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Investigating the relationship between TaqI polymorphism of vitamin D receptor (VDR) gene and breast cancer

Abstract

Scientific evidence indicates a relationship between the occurrence and rate of tumor progression, impaired calciferol metabolism, and VDR gene polymorphisms. The TaqI polymorphism of the VDR gene has no effect on the protein product because its position in exon 9 is in a way that both alleles encode the same amino acid (Isoleucine). This polymorphism probably modulates the function of this gene by affecting transcript stability, regulation of transcription activity, and translation process efficiency. In this study, 100 women with breast tumors and 100 women as the control group in Dezful (Khuzestan, Iran) were sampled. DNA extraction through the salting-out method was conducted on peripheral venous blood of control individuals and breast tumor tissue of patients. In the next step, analysis of *TaqI polymorphism of the VDR gene* was implemented by PCR-RFLP. Statistical analysis showed that regarding P-value = 0.16 for genotype distribution, there is no significant difference between control and case groups; So, P-value = 0.54 indicates no significant difference in terms of allele frequencies. Besides, CI = 95%, OR = 1,500, and P-value = 0.060 do not represent any correlation between the risk of disease and genotypes; thus, CI = 95%, OR = 1.515, and P-value = 0.54 do not illustrate any association between breast cancer and the type of allele. No significant connection exists between the risk of disease and the TaqI polymorphism of the VDR gene.

Keywords: *TaqI, polymorphism, vitamin D receptor (VDR) gene, breast cancer*

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Introduction

The effect of calciferol, vitamin D, and its receptor (VDR) on the cell cycle has revealed that the active form of calciferol, 1, 25 (OH) 2 D3, is an important controller in regulating cell growth and differentiation, and new evidence suggests they also play an important role in angiogenesis, tumor invasion, and cell death, so VDR could play an important role in controlling the development and regression of cancer (1).

Growing evidence demonstrates the inverse correlation between vitamin D and the development of breast cancer (2). Several evidence-based studies have shown that calcitriol, an active form of vitamin D, plays a crucial role in inhibiting cell proliferation and angiogenesis and inducing cell differentiation and cell death in breast cancer through the intervention of VDR (3, 4, 5). Calciferol receptor, or VDR, is expressed in normal mammary glands where it acts as an inhibitor of cell proliferation and induces cell differentiation, revealing that 1,25 (OH) 2D3 plays a role in controlling the growth and inhibition of breast epithelial cells (6).

VDR gene is located in chromosome 12 (12q13.11) which comprised 11 exons with some common single nucleotide polymorphisms such as TaqI (7). TaqI polymorphism has no effect on the protein product of the VDR gene; because its position in exon 9 is in a way that both alleles encode the same amino acid (i.e. isoleucine) (8). The mechanism by which this polymorphism affects the action of protein derived from the VDR gene is unknown. However, this polymorphism probably modulates the function of this gene by affecting transcript stability, regulation of transcription activity, and translation process efficiency (9,10).

So far, various studies have been conducted to investigate the relationship between VDR gene polymorphism and breast tumors in some human societies, but the results have varied according to the type of population. Therefore, studies in other communities, including Iran, seem to be necessary to achieve more accurate results, so this study was designed to observe the polymorphic relationship of TaqI with breast tumors.

Materials and methods

Sample collection

A total of 3 mL of the peripheral venous blood from the control samples was collected in a sterile ethylene-diamine-tetra-acetic acid (EDTA) tube, and tumor samples were collected from the breast tissues of females with breast cancer. They were stored at −80°C for the following analysis.

DNA extraction and genotyping

DNA Extraction from blood and tissue samples was processed through salting-out method, and then DNA samples were stored at −20°C till the time of use.

Analysis of *TaqI polymorphism of the VDR gene* was implemented by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The TaqI region, located in the VDR gene, was multiplied through PCR using a forward primer, 5’ CAG AGC ATG GAC AGG GAG CAA 3’, and a reveres primer, 5’ CGG CAG CGG ATG TAC GTC TGC AG 3’, previously published (11).

The amplification was accomplished in a mixture of 2 µL of 50 ng template DNA, 1 µL of F and R primer (10 pmol), and 25 µL master mix *Taq* polymerase (Promega). The thermo-cycler temperature program was as follows: initial

denaturation at 94°C for 3 min, followed by denaturation at 94°C for 30 s, then annealing at 60°C for 30 s, and subsequently extension at 72°C for 30 s for 30 cycles, and final elongation at 72°C for 5 min, and finally holding at 4°C. The PCR products were digested with *TaqI*(Fermentas GmbH, Leon-Rot, Germany) restriction endonuclease according to the manufacturer's protocol. The PCR products were documented with the use of agarose gel 2% containing ethidium bromide under ultraviolet trans-illumination. The DNA fragments' sizes were as follows: T allele (489 bp), C allele (193-296 bp). To confirm the PCR-RFLP results, 5% of PCR products were sequenced by an ABI sequencer machine. MEGA software version 16 was used to analyze the sequence data

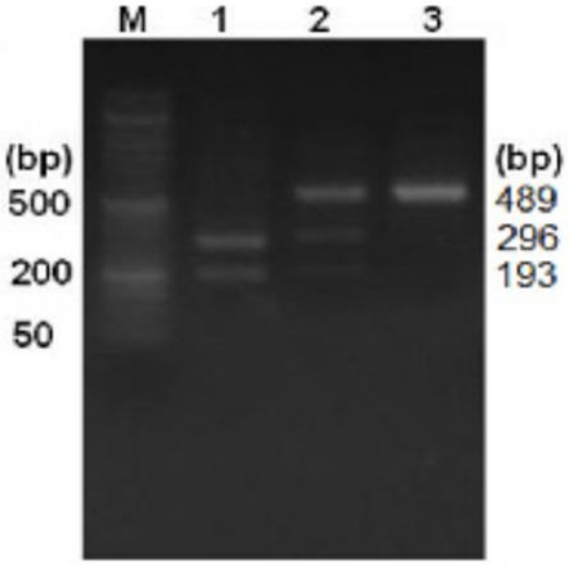

Figure 1- Genotyping of TaqI. *The electrophoretic pattern, after digestion of the amplified part of* VDR *gene, using restriction enzymes, shows homozygous genotype CC (lane 1), heterozygous genotype TC (lane 2), and homozygous genotype TT (lane 3), where T allele appears at 489 bp and C allele appears at 193–296 bp. M: 100 bp ladder marker.*

Results

Examination of electrophoretic bands, produced through enzymatic digestion, showed that in the control population, TT, TC, and CC genotypes accounted for 53 (53%), 39 (39%), and 8 (8%), respectively; and in the patient population, the TC genotype constituted the highest proportion with 45 (45%), then TT with 41 (41%), and finally CC with 14 (14%). Statistical analysis, using SPSS Ver. 16, showed that there is no significant difference between the frequency of genotypes in the control and patient populations due to a larger P-value than 0.05 (P-value = 0.16).

According to the frequency of each genotype, it can be concluded that in the control group, the number of T and C alleles are 145 and 55, respectively, and in the patient population, these numbers are equal to 127 and 73, in the order given. Thus, It could be concluded that there is no significant difference between the frequency of alleles in the two observed communities due to the close proximity of P-value (P-value = 0.054) to 0.05, and additionally, the P-value of the difference in the distribution of genotypes between the two different groups (P-value = 0.16).

Furthermore, statistical analysis with CI = 95% could indicate the fact that between genotype and the risk of disease (OR = 1,500), due to the close and higher proximity of P-value = 0.060 in relation to 0.05, and no significant difference between the frequency and distribution of genotypes between the cases and controls, no significant difference exists; in other words, the risk of disease could not be dependent on the genotype. Similarly, in terms of alleles, with CI = 95%, it could be concluded that there is not a significant difference between the risk of disease (OR = 1.515) and the type of allele due to the close proximity of P-value = 0.054 to 0.05, plus, there is no significant difference between the frequency of genotypes between the two communities; in other words, the risk of disease could not be related to the type of allele.

Table 1- Association between polymorphism of TaqI region of VDR gene and breast cancer

Genotype/ Allele	No. and percentage		OR (95% CI)	P_{Value}
	Control (100)	Case (100)		
TT	53 (53%)	41 (41%)	1.500 (0.984 to 2.286)	0.060
TC	39 (39%)	45 (45%)		

CC	8 (8%)	14 (14%)		
T	145 (72.5%)	127 (63.5%)	1.515 (0.992 to 2.314)	0.054
C	55 (27.5%)	73 (36.5%)		

Discussion

Biological and epidemiological data indicate that the levels of vitamin D maybe affect the breast cancer risk. Vitamin D plays an important role in cell proliferation, apoptosis and tumor growth suppression. Vitamin D receptor is a critical mediator for the cellular reactions of vitamin D (12). The consequences of proper control of VDR signaling pathway correlate with the inhibition of tumor invasion and inhibition of cell growth, so any disruption in it leads to a tumor (13).

TaqI polymorphism has no effect on the protein product of the VDR gene; because its position in exon 9 is in a way that both alleles encode the same amino acid (i.e. isoleucine) (8). The mechanism by which this polymorphism affects the action of protein derived from the VDR gene is unknown. However, this polymorphism probably modulates the function of this gene by affecting transcript stability, regulation of transcription activity, and translation process efficiency (9,10).

Various studies have been performed to investigate the relationship between TaqI polymorphism of the VDR gene and breast tumors in some human communities. Some research has shown that there is no significant association between TaqI SNP and breast tumors, but others have confirmed this connection.

Schondorf et al. (2013) found that there was a significant correlation between ApaI and TaqI polymorphic loci and bone metastasis in breast cancer patients, but no such correlation was observed for the BsmI polymorphic locus of the VDR gene (14); Sillanpaa et al. (2014) in their study concluded that genotypes containing T allele (Tt and TT) with OR = 0.68 reduce the risk of breast cancer in women. However, they stated that in this study, the potential effect of TaqI polymorphism on breast cancer in the control and case groups could not be properly evaluated (15); El-Shorbagy et al. (2017) found that TC in rs731236, and TG in KY859868 single-nucleotide polymorphism showed significant distribution differences with an increased risk of breast cancer ($p<0.05$, odds ratio=3.71, 95% confidence interval: 1.04–13.28 and $p<0.001$, odds ratio=7.05, 95% confidence interval: 2.02–24, respectively) compared with the wild-type TT genotype carriers in both single-nucleotide polymorphisms (16); and In the Study by Mozaffarizadeh et al. (2018), 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). (17).

Contrary to the findings of the researchers mentioned above, Iqbal and Khan (2017) in a meta-analysis, literature searched through PubMed database, Google scholar, and the web of knowledge from December 2015 to January 2017, concluded that VDR Bsm1, Apa1, Fok1, and Poly (A) gene polymorphisms may be susceptible to breast cancer development, while Cdx2, Bgl1, and Taq1 do not show association with breast cancer (18); Amira et al. (2021) reported that the three genotypes for TaqI SNP (CC, TC, and TT) were evenly distributed throughout cases and controls, so according to these findings, there is no statistically significant association of this SNP with breast cancer risk (p. value 0.650) (OR(95%CI) 1.39 (0.64-3.00), 1.00 (0.54-1.87), 1.00). (19); and the research by Dogra et al. (2022), a systematic analysis of published literature using the National Library of Medicine (NLM) of PubMed database search from the year 2010–2020, has revealed that Fok1, Bsm1, Apa1were to some extent associated with breast cancer risk, Taq1 shows no association, and Cdx2, poly(A), Tru91 gene polymorphisms may be susceptible for breast cancer development (20).

In the current study, performed on 100 women with breast tumors and 100 control women in Iran, statistical analysis showed that there is no significant difference between the distribution of genotypes and alleles in these two groups. Considering the lack of difference in genotypic and allelic frequency, statistical analysis with CI = 95% indicates that the difference between the risk of disease in two groups in terms of genotypes and, also, alleles is not significant; in other words, this polymorphism does not affect the risk of breast cancer.

Conclusion

The mechanism by which this polymorphism affects the action of protein derived from the VDR gene is unknown. However, this polymorphism probably modulates the function of this gene by affecting transcript stability, regulation of transcription activity, and translation process efficiency (9,10). The findings of a number of studies also support the effectiveness of this type of polymorphism in increasing the risk of breast cancer. In a few other studies and also this current one, this relationship is not significant and does not increase the occurrence of breast tumors. However, the effect of the number of subjects, VDR gene interactions with other genes, epigenetic factors, the effect of sunlight, as well as a family history of breast cancer, which were not examined in this

study, should not be ignored. Therefore, in order to achieve more accurate results, it is necessary to conduct more research by considering the other effective factors mentioned earlier.

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Conflict of interest

None

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Ethical statement

A written informed consent was taken from the patients

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