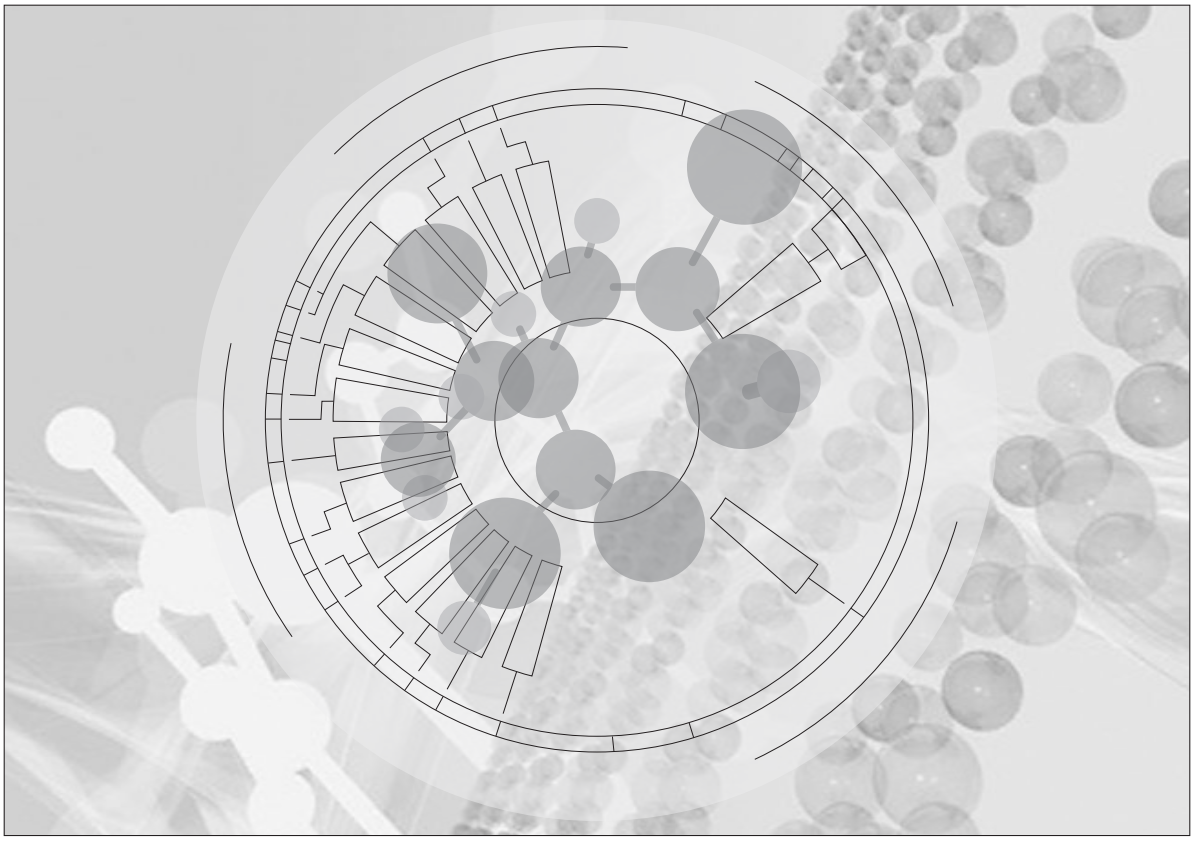

Trends in Molecular Sciences

ISSN 1994-5469

science alert

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Trends in Molecular Sciences 1 (1): 1-10, 2009
ISSN 1994-5469

Estrogen Receptor-α Gene Codon 10 (T392C) Polymorphism in Iranian Women with Breast Cancer: A Case Study

[1,2]S. Abbasi, [3]C. Azimi, [4]F. Othman, [5]M.R. Noori Daloii, [5]Z.O. Ashtiani,
[5]M. Mojarrad, [5]S.A. Oskouei, [5]F.M. Nejad and [1]P. Ismail
[1]Department of Biomedical Science,
Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Malaysia
[2]Department of Medical Laboratory Sciences, Faculty of Allied Medicine,
Tehran University of Medical Sciences, Tehran, Iran
[3]Department of Genetics, Cancer Institute, School of Medicine,
Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran
[4]Department of Biomedical Science, Faculty of Medicine and Health Sciences,
Universiti Putra Malaysia, Malaysia
[5]Department of Medical Genetics, School of Medicine,
Medical Sciences/University of Tehran, Tehran, Iran

Abstract: A case study was conducted to establish a database of polymorphisms in Iranian population in order to compare Western and Iranian (Middle East) distributions and to evaluate ESR1 polymorphism as an indicator of clinical outcome. The *ESR1* gene was scanned in Iranian patients newly diagnosed invasive breast tumors, (150 patients) and in healthy individuals (147 healthy control individuals). PCR single-strand conformation polymorphism methodology and direct sequencing were performed. The silent single nucleotide polymorphism (SNPs) was performed, as reported previously in other studies, but at significantly different frequencies, with further increasing predictive accuracy in Iranian population. Data suggest that ESR1 polymorphisms are correlated with various aspects of breast cancer in Iranian *ESR1* genotype, as determined during pre-surgical evaluation, might represent a surrogate marker for predicting breast cancer.

Key words: Estrogen receptor polymorphisms, LN metastases, SSCP-PCR

INTRODUCTION

Breast cancer is the most common malignancy among women in Iran and is also the number one female cancer, with more than 7000 new diagnosed in each year. Unfortunately, the current criteria can only help 60% of women with breast cancer in diagnosis and a long-term treatment. Breast cancer accounted for 25% of all female cancers (Behjati *et al*., 2005). Although breast cancer at one of the lowest incidence rates in Iran as compared to that in other Asian countries, but during last four decades, increasing its incidence rate has made breast cancer one of the most frequent malignancies among Iranian women (Behjati *et al*., 2005). Breast cancer affects Iranian women at least one decade younger than their counterparts in developed countries (Harirchi *et al*., 2000; Lin *et al*., 2008). The mortality rate of breast cancer was about 6 per 100,000 women in Tehran in 1998 (Mousavi *et al*., 2007), 2.5 per 100,000 for female population and 7762 life lost in the 18 provinces of Iran in 2001 (Najafi *et al*., 2005). The present clinical-histological parameters, however, can ouly help 60% of patients with breast cancer to achieve long-term disease-free status (Bertucci *et al*., 2002). The genetic

Corresponding Author: Patimah Ismail, Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Malaysia

markers both at the level of single genes, such as oncogenes and tumor suppressor genes, as well as that of chromosomes can, therefore, be of much significance in improving the diagnosis and prognosis of breast cancer patients (Montazeri *et al*., 2003).

The biological effect of estrogens such as stimulating growth and differentiation of normal mammary tissue is mediated primarily through high-affinity binding to ESRs (Roodi *et al*., 1995). There are two types of *ESRs*, *ESR1* (*ESR-α*) and *ESR-2*(*ESR-β*). The *ESR1* gene is localized on chromosome 6q25.1 and the *ESR-2* gene is localized on chromosome 14q22-24 (Enmark *et al*., 1997; Shin *et al*., 2003). Genetic factors such as *ER* genes polymorphisms also considered before as an effective risk factor with positive effects (Vasconcelos *et al*., 2002; Heldring *et al*., 2007; Wang *et al*., 2007; Holst *et al*., 2007) and negative effects (Slattery *et al*., 2007; Gonzalez-Zuloeta Ladd *et al*., 2008; Einarsdóttir *et al*., 2008) in the different studies.

Asian-Americans had traditionally the lowest risk for breast cancer in the USA, although the difference is diminished over a couple of generations (Brinton *et al*., 2002). Comparison of incidence-age curves for breast cancer in Asian and Western populations in their native countries reveals an additional interesting difference. Breast cancer onset age distributions for East Asian groups show the age range of 40-50 years, contrasting with the continued increasing incidence beyond age of 50 years in Western women. In Iran too, breast cancer patients are relatively younger than their Western counterparts. The similar and apparently unique manifestation of breast cancer in genetically similar but geographically separated Middle East groups suggests the involvement of an unusual genetic factor (Hsiao *et al*., 2004).

The association of genetic polymorphisms in the ESR-genes and the risk of diseases, including breast cancer, have been the subject of increasing interest. Several DNA sequence variations in the ESR-gene have been reported by Brinton *et al*. (2002) and Roodi *et al*. (1995).

At present the literature contains little information regarding ESR1 gene expression, mutational frequency and allelic variants in breast cancer among Asians and Middle East, especially those who reside in their native country. Thus, the present study examined ESR1 polymorphisms in Iranian breast cancer patients in order to establish a genetic polymorphism database for the ESR1 encoding region of the Iranian, (Asian Caucasian in Middle East) women, to compare this distribution with that reported for Western and Eastern study groups and to test for any correlation between ESR1 polymorphisms and breast cancer risk among Iranian women.

MATERIALS AND METHODS

Study Population

A case study was conducted from April 2004 to September 2007 in Tehran, Iran. The breast cancer patients (n = 150; median age 47.49±11.43 years) were newly diagnosed and mostly living in Tehran. They were entered into the study if they had a confirmed pathological breast cancer diagnosis at the Imam Khomeini Hospital Complex (a large teaching and general hospital in the central district of Tehran) and were referred to our several breast surgery clinics of the Cancer Institute. The control group (n = 147; median age 40.75±10.54 years) included healthy women neither with any history of breast cancer nor any other neoplastic diseases and also none of their relatives had a history of breast cancer. 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 provided with written informed consent to participate in that protocol before entering into the present study.

Demographical and risk factor data were collected using a short structured questionnaire, during survey, including information on age, weight, height, race, religion, marital status, number of pregnancies and children, age at the first child birth, average lactation term, family history of breast cancer (first-degree relatives), age at menarche, age at marriage, parity, age at first pregnancy,

menopausal status and age at menopause, blood groups, race, age at onset, lymph node metastases, cancer stage at the time of testing and ER expression I breast cancer tissue. An ongoing protocol to collect and store blood samples for future genomic tests has been approved by the institutional review board. Peripheral whole blood was collected and stored at -80°C until genotyping analysis. This information was obtained by interview with patients and family members.

Screening for ESR1 Variants by Single Strand Conformation Polymorphism Analysis

In order to identify any mutation or variant sites in the Iranian population, the strategy was to screen initial samples for the entire coding region of *ESR1* using the PCR Single-Strand Conformation Polymorphism (SSCP) method. 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/ mutations. Genomic DNA was extracted from whole blood cells using DNG™-Plus extraction solution kit (Cinnagen Inc., Tehran, Iran) according to with the manufacturer's instructions. Genomic DNA (50 ng) was used for each run of PCR-based genotyping.

Exon 1 of the *ESR-α* gene was amplified by PCR methods, using set of primers according to the oligonucleotide sequences by Hsiao *et al*. (2004):

Forward primer 5'- GGTTTCTGAGCCTTCTGCCCTG -3' (301-322)
Reverse primer 5'- AGGCCGGTCTGACCGTAGA -3'(593-575)

PCR was performed for 30 cycles of 30 sec at 95°C, 30 sec at 58°C and 40 sec at 72°C. Optimal electrophoretic separation for SSCP was conducted in 8% polyacrylamide gel (19:1 Acrylamide: Bisacrylamide) in buffer (90 mmol L^{-1} Tris-borate and 2 mmol/l EDTA) at 200 V for 2 h followed with 250 V for 24 h at 16°C. After electrophoresis, the bands on gel were visualized using 0.1% silver nitrate stain. PCR samples exhibiting varying band shifting patterns as the result of first sequencing with forward primer, re-purified on agarose gel using a DNA Extraction Kit , Fermentas # K0153, Germany and directly sequenced by big dye Terminator V3.1 Cycle Sequencing kit protocol' (Applied Biosystem Kit, Microgen Co.,USA), on a sequencer ABI 3130XL (16 capillaries).

The PCR products purification method was used in order to confirm sequencing by reverse primer. The PCR products were purified using QIAquick PCR purification Kit (QIAGEN cat. No. 28104, USA).

Statistical Analysis

χ^2 testing was employed to assess the influence of polymorphism status on features of breast cancer. Unconditional logistic regression analysis was performed using SPSS software (version 11.5 for Windows XP; SPSS Inc., Cary, NC, USA) to calculate odds ratios (ORs) with 95% confidence intervals (CIs) and to examine the predictive effect of each factor on risk for breast cancer. $p<0.05$ was considered as a statistically significant.

RESULTS

Allelic frequencies of exon 1 in the *ESR1* gene among 297 Iranian women (150 breast cancer patients and 147 healthy control individnals) was screened for mutation or variant sites of single nucleotide polymorphisms (SNPs) by PCR-SSCP and DNA sequencing. The observed numbers of individnals with different genotypes showed that SNP fitted the Hardy-Weinberg equilibrium for both control and patient groups ($p>0.05$) (Table 1).

Table 2 represents frequencies distribution of selected demographic characteristics and major risk factors such as BMI, age at menarche race, blood groups and Rh in the study population comprising between breast cancer and control groups. All these characteristics with different frequencies distribution between breast cancer and control groups were statistically significant ($p<0.05$).

Table 1: The distributions of selected demographic characteristics and major risk factors for breast cancer of whole study population: Breast cancer versus control groups

Characteristics	Case	Control
	No. (%)	
Age (years)		
</=40	52(41.3)	98(57.3)
>40	74(58.7)	73(42.7)
BMI (kg m^{-2})		
</=18.5 (underweight)	5(3.3)	9(6.1)
18.6-24.9 (normal)	57(38.0)	90(61.2)
25.0-29.9 (overweight)	55(36.7)	35(23.8)
>30.0 (obese)	33(22.0)	13(8.9)
Profession		
Housewife	129(86.0)	27(18.3)
Student	2(1.3)	32(21.8)
Others	19(12.7)	88(59.9)
Religion		
Moslem	148(98.7)	146(99.3)
Non-Moslem	2(1.3)	1(0.7)
Age at menarche (years)		
</=12	60(40.0)	36(24.5)
>12	90(60.0)	111(75.5)
No. of deliveries in married individuals		
0	6(4.3)	5(5.1)
1	9(6.4)	37(37.4)
2	21(15.0)	29(29.2)
>/=3	104(74.3)	28(28.3)
No. of children in married individuals		
0	6(4.3)	5(5.1)
1	10(7.1)	38(38.4)
2	30(21.4)	31(31.3)
>/=3	94(67.2)	25(25.2)
Menopause status		
Yes	59(39.3)	18(12.2)
No	91(60.7)	129(87.8)
Age at menopause (years)		
</=50	47(79.7)	11(61.1)
> 50	12(20.3)	7(38.9)
Race		
Arbs and Armani	3(2.0)	-
Fars	60(40.0)	88(59.9)
Lor and Kurdish	18(12.0)	9(6.1)
Turkish	46(30.7)	39(26.5)
Gilaki and Mazani	23(15.3)	11(7.5)
ABO and Rh blood groups		
A^+	27(18.0)	39(26.5)
B^+	12(8.0)	31(21.1)
AB^+	6(4.0)	15(10.2)
O^+	100(66.7)	47(32.0)
A^-	-	4(2.7)
B^-	2(1.3)	4(2.7)
AB^-	-	1(0.7)
O^-	3(2.0)	6(4.1)
ABO blood groups		
A	27(18.0)	43(29.2)
B	14(9.3)	35(23.8)
AB	6(4.0)	16(10.9)
O	103(68.7)	53(36.1)
Family history of breast cancer		
First-degree family affected	19(12.7)	-
Not affected	131(87.3)	147(100.0)

Table 1: Continued

Characteristics	Case	Control
	No. (%)	
First-degree family history of breast cancer		
Mother	8(42.1)	-
Sister	6(31.6)	-
Daughter	4(21.0)	-
Mother and sister	1(5.3)	-
Lymph node metastases		
Yes	23(15.3)	-
No	127(84.7)	-

Table 2: Frequencies distribution of selected demographic characteristics and major risk factors in the study population: Breast cancer versus control groups

	Case		Control		Total		
Characteristics	Frequency	Percent	Frequency	Percent	Frequency	Percent	Test result
Age (years)							
</=40	52	41.3	98	57.3	150	50.5	$\chi^2 = 7.417$
>40	74	58.7	73	42.7	147	49.5	$p = 0.006$
Total	126	100.0	171	100.0	297	100.0	
BMI (kg m^{-2})							
</=18.5 (underweight)	5	35.7	9	64.3	14	100.0	$\chi^2 = 21.663$
18.6-24.9 (normal)	57	38.8	90	61.2	147	100.0	$p = 0.001$
25-29.9 (overweight)	55	61.1	35	38.9	90	100.0	
>30 (obese)	33	71.7	13	28.3	46	100.0	
Total	150	50.5	147	49.5	297	100.0	
Profession							
Housewife	129	86.0	27	18.3	156	52.5	$\chi^2 = 137.642$
Student	2	1.3	32	21.8	34	11.5	$p = 0.001$
Others	19	12.7	88	59.9	107	36.0	
Total	150	100.0	147	100.0	297	100.0	
Age at menarche (years)							
</=12	60	40.0	36	24.5	96	33.3	$\chi^2 = 8.165$
>12	90	60.0	111	75.5	201	67.7	$p = 0.004$
Total	150	100.0	147	100.0	297	100.0	
Marital status							
Married	140	93.3	99	67.3	239	80.5	$\chi^2 = 11.992$
Single	10	6.7	48	32.7	58	19.5	$p = 0.001$
Total	150	100.0	147	100.0	297	100.0	
Age at marriage (years)							
</=20	92	65.7	40	40.4	132	55.2	$\chi^2 = 14.962$
>20	48	34.3	59	59.6	107	46.8	$p = 0.001$
Total	140	100.0	99	100.0	239	100.0	
No. of delivery (married individuals)							
0	6	4.3	5	5.1	11	4.6	$\chi^2 = 41.493$
1	9	6.4	37	37.4	46	19.2	$p = 0.001$
2	21	15.0	29	29.2	50	20.9	
>/=3	104	74.3	28	28.3	132	55.2	
Total	140	100.0	99	100.0	239	100.0	
No. of children							
0	6	4.3	5	5.1	11	4.6	$\chi^2 = 38.285$
1	10	7.1	38	38.4	48	20.1	$p = 0.001$
2	30	21.4	31	31.3	61	25.5	
>/=3	94	67.2	25	25.2	119	49.8	
Total	140	100.0	99	100.0	239	100.0	
Menopause status							
Yes	59	39.3	18	12.2	77	25.9	$\chi^2 = 28.367$
No	91	60.7	129	87.8	220	74.1	$p = 0.001$
Total	150	100.0	147	100.0	297	100.0	

Table 2: Continued

Characteristics	Case Frequency	Case Percent	Control Frequency	Control Percent	Total Frequency	Total Percent	Test result
Race							
Arab and Armani	3	2.0	-	-	3	1.0	$\chi^2 = 7.351$
Fars	60	40.0	88	59.9	148	49.8	$p = 0.007$
Lor and Kurdish	18	12.0	9	6.1	27	9.1	
Turkish	46	30.7	39	26.5	85	28.6	
Gilaki and Mazani	23	15.3	11	7.5	34	11.4	
Total	150	100.0	147	100.0	297	100.0	
ABO and Rh blood groups							
A^+	27	18.0	39	26.5	66	22.2	$\chi^2 = 25.144$
B^+	12	8.0	31	21.1	43	14.5	$p = 0.023$
AB^+	6	4.0	15	10.2	21	7.1	
O^+	100	66.7	47	32.0	147	49.5	
A^-	-	-	4	2.7	4	1.4	
B^-	2	1.3	4	2.7	6	2.0	
AB^-	-	-	1	0.7	1	0.3	
O^-	3	2.0	6	4.1	9	3.0	
Total	150	100.0	147	100.0	297	100.0	
ABO blood groups							
A	27	18.0	43	29.2	70	23.6	$\chi^2 = 33.201$
B	14	9.3	35	23.8	49	16.5	$p = 0.001$
AB	6	4.0	16	10.9	22	7.4	
O	103	68.7	53	36.1	156	52.5	
Total	150	100.0	147	100.0	297	100.0	
Family history of breast cancer							
First-degree family effected	19	12.7	-	-	19	6.4	$\chi^2 = 19.893$
Not effected	131	87.3	147	100.0	278	93.6	$p = 0.001$
Total	150	100.0	147	100.0	297	100.0	
Family history of breast cancer							
Mother	8	5.3	-	-	8	2.7	$\chi^2 = 27.231$
Sister	6	4.0	-	-	6	2.0	$p = 0.001$
Daughter	4	2.7	-	-	4	1.3	
Mother and sister	1	0.7	-	-	1	0.3	
Not effecter	131	87.3	147	100.0	278	93.7	
Total	150	100.0	147	100.0	297	100.0	

Table 3: Genotypic and allelic frequencies of estrogen receptor-α exon 1, codon 10 (TCT/TCC) in the study population: Breast cancer versus control groups and breast cancer cases in the presence versus the absence of major risk factors

Characteristic		ER-α genotypes 00[a]	01[b]	11[c]	ER-α alleles 0[d]	1[e]
Breast cancer						
Case	(n = 150)	26(17.3%)	111(74.0%)	13(8.7%)	163(54.3%)	137(45.7%)
Control	(n = 147)	32(21.8%)	113(76.9%)	2(1.3%)	177(60.2%)	117(39.8%)
Test result		$\chi^2 = 8.67$, $p = 0.013$			$\chi^2 = 2.091$, $p = 0.148$	
Family history of breast cancer						
First-degree family affected	(n = 19)	2(10.5%)	4(21.1%)	13(68.4%)	8(21.1%)	30(78.9%)
Not effected	(n = 131)	24(18.3%)	107(81.7%)	-	155(59.2%)	107(40.8%)
Test result		$\chi^2 = 94.423$, $p = 0.001$			$\chi^2 = 19.42$, $p = 0.001$	
Lymph node metastases						
Yes	(n = 23)	2(8.7%)	15(65.2%)	6(26.1%)	19(41.3%)	27(58.7%)
No	(n = 127)	24(18.9%)	96(75.6%)	7(5.5%)	144(56.7%)	110(43.3%)
Test result		$\chi^2 = 8.568$, $p = 0.014$			$\chi^2 = 3.72$, $p = 0.054$	

[a]Genotype 00 (normal), TCT/TCT; [b]Genotype 01 (heterozygote); TCT/TCC, [c]Genotype 11 (homozygote), TCC/TCC; [d]Allele 0, TCT; [e]Allele 1, TCC

The results showed novel mutations but it did reveal the presence, in the Iranian population studied, a silent common Single Nucleotide Polymorphisms (SNP) rs2077647 (dbSNP128), in codon 10. The genotypic and allelic frequencies within the population studied comprising between breast cancer and control groups are shown in Table 3. The frequency of allele 1 in codon 10 (TCT→TCC) (T/C, S392S), was higher in cancer patients (about 50%) than in control individuals (about 40%);

Table 4: Estimated risk for selected demographic characteristic and major risk factors with estrogen receptor-α exon 1, codon 10 in different genotypes

Characteristic			p-value	OR (95% CI)
Breast cancer	Yes (n = 150)	No (n = 147)		
Normal[a]	26(44.8%)	32(55.2%)	0.013	1.0 (reference)
Heterozygote[b]	111(49.6%)	113(50.4%)		0.826 (0.463-1.477)
Homozygote[c]	13(86.7%)	2(13.3%)		0.148 (0.30-0.727)
Age at menarche (years)	</=12 (n = 60)	>12 (n = 90)	p-value	OR (95% CI)
Normal	9(34.6%)	17(65.4%)	0.973	1.0 (reference)
Heterozygote	46(41.4%)	65(58.6%)		0.748 (0.307-1.825)
Homozygote	5(38.5%)	8(61.5%)		0.847 (0.213-3.363)
First-degree family history of breast cancer	Affected (n = 19)	Not affected (n = 131)	p-value	OR (95% CI)
Normal	2(7.7%)	24(92.3%)	0.005	1.0 (reference)
Heterozygote	4(3.6%)	107(96.4%)		2.229 (0.386-12.881)
Homozygote	13(100%)	-		-
Lymph node metastases	Yes (n = 23)	No (n = 127)	p-value	OR (95% CI)
Normal	2(7.7%)	24(92.3%)	0.001	1.0 (reference)
Heterozygote	15(13.5%)	96(86.5%)		0.533 (0.114-2.492)
Homozygote	6(46.2%)	7(53.8%)		0.097 (0.016-0.593)

[a]Genotype normal or 00, TCT/TCT; [b]Genotype heterozygote or 01, TCT/TCC; [c]Genotype homozygote or 11; TCC/TCC

although the difference was not statistically significant (p = 0.148). For risk factor, first-degree family affected breast cancer, the frequency of allele 1 in codon 10 (TCT→TCC) was significantly (p = 0.001) two fold higher in cancer patients with family history (approximately 80%) than in those without family history (about 40%). Those samples with SNPs results from first sequencing with sense primer were performed for re-sequencing with anti-sense primer.

ER-α genotypes were compared with selected clinical breast cancer features, including; age at menarche, marital status, age at onset, LN metastasis and the presence or absence of the family history of cancer. The only significant correlation was found for LN metastasis and family history of breast cancer as indicated by the ORs presented in Table 4.

Genotype frequencies exhibited different distributions in the presence and absence of breast cancer in family, with statistical significance for codon 10 (p = 0.005). Although, the estimated risk much higher for individuals who were 11 homozygote in codon 10 (OR 0.826, 95% CI 0.463-1.477), with OR less than 1, the results demonstrated that codon 10 SNP may have protective against breast cancer. In first-degree family history of breast cancer the higher the frequency of allele 1, approximately 80% in patients with family history of breast cancer in compare with the frequency of allele 1, nearly 40% in patients with no family history of breast cancer, the higher the likelihood of breast cancer with 11 homozygote genotype. Also, the estimated risk for first- degree family history of breast cancer was much greater in 11 homozygote individuals than for the corresponding and 01 heterozygote (OR 2.229, 95% CI 0.386-12.881). The genotype frequencies exhibited different distributions in the presence and absence of LN metastasis, with statistical significance for codon 10 (p = 0.001). The estimated risk was much more lower for individuals who were 00 heterozygote in codon 10 or sixth fold lower for individuals who were 01 heterozygote in codon 10 (OR 0.533, 95% CI 0.114-2.492) than for the corresponding 11 homozygote (OR 0.097, 95% CI 0.016-0.593). So, these results demonstrated that especially the 01 heterozygote in codon 10 SNP may decrease accuracy in predicting LN metastasis and this SNP is protective against LN metastases in breast cancer patients. Finally, in cooperation the known global geographical distributions of ER-α polymorphism in codon 10, reveals that exon 1 is significantly different in comparison with reported Western genomic studies. Comparison of the data indicates the following. The frequency of allele 1 in codon 10 in Iran (46%) matches that in the USA (45%) and lower than in Australia (51%), higher than England (41%) and much greater than in Taiwan (32%). Thus, the Iranian population exhibited a similar pattern of ER-α polymorphism with other Caucasian rather than Asians (Hsiao *et al*., 2004).

DISCUSSION

The association of ESR1 genetic polymorphisms with breast cancer risk attracts much attention because *ESRs* acts as a hormone-dependent transcriptional regulator, which, in turn, plays a pivotal role in the development of breast cancer (Clark *et al*., 1992; Beato *et al*., 1995). Several *ESR1* investigated gene polymorphisms have been reported including exon 1 polymorphisms (Hsiao *et al*., 2004; Wedren *et al*., 2004; Vasconcelos *et al*., 2002). Breast cancer associated ESR1 polymorphisms were in earlier studies (Iwase *et al*., 1996; Southey *et al*., 1998; Curran *et al*., 2001; Kang *et al*., 2002). Somatic mutation of the *ESR1* gene has been identified (Murphy *et al*., 1997), but *ESR1* germ-line mutation rarely occurs in breast cancer patients. Unexplained differences between Asian Caucasians and Western breast cancer symptomatology and demographics led us to study whether unknown genetic factors within the Iranian genome are involved and this prompting us to conduct the present PCR analysis of ESR1 polymorphism.

ESR1 (exon 1) screening was conducted in 150 consecutive breast cancer patients and 147 healthy women. PCR primers used in the initial screening in a US study conducted in Caucasians (Clark *et al*., 1992). However, the PCR-SSCP screening revealed the presence of the SNP - in 10 (TCT→TCC) (T/C, S392S) in the Iranian population that were previously reported for USA (45%), UK (41%), Australian (51%), Taiwanese (32%) populations (Hsiao *et al*., 2004).

PCR primers used in screening were from a US study conducted in Caucasians (Clark *et al*., 1992). The PCR-based genotyping was able to detect new mutations, but none was found. The frequency of *ESR1* exon 1 SNP exhibited a different pattern from that in Asian study groups. Comparison of the local Iranian *ESR1* genotype in breast cancer patients with findings from other countries indicates the following: allele 1 in codon 10 (T/C, S392S) is the same frequent in Iranian (Asian- Caucasians) breast cancer patients (46%) with those reported from the West, but much higher than Asian areas, including Taiwan (Hsiao *et al*., 2004) and Korea (Kang *et al*., 2002). This finding, together with the relatively low incidence of breast cancer in Iran in compare with western population, suggests that this SNP has protective effects in developing breast cancer and LN metastases.

In terms of practical utility, the relation between codon 10 and probability of LN metastasis deserves further consideration as a clinical indicator during presurgical evaluation, at least in the Iranian population. Such a test is of interest because lymphatic invasion is associated with local recurrence and disease progression and LN metastasis is considered an important indicator when deciding whether chemotherapy should be given (Fisher *et al*., 1993; Goldhirsch *et al*., 1995) . Various studies of LN metastasis have considered factors such as intrinsic genetic factors involving cell mobility, vascular invasion and angiogenesis. Data reported in the present study show that there is a positive correlation between allele 1 in codon 10 and LN metastasis, indicating that presence of both alleles 0 and 1 may be dependent parameters for node positively (Table 4).

Conclusively, ESR1 polymorphisms in a Iranian clinical breast cancer group (150 breast cancer patients and 147 control individnals) were established using PCR SSCP of peripheral blood. The same SNP in exon1 of *ESR1* gene reported in Western and Eastern studies was found in the Iranian population studied, but at different frequencies than in Eastern studies. Statistically significant correlations were found between allele distribution and individnal and familial manifestation of breast cancer in allele 1 of codon 10 T/C (S392S). Because of the limited sample size in the present study, this findings will require further confirmation. This is planned as part of our future study, because SNP determination from peripheral blood represents a highly feasible and noninvasive option for preoperative evaluation.

ACKNOWLEDGMENTS

This research has been supported by Tehran University of Medical Sciences and Health Services grant No. 2850. The authors would like to thank Ms. Elham Farazandeh and Ms. Maasumeh Jafari Eftekhar from Central Clinic of 1, Cancer Institute Imam Khomeini Hospital Complex, who made blood samples and clinical information available from the patients. We are grateful to Ms. Roya Sharifiean for her knowledge in statistical analysis. The authors also, wish to thank the anonymous referees of the Journal for their helpful comments on a earlier version of the study.

REFERENCES

Beato, M., P. Herrlich and G. Schultz, 1995. Steroid hormone receptors: Many actors in search of a plot. Cell, 83: 851-857.

Behjati, F., M. Atri, H. Najmabadi, K. Nouri, M. Zamani and P. Mehdipour, 2005. Prognostic value of chromosome 1 and 8 copy number in invasive ductal breast carcinoma among Iranian women: An interphase fish analysis. Pathol. Oncol. Res., 11: 157-163.

Bertucci, F., V. Nasser, S. Granjeaud, F. Eisinger and J. Adelaïde *et al*., 2002. Gene expression profiles of poor prognosis primary breast cancer correlate with survival. Hum. Mol. Genet., 11: 863-872.

Brinton, L., J. Lacey and S.S. Devesa, 2002. Epidemiology of Breast Cancer. In: Cancer of the Breast, Donegan, W.L. and J.S. Spratt (Eds.). WB Saunders, Philadelphia, ISBN: 13: 978-0-7216-8951-7, pp: 111-132.

Clark, J.H., W.T. Schrader and B.W. O'Malley, 1992. Mechanism of Action of Steroid Hormone. In: Textbook of Endocrinology, Wilson, J.D. and D.W. Foster (Eds.). WB Saunders, New York,, pp: 35-90.

Curran, J.E., RA. Lea, S. Rutherford, S.R. Weinstein and L.R. Griffiths, 2001. Association of estrogen receptor and glucocorticoid receptor gene polymorphisms with sporadic breast cancer. Int. J. Cancer, 95: 271-275.

Einarsdóttir, K., H. Darabi, Y. Li, Y.L. Low and Y.Q. Li *et al*., 2008. *ESR1* and EGF genetic variation in relation to breast cancer risk and survival. Breast Cancer Res., 10: R15-R15.

Eumark, E., M. Pelto-Huikko, K. Grandien, S. Lagercrantz and J. Lagercrantz *et al*., 1997. Human estrogen receptor β gene structure, chromosomal localization and expression pattern. J. Clin. Endocrinol. Metab., 82: 4258-4265.

Fisher, E.R., S. Anderson, C. Redmond and B. Fisher, 1993. Pathologic findings from the national surgical adjuvant breast project protocol B-06. 10-year pathologic and clinical prognostic discriminants. Cancer, 71: 2507-2514.

Goldhirsch, A., W.C. Wood, H.J. Senn, J.H. Glick and R.D. Gelber, 1995. Meeting highlights: International consensus panel on the treatment of primary breast cancer. J. Natl. Cancer Inst., 87: 1441-1445.

Gonzalez-Zuloeta Ladd, A.M., A. Vαsquez, A. Rivadeneira and F. Siemes *et al*., 2008. Estrogen receptor α polymorphisms and postmenopausal breast cancer risk. Breast Cancer Res. Treat., 107: 415-419.

Harirchi, I., M. Ebrahimi, N. Zamani, S. Jarvandi and A. Montazeri, 2000. Breast cancer in Iran: A review of 903 case records. Public Health, 114: 143-145.

Heldring, N., A. Pike, S. Andersson, J. Matthews, G. Cheng and J. Hartman *et al*., 2007. Estrogen receptors: How do they signal and what are their targets. Physiol. Rev., 87: 905-931.

Holst, F., P.R. Stahl, C. Ruiz, O. Hellwinkel and Z. Jehan *et al*., 2007. Estrogen receptor alpha (*ESR1*) gene amplification is frequent in breast cancer. Nature Genet., 39: 655-600.

Hsiao, W.C., K.C. Young, S.L. Lin and P.W. Lin, 2004. Estrogen receptor-α polymorphism in a Taiwanese clinical breast cancer population: A case-control study. Breast Cancer Res., 6: R180-R186.

Iwase, H., J.M. Greenman, D.M. Barnes, S. Hodgson, L. Bobrow C.G. Mathew, 1996. Sequence variants of the estrogen receptor (*ER*) gene found in breast cancer patients with *ER* negative and progesterone receptor positive tumors. Cancer Lett., 108: 179-184.

Kang, H.J., S.W. Kim, H.J. Kim, S.J. Ahn and J.Y. Bae *et al*., 2002. Polymorphisms in the estrogen receptor-alpha gene and breast cancer risk. Cancer Lett., 178: 175-180.

Lin, Y., S. Kikuchi, K. Tamakoshi, K. Wakai and T. Kondo *et al*., 2008. Active smoking, passive smoking and breast cancer risk: Findings from the Japan collaborative cohort study for evaluation of cancer risk. J. Epidemiol., 18: 77-83.

Montazeri, A., M. Ebrahimi and N. Mehrdad, 2003. Delayed presentation in breast cancer: A study in Iranian women. BMC Womens Health, 3: 4-4.

Mousavi, S.M., A. Montazeri, M.A. Mohagheghi, A. Mosavi Jarrahi and I. Harirchi *et al*., 2007. Breast cancer in Iran: An epidemiological review. Breast J., 13: 383-391.

Murphy, L.C., H. Dotzlaw, E. Leygue, D. Douglas, A. Coutts and P.H. Watson, 1997. Estrogen receptor variants and mutations. J. Steroid Biochem. Mol. Biol., 62: 363-372.

Najafi, M., M. Ebrahimi, A. Kaviani, E. Hashemi and A. Montazeri, 2005. Breast conserving surgery versus mastectomy: Cancer practice by general surgeons in Iran. BMC Cancer, 5: 35-35.

Roodi, N., L.R. Bailey, W.Y. Kao, C.S. Verrier, C.J. Yee, W.D. Dupont and F.F. Parl, 1995. Estrogen receptor gene analysis in estrogen receptor-positive and receptor-negative primary breast cancer. J. Natl. Cancer Inst., 87: 446-451.

Shin, A., D. Kang, H. Nishio, M.J. Lee and S.K. Park *et al*., 2003. Estrogen receptor alpha gene polymorphisms and breast cancer risk. Breast Cancer Res. Treat., 80: 127-131.

Slattery, M.L., C. Sweeney, J. Herrick, R. Wolff, K. Banmgartner, A. Giuliano T. Byers, 2007. *ESR1*, AR, body size and breast cancer risk in Hispanic and non-Hispanic white women living in the Southwestern United States. Breast Cancer Res. Treat., 105: 327-335.

Southey, M.C., L.E. Batten, M.R. McCredie, G.G. Giles and G. Dite *et al*., 1998. Estrogen receptor polymorphism at codon 325 and risk of breast cancer in women before age forty. J. Natl. Cancer Inst., 90: 532-536.

Vasconcelos, A., R. Medeiros, I. Veiga, D. Pereira and S. Carrilho *et al*., 2002. Analysis of estrogen receptor polymorphism in codon 325 by PCR-SSCP in breast cancer: Association with lymph node metastasis. Breast J., 8: 226-229.

Wang, J., R. Higuchi, F. Moduguo, J. Li and N. Umblas *et al*., 2007. Estrogen receptor alpha haplotypes and breast cancer risk in older Caucasian women. Breast Cancer Res. Treat., 106: 273-280.

Wedren, S., L. Lovmar, K. Humphreys, C. Magnusson and H. Melhus *et al*., 2004. Oestrogen receptor-α gene haplotype and postmenopausal breast cancer risk: A case control study. Breast Cancer Res., 6: R437-R449.