Research Article

Hülya Çiçek*, Nesli Güleken, Serdar Öztuzcu and Alper Sevinç

# Vitamin D receptor polymorphisms and related biochemical parameters in various cancer species

# Çeşitli kanser türlerinde D vitamini reseptör polimorfizmleri ve ilgili biyokimyasal parametreler

DOI 10.1515/tjb-2016-0215
Received July 3, 2015; accepted June 21, 2016; previously published online January 20, 2017

**Abstract**

**Objective:** Certain cancer types have been shown to be associated with vitamin D deficiency. The aim of this study was to appraise the relationship between the vitamin D receptor (VDR) gene single nucleotide polymorphisms (SNPs) of VDR Fok1 and Bsm1 with serum vitamin D, calcium and phosphorus levels among patients of lung, colon, breast and pancreatic cancer patients.
**Materials and methods:** Groups; lung, colon, breast and pancreatic cancer patients (n = 212) and 58 age-matched healthy controls. Serum levels of vitamin D were measured by immunochemiluminesence method and Fok1 and Bsm1 genotypes were assessed with Real-Time Polymerase Chain Reaction.
**Results:** VDR Fok1 and Bsm1 genetic polymorphisms have a significant difference between lung cancer and control group subjects ($p = 0.042$, $p = 0.040$, respectively). VDR Bsm1 genetic polymorphism has a significant association between breast cancer and control group according to the logistic regression model ($p = 0.038$). Vitamin D levels were found significantly lower in all cancer groups ($p < 0.01$). Phosphorus levels of lung cancer and calcium levels of pancreatic cancer patients were statistically significantly lower than control group ($p < 0.02$, $p < 0.01$).
**Conclusion:** This study indicates that VDR genetic polymorphisms, calcium, phosphorus and vitamin D status of individuals were associated with certain cancer species.

**Keywords:** Vitamin D; Cancer; VDR polymorphism; Calcium; Phosphorus.

***Corresponding author: Hülya Çiçek,** Faculty of Medicine, Department of Medical Biochemistry, Gaziantep University, Gaziantep, Turkey, e-mail: drhulyacicek@hotmail.com
**Nesli Güleken:** Faculty of Medicine, Department of Medical Biochemistry, Gaziantep University, Gaziantep, Turkey
**Serdar Öztuzcu:** Faculty of Medicine, Department of Medical Biology, Gaziantep University, Gaziantep, Turkey
**Alper Sevinç:** Faculty of Medicine, Department of Medical Oncology, Gaziantep University, Gaziantep, Turkey

**Özet**

**Amaç:** Belirli kanser türlerinin D vitamini eksikliği ile ilişkili olduğu gösterilmiştir. Bu çalışmanın amacı, akciğer, kolon, meme ve pankreas kanserli hastalarda D vitamini reseptör (VDR) geni tek nükleotid polimorfizmleri (SNPs) olan VDR Fok1 ve Bsm1 ile serum vitamin D, kalsiyum ve fosfor düzeyleri arasındaki ilişkiyi değerlendirmektir.
**Gereç ve Yöntem:** Gruplar; akciğer, kolon, meme ve pankreas kanseri hastası (n = 212) ve yaş uyumlu 58 sağlıklı kontrol. Serum D vitamin düzeyleri immünkemilüminesans yöntem ile, Fok1 ve Bsm1 genotipleri gerçek zamanlı polimeraz zincir reaksiyonu ile ölçülmüştür.
**Bulgular:** VDR Fok1 ve Bsm1 genetik polimorfizmlerinde akciğer kanseri ve kontrol grubu arasında anlamlı fark belirlenmiştir (sırasıyla, $p = 0.042$, $p = 0.040$). VDR Bsm1 genetik polimorfizminde ise lojistik regresyon modeline göre meme kanseri ve kontrol grubu arasında anlamlı bir ilişki saptanmıştır ($p = 0.038$). D vitamini seviyeleri tüm kanser gruplarında belirgin olarak düşük bulunmuştur ($p < 0.01$). Akciğer kanserli hastaların fosfor seviyeleri pankreas kanserli hastaların ise kalsiyum seviyeleri kontrol grubuna göre istatistiksel olarak önemli düzeyde düşük bulunmuştur ($p < 0.02$ ve $p < 0.01$).
**Sonuç:** Bu çalışma, bireylerin VDR gen polimorfizmleri, kalsiyum, fosfor ve D vitamini durumunun belirli kanser türleri ile ilişkili olduğunu göstermektedir.

**Anahtar kelimeler:** D vitamini; Kanser; VDR polimorfizmi; Kalsiyum; Fosfor.

Brought to you by | Turkish Biochemical Society (TBD)
Authenticated
Download Date | 10/3/17 3:55 PM

## Introduction

Vitamin D is a steroid hormone that plays a central role in regulating calcium, phosphate and bone metabolism. 25-Hydroxyvitamin D is a preferred biomarker circulating form of vitamin D from diet and sun exposure that undergoes hydroxylation in the liver. It has antitumor effects on cell differentiation induction, apoptosis and inhibits cell proliferation, angiogenesis and metastasis [1]. Up-regulation of adherence and signaling between epithelial cells, contact inhibition of cell cycle stabilization, differentiation, proliferation, promotion of apoptosis, anti-neoangiogenesis are cellular mechanisms for cancer formation that have been determined in vitro conditions. Mechanisms such as down-regulation of glycogen synthase kinase 3 reduce the proliferation of pancreatic, prostate and colorectal cancers. The Wnt/β-catenin signaling pathway plays crucial roles in various biological processes, including cell proliferation and differentiation, regulation of cell cycle, cell-cell and cell-matrix interactions, angiogenesis, apoptosis and adipogenesis. 1,25-dihydroxyvitamin $D_3$ [$1,25(OH)_2D_3$] exerts a complex set of regulatory actions leading to the inhibition of the Wnt/β-catenin pathway in line with its protective effect against several types of cancer [2]. Vitamin D deficiency is associated with a number of severe diseases, such as cancer and autoimmune disorders. This association has led the researchers to focus on the vitamin D receptor, its ligand $1,25(OH)_2D_3$, and genetic differences and transactions between these molecules [3]. Studies of vitamin D levels and incidences of various cancer species such as breast, pancreatic, ovarian, colon, renal, prostate, bladder, esophageal, skin, hepatocellular cancer have suggested an inverse relationship between them [1]. Also this observation has directed researchers to investigate the biological effects of vitamin D on cancer prevention.

Vitamin D receptor (VDR) is a hormone receptor for $1,25(OH)_2D_3$ which acts as a nuclear transcription factor and regulates the biological activities such as cell differentiation and growth by affecting related nucleotide sequences. The VDR gene is known to contain a number of polymorphisms located on chromosome 12q12–q14. The alleles of Fok1 (T/C rs2228570) are located in exon 2 at the 5′-end of the VDR gene and those of Bsm1 (G/A rs1544410) are located in intron 8 at the 3′-end of the VDR gene. The Fok1 restriction fragment length polymorphism generates three amino acids longer VDR protein than Bsm1. Although no significant differences in ligand affinity, DNA binding or transactivation activity have been found between the two allelic forms of Fok1, the shorter restriction fragment length polymorphism variant displays higher receptor activity than the longer one [4].

VDR has a substantial effect for cellular signal mechanisms of vitamin D in the cancer cell development. Since its discovery in 1969, researchers have reported that VDR is related to insulin-like growth factor signaling, inflammation and estrogen, calcium and phosporus metabolism because of its presence in more than 30 tissues or organs in the human body and genetic relationship with the immune system [5].

Various genes and polymorphisms contributing to vitamin D activity have been observed to be associated with $1,25(OH)_2D_3$ concentrations. Genetic variants of vitamin D and relation with the risk of various cancers such as pancreas, prostate, breast and colon cancer have been investigated in earlier studies but, incoherent results were reported [6]. However, the long-term serum levels and genetic variants of vitamin D-related genes were related with the risk of several cancer species [7].

In this study, we have hypothesized that lung, colon, breast and pancreatic cancers may be associated with two frequently analyzed VDR gene polymorphisms (Fok1 and Bsm1). We also measured related biochemical parameters such as calcium and phosphorus levels in these patients.

## Materials and methods

### Subjects

This study consisted of 212 patients; 59 lung, 52 colon, 51 breast and 50 pancreatic cancer patients who were undergoing treatment at the Oncology Hospital of the Medical School of Gaziantep University and as control group 58 age-matched subjects were selected from healthy people without any family history of malignancy, acute or chronic illness, or autoimmune diseases and who were not receiving hormonal therapy, vitamin D or calcium supplements.

The study was performed after approval of the study protocol by the Local Human Ethics Committee and it was conducted in accordance with the Declaration of Helsinki. In addition, written informed consent was obtained from all participants.

### Blood samples

Blood samples were obtained after overnight fasting for at least 12 h. Three milliliter of venous blood was collected by venipuncture into sterile vacutainer tubes containing 2 mg/dL disodium ethylene diamine tetra acetic acid for

Brought to you by | Turkish Biochemical Society (TBD)
Authenticated
Download Date | 10/3/17 3:55 PM

genetic studies and 2 mL into tubes without additives for serum samples. Serum was separated from blood samples by centrifuging at 5000 rpm for 10 min and stored at – 70°C until analysis.

## Serum biochemical parameters

Vitamin D levels were analyzed using the Architect 25-OH Vitamin D assay (Abbott Laboratories, Abbott Park, IL, USA) on the Abbott Architect i2000 immunoassay analyzer. The method was used for the quantitative measurement of 25-OH Vitamin D in human serum samples. Chemiluminescent Microparticle Immunoassay technology, referred to as Chemiflex in the flexible assay protocol, was used for measurement, according to the manufacturer's instructions. Calcium and phosphorus levels were determined spectrophotometrically using automated clinical chemistry analyzers: the Aeroset system and the Architect c8000 system (Abbott Laboratories, Abbott Park, IL, USA). The inter-assay and intra-assay CV values were 7% and 6%, respectively for calcium and phosphorus tests.

## DNA isolation and genotyping for VDR polymorphisms

Genomic DNA was obtained from whole blood according to the currently available DNA extraction protocol using the salting-out method and stored at – 20°C [8].

Genotyping of Fok1 and Bsm1 VDR polymorphisms by real-time PCR was carried out using 2X Multiplex PCR Master Mix (206143) (Qiagen GmbH, Hilden, Germany) and TaqMan Universal PCR Master Mix (PN 4304437). The rs1544410 and rs2228570 VDR single nucleotide polymorphisms (SNPs) were evaluated by allelic discrimination real-time PCR using pre-designed TaqMan probes (Applied Biosystems, Foster City, CA, USA). The following flanking primers were used to analyze the Bsm1 (rs1544410 G > A) polymorphism, 5′GTGGCCTGCTTGCTGTTC3′ and 5′GCCGCCATTGCCTCC3′. The amplification protocol was as follows. Initial enzyme activation was achieved by incubation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s and annealing/extension at 60°C for 1 min. In order to investigate Fok1 (rs2228570, 27823 T > C) polymorphism, the primer pair 5′GCCCAGTTCACGCAAGAG3′, 5′CCACAGACAGGCCTGC3′ was used following the same protocol for Bsm1 (rs1544410, 60890 G > A) polymorphism. Specific Target Amplification (STA) DNA materials were stored at – 20°C for use in Real Time PCR.

Primers for Bsm1:
Short primer 5′-CGCCGCCATTGCCTCCA-3′
Long primer
5′-TGCCGCCTGCCTGCGCGCCATTGCCTCCG-3′
Constant primer 5′-GTGGCCTGCTTGCTGTTC-3′

Primers for Fok1:
Short primer 5′-CGCACAGACAGGCCTGCA-3′
Long primer
5′-TGCCGCCTGCCTGCGCAGACAGGCCTGCG-3′
Constant primer 5′-GCCCAGTTCACGCAAGAG-3′

TaqMan® Universal PCR Master Mix (Applied Biosystems, PN 4304437) and 20X SNP Genotyping Assay were used for Real Time PCR mix preparation. After treatment of STA DNA materials and this mixture, Bsm1 and Fok1 polymorphisms of VDR gene were detected using Light Cycler ®480 Real-Time PCR System. The VDR SNPs were genotyped by Taqman methodology using the Sequence Detection Software on an ABI-Prism 7900 instrument, according to the manufacturer's instructions (Applied Biosystems) as described by Fedirko et al. [9]. Primers and probes were supplied by Applied Biosystems so each plate included a negative control and positive controls were duplicated on a separate plate.

## Statistical analysis

Statistical analysis was performed using SPSS for Windows version 15. All statistical significance tests were two way and $p < 0.05$ was considered statistically significant. Frequency, percentage and mean ± SD values were given for descriptive statistics. Kolmogorov-Smirnov test was used for checking the equality of continuous, one-dimensional probability distributions. Student's t-test was performed for comparison of two independent groups consisting of normally distributed variables, and the Mann-Whitney U-test for non-normally distributed variables. The relationship between categorical variables was tested with $\chi^2$ analysis. Logistic regression analysis was used for detection analysis of risk factors.

# Results

Mean ages and levels of biochemical parameters for patient and healthy control groups and comparison of parameters were shown in Table 1 with statistical significance values (p-values). Serum calcium levels (8.95 ± 1.41 mg/dL) of

Brought to you by | Turkish Biochemical Society (TBD)
Authenticated
Download Date | 10/3/17 3:55 PM

lung cancer patients were not significantly different than control group levels (9.27 ± 0.53 mg/dL) (p = 0.10). However, serum phosphorus levels of lung cancer patients (3.25 ± 0.70 mg/dL) were found to be significantly lower than those of the control group (3.53 ± 0.57 mg/dL) (p = 0.02) and vitamin D levels of the lung cancer patients (10.97 ± 5.60 ng/mL) were significantly decreased when compared with controls (20.94 ± 5.28 ng/mL) (p < 0.01). Serum calcium (9.38 ± 0.97 mg/dL) and phosphorus (3.56 ± 0.94 mg/dL) levels of colon cancer patients were not significantly different when compared with control group (9.27 ± 0.53 mg/dL and 3.53 ± 0.57 mg/dL), (p = 0.44 and p = 0.82), respectively. But vitamin D (10.04 ± 5.04 ng/mL) levels of colon cancer group were significantly lower than controls (20.94 ± 5.28 ng/mL), (p < 0.01). Serum calcium (9.21 ± 0.80 mg/dL) and phosphorus (3.44 ± 0.66 mg/dL) levels of the breast cancer group were not significantly different when compared with controls (9.27 ± 0.53 mg/dL and 3.53 ± 0.57 mg/dL) (p = 0.64 and p = 0.45), respectively. However, vitamin D levels were (9.85 ± 2.71 ng/mL) found to be lower than controls (20.94 ± 5.28 ng/mL) (p < 0.01). Serum calcium levels (8.52 ± 0.92 mg/dL) were significantly lower in pancreas cancer patients, than in controls (p = 0.01). But phosphorus levels (3.34 ± 0.75 mg/dL) were not significantly different when compared with control group (3.53 ± 0.57 mg/dL), (p = 0.14). Serum vitamin D levels were significantly decreased in pancreas cancer group (8.04 ± 6.00 ng/mL) when compared with controls (20.94 ± 5.28 ng/mL) (p < 0.01).

Genotype and allele frequencies of the VDR Fok1 gene SNPs (CC, CT or TT) for patients and control subjects are given in Table 2. The CC allele was observed in 30 subjects in the control group (54.5%) and 31 patients in the lung cancer group (52.5%). The CT allele was detected in 23 subjects in the control group (41.8%) and 20 patients in the lung cancer group (33.9%). The TT allele was only present in two subjects in the control group (3.6%) and eight patients in the lung cancer group (13.6%). This study showed that the VDR Fok1 allele frequencies had a significant difference between lung cancer and control group subjects according to the logistic regression model (p = 0.042). TT allele frequencies of the VDR Fok1 gene polymorphism were detected to be higher in lung cancer patients compared with CC allele frequencies; results were significantly different at the 95% confidence interval (p = 0.03). The CC allele was detected in 30 subjects in the control group (54.5%) and 29 patients in colon cancer group (55.8%). CT allele was observed in 23 subjects in the control group (41.8%) and 19 patients in the colon cancer group (36.5%). The TT allele was observed in two subjects in the control group (3.6%) and four patients in the colon cancer group (7.7%). The CC allele was observed in 30 subjects in the control group (54.5%) and 33 patients in the breast cancer group (64.7%). The CT allele was observed in 23 subjects in the control group (41.8%) and 16 patients in the breast cancer group (31.4%). The TT allele was observed in two subjects in the control group (3.6%) and two patients in the breast cancer group (3.9%). The CC allele was observed in 30 subjects in the control group (54.5%) and 23 patients in the pancreas cancer group in (46.0%). The CT allele was observed in 23 subjects in the control group (41.8%) and 21 patients in the pancreas cancer group (42.0%). The TT allele was observed in two subjects in the control group (3.6%) and six patients in the pancreatic cancer group (12.0%).

Genotype and allele frequencies of VDR Bsm1 gene SNPs (AA, AG or GG) for patients and control subjects are given in Table 2. The AA allele was observed in three subjects in the control group (5.5%) and three patients in the lung cancer group (5.1%). The AG allele was observed in 23 subjects in the control group (41.8%) and 19 patients in the lung cancer group (32.2%). The GG allele was observed in 29 subjects in the control group (52.7%) and 37 patients in the lung cancer group (62.7%). This study showed that the VDR Bsm1 gene polymorphism has a significant difference in the GG/AA allele ratio between lung cancer and control group subjects (p = 0.04). The AA allele was observed in three subjects in the control

**Table 1:** Mean ages and levels of biochemical parameters according to groups.

| Group | Age (years) (mean ± SD) | Vitamin D (ng/mL) (mean ± SD) | p-Value[a] | Calcium (mg/dL) (Mean ± SD) | p-Value[a] | Phosphorus (mg/dL) (mean ± SD) | p-Value[a] |
|---|---|---|---|---|---|---|---|
| Lung cancer (n = 59) | 60.41 ± 11.42 | 10.97 ± 5.60 | **< 0.01** | 8.95 ± 1.41 | 0.10 | 3.25 ± 0.70 | **0.02** |
| Colon cancer (n = 52) | 55.00 ± 14.48 | 10.04 ± 5.04 | **< 0.01** | 9.38 ± 0.97 | 0.44 | 3.56 ± 0.94 | 0.82 |
| Breast cancer (n = 51) | 50.70 ± 13.17 | 9.85 ± 2.71 | **< 0.01** | 9.21 ± 0.80 | 0.64 | 3.44 ± 0.66 | 0.45 |
| Pancreatic cancer (n = 50) | 61.98 ± 12.52 | 8.94 ± 6.00 | **< 0.01** | 8.52 ± 0.92 | **0.01** | 3.34 ± 0.75 | 0.14 |
| Control (n = 55) | 55.71 ± 8.60 | 20.94 ± 5.28 | | 9.47 ± 0.53 | | 3.53 ± 0.57 | |

Bold values are statistically significant values.

[a]p-Value < 0.05 is significant. Patient groups compared with control group.

Brought to you by | Turkish Biochemical Society (TBD)
Authenticated
Download Date | 10/3/17 3:55 PM

**Table 2:** Genotype and allele frequencies for VDR Fok1 and Bsm1 polymorphisms.

| | Controls n (%) | Allele frequency | Lung cancer n (%) | Allele frequency | Colon cancer n (%) | Allele frequency | Breast cancer n (%) | Allele frequency | Pancreas cancer n (%) | Allele frequency |
|---|---|---|---|---|---|---|---|---|---|---|
| Fok1 | | | | | | | | | | |
| CC | 30 (54.5) | C (0.75) | 31 (52.5) | C (0.70) | 29 (55.8) | C (0.74) | 33 (64.7) | C (0.80) | 23 (46) | C (0.67) |
| CT | 23 (41.8) | | 20 (33.9) | | 19 (36.5) | | 16 (31.4) | | 21 (42) | |
| TT | 2 (3.6) | T (0.25) | 8 (13.6) | T (0.30) | 4 (7.7) | T (0.26) | 2 (3.9) | T (0.20) | 6 (12) | T (0.33) |
| Bsm1 | | | | | | | | | | |
| AA | 3 (5.5) | A (0.26) | 3 (5.1) | A (0.21) | 2 (3.8) | A (0.14) | 4 (7.8) | A (0.20) | 3 (6) | A (0.23) |
| AG | 23 (41.8) | | 19 (32.2) | | 11 (21.2) | | 12 (23.5) | | 17 (34) | |
| GG | 29 (52.7) | G (0.74) | 37 (62.7) | G (0.79) | 39 (75) | G (0.86) | 35 (68.6) | G (0.80) | 30 (60) | G (0.77) |

group (5.5%) and two patients in the colon cancer group (3.8%). The AG allele was observed in 23 subjects in the control group (41.8%) and 11 patients in the colon cancer group (21.2%). GG allele was observed in 29 subjects in the control group (52.7%) and 39 patients in the colon cancer group (75.0%). The VDR Bsm1 gene polymorphism allele frequencies were not significantly different between the colon cancer and control group subjects ($p > 0.05$). The AA allele was observed in three subjects in the control group (5.5%) and four patients in the breast cancer group (7.8%). The AG allele was detected in 23 subjects in the control group (23.5%) and 12 patients in the breast cancer group (23.5%). The GG allele was observed in 29 subjects in the control group (52.7%) and 35 patients in the breast cancer group (68.6%). The VDR Bsm1 gene polymorphism had a significant difference in allele frequency between the breast cancer and control group subjects according to the logistic regression model ($p = 0.038$). The VDR Bsm1 gene polymorphism AG/GG allelle ratios were found to be significantly different ($p = 0.012$) and GG allele frequency was higher than AG allelle ($p = 0.012$) in 95% confidence interval in the breast cancer group. The AA allele was observed in three subjects in the control group (5.5%) and three patients in pancreatic cancer group (6.0%). The AG allele was observed in 23 subjects in the control group (41.8%) and 17 patients in the pancreatic cancer group (34.0%). GG allele was observed in 29 subjects in the control group (52.7%) and 30 patients in the pancreatic cancer group (60.0%). No significant difference was observed in the VDR Bsm1 gene polymorphism allele frequencies between pancreatic cancer and control group subjects ($p > 0.05$).

## Discussion

Epidemiological studies have shown that there is a relationship between vitamin D status and the risk of various malignencies [10]. It is also associated with autoimmune diseases, inflammatory bowel diseases, rheumatoid arthritis, multiple sclerosis, diabetes, heart and brain diseases, obesity and respiratory diseases. The presence of vitamin D receptors in most tissues and organs such as the ovary, skin, stomach, lung, pancreas, pituitary gland, breast, kidney, thymus, leukocytes and parathyroid gland indicates that vitamin D has crucial roles in the body [3].

According to the International Agency for Research on Cancer, by the year 2025, there will be an estimated 19.3 million new cancer cases and 11.4 million cancer deaths. Lung and prostate cancers are the most common cancers in men, followed by colorectal, stomach, and liver cancers. In terms of mortality in men, lung cancer has the highest rates followed by liver and stomach cancers. Breast cancer is by far the most frequently diagnosed and has the highest mortality in females, followed by colorectal, cervix, and lung cancer [11].

Nakagawa et al. [12] carried out studies on rats and reported that calcitriol is thought to inhibit the metastasis of lung cancer due to antiproliferative effect of vitamin D. People living in northern latitudes have higher mortality from lung cancer probably due to the lower levels of circulating $1,25(OH)_2D_3$ resulting from inadequate sun light exposure [13].

In our study, calcium levels were similar for lung cancer patients and controls in the reference range. The data showed that vitamin D and serum phosphorus levels in lung cancer patients were significantly lower ($p < 0.01$ and $p = 0.02$, respectively), than healthy individuals, these results impelled us to consider their possible roles in pathogenesis.

Although there have been many studies on the effect of VDR polymorphisms for risk of developing cancer, there is very little information about prognostic value. The VDR G-T-C (Cdx-2-Fok1-Bsm1) haplotype, which is thought to represent lower receptor activity, is related to worse prognosis in lung cancer [14]. A study in Tunisia showed

Brought to you by | Turkish Biochemical Society (TBD)
Authenticated
Download Date | 10/3/17 3:55 PM

that, VDR Fok1 and Apa1 polymorphisms might be risk factors for lung cancer development but Bsm1 polymorphism was not a risk factor [15]. According to our findings, lung cancer risk was influenced by VDR Fok1 and Bsm1 polymorphisms. There were associations between Fok1 polymorphisms ($p = 0.042$), VDR Fok1 TT/CC allele frequencies ($p = 0.03$) and VDR Bsm1 GG/AA allele frequencies ($p = 0.04$) when compared with control subjects.

A previous study in Japan reported that higher $1,25(OH)_2D_3$ levels were associated with a better survival status in operated colorectal cancer patients, but they reported that 97% of patients had insufficient vitamin D concentrations [16]. On the other hand a meta-analysis has suggested that $1,25(OH)_2D_3$ levels were inversely proportional with colorectal adenoma risk [17]. As reported by Rasool et al. [18] the Bsm1 SNPs may lead to 2.7-fold raising of colorectal cancer risk in individuals found homozygous for the presence of the 'b' allele, in comparison to subjects homozygous for the 'B' allele (Bsm1).

A recent review reported that increasing $1,25(OH)_2D_3$ levels to the normal range, by supplementation with an 1000 IU daily dose of vitamin D has provided reduced colorectal cancer risk [19]. In another study on 1248 colorectal cancer patients and 1248 controls the Bsm1 (rs1544410) and the Fok1 (rs2228570) polymorphisms have been investigated. The results of that study showed an inverse correlation between colorectal cancer risk and the BB genotype of the Bsm1 (rs1544410) polymorphism; but, no colorectal cancer risk associations were observed for the Fok1 (rs2228570) [20]. According to our research there was no association between calcium and phosphorus levels or VDR Fok1 and Bsm1 polymorphisms in colon cancer patients, but vitamin D concentrations were significantly lower.

Evidence is accumulating proofs that vitamin D is protective against breast cancer through effects carried out via the VDR. A study from the Roswell Park Cancer Institute examined 579 women with breast cancer and 574 healthy women between 2003 and 2008 and detected a correlation between higher $1,25(OH)_2D_3$ serum levels and decreased breast cancer risk [21].

In many meta-analyses a significant association has been declared between VDR Fok1 polymorphisms and most cancer species across multiple ethnic studies. Although the published findings are conflicting, it is usually reported that breast cancer is influenced by some VDR gene polymorphisms [22]. A study has found no significant correlation between the Taq1, Bsm1 and Apa1 variants and the risk of breast cancer. Also, a huge meta-analysis including 23.020 subjects found no association between the Bsm1 polymorphism and breast cancer [23]. Another meta-analysis by Tang et al. suggested that Fok1 could be a sensitive biomarker for breast cancer in the European population. However, dietary factors may alter the effects of VDR genotype for women under low risk of breast cancer who consume higher calcium [24]. On the other hand, according to research by Bretherton-Watt et al. [25] among VDR gene polymorphisms the poly (A) variant was related but, Fok1 was not related with breast cancer risk in the United Kingdom Caucasian population. A previous meta-analysis, after evaluation of 19 SNPs of the VDR, concluded that several polymorphisms may affect breast cancer risk. A poor association was found between breast cancer risk and Fok1, Cdx2, Apa1 and Poly-A, but strong association with Taq1 and Bsm1 polymorphisms [26]. The Bsm1 polymorphism was also related with breast cancer risk in the current study ($p = 0.038$). Another study also supports an increased risk of breast cancer associated with the VDR Bsm1 polymorphism [27].

Vitamin D deficiency is commonly detected in pancreatic adenocarcinoma patients and vitamin D levels may be a prognostic factor for patients with advanced pancreatic adenocarcinoma [28]. Although numerous studies of vitamin D status showed that patients taking vitamin D supplements were protected against the risk of pancreatic cancer with increased vitamin D levels, conversely another case-control study showed a significant threefold increased risk for pancreatic cancer patients with higher vitamin D levels [29]. VDR gene expression may be a potential prognostic factor for individuals with pancreatic adenocarcinoma and this receptor has been studied widely on pancreatic cancer patients [30]. When the cell lines obtained from these patients were treated with vitamin D analogs at high concentrations, they responded with a reduction in the cell number [31]. Our results suggested that serum calcium and vitamin D levels of pancreatic cancer patients were significantly lower then controls ($p = 0.01$ and $p < 0.01$, respectively). Also, no association has been found between VDR Fok1 and Bsm1 polymorphisms and pancreatic cancer.

It has been reported that VDR polymorphisms were significantly associated with most cancer species such as breast [Fok1, Bsm1, Taq1, Apa1, poly (A)], skin (Fok1, Bsm1, A-1210), colorectal (Fok1, Bsm1), prostate [Fok1, Bsm1, Taq1, poly (A)], bladder (Fok1), ovary (Fok1, Apa1), malignant melanoma (Fok1, Bsm1) and in renal cell carcinoma (Taq1, Apa1). However, the indicated associations between VDR polymorphisms and cancer prognosis were strongest for prostate cancer (Fok1), breast cancer (Bsm1, Taq1), malignant melanoma (Bsm1) and renal cell carcinoma (Taq1) [32]. Thus, a study has suggested that these polymorphisms may alter the polyadenylation of the VDR

Brought to you by | Turkish Biochemical Society (TBD)
Authenticated
Download Date | 10/3/17 3:55 PM

mRNA transcript, and thereby affects mRNA stability [33]. Certain VDR gene polymorphisms alter the translational process, which may cause vitamin D resistance. Isojima et al. reported that the Fok1 polymorphism (F/f) affects both VDR translation and VDR activation [34].

Serum levels of Vitamin D under 12 ng/mL were defined as hypovitaminosis D, 12–20 ng/mL as deficiency, 20–30 ng/mL as insufficiency and concentrations above 30 ng/mL as sufficent for adults [28]. In our study groups, cancer patients had lower vitamin D levels that are probably associated with a higher incidence of cancer, while the healthy group were in the range of insufficiency, but not hypovitaminosis. We collected samples from indoor patients who were taking prolonged bedrest and not frequently exposed to the sun, in winter months. So, the healthy subjects were affected by the lack of sunlight and had lower vitamin D concentrations than might be expected in summer months. However, different types of dressing may also affect vitamin D levels in individuals.

Limitations of the current study include a lower number of patients, possible different forms of cancer in each organ, the need for a separate female control group for breast cancer, and possible differences in sun exposure and life-style between patients and healthy individuals. Therefore, further studies should be carried out to assess the diagnostic and prognostic value of vitamin D levels and VDR expression.

## Conclusion

Although a lot of studies about the protection mechanisms of vitamin D have not clearly demonstrated that it has a role in improvement of malignencies, evidence suggest that vitamin D contributes to cell growth regulation, apoptosis and differentiation. Also, vitamin D has been shown to be related to invasion, angiogenesis and metastasis of cancer cells.

Vitamin D has anti-cancer effects through promotion of differentiation and inhibition of proliferation, inflammation, angiogenesis, etc. Vitamin D expresses its biological activities via vitamin D receptors that are found in many healthy human tissues and also cancer tissues derived from various locations. The VDR regulates both transcriptional responses and post transcriptional mechanisms. The role of vitamin D in calcium and bone metabolism has been proven and its impact on carcinogenesis in many cellular levels such as apoptosis, angiogenesis, proliferation and metastasis has also been demonstrated at the genomic level. We investigated the VDR gene polymorphism Fok1 and Bsm1 and levels of vitamin D and related biochemical parameters such as calcium and phosphorus in lung, colon, breast and pancreatic cancer. In the present study, there were significant valuable results that may contribute to literature and shed light on ongoing researchs.

To conclude, our findings suggest that VDR polymorphisms may modulate the risk of cancer in some tissues and in future studies VDR genetic variations should be integrated with vitamin D status as a prediagnostic indicator. All cancer patients have had lower vitamin D levels than the healthy group.

**Acknowledgments:** This study was supported financially by Scientific Research Projects Management Unit of Gaziantep University.

**Conflict of interest statement:** There are no conflicts of interest among the authors.

## References

1. Shui I, Giovannucci E. Vitamin D status and cancer incidence and mortality. Adv Exp Med Biol 2014;810:33–51.
2. Pendás-Franco N, Aguilera O, Pereira F, González-Sancho JM, Muñoz A. Vitamin D and Wnt/beta catenin pathway in colon cancer: role of regulation of DICKKOPF genes. Anticancer Res 2008;28:2613–23.
3. Carlberg C. What do we learn from the genome-wide perspective on Vitamin D3? Anticancer Res 2015;35:1143–51.
4. Raimondi S, Johansson H, Maisonneuve P, Gandini S. Review and meta-analysis on vitamin D receptor polymorphisms and cancer risk. Carcinogenesis 2009;30:1170–80.
5. Norman AW. Minireview: vitamin D receptor: new assignments for an already busy receptor. Endocrinology 2006;147:5542–8.
6. McCullough ML, Bostick RM, Mayo TL. Vitamin D gene pathway polymorphisms and risk of colorectal, breast, and prostate cancer. Annu Rev Nutr 2009;29:111–32.
7. Bjelakovic G, Gluud LL, Nikolova D, Whitfield K, Krstic G, Wetterslev J, et al. Vitamin D supplementation for prevention of cancer in adults. Cochrane Database Syst Rev 2014;6:CD007469.
8. Sambrook J, Russel DW. Molecular Cloninga laboratory manual publishing, 3rd ed. New York, USA: Cold Spring Harbour Laboratory Press, 2001.
9. Fedirko V, Riboli E, Tjønneland A, Ferrari P, Olsen A, Bueno-de-Mesquita HB, et al. Pre-diagnostic 25-hydroxyvitamin D, VDR and CASR polymorphisms and survival in patients with colorectal cancer in Western European Populations. Cancer Epidemiol Biomarkers Prev 2012;21:582–93.
10. Pilz S, Gaksch M, Hartaigh BO, Tomaschitz A, März W. Vitamin D in preventive medicine. Anticancer Res 2015;35:1161–70.
11. http://www.cancer.org/acs/groups/content/@editorial/documents/document/acspc-044552.pdf (Last accessed: April 2015).

Brought to you by | Turkish Biochemical Society (TBD)
Authenticated
Download Date | 10/3/17 3:55 PM

12. Nakagawa K, Kawaura A, Kato S, Takeda E, Okano T. 1 alpha, 25-Dihydroxyvitamin D 3 is a preventive factor in the metastasis of lung cancer. Carcinogenesis 2005;26:429–40.
13. Huotari A, Herzig KH. Vitamin D and living in northern latitudes–an endemic risk area for vitamin D deficiency. Int J Circumpolar Health 2008;67:164–78.
14. Heist RS. Circulating 25-hydroxyvitamin D, VDR polymorphisms, and survival in advanced non-small-cell lung cancer. J Clin Oncol 2008;26:5596–602.
15. Kaabachi W, Kaabachi S, Rafrafi A, Amor AB, Tizaoui K, Haj Sassi F, et al. Association of vitamin D receptor FokI and ApaI polymorphisms with lung cancer risk in Tunisian population. Mol Biol Rep 2014;41:6545–53.
16. Mezawa H, Sugiura T, Watanabe M, Norizoe C, Takahashi D, Shimojima A, et al. Serum vitamin D levels and survival of patients with colorectal cancer: Post-hoc analysis of a prospective cohort study. BMC Cancer 2010;10:347.
17. Yin L, Grandi N, Raum E, Haug U, Arndt V, Brenner H, et al. Meta-analysis: serum vitamin D and colorectal adenoma risk. Prev Med 2011;53:10–6.
18. Rasool S, Kadla SA, Rasool V, Qazi F, Khan T, Shah NA, et al. Role of the VDR Bsm I and Apa I polymorphisms in the risk of colorectal cancer in Kashmir. Oncol Res Treat 2014;37:345–9.
19. Zhou G, Stoitzfus J. Optimizing vitamin D status to reduce colorectal cancer risk: an evidentary review. Clin J Oncol Nurs 2009;13:E3–17.
20. Jenab M, McKay J, Bueno-de-Mesquita HB, van Duijnhoven FJ, Ferrari P, Slimani N, et al. Vitamin D receptor and calcium sensing receptor polymorphisms and the risk of colorectal cancer in European populations. Cancer Epidemiol Biomarkers Prev 2009;18:2485–91.
21. Yao S, Sucheston LE, Millen AE, Johnson CS, Trump DL, Nesline MK, et al. Pretreatment serum concentrations of 25-hydroxyvitamin D and breast cancer prognostic characteristics: a case-control and a case-series study. PLoS One 2011;6:e17251.
22. Xu Y, He B, Pan Y, Deng Q, Sun H, Li R, et al. Systematic review and meta-analysis on vitamin D receptor polymorphisms and cancer risk. Tumour Biol 2014;35:4153–69.
23. Du Y, Hu L, Kong F, Pan Y. Lack of association between vitamin D receptor gene BsmI polymorphism and breast cancer risk: an updated meta-analysis involving 23,020 subjects. Tumour Biol 2014;35:2087–93.
24. Tang C, Chen N, Wu M, Yuan H, Du Y. Fok I polymorphism of vitamin D receptor gene contributes to breast cancer susceptibility: a meta-analysis. Breast Cancer Res Treat 2009;117:391–9.
25. Bretherton-Watt D, Given-Wilson R, Mansi JL, Thomas V, Carter N, Colston KW. Vitamin D receptor gene polymorphisms are associated with breast cancer risk in a UK Caucasian population. Br J Cancer 2001;85:171–5.
26. Huang QQ, Liao YY, Ye XH, Fu JJ, Chen SD. Association between VDR polymorphisms and breast cancer: an updated and comparative meta-analysis of crude and adjusted odd ratios. Asian Pac J Cancer Prev 2014;15:847–53.
27. Shahbazi S, Alavi S, Majidzadeh-A K, Ghaffarpour M, Soleimani A, Mahdian R. Bsm I but not Fok I polymorphism of VDR gene is contributed in breast cancer. Med Oncol 2013;30:393.
28. Cho M, Peddi PF, Ding K, Chen L, Thomas D, Wang J, et al. Vitamin D deficiency and prognostics among patients with pancreatic adenocarcinoma. J Transl Med 2013;8:206.
29. Stolzenberg-Solomon RZ, Vieth R, Azad A, Pietinen P, Taylor PR, Virtamo J, et al. A prospective nested case-control study of vitamin D status and pancreatic cancer risk in male smokers. Cancer Res 2006;66:10213–9.
30. Wang K, Dong M, Sheng W, Liu Q, Yu D, Dong Q, et al. Expression of vitamin D receptor as a potential prognostic factor and therapeutic target in pancreatic cancer. Histopathology 2015;67:386–97.
31. Albrechtsson E, Jonsson T, Möller S, Höglund M, Ohlsson B, Axelson J. Vitamin D receptor is expressed in pancreatic cancer cells and a vitamin D3 analogue decreases cell number. Pancreatology 2003;3:41–6.
32. Köstner K, Denzer N, Müller CS, Klein R, Tilgen W, Reichrath J. The relevance of vitamin D receptor (VDR) gene polymorphisms for cancer: a review of the literature. Anticancer Res 2009;29:3511–36.
33. Uitterlinden AG, Fang Y, Van Meurs JB, Pols HA, Van Leeuwen JP. Genetics and biology of vitamin D receptor polymorphisms. Gene 2004;338:143–56.
34. Okazaki R. Vitamin D and cancer. Clin Calcium 2014;24:1193–9.

Brought to you by | Turkish Biochemical Society (TBD)
Authenticated
Download Date | 10/3/17 3:55 PM