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FokI and *BsmI* Polymorphisms of the *VDR* Gene and Breast Cancer Risk

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Abstract

Introduction: Vitamin D fulfills its crucial role in cell proliferation and death through signal transduction into the nucleus by vitamin D receptor (VDR). Recent studies have depicted the association between *VDR* gene polymorphisms and different cancers, including breast cancer. This study attempted to consider the relationship between *VDR* gene polymorphisms and breast cancer risk among women in West Azerbaijan Province, Iran.

Methods: *FokI* and *BsmI* polymorphisms were studied through the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique on blood samples taken from 95 cancer and 71 healthy participants from Urmia City. The association between the mentioned polymorphisms and breast cancer risk was considered using calculated odds ratio (OR).

Results: The findings showed the protective effect of the *FokI* polymorphism in breast cancer development (OR = 0.2731), while no statistically significant correlation was observed between breast cancer and *BsmI* polymorphism (P > 0.05).

Conclusions: This study identified the protective effect of the *FokI* polymorphism in breast cancer, and the absence of statistically significant association between *BsmI* polymorphism and breast cancer.

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INTRODUCTION

Breast cancer is considered to be the most common cancer among women in the world, and accounts for 24.4% of total cases of cancer in Iran [1]. In Iran, the age of onset for breast cancer (45 to 55 years in average) is about 10 years less than developed countries (55 to 65 years) [2]. Despite huge progresses in breast cancer diagnosis and treatment, it still remains a major challenge due to its late diagnosis and lack of a definitive treatment.

Vitamin D is a fat-soluble vitamin that performs its biological role in the body through its bioactive form (1, 25-dihydroxyvitamin D). Vitamin D insufficiency is associated with different diseases including cancer [3]. Research on cell lines and animal models has pointed toward the inhibitory effect of vitamin D on breast tumors incidence and metastasis [4].

The interaction between Vitamin D and its receptor is necessary for its activity in cells. Vitamin D receptor (VDR) belongs to the large family of steroid receptors expressed in various tissues, including breast cells [4]. It transduces signals from the vitamin D cycle to the cell nucleus, which can affect the expression of some genes [5, 6]. Some studies have shown that breast cancer cells express lower levels of *VDR* than normal cells. Zinser et al. showed that *VDR* inactivity causes breast tumors in mice [7]. The level of *VDR* could diminish due to several reasons, such as polymorphisms of the *VDR* gene [5], which has a number of important single nucleotide polymorphisms (SNP). The most common SNPs of the *VDR* gene tested among different populations were rs2228570 and rs1544410, which are cut by the *FokI* and *BsmI* restriction enzymes, respectively. The Rs1544410 resides in intron 8, near the

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3'UTR, and rs2228570 is located in the 5' promoter region of the *VDR* gene [4, 8, 9]. Analyses have established an association between *FokI*, the 3'UTR region polymorphisms and breast cancer risk [10].
The association between different *VDR* SNPs and breast cancer has been studied in a number of populations. However, their findings require to be further processed in order to clarify the role of different *VDR* polymorphisms in breast cancer risk. In this way, the present study examined *FokI* and *BsmI* polymorphisms for their impact on breast cancer occurrence among a group of women in Urmia City, Iran.

METHODS

Moral approval

This research was conducted after receiving a moral approval from the Ethics Committee of the Medical Faculty, Urmia University. The participants in the study also completed and signed an informed consent.

Research population

The study included 166 participants, divided to one patients group (95 patients from Omid Hospital in Urmia City) and one control group (71 person from Shahid Motahari Hospital in Urmia City). The control group comprised of women, whose initial clinical examinations did not show any breast tumors. The participants were selected from women, who had not been taking vitamin D on a regular basis.
The participants' information, including age, body mass index (BMI), number of pregnancies, pregnancy age, breast-feeding age, use of oral contraceptive pills (OCP) and menopause age, was collected from their medical records. Some of the healthy participants did not refer to the hospital during the study and their results were not complete and thus were omitted from the analysis.

DNA extraction

Genomic DNA of participants was extracted from 4 cc of whole blood samples, using the improved phenol-chloroform method (Barzegar et al.). Agarose gel (0.7%) was also employed to determine the quality and quantity of the extracted DNA, and the SNPs were identified through the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) technique [11]. The exon 2 segment related to the *FokI*, and the intron 8 segment targeted by *BsmI* were proliferated by the PCR, using special primers (Table 1).
PCR was conducted under the following conditions: denaturation at 94°C for five minutes, followed by 40 cycles at 94°C (for 30 seconds), 61°C (for 30 seconds) and 72°C (for 30 seconds).
The PCR products were digested by *FokI* and *BsmI* enzymes and in accordance with the enzyme manufacturer's protocol (Fermentas from Vilnius, Lithuania). The presence or absence of enzyme restriction sites was shown with Safe Stain on Agarose gel (2 %). Each test was repeated at least for three times.
FokI and *BsmI* alleles were marked with capitals letters (F = C and B = A, respectively) for the absence of enzyme restriction sites, and with small letters (f = T and b = G, respectively) for the presence of enzyme restriction sites.

Statistical analysis

The data was analyzed by the SPSS18, and the genotypic and allelic frequencies were determined by Pop Gene Slope and the Hardy-Weinberg Equilibrium test. In order to determine the correlation between the polymorphisms and breast cancer incidence, the odds ratio (OR) and the corresponding 95% confidence intervals were calculated.

RESULTS

Association Between *VDR* Gene SNPs and Breast Cancer Risk

The *FokI* enzyme digested 168 base pair segments and resulted in the three groups; FF homozygous genotypes with 168 base pairs, Ff homozygous genotypes with 168, 140 and 28 base pairs, and ff genotypes with 140 and 28 base pairs.
The frequency of various genotypes of the population depicted that the ff genotype (with a frequency of 81.9%) and the *f* allele (with a frequency of 85.1%) were the most common among the participants in this study. Moreover, the chi-square of 90.07 meant the population was not in the Hardy-Weinberg equilibrium (Table 2).

Table 1: Primer Sequences

	Primers
Exon 2	
FokI (46559145-46559162)	
	F: 5'-TGGCCGCCATTGCCTCCG-3'
	R: 3'-CGGAGGCAATGGCGGCCA-5'
Intron 8	
BsmI **(46526083-46526102)**	
	F: 5'-AGCCTGAGTACTGGGAATGT-3'
	R: 3'-ACATTCCCAGTACTCAGGCT-5'

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Table 2: Allelic and Genotypic Frequency, Along With Chi-Square for the Hardy-Weinberg Equilibrium After Enzyme Digestion of 168 base pair Segments by the FokI Enzyme

Observed Allele	Chi-Square	Expected	Observed	Value	Genotype
F = 0.147		0.021	0.114	19	FF
	90.07	0.0251	0.066	11	Ff
f = 0.85		0.726	0.819	136	ff

Table 3: Genotypic Frequencies Related to *FokI* Polymorphism

Genotypes	Cases (n = 95), No. (%)	Controls (n = 71), No. (%)	OR, (95% CI)	P Value
FF	16 (16.8)	3 (4.2)	1 (Reference)	
Ff	8 (8.4)	3 (4.2)	0.5000 (0.081-3.05)	0.4533
ff	71 (74.7)	65 (91.5)	0.2731 (0.057-0.735)	0.0078

Table 4: Allelic and Genotypic Frequency, Along With Chi-Square for the Hardy-Weinberg Equilibrium After Enzyme Digestion of 823 Base Pair Segments by the *BsmI* Enzyme

Observed Allele	Chi-Square	Expected	Observed	Value	Genotype
A = 0.127		0.160	0.663	11	BB
	34.34	0.221	0.120	20	Bb
G = 0.873		0.763	0.813	135	bb

Table 5: Genotypic Frequencies Related to *BsmI* Polymorphism

Genotypes	Cases (n = 95), No. (%)	Controls (n = 71), No. (%)	OR, (95% CI)	P Value
BB	10 (10.5)	1 (1.4)	1 (Reference)	
Bb	12 (12.6)	8 (11.2)	0.15 (0.0159-1.411)	0.0972
bb	73 (76.8)	62 (87.3)	0.11 (0.014-0.945)	0.0906

Equilibrium After Enzyme Digestion of 168 Base Pair Segments by the *FokI* Enzyme

Table 3 depicts the distribution of *VDR-FokI* polymorphisms in the patient and control groups. The OR assessments by comparing FF and ff genotypes pointed toward the protective role of the ff genotype against breast cancer incidence.

Three genotypes of BB with 823 base pairs, bb with 177 and 646 base pairs, and the heterozygous Bb with 823, 646 and 177 base pairs were observed and categorized after enzyme digestion of PCR products with the *BsmI* enzyme.

The results also identified *b* allele with 87.3% and *B* allele with 12.7% to possess the highest and lowest frequencies, respectively. A comparison of observed and expected frequencies attested that the population was not in the Hardy-Weinberg equilibrium (Table 4).

Table 5 depicts the distribution of *VDR-BsmI* polymorphisms in the patient and control groups. The calculated P > 0.05 for the bb genotype, compared to the BB genotype meant that *BsmI* polymorphism had no relationship with breast cancer in the population under study.

DISCUSSION

Several studies have considered the association between vitamin D and cancers, especially in the recent years. Research on various populations has pointed toward the impact of low vitamin D levels and breast cancer risk [12, 13]. Researchers have also identified a positive relationship between the gene polymorphisms, which are associated with vitamin D performance and breast cancer occurrence. According to these studies, different polymorphisms in the *VDR* gene have been identified to affect the risk of the breast cancer [14]. The presence of the restriction site of *FokI* enzyme on the *VDR* gene (*f* allele = T) produces a protein that performs less efficiently than the natural protein [8]. The present study compared two healthy and cancerous groups, and established that the presence of the *VDR* polymorphism would play a protective role against breast cancer incidence. A research by Ingles et al. on allelic variation located at or near the 3' end of the *VDR* gene, as well as the *FokI* polymorphism located at the 5' end of this gene depicted a correlation of the *BsmI* polymorphism and other polymorphisms at the poly (A) tail, with breast cancer risk. According to this study, no correlation was observed between the start codon polymorphism (*FokI*) and the risk of breast cancer incidence [15]. Another study by McKay et al. on 405 women with breast cancer in comparison with 405 healthy women in a Japanese-American population

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reported that ff genotype is related to breast cancer occurrence [16]. However, studies on a group of women affected by breast cancer in China and Iran failed to report a similar relationship between the *FokI* polymorphism and breast cancer risk [17, 18].

The *BsmI* polymorphism in intron 8 region of the *VDR* gene changes the stability of *VDR* mRNA, and subsequently, the *VDR* protein levels [8]. Several studies have concentrated on the association between this polymorphism and breast cancer, which have shown various outcomes. Some reported that BB genotype (no enzyme restriction site) correlates whit occurrence risk, while others identified the presence of polymorphisms and the bb genotype as a factor increasing the risk. In a study by Lowe et al., it was concluded that a low level of vitamin D in plasma and/or along with the *BsmI* polymorphisms of the *VDR* gene could heighten the risk of breast cancer incidence [19]. The present study identified no significant association between the *BsmI* polymorphism and breast cancer risk in the participating population. Similarly, studies on Chinese and Pakistani women with breast cancer did not observe any relationship between the mentioned factors [4]. That is while a study on a population of Iranian women reported a correlation between the presence of the restriction site (*b* allele) and breast cancer occurrence [18]. Furthermore, a research on the previously mentioned Japanese-American population confirmed the decrease of breast cancer risk by the presence of allele B [16].

The discrepancies observed in the results of the mentioned studies could be attributed to the differences in the number of participants, their races and the impact of factors not simultaneously considered in those cases. On the other hand, studies in the recent years have pointed toward the association between concentration of vitamin D and breast cancer [19]. Confirming the role of vitamin D in preventing breast cancer, and the development of a novel treatment method definitely requires further investigations on different polymorphisms of the *VDR* gene, and simultaneous consideration of the presence of these polymorphisms along with other factors, including genetic factors (such as mutation in *BRCA1/2* genes) and environmental factors (the nutritional diet and vitamin D concentrations in the participants' bodies, etc.).

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CONFLICT OF INTEREST

The authors declared that there was no conflict of interests.

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