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Research Article

Evaluation of Vitamin D-Binding Protein Gene Polymorphism and its Plasma Concentration in Kurdish Patients With Breast Cancer in Sanandaj, Iran

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Abstract

Background: Several studies have indicated that polymorphism in vitamin D pathway genes is associated with breast cancer (BC) risk. Vitamin D-binding protein (VDBP) is a vital element in the metabolism of the vitamin D. VDBP carries the serum 25(OH) D3 to cells to promote vitamin D biological functions, such as cell proliferation and apoptosis. Missense SNP (rs.7041) is a common polymorphism in VDBP gene, which shows ethnic-specific allele frequencies.

Objectives: This study presents the correlation of the rs7041 (Asp432Glu) gene polymorphism and plasma concentrations of VDBP in Kurdish patients with BC in Sanandaj, Iran.

Methods: This cross-sectional study included 44 premenopausal BC patients and 44 healthy subjects. Plasma VDBP concentration was measured by enzyme-linked immunosorbent assay (ELISA). The VDBP (rs7041) was genotyped by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP).

Results: VDBP level was associated with a non-significant risk of BC ($P=0.397$). Frequencies of individuals with VDBP (rs7041) TT, TG, and GG genotypes were 13.6%, 52.2%, and 34.09% in case group and 11.3%, 79.5%, and 9.9% in control group, respectively. Genotype GG associated with increased susceptibility to developing BC (odds ratio [OR] = 5.172, CI: 1.555-17.2, $P=0.007$). There was a significant reverse correlation between GT genotype and BC (OR = 0.282, 95% CI: 0.110-0.722, $P=0.008$).

Conclusion: The changes in the vitamin D pathway may increase susceptibility to develop BC in the Iranian Kurdish population.

Keywords: Polymorphism, Vitamin D binding protein, Breast cancer

Background

Breast cancer (BC) is the most prevalent cancer among women in the worldwide (1). The incidence rate of BC is 33.2 per 100 000 people among Iranian females (2). Genes and environmental elements are the most influential causes controlling susceptibility to BC (3,4). Vitamin D3 is an important factor for regulating cell proliferation, and impairment of vitamin D3 can be considered as a risk factor for susceptibility to various cancers such as BC (5). It seems that a range of genes can alter the stimulation of cancerous cells to vitamin D in BC (6,7). Several studies indicated that polymorphism in vitamin D pathway genes can increase susceptibility to BC (8-11). Vitamin D-binding protein (VDBP) is an essential factor for the metabolism of vitamin D, encoded by the *GC* gene. This gene contains 13 exons and 12 introns on chromosome 4 (4q12-q13) (12,13). Two missense single nucleotide polymorphisms (SNPs), rs.7041 and rs.4588 are the common polymorphisms in the VDBP gene (13). The frequency distribution of VDBP alleles is ethnically diverse (14,15). While rs7041(T) is the common allele in the world populations encoding an aspartic acid (Asp) at position 432 in VDBP, rs7041(G) is the rarer allele encoding a glutamic acid (Glu) at this position (16). VDBP carries the serum 25(OH) D3 to cells to promote vitamin D biological functions, such as cell proliferation and apoptosis (17). Regarding the normal subjects, the plasma concentration of VDBP is 200–600 µg/mL, which may correlate with genetic variation in VDBP (18,19). In the present study, the relationship between plasma VDBP concentrations and SNP located in the VDBP gene (rs7041) was assessed in Iranian Kurdish women with BC.

Materials and Methods

Blood samples were collected from 88 Iranian women in Kurdistan province from October 2016 to July 2018. The

samples were categorized into two equal groups (n = 44 in each group) of case and control. The first group included 44 patients diagnosed with BC and the control group included 44 healthy individuals with normal mammography results. The mean age of case and control groups was 47.5±7.7 and 46.8±7.3 years, respectively. All participants signed an informed consent letter to participate in the study.

Whole blood was collected into 2 tubes: one tube with and the other without EDTA (Sigma-Aldrich, USA). After centrifugation of the blood from the first tube at 3000 rpm at 4°C for 10 minutes, plasma was immediately aliquoted and stored at -80°C until the tests were carried out. Blood from the other tube was utilized for DNA extraction and stored at -20°C.

Measurement of Plasma VDBP Concentrations

Enzyme-linked immunosorbent assay (ELISA, BioVendor, Czech) was performed for measuring the plasma VDBP concentrations based on the standard protocols.

Genotyping

The whole blood samples were used for DNA extraction and the DNA was isolated by the DNA extraction kit according to the manufacturer's protocol (Qiagen, GmbH, Germany). Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was utilized for genotyping. The PCR primers were designed using Gene Runner and synthesized by CinnaGen Co. (Iran) (Table 1).

PCR was performed in a Thermal Cycler (Eppendorf, Germany); the conditions and programs for PCR are summarized in Table 2. Deionized water was utilized as the negative control. After amplification, RFLP analysis was performed by digesting PCR products with HaeIII (Fermentas) at 37°C overnight. For separating the restriction fragments, 2% agarose gel was prepared. PCR amplicon fragments digested by HaeIII, the 570 bp PCR product was uncleaved in rs7041T and then cleaved into two fragments of 161 bp and 409 bp in rs7041G (two bands: 409 and 161 bp for GG genotype; one band: 570bp for TT genotype; three bands: 570 bp, 409 bp, and 161 bp for heterozygous TG genotype) (Figures 1 and 2). Allele and genotype frequencies were determined in patients with BC and control group by direct gene counting.

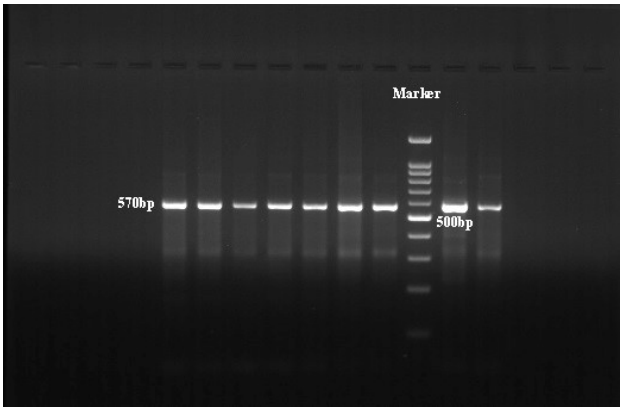

Figure 1. The PCR Product Band Pattern on Agarose Gel. The band size is 570bp.

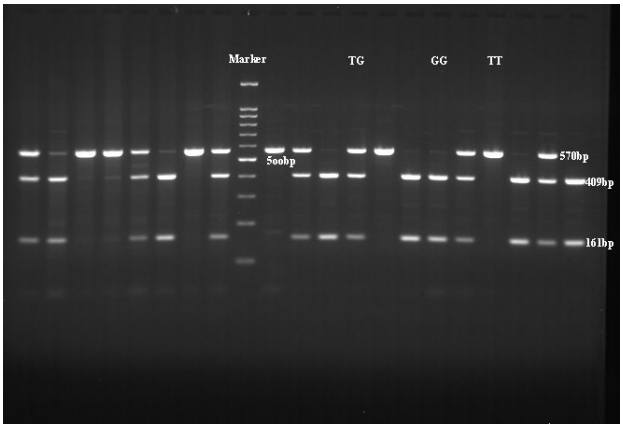

Figure 2. The RFLP Band Patterns on an Agarose Gel. TT genotype yields one band with size of 570 bp. GG genotype produces two bands with sizes of 161 bp and 409 bp. TG genotype yields three bands with sizes of 570, 409, and 161 bp.

Statistical Analysis

Data were analyzed by SPSS (version 19). To assess the normality, the non-parametric one Sample Kolmogorov–Smirnov test was utilized. In addition, Fisher's exact test and chi-square test were run to evaluate the correlation between GC genotypes and allelic distribution. To evaluate the association between GC genotypes and alleles with susceptibility to BC, the odds ratio (OR) and its 95%

Table 1. Primer Sequence and Reaction Condition

SNPs	Primer Sequences	Annealing Temperature (°C)	Restriction Enzyme	Product Size (bp)
VDBP rs7041	F: 5`-TAAGCTGGTATGAGGTCCTG -3` R: 5`-*GATTGGAGTGCATACGTTC* -3`	58	Hea III (BsuRI)	TT: 570 bp TG: 570 bp, 409 bp and 161 GG: 409 bp and 161

Table 2. PCR Conditions and Program

PCR Conditions	PCR Program
100 ng DNA 12.5 µL of PCR Master Mix 2X (Roche, Germany) 1 µL of each primer	95°C for 5 minutes 94°C for 45 seconds, 58°C for 30 seconds, 72°C for 30 seconds (38 cycles) 72°C for 5 minutes

confidence interval (CI) were determined (P<0.05 was regarded as statistically significant). The Hardy-Weinberg equilibrium in control group was assessed by chi-square test (20)

Results

Associations between VDBP (rs7041) Gene and BC Risk

A total of 88 subjects (44 cases, 44 controls) were analyzed for the rs7041 SNP with the PCR-RFLP technique. The Hardy-Weinberg equilibrium of genotype distribution was seen for control group (P>0.05). The frequencies of subjects with VDBP (rs7041) TT, GT, and GG genotypes were 13.63%, 52.27%, and 34.09% in case group, and 11.36%, 79.54%, and 9.9% in controls, respectively (Table 3). Genotype GG was associated with increased susceptibility to developing BC (OR = 5.172, 95% CI: 1.555-17.2, P = 0.007). A significant reverse correlation was observed between susceptibility to BC and TG genotype (OR = 0.0282, 95% CI: 0.110-0.722, P = 0.008). However, the correlation between TT genotype and BC was not significant (OR = 1.232, CI: 0.347-4.377, P = 0.7). No significant association was observed between serum VDBP and GC genotypes (P = 0.397).

Associations between Plasma VDBP Concentration and BC Risk

Plasma VDBP concentrations were measured by the ELISA technique in the studied groups. The mean serum concentrations of VDBP were 215.4±12.9 and 124.3±18.4 µg/mL in cases and controls, respectively (Table 4). Having compared the serum concentration mean score of VDBP in the case group with the healthy subjects, we found the greater rate of VDBP in case group, even though it was not significant (P = .0.2). In addition, the association between serum concentration of VDBP and BC risk was not statistically significant (P = 0.397).

Discussion

This study investigated the correlation between VDBP levels and GC SNP gene (rs7041) with susceptibility to BC among Iranian Kurdish women. The frequency of allele was 39.7% for T allele and 60.2% for G allele in patients, and it was 51.1% for T allele and 48.8% for G allele in healthy subjects. The collected data showed a greatly reduced risk of BC for TG genotype (rs7041) (OR = 0.0282). The GG genotype was more frequently observed in patients compared to controls (34.09% vs. 9.9%). Therefore, GG genotype could act as a risk factor in our population (OR = 5.172). On the other hand, the cases were found to have a VDBP mean score higher than that of controls, even though the correlation between the concentration of serum VDBP and susceptibility to BC was not significant (P = 0.39).

The association between plasma VDBP concentrations and SNPs located in the VDBP gene has previously been indicated, though the results have been contradictory. Powe et al (21) indicated that the frequency of T allele at rs7041 is 91% in black American population, and VDBP serum levels are lower in Blacks than Whites. Similarly, Amadori et al (19) found that TT genotype for rs7041 was significantly more common in African normal population, and that there was no difference in DBP levels with polymorphisms in rs7041 of GC gene in the case and control groups. Moreover, Abbas et al (18) observed a significantly reduced risk of BC in TT genotype in the VDBP gene and the correlation between vitamin D concentration and genotypes was not significant. In the current study, a significant reverse correlation was observed between susceptibility to BC and TG genotype.

In a study conducted by Sinotte et al (22), rs7041 and rs4588 were genotyped in 741 Canadian premenopausal women. Both SNPs were associated with 25(OH) D concentrations. The frequencies of subjects with rs7041 GG, GT, and TT genotypes were 31.1%, 51.4%, and 17.5% in healthy premenopausal women. Being in line with this study, several larger reports revealed a correlation between lower 25(OH) D levels and SNPs rs2282679 and rs7041 (23,24).

Francis et al (25) investigated 120 women with BC frozen tissue in Kuwait and indicated that the VDBP rs7041 associated to susceptibility to BC. However, McCullough et al (6) reported no correlation between genotypes of

Table 3. Genotypes Frequencies in Control and Breast Cancer Patients

GC Variant	Control Group (n = 44)	Breast Cancer Patients (Case) (n = 44)	*P* Value	OR (95% CI)
VDBP rs7041	TT 5 (11.36%)	TT 6 (13.63%)	0.748	1.232 (0.347-4.377)
	TG 35 (79.54%)	TG 23 (52.27%)	0.008	0.0282 (0.110-0.722)
	GG 4 (9.9%)	GG 15 (34.09%)	0.007	5.172 (1.555-17.209)

Table 4. VDP Concentration in Control and Breast Cancer Groups

Serum VDBP Concentration	Breast Cancer Patients (n = 44)	Control Group (n = 44)	*P* Value
VDBP (µg/mL) (mean ± SEM)	215.4 ± 12.9	124.3 ± 18.4	0.397

VDBP SNPs rs7041 and susceptibility to BC.

On the other hand, Larcombe et al (26) reported that VDBP is generally present in high concentrations among the Dene population in Canada; however, the serum concentration mean of VDBP was found to be greatly lower for all studied groups in summer, compared to winter. Subjects with G/G genotype for rs7041 of VDBP showed a high concentration of VDBP in the serum. The other polymorphisms situated in VDBP gene have been indicated to be correlated with vitamin D metabolite levels in plasma.

Chen et al (27) evaluated the association between different polymorphisms in GC gene with BC risk and indicated a significant association between polymorphisms of rs2298850 and rs3755967 GC genes and BC risk. They indicated that these SNPs significantly affect BC development.

In a nutshell, our results indicated an increased risk of BC for individuals with GG genotype of VDBP gene (rs7041) among a sample from Kurdish population in Iran. Furthermore, no significant correlation was found between rs7041 SNP and VDBP serum concentration in the studied groups.

Authors' Contributions

All authors contributed equally in this work.

Conflict of Interest Disclosures

None declared.

Ethical Issues

All procedures performed in studies involving human participants were in accordance with the ethical standards of the ethics committee of Kurdistan University of Medical Sciences and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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Informed Consent

Informed consent was obtained from all individual participants included in the study.

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