausal patients. This result, to the best of our knowledge, has not been reported previously and needs to be repeated in future studies.

We found that among premenopausal patients, women who were obese (BMI ≥ 30) were less likely to have luminal B and HER2-overexpression tumors than a luminal A subtype. Moreover, women who had ever used the OCP had a higher risk of luminal B breast cancer compared to luminal A cancer. Among postmenopausal women, those who reported late-onset menarche had a higher risk of HER2-overexpression cancer compared to luminal A cancer. In a study conducted in the USA, higher age at first pregnancy was negatively associated with triple negative tumors; however, this variable was not analyzed according to menopausal status[22]. In addition, they reported that a high BMI was positively associated with triple negative tumors in premenopausal patients, while other studies found no association between BMI and tumor subtypes with menopausal status[21].

There is further inconsistency in the results of studies that analyzed their data without considering menopausal status[21, 22, 34, 35]. In 2008, Millikan et al. found that patients with basal like tumors tended to be; younger at menarche, had higher parity, shorter duration of breast feeding, and younger age at first pregnancy[34]. However, in 2009, Kwan et al. and Carmon et al. found a significant association between triple negative tumors and breastfeeding[21,35]. In a large pooled analysis, Yang et al. demonstrated that triple negative tumors were strongly associated with lower parity and lower age at first full-term pregnancy[22].

Our results reveal that breast cancer subtypes are associated with environmental risk factors and also with menopausal status. These findings mean that the presence of etiological heterogeneity in the occurrence of breast cancer could be the result of either the presence of distinct casual mechanisms, or a different strength of effect via the same mechanism[36,37]. Response to the treatment of breast cancer is related to the presentation of different tumor markers. Therefore, control of environmental risk factors would affect both the prevention of cancer and presentation of the tumors and required treatments. Analyses of the interaction between breast tumor markers and environmental risk factors may also shed further light on the mechanism of carcinogenesis in breast cancer. Future studies should explore potential associations between the presentation of different tumor markers and other risk factors, including modifiable risk factors like; nutrition, physical activity, and alcohol consumption.

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