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Original Article

Association of estrogen receptor-α A908G (K303R) mutation with breast cancer risk

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Abstract: Genetic mutations in premalignant breast lesions may have a role in malignancy progression or influence the behavior of subsequent disease. A point mutation in estrogen receptor-α (ER-α) as A908G (Lys303→Arg) was originally involved to hypersensitive to estrogen breast hyperplasia. We detected this mutation among Iranian women with invasive breast cancer. A population-based case-control study was conducted in 150 newly diagnosed invasive breast cancer and 147 healthy control individuals controls to screen for presence of the ER-α A908G mutation by using single-strand conformation polymorphism (SSCP) analysis and 33Pcycle DNA sequencing. We detected the 10.7% ER-α A908G mutation in the form of heterozygote genotype only among cancer patients (χ^2=22.752, *P*=0.00). The allelic frequency of mutant allele AGG in codon 303 was significantly (χ^2=29.709, *P*=0.001) higher in patients with the family history of breast cancer (28.9%) than those without the family history of breast cancer (1.9%). Our data suggest that ER-α codon 303 mutation is correlated with various aspects of breast cancer in Iran. ER-α genotype might represent a surrogate marker for predicting breast cancer developing later in life.

Keywords: Breast cancer, mutation, estrogen receptor, PCR-SSCP, lymph node metastasis

Introduction

Breast cancer is the most common cancer among women worldwide [1]. Unfortunately, during last four decades, increasing its incidence rate has made breast cancer one of the most frequent malignancies among Iranian women [2] and affects Iranian women at least one decade younger than their counterparts in developed countries [3, 4]. The principal risk factor for breast cancer is hormonal that increase exposure to estrogen [5] and a portion of this increase can be attributed to changes in reproductive patterns, such as delayed childbearing and having fewer children (increased life expectancy). However, the importance of estrogen in breast cancer development is due to the changes in estrogen signaling and expression of the two estrogen receptors (ERs) ER -α and ER-β during breast tumorigenesis and progression [6-10, 12].

Early detection of breast cancer remains an important challenge to health professionals. Mutation and polymorphism of cancer-associated genes have been found to predict tumor formation and prognosis subsequent response to treatment. The human ER-α gene exhibits low mutational frequency in breast cancer tissue [6, 11-12]. However, ER-α allelic variant has been associated with breast cancer risk [12-17], in Caucasians, with certain clinical features including presence of a family history [11] and lymph node (LN) metastasis [18] However, no association was found between common genetic variations in the ER-α gene in relation to breast cancer risk in some studies [19-21]. The suggestion that mutation of ER-α might have a role in the formation of breast cancer and subsequent response to treatment was raised by the detection of a somatic A908G or K303R mutation in the gene encoding ER-α exon 4, results in an amino acid change of Lysine to Arginine. This mutation was reported in a significant proportion of breast hyperplasia [22] and also in the majority of invasive cancers and all metastases tested [23, 24] The K303R ER-α variant apparently exhibits a hypersensitivity to estradiol [22] a characteristic that might allow breast cancers to respond to much lower levels

of estrogenic stimulation with a subsequent impact on malignant progression and the effectiveness of anti-estrogen treatment.

At present the literature contains little information regarding ER-α gene expression, mutational frequency, and allelic variants in breast cancer among Asian-Caucasians (Iranian), especially those who reside in their native country. Thus, the present study we screened a series of newly diagnosed invasive breast cancer from patients referred to Imam Khomeini Hospital Complex a population-based case-control study of breast cancer in Iranian for ER-α A908G point mutations by using a combination of single-strand conformational polymorphism (SSCP) analysis and ^{33}P-cycle DNA sequencing.

Materials and methods

Study population

After Imam Khomeini Hospital Complex the ethic committee permission letter we selected our study population; newly pathological breast cancer diagnosed breast cancer patients at the Imam Khomeini Hospital Complex (a large teaching and general hospital in the central district of Tehran) and were referred to our several clinics of the Cancer Institute (*n*=150) and mostly living in Tehran. The control group (*n*=147) included healthy women neither with any history of breast cancer nor any other neoplastic diseases, and with no family history of breast cancer diagnosed at the same clinics. Women with hysterectomy and artificial menopause or exposed to any kind of radiation and chemotherapy in their life time were excluded from the study. By the permission from the hospital ethics committee, all the patients were provided written informed consent to participate in that protocol before entering into the present study.

Demographical and epidemiological risk factor data were collected from questionnaire, including information on family history of breast cancer (first-degree relatives), age at menarche, age at menopause, ABO and Rhesus blood groups, race, age at onset, lymph node metastases, and ER expression in breast cancer tissue were administered by trained nurse interviewers. An ongoing protocol to collect and store formalin-fixed paraffin-embedded tumor tissue samples for future genomic tests had been approved by the institutional review board. Tumors were sectioned and underwent standardized histopathologic review as previously described [29]. With the hematoxylin-and-eosin-stained slide as a guide, the area of tumor was microdissected away from other surrounding nontumor tissue, and DNA lysates were prepared using extraction kit (QIAamp DNA FFPE Tissue Kit (50) # 56404). Peripheral whole blood was collected and kept until genotyping analysis. This information was obtained by interviews with patients and family members. Individuals with at least one first-degree or second-degree female relative affected by breast cancer were considered to have a family history. Women with hysterectomy and artificial menopause or exposed to any kind of radiation and chemotherapy in their life time were excluded from the study. By permission from the hospital ethics committee, all patients provided to sign the Informed Consent before entering into the present study. The clinical characteristics of the 150 breast cancer patients are shown in **Table 1**.

DNA extraction ER-α exon 4 PCR

A total of 150 breast cancer patients were screened at this stage and compared with 147 control individuals in order to identify disease-associated variants. Genomic DNA was extracted from embedded tumor samples by extraction kit (QIAamp DNA FFPE Tissue Kit (50) # 56404) in accordance with the manufacturer's instructions. Genomic DNA (50 ng) was used for each run of PCR-based genotyping.

A 329-bp fragment of 336bp of exon 4 was amplified with forward (5'-ACC TGT GTT TTC AGG GAT ACG A-3') and reverse (5'-GCT GCG CTT CGC ATT CTT AC-3') primers. Reactions were performed in 1 × PCR buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.3, 1.5 mM MgCl2, 0.001% gelatin), with 100 μM each of the four deoxyribonucleotide triphosphates, 1.25 units of AmpliTaq Gold DNA Polymerase (ABI), 0.6 μM of each primer, and 1 μl DNA lysate under the following cycle conditions: one cycle of 95°C for 5 min, 30 cycles of 95°C for 30$_S$, 65°C for 30$_S$ and 72°C for 40$_S$, and a final extension at 72°C for 6 min [18].

Screening for ER-α A908G mutation By SSCP-PCR

In order to identify mutation at codon 303 of ER -α among Iranian population, the strategy was

Table 1. Clinical characteristics of the 150 breast cancer patients in the study

Characteristics	Case	
	Frequency	*Percent*
Onset age of breast cancer (years)		
<40	48	32.0
>/=40	66	44.0
After menopause	36	24.0
Total	150	100
Lymph node metastasis		
Yes	23	15.3
No	127	84.7
Total	150	100
Type of breast cancer		
Unilateral	142	94.7
Bilateral	8	5.3
Total	150	100
Stage of breast cancer at the time of testing		
Stage II	133	88.7
Stage III	15	10.0
Stage IV	2	1.3
Total	150	100
ER expression in breast cancer tissue		
Positive	40	26.7
Negative	92	61.3
Not studied	18	12.0
Total	150	100

to screen samples by using the PCR single-strand conformation polymorphism (SSCP) method.

First-round PCR product was diluted in distilled H_2O (about 1:25) and 1 μl was used in a 20 μl SSCP-PCR reaction containing primers at 600 nM, 1 × PCR buffer, each dNTP at 150 μM except for labeled 32P-dCTP (22.5 μM dCTP and 0.2 μl), and 0.5 unit of AmpliTaq Gold DNA Polymerase (ABI). Cycling parameters were one cycle of 95°C for 4 min, 60°C for 30_S and 72°C for 40s, 30 cycles of 94°C for 30_S, 60°C for 30_S and 72°C for 1 min, and a final extension of 94°C for 1 min followed by 60°C for 8 min. The SSCP-amplified PCR product was diluted 1:50 in 0.1% SDS and 10 mM EDTA, mixed with 92% formamide and 40 mM EDTA stop dye at a 1:1 ratio, denatured, and analyzed by Optimal electrophoretic separation for SSCP was conducted in 19:1 Polyacrylamide: Bisacrylamide and 8% Polyacrylamide gel in buffer (90 mmol/l Tris-borate and 2 mmol/l EDTA) along with positive, negative, and unsaturated control samples. Geld at were runat 200 V for 2 hours followed with 250 V for 24 hours at 16°C. After electrophoresis, the bands on gel were visualized using 0.1% silver nitrate stain.

^{33}P- cycle sequencing

PCR samples exhibiting varying band shifting patterns by SSCP, were purified on agarose gel using a DNA Extraction Kit, Fermentas # K0153, Germany, were sequenced on both the forward and reverse DNA strands by using ^{33}P- cycle sequencing methods. The purified PCR products mixed with ExoSAP-IT (2 μl per 5 μl PCR product) and incubated at 37°C for 15 min. Cycle sequencing was performed with the Thermo Sequenase Radiolabeled Terminator Cycle Sequencing Kit (USB), using either primer for 30 cycles of 95°C for 30 s, 62°C for 30s, and 72°C for 1 min. Stop solution (95% formamide, 20

Table 2. Genotypic distribution frequencies of codon 303 in exon 4 mutation of estrogen receptor-α gene in the study population: breast caner versus control groups

	Normal[a]		Heterozygote[b]		Homozygote[c]		Total		Test result
Group	Frequency	Percent	Frequency	Percent	Frequency	Percent	Frequency	Percent	
Case	134	89.3	16	10.7	-	-	150	100	χ^2 =22.752 p=0.001
Control	147	100	-	-	-	-	147	100	
Total	281	94.6	16	5.4	-	-	297	100	

[a]Genotype normal, AAG/AAG, [b]Genotype heterozygote, AAG/AGG, [c]Genotype homozygote AGG/AGG.

mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol FF) was added and samples were heated to 70°C for 5 to 10 min before being run on an 8% polyacrylamide standard sequencing gel. All mutations were confirmed (and the possibility of mutation artifacts was ruled out) by sequencing of a second, separately amplified PCR product. Additionally, at least 5% of SSCP-negative samples were sequenced (*n*=46), but no mutations were found.

Automated fluorescent sequencing

Sequencing was conducted at the UNC DNA Sequencing Core Facility on a 219-bp PCR product amplified from ER-α exon 4. The PCR products were purified using QIAquick PCR purification Kit (QIAGEN cat. #28104, USA), and was cycle sequenced with fluorescently labeled Big Dye v1.1 terminators (ABI) on a 3730 DNA Analyzer (ABI) with a 48-capillary array.

Positive control

A DNA positive sample was confirmed by $_{33}$P and $_{35}$S sequencing to carry the ER-α A908G mutation from Iran pasture Institute was used as a positive control throughout the screening studies. This sample also produced a prominent band shift on SSCP and was positive for the mutation by SNaPshot. Mutant ER-α exon 4 PCR product was cloned from this control sample and several clones were sequenced for further confirmation of the presence of the mutation in this sample.

Ethical considerations

The study and signed informed consent were approved by the Ethics Committee of Research of Institute of Cancer, Imam Khomeini Hospital complex, Tehran University of Medical Sciences.

Statistical analysis

To assess the influence of mutation status on features of breast cancer, unconditional logistic regression analysis was performed using SPSS software (version 14 for Windows 7; SPSS Inc., Cary, NC, USA). To calculate odds ratios (ORs) with 95% confidence intervals (CIs) to examine the predictive association between ER-α A908G mutation and each factor on risk for breast cancer. ORs were calculated by logistic regression as implemented in the SAS software program (version 8.2; SAS Institute Inc., Cary, NC, USA). *P* values were calculated using Wald χ^2 testing. All *P* values were two sided and the $P < 0.05$ was considered as a statistically significant.

Results

The cases and controls characteristics

For the present study, a total of 150 (median age 47.49 ± 11.43 years) invasive breast cancer cases and 147 (median age 40.75 ± 10.54 years) healthy women were screened for mutation in a 329 bp region of exon 4 including codon 303 of ER-α gene.

Some clinical characteristics of cases evaluated for the ER-α mutation have been reported before [30]. All cases were diagnosed with invasive ductal carcinoma. The majority (76%) of cases had onset of breast cancer before menopause and unilateral breast cancer (94.7%). Among cases, 84% were lymph node metastasis negative, 88.7% were at stage II, 61.3% were ER- negative (**Table 1**). Of the 150 breast cancer cases screened 16 (10.7%) were positive for the ER-α A809G mutation, with heterozygote genotype (AAG/AGG), consequently all controls (147) were negative for the same mutation (χ^2=22.752, *P*=0.001) (**Table 2**). The observed numbers of individuals with different genotypes

Table 3. Genotypic distribution frequencies of codon 303 in exon 4 mutation of estrogen receptor-α gene and selected demographic characteristics and major risk factors in the study population: breast cancer versus control groups

Characteristic	Group	Normal[a]		Heterozygote[b]		Test result
		Frequency	Percent	Frequency	Percent	
Age at menarche (years)						
</=12	Case	51	85.0	9	15.0	χ^2=9.012
	control	36	100	-	-	*P*=0.003
	total	87	90.6	9	9.4	
>12	Case	83	92.2	7	7.8	χ^2=11.561
	control	111	100	-	-	*P*=0.001
	total	194	96.5	7	3.5	
Total	Case	134	89.3	16	10.7	
	control	147	100	-	-	
	total	281	94.6	16	5.4	
ABO blood groups						
A	Case	24	88.9	3	11.1	χ^2=5.932
	control	43	100	-	-	*P*=0.015
	total	67	95.7	3	4.3	
B	Case	10	71.4	4	28.6	χ^2=10.957
	control	35	100	-	-	*P*=0.001
	total	45	91.8	4	8.2	
AB	Case	6	100	-	-	
	control	16	100	-	-	-
	total	22	100	-	-	
O	Case	94	91.3	9	8.7	χ^2=7.753
	control	53	100	-	-	*P*=0.005
	total	147	94.2	9	5.8	
Total	Case	134	89.3	16	10.7	
	control	147	100	-	-	
	total	281	94.6	16	5.4	
Race						
Arab-Armani	Case	3	100	-	-	
	control	-	-	-	-	-
	total	3	100	-	-	
Fars	Case	50	83.3	10	16.7	χ^2=19.134
	control	88	100	-	-	*P*=0.001
	total	138	93.2	10	6.8	
Lor – Kurdish	Case	16	88.9	2	11.1	χ^2=1.701
	control	9	100	-	-	*P*=0.192
	total	25	92.6	2	7.4	
Turkish	Case	44	95.7	2	4.3	χ^2=2.497
	control	39	100	-	-	*P*=0.114
	total	83	97.6	2	2.4	
Gilaki-Mazani	Case	21	91.3	2	8.7	χ^2=1.623
	control	11	100	-	-	*P*=0.203
	total	32	94.1	2	5.9	
Total	Case	134	89.3	16	10.7	
	control	147	100	-	-	
	total	281	94.6	16	5.4	

[a]Genotype normal, AAG/AAG, [b]Genotype heterozygote, AAG/AGG

showed this mutation fitted the Hardy–Weinberg equilibrium for ($P > 0.05$).

Risk factors for breast cancer according to ESR1 A908G mutation status

This mutation was observed only in heterozygote genotype in breast cancer patients but not in healthy individuals. The genotypic distribution frequencies of mutation of estrogen receptor- α gene codon 303 and major risk factors in the breast cancer versus control groups are shown in **Table 3**. The statistically significant frequencies were achieved only for risk factors, age at

Table 4. Genotypic frequencies of codon 303 in exon 4 mutation of estrogen receptor-α gene and selected demographic characteristics and major risk factors in the breast cancer group

Characteristic	Normal[a]		Heterozygote[b]		Test result
	Frequency	Percent	Frequency	Percent	
Onset age of breast cancer (years)					
<40	43	89.6	5	10.4	χ^2=0.005
>/=40	91	89.2	11	10.8	*P*=0.946
Total	134	89.3	16	10.7	
Family history of breast cancer					
First-degree family affected	8	42.1	11	57.9	c^2=33.518
Not affected	126	96.2	5	3.8	*P*=0.001
Total	134	89.3	16	10.7	
Lymph node metastases					
Yes	23	100	-	-	χ^2=5.662
No	111	87.4	16	12.6	*P*=0.017
Total	134	89.3-	16	10.7	
ER expression in breast cancer tissue					
Positive	34	85.0	6	15.0	χ^2=4.6
Negative	86	93.5	6	6.5	*P*=0.1
Not studied	14	77.8	4	22.2	
Total	134	89.3	16	10.7	

[a]Genotype normal, AAG/AAG, [b]Genotype heterozygote, AAG/AGG

menarche 12 and below 12- years- old, among different races, Fars, who inhabit the near southern region of Iran and A, B and O blood groups, in ABO blood groups, (χ^2=9.012, *P*=0.003; χ^2=19.134, *P*=0.001; (χ^2=5.932, *P*=0.015; χ^2=10.957 *P*=0.001 and χ^2=7.753, *P*=0.005, respectively).

Table 4 presents the genotypic distribution frequencies of codon 303 mutation and major potential risk factor in breast cancer cases. Among all these risk factors only family history of breast cancer and lymph node metastases presented statistically significant differences (*P*<0.05) between different genotypes (normal and heterozygote genotypes) (*P*=0.001, χ^2 =33.518; χ^2 =5.662, *P*=0.017 respectively).

The genotypic and allelic frequencies within the group studied, are shown in **Table 5**. The heterozygote genotype (AAG/AGG) was found only among cancer patients (10.7%) (χ^2=16.573, *P*=0.001). As a result, the allele 1 (AGG) in codon 303 was presented only in cancer patients (5.3%), (χ^2=16.114, *P* = 0.001). The allelic frequency of allele 1 (AGG) in codon 303 was significantly (χ^2 = 29.709, *P* = 0.001) much higher (fourteen- fold) in the cancer patients with a family history of breast cancer (28.9%) than those without family history of breast cancer (1.9%).

When we consider the effects of different genotypes on developing breast cancer, the frequencies distribution for genotypes heterozygote was (χ^2=22.752, *P*=0.001) observed in only cases (100.0%). However, the estimated risk was higher in cases (47.7%) than in controls (52.3%) for normal genotype in codon 303, but corresponding heterozygote genotypes are found only in breast cancer patients (**Table 6**).

Genotype frequencies exhibited different distributions in the presence and absence of first-degree family history of breast cancer, with statistical significance for codon 303 mutation (*P* = 0.001). The estimated risk was higher for normal genotype patients without family history of breast cancer (94.0%) than patients with family history of breast cancer (6.0%) but the esti-

Table 5. Allelic frequencies of estrogen receptor-α exon 4 mutation at codon 303 (AAG→AGG) in the study population: breast cancer cases versus control groups and breast cancer cases in the presence versus the absence of major risk factors

Characteristic		ER-α Alleles	
		0[c]	1[d]
Breast cancer			
Case	(n=150)	284(94.7%)	16(5.3%)
Control	(n=147)	294(100%)	-
		χ^2=16.114, *P*=0.001	
Age at menarche at (years)			
</=12	(n=60)	111(92.5%)	9(7.5%)
>12	(n=90)	173(96.1%)	7(3.9%)
		χ^2=1.86, *P*=0.173	
Onset age of breast cancer			
</=40	(n=48)	91(94.8%)	5(5.2%)
>40	(n=66)	126(95.5%)	6(4.5%)
After menopause	(n=36)	67(93.1%)	5(6.9%)
		χ^2=0.535, *P*=0.765	
ABO blood groups			
A	(n=27)	51(94.4%)	3(5.6%)
B	(n=14)	24(85.7%)	4(14.3%)
AB	(n=6)	12(100%)	-
O	(n=103)	197(95.6%)	9(4.4%)
		χ^2=4.838, *P*=0.184	
Arab & Armani	(n=3)	6(100%)	
Fars	(n=60)	110(91.7%)	10(8.3%)
Lor & Kurdish	(n=18)	34(94.4%)	2(5.6%)
Turkish	(n=46)	90(97.8%)	2(2.2%)
Gilaki & Mazani	(n=23)	44(95.7%)	2(4.3%)
		χ^2=4.916, *P*=0.296	
Family history of breast cancer			
First-degree family affected	(n=19)	27(71.1%)	11(28.9%)
Not affected	(n=131)	257(98.1%)	5(1.9%)
		χ^2=29.709, *P*=0.001	
Other cancer affected status			
Yes	(n=3)	6(100%)	-
No	(n=147)	278(94.6%)	16(5.4%)
		χ^2=0.665, *P*=0.415	
Lymph node metastases			
Yes	(n=23)	46(100%)	-
No	(n=127)	238(93.7%)	16(6.3%)
		χ^2=5.487, *P*=0.091	
ER expression in breast cancer tissue			
Positive	(n=40)	74(92.5%)	6(7.5%)
Negative	(n=92)	178(96.7)	6(3.3%)
Not studied	(n=18)	32(88.9%)	4(11.1%)
		χ^2=4.312, *P*=0.116	

[a]Genotype normal, AAG/AAG, [b]Genotype heterozygote, AAG/AGG, [e]Allele 0, AAG, [d]Allele 1, AGG

mated risk was lower (more than twofold) for heterozygote individuals in codon 303 mutation, without family history of breast cancer (31.2%) than individuals with family history of breast cancer (68.8%) (OR 0.029, 95% CI 0.008-0.103). Furthermore, statistical significance

Table 6. Genotypic distribution frequencies of codon 303 in exon 4 of estrogen receptor-α gene breast cancer in the study population: breast caner versus control groups

Codon 303	Yes		No		Total		Test result
	Frequency	Percent	Frequency	Percent	Frequency	Percent	
Normal[a]	134	47.7	147	52.3	281	100	χ^2=22.752 *P*=0.001
Heterozygote[b]	16	100	-	-	16	100	
Homozygote[c]	-	-	-	-	-	-	
Total	150	50.5	147	49.5	297	100	

[a]Genotype normal, AAG/AAG, [b]Genotype heterozygote, AAG/AGG, [c]Genotype homozygote AGG/AGG

Table 7. Estimated risk for selected demographic characteristics and major risk factors with estrogen receptor-α exon 4 mutation at codon 303 in different genotypes

Genotype		Yes n=150	No n=147	*P* value	OR (95% CI)
Normal [a]		134(47.7%)	147(52.3%)	0.001	1.0(reference)
Heterozygote [b]		16(100%)	-		-
	First- degree family history of breast cancer				
Genotype		Affected n=19	Not affected n=131	*P* value	OR (95% CI)
Normal		8(6.0%)	126(94.0%)	0.001	1.0(reference)
Heterozygote		11(68.8%)	5(31.3%)		0.029(0.008-0.103)
	Lymph node metastases				
Genotype		Yes n=23	No n=127	*P* value	OR (95% CI)
Normal		23(17.2%)	111(82.8%)	0.017	1.0(reference)
Heterozygote		-	16(100%)		-

[a]Genotype normal, AAG/AAG, [b]Genotype heterozygote, AAG/AGG.

was achieved in the presence and absence of LN metastases for the same codon (*P* = 0.017). The estimated risk was fivefold higher for normal patients (for codon 303) without LN metastases, but all heterozygote patients for mutation at codon 303 were LN metastases- negative (**Table 7**).

Discussion

Evidence suggests that breast cancer is characterized by unique gene expression profiles, hormonal or reproductive factors that increase exposure to estrogen as well-documented molecular or protein markers, and as exhibit variable of clinical behavior, prognosis, and response to therapies [27, 31-40]. The amount of ER-α expression in most breast cancer is fundamental to our understanding of this disease and its treatment. The observations that ER-α is over expressed in a proportion of premalignant lesions and is possibly related to an increased risk of progression further raise the importance of estrogenic activity in the establishment and behavior of breast carcinoma [41-43].

The principal risk factors for breast cancer are hormonal or reproductive factors that increase exposure to estrogen as well-documented [25-34]. The importance of estrogen in breast cancer development is further supported by studies demonstrating the occurrence of marked changes in estrogen signaling and expression of the two estrogen receptors (ERs) ER-α and ER-β during breast tumorigenesis and progression [6-10, 35-38].

Data obtained from some epidemiologic studies of breast cancer suggest that tumor subsets classified according to certain somatic or protein expression changes may be associated with specific etiologic risk factors [12, 14-17, 30, 39-

43]. Consistent with this, our study has revealed that first-degree family history of breast cancer may be a risk factor for breast tumors carrying the *ER*- α gene A908G mutation. Consequently, the allelic frequency of mutant allele (AGG) in codon 303 was significantly (χ^2 = 29.709, *P* = 0.001) also very higher (fourteen fold) in the cancer patients with the family history of breast cancer (28.9%) than those without family history of breast cancer (1.9%).

Our results confirm the presence of the *ER*- α gene A908G mutation among Iranian women invasive breast cancer. However, the frequency of A908G or K303R mutation was greater (10.7%) in comparison with the literature, which indicates that this mutations *ER*- α gene K303R occur at lower frequency in breast tumor (around 6%) [6, 44].

In our first evaluation of association of the *ER*- α gene A908G mutation with demographic and clinical characteristics of breast cancer cases among Iranian, such as age at menarche below 12 years old in compression with age at menarche above 12 years old, from ABO blood groups, groups of B and O and among all eight different races race of Fars (is located the southern part of Iran), were observed only in heterozygote cancer patients with a statistically significant frequencies in heterozygote cancer patients.

The estimated risk was very much higher for normal genotype individuals without family history of breast cancer (94.0%) than individuals with family history of breast cancer (6.0%) but the estimated risk was very lower heterozygote patients in codon 303 mutation, without family history of breast cancer (31.2%) than patients with family history of breast cancer (68.8%) (OR 0.029, 95% CI 0.008-0.103). Compared with controls, Iranian breast cancer cases with *ER*- α A908G mutation-positive tumors were more likely to have a first-degree family history of breast cancer whereas the mutation-negative cases were not; this finding was supported by case-case comparisons [45].

Finally, taking these results together, it was noted that: 1) The presence of the ER-α A908G point mutation in invasive breast tumors may have important implications for etiology, prognosis and directly association for increasing risk of developing breast cancer, even in heterozygote genotype; 2) the cancer patients with the family history of breast cancer are more likely to reveal this point mutation (A→G) than those without family history of breast cancer, and also; 3) the greater the frequency of mutant allele, the lesser the likelihood of LN metastasis in Iranian population. Small but statistically significant correlations were found between allelic distribution and familial manifestation of breast cancer. Because of the limited sample size in the present study, our finding of a correlation between LN metastasis and mutant allele of codon 303 will require further confirmation. This is planned as part of our future work, because mutation determination from peripheral blood represents a highly feasible and noninvasive option for preoperative evaluation.

Conclusion

This is the first study focusing on a comprehensive breast cancer genetic mutation in Iran. Characterization of breast tumors for the *ER*- α A908G point mutation, shown by both Fuqua and Conway [23, 45] to be hypersensitive to estrogen, may reveal important etiologic clues. Furthermore, *ER*- α A908G mutation-positive breast cancer was significantly associated with a first-degree family history of breast cancer suggesting that this mutation is associated with familial breast cancer. Some reproductive factors linked to greater exposure to endogenous hormones, including younger age at menarche was associated with the mutation-negative subgroup, suggesting that endogenous hormonal factors may be more important for mutation-negative cancer. Furthermore, additional studies are required to confirm these findings.

Abbreviations

BMI = Body Mass Index; CI = confidence interval; ESR = estrogen receptor; LN = lymph node; OR = odd ratio; PCR = polymerase chain reaction; Rh = Rhesus blood group system; SNP = single nucleotide polymorphism; SSCP = single-strand conformational polymorphism.

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