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The Relationship Between Breast Cancer and VDR Gene Polymorphisms

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ABSTRACT

Background: Vitamin D serves several cancer protective roles within the human body. Vitamin D functions through binding with the VDR encoded by VDR gene. It has been demonstrated that polymorphism in VDR gene would influence expression and/or function of the VDR protein. The researchers found that the most important VDR gene polymorphisms that are associated with tumorigenesis include Fok1 (rs2228570), Bsm1 (rs1544410), Taq1 (rs771236), and Apa1 (rs7975232). The purpose of this study was to assess the association between Fok1, Bsm1, and Taq1 polymorphisms and breast cancer development.

Methods: In this study, 50 patients suffering from breast cancer with less than 6 months after the diagnosis of breast cancer and 50 healthy control individuals were included. Restriction fragment length polymorphism PCR (RFLP-PCR) was used to determine the genotype of polymorphisms.

Results: Statistical results showed that among the studied polymorphisms, Tt genotypes of Taq1 polymorphism have correlations with breast cancer development ($P < 0.001$, OR= 5.51, 0.95 CI= 2.30-13.21).

Conclusion: The results of the present clearly demonstrated that there is a relationship between Taq1 polymorphism in VDR gene and development of breast cancer.

Introduction

Breast cancer is the most common cancer among women worldwide and, after lung cancer, it is the second main cause of death among them.[1] There are various risk factors in breast cancer including age, gender, benign tumors of the breast, early menopause, late menarche, hormone therapy, chest exposure to radiation, alcohol consumption, combined use of estrogen and progesterone, diethylstilbestrol consumption, genetic factors, postmenopausal obesity, first pregnancy after the age of 30, lack of breastfeeding, and environmental factors.[2] Genetic factors in breast cancer include changing the level of gene expression, epigenetic modifications, and polymorphisms (DNA Sequence alterations).[3]

The role of vitamin D among the various environmental factors that play a key role in cancer progression is noticeable. Vitamin D is available in 2 forms: vitamin D2 in plants and Vitamin D3 in the human skin. Vitamin D in body comes from sun exposure (up to 90%) and food supplements.[4] Several enzymatic steps and genes are involved in vitamin D metabolism.[5] The first occurs in the liver and the second in the kidneys, which construct the most common biologically active form.[6] The most

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important role of vitamin D is the regulation of bone metabolism. Also, it has a protective function on cancer through the regulation of gene expression, the reduction of invasiveness and angiogenesis, proliferation, differentiation, and the apoptosis of several cancer cell lines.[7-9] This process occurs through binding vitamin D to its receptor. Vitamin D receptor (VDR) is expressed in more than 30 human tissues.[10]

The VDR gene is on the long arm of chromosome 12 with at least 5 promoters and 11 exons.[11] Previous studies have demonstrated the impact of polymorphisms in VDR gene on the expression and/or function of the VD protein.[12] Among 200 polymorphisms described in VDR, Fok1, Bsm1, Taq1, Apa1, EcoRV, and Cdx2 are more frequently associated with tumorigenesis, although data are yet controversial in this filed.[11] Epidemiological studies and laboratory investigations have proposed the increased cancer risk would be related with the level of vitamin D and its expressed receptor (VDR).[13]

Polymorphism Fok1

This polymorphism is located in exon 2, next to the 5'-UTR region of VDR gene and causes the transition of C to T at this position. The Fok1 polymorphism has 2 alleles; the shorter VDR (C allele) has higher transcription activity compared to the longer type (T allele).[12] Despite no significant difference in DNA binding, ligand affinity, and transactivation affinity between 2 VDR forms, the shortened VDR variant showed higher potency than the longer one.

Polymorphism Bsm1

Polymorphism Bsm1 is in intron 8, where guanosine converts to adenosine and it may be associated with poly-A sequence in 3'-UTR region; hence, it may affect VDR gene expression via the regulation of mRNA stability.[14]

Polymorphism Taq1

This polymorphism is located in exon 9 and, instead of T nucleotide, has a C nucleotide. The polymorphisms in the 3'-UTR region of the gene are associated with linkage disequilibrium (LD) and allele frequencies of these polymorphisms seem to differ within populations.[13]

In this study, we have concentrated on the distribution of VDR *Fok1*, *Bsm1*, and *Taq1* polymorphisms in patients with breast cancer in Isfahan, compared with a healthy population.

Methods

Study population

The present study includes case and control groups. In this study, 50 patients suffering from breast cancer with less that 6 months after the diagnosis of breast cancer were referred to two breast cancer centers in Isfahan, Iran. Also, 50 healthy control individuals were included. Healthy women without breast cancer were randomly selected among the women who, visited Alzahra hospital for routine mother and child healthcare. The study was approved by the Ethics Committee of Iran National Science Foundation and the questionnaire and informed consent forms were completed by the study subjects. Demographic data such as age, familial, or sporadic status and types of breast tumors were obtained (Table 1).

DNA extraction, SNP selection, and genotyping

In this survey, 3-5cc peripheral blood was taken from participants. Genomic DNA was extracted from samples by the salting-out method. The quality and concentration of extracted DNA were measured, using NanoDrop® ND-1000 spectrophotometer at 260nm and 280nm wavelengths. Specific PCR primers were designed through BLAST website (http:// blast.ncbi.nlm.nih.gov/Blast.cgi) and single nucleotide polymorphisms (SNPs) database (dbSNP 129; http://www.ncbi.nlm.nih.gov/projects/SNP/) and Genomic DNA was amplified by PCR protocol as follow (Table 2).

We focused on 3 well-characterized polymorphisms. PCR (RFLP) was used to determine the genotype of polymorphisms. PCR-RFLP analysis was performed in 10µL of reaction volume, containing 1x PCR buffer (75 mM Tris-HCl, pH8.8, 20 mM $(NH_4)_2SO_4$, 0.01% (v/v) Tween 20), 2 mM $MgCl_2$, 0.2 mM dNTP , 0.2 mM of each primer (Table 2), 40 ng of DNA, and 0.3 U of Taq DNA polymerase. The touchdown PCR program was performed for Fok1(cycle:30 s, initial denaturation:95$^{\circ C}$ for 4 min, Denaturation: 95$^{\circ C}$ for 1 min, Anneling: 70$^{\circ C}$ for 1 min), Bsm1(cycle: 30s, initial denaturation: 94$^{\circ C}$ for 4 min, Denaturation: 94$^{\circ C}$ for 1 min, Anneling: 66$^{\circ C}$ for 1:30 min), and Taq1(cycle: 30 s, initial denaturation: 95$^{\circ C}$ for 4 min, Denaturation: 95$^{\circ C}$ for 1 min, Anneling: 65$^{\circ C}$ for 1 min, and 72$^{\circ C}$ for 5 min for each primer [final extension]).

The PCR products (5 µL) of VDR gene were digested in a 20 µL reaction volume for 7h with 1.5 U of Fok1, Bsm1, and Taq1 restriction endonucleases (Fermentas, St. Leon-Rot, Germany) at 37$^{\circ C}$. The digested PCR product was separated, using electrophoresis on 3% agarose gel by ethidium bromide and it was analyzed, using UV light after staining.

The product alleles of the VDR gene at the restriction enzyme site Fok1, Bsm1, and Taq1 involved a T→C (Fok1), a C→T (Bsm1), and a C→T (Taq1) transition. The PCR products were digested with appropriate restriction endonuclease per manufacturer's instruction (Macrogene, South Korea). Digested products of the product alleles of cutting by Fok1, Bsm1, and Taq1 enzymes were indicated by f, b, and t, while undigested alleles were

Table 1. The demographic parameters for patients and control group

Variations	Case	Control	P-Value(p<0.05)
N	50	50	
Age	49.61±12.21	42.70±14.05	0.012
Family history			
Yes	9(19.6%)		0.306
No	37(80.4%)		0.306
Types of breast tumors			
Lobular Carcinoma Insitu	8(15%)		0.215
Ductal Carcinoma Insitu	8(15%)		0.112
Invasive Lobular Carcinoma	5(10%)		0.304
Invasive Ductal Carcinoma	12(25%)		0.257

assigned as F, B, and T, respectively. In order to confirm the RFLP results, randomly selected PCR products were sequenced.

Statistical analysis

The data were analyzed by SPSS (Version.18), using Chi-square ,independent sample t test, and logistic regressionwith 95% confidence intervals (CIs). Chi-square test and independent t test were performed. P value less than 0.05 was considered statistically significant.

Results

To investigate the VDR gene polymorphisms, we randomly selected 50 patients with breast cancer, with the mean age of 47.18 ± 14.36 years and 50 healthy subjects, with the mean age of 43.70 ± 14.70 years. Independent t test showed that the average age of the two groups did not have any difference (P = 0.23). Moreover, Chi-square test did not show any differences between the two groups regarding menopausal status (P=0.17).

The histopathology reports of the patients' tumors showed 62% Positive and 38% Negative estrogen receptor expression. Progesterone receptor (PR) expression was positive in 62% of patients. The Her-2 over expression was detected in 30 % of the breast tumors.

The frequencies of each allele for both groups are shown in Table 3.

The data suggest that the T allele may contribute in susceptibility to breast cancer, either in heterozygote or homozygote state. There were no association between Taq1 SNP and tumor characteristics including ER, PR, and Her-2 status.

Table 2. Sequence of the primers and PCR–RFLP product characteristics

Primer name	Sequence	PCR product length (bp)	Digested length (bp)
Fok1-F	GCACTGACTCTGGCTCTGAC	341	60 and 281
Fok1-R	ACCCTCCTGCTCCTGTGGCT		
Bsm1-F	GCAACCAAGACTACAAGTACCGCGTCA	845	194 and 651
Bsm1-R	TTTTCTCCCTCTTCTCACCTCTAACCA		
Taq1-F	CTGGCACTGACTCTGGCTCT	634	207 and 427
Taq1-R	GGGCTCACCTGAAGAAGCCT		

Table 3. Fok1, Bsm1, and Taq1 polymorphisms and breast cancer

Genotype		Breast cancer (N=50)		Control (N= 50)	P value	OR (95%CI)
		Breast Cancer	Relative Frequency (%)	Relative Frequency (%)		
Fok1					0.14	0.67(0.11-4.17)
	FF	34(68)	F 0.94	F 0.96		
	Ff	13(26)	f 0.06	f 0.04		
	ff	3(6)				
Bsm1					0.50	0.91(0.40-2.10)
	BB	12(24)	B 0.66	B 0.68		
	Bb	21(42)	b 0.34	b 0.32		
	bb	17(34)				
Taq1					**<0.001**	5.51(2.30-13.21)
	TT	2(4)	T 0.74	T 0.34		
	Tt	35(70)	t 0.26	t 0.66		
	tt	31(26)				

Discussion

Breast cancer is a common cancer with major public health implications. In 1919, vitamin D was originally discovered by Edward Mellanby [15] and in 1969, the vitamin D receptor (VDR) was discovered by Norman.[16] Recent studies demonstrate that not only vitamin D has the main function in bones, but also it significantly affects differentiation and cell proliferation. The metabolite of vitamin D, 1,25-dihydroxycholecalciferol (1,25[OH]2D3) can suppress cell proliferation in cancer cells.[17] One study suggested that vitamin D effects, for the most part, are correlated with nuclear VDR.[18]

Many human tissues express VDR like breast, prostate, bone, etc.[19] In recent years, many molecular epidemiological studies (case-control studies and nested case-control studies) were conducted on women to investigate the associations of different VDR polymorphisms with breast cancer. The association of all mentioned polymorphisms (Fok1, Bsm1, and Taq1) and breast cancer were investigated in different studies.[9, 20-23] Taq1 was associated with breast cancer.[21]

In a study, Abbas *et al.* showed a significant association between the Taq1 polymorphism and increased breast cancer risk in estrogen receptor positive patients. They emphasized concerning the strong linkage disequilibrium of 3 polymorphisms; the combinations of 3 variants may be more discriminating as risk factors than a single one.[24]
Perna *et al*. demonstrated a significant prognostic value of Taq1 in patients with breast cancer without any risk for women both heterozygous and homozygous for t allele.[25]

In a Swedish study in the field of breast cancer, a trend was found towards a higher survival rate, especially among those tamoxifen-treated estrogen receptor positive patients and homozygous for the rare Taq1 allele.[26] However, Perna *et al.* reported increased death rate in rare homozygous carriers Taq1 homozygous genotype compared with homozygous carriers with the common allele.[25] This study confirms our findings. In the present study, the statistical results showed that among the studied polymorphisms, Tt genotypes of Taq1 polymorphism correlate with breast cancer (P< 0.001, OR=5.51, 0.95 CI= 2.30-13.21). In the mentioned polymorphism, Fok1 is the one that has most frequently been studied regarding its association with different types of cancer.

Several lines of studies have reported a significant association between the Fok1 polymorphism and different types of cancer including multiple myeloma, breast, prostate, and ovarian cancer,[11] while others did not observe any significant associations.[11] For example, Sinotte *et al.*, Gapska *et al.*, and McKay *et al.* reported increased risk among ff carriers on Fok1,[27-29] whereas, Anderson *et al.* reported decreased risk among ff carriers.[30] Curran *et al.*, Guy et al., Abbas *et al.*, Engel *et al.*, Rollison *et al.,* Fuhrman *et al.*, Mishra *et al.*, and Shahbazi *et al.* reported no association between ff carriers and breast cancer risk.[21-24, 31-34] As the last polymorphism, Guy *et al.* reported an increased risk of breast cancer for bb genotype.[22]
Another study reported Bsm1 polymorphism in LD with the poly-A tail sequence in the 3' UTR with a higher risk of breast cancer and bb genotype.[35] Ruggiero *et al.* showed no statistically different distribution of Bsm1 polymorphism in case and control groups,[33] while two-fold higher prevalence of the bb genotype was found in metastatic cancer group and percentage of BB women with metastases was half in control group.[22, 36] On the other hand, no significant association between the Bsm1 polymorphism and breast cancer risk made a different study, but we should note that this study included only a limited number of Turkish cases. Three other studies referring to Taiwanese women[37] and Caucasian women were associated with BB genotype with increased breast cancer risk.[5] Hou *et al.* showed an association between Bsm1 B allele and increased breast cancer risk. [37]

As the results of studies in various cancers are inconsistent, the role of vitamin D in the development and progression of cancer is still unknown and further studies are required. In conclusion, as the result of the present study showed, VDR Taq1 RFLP seems to be associated with breast cancer. T allele could be considered as risk allele and t allele as the protective allele. On the other hand, we found a higher prevalence of Tt genotype among patients with breast cancer in comparison with health controls. The VDR may represent an important target for breast cancer prevention.

Conflict of Interest

The authors declare that they have no conflict of interests.

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