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Investigation of Critical Genetic Variations of Vitamin D Metabolism and Vitamin D Serum Levels in Brain Cancer

D Vitamini Serum Düzeyleri ile D Vitamini Metabolizmasındaki Kritik Genlere Ait Varyasyonların Beyin Kanserinde İncelenmesi

Murat Özdemir[1], M. Tolgahan Hakan[1], Dilara Sönmez[1], Ali Kafadar[2], İlhan Yaylım[1], Özlem Küçükhüseyin[1]

[1]Department of Molecular Medicine, Istanbul University Aziz Sancar Institute of Experimental Medicine, İstanbul, Turkey
[2]Department of Neurosurgery, Istanbul University-Cerrahpasa Medical Faculty, İstanbul, Turkey

ORCID ID: M.Ö. 0000-0002-4081-7096; M.T.H. 0000-0002-0622-7148; D.S. 0000-0002-4962-5330; A.K. 0000-0002-3312-3192; İ.Y. 0000-0003-2615-0202; Ö. K. 0000-0001-6298-5026

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ABSTRACT

Recent studies imply the effects of micronutrient intake on the development of several cancers including primary brain cancer (PBC). The biological effects of vitamin D, a member of the fat-soluble vitamin family acting as a steroid hormone, was carried out by binding its receptor (VDR) through vitamin D-binding-protein (VDBP). The present study aims to investigate the effects of vitamin D levels and VDR rs2228570, VDR rs731236, VDBP 7041 polymorphisms on PBC development. The study group consisted of 71 patients and 84 controls. Vitamin D levels were determined by high-pressure liquid chromatography where polymorphisms by polymerase-chain-reaction and restriction fragment length polymorphism methods. The distribution of VDR rs2228570 variants in PBC and its subgroups were determined as FF>Ff>ff; VDBP rs7041 variants were TG> GG>TT, however, VDR rs731236 variants were Tt>TT>tt in PBC and meningioma and TT>Tt>tt in glioma. Vitamin D levels were measured below normal levels in all patients and control groups, which shows the deficiency in Turkish society in line with the literature. Our results show that low serum vitamin D level may be an individual risk factor in the development of brain tumors, however, VDR rs2228570 and rs731236 and VDBP rs7041 polymorphisms have no effect on the risk of disease development.

Keywords: Brain tumors, glioma, meningioma, VDR, VDBP, vitamin D

ÖZ

Son çalışmalar, mikrobesin alımının primer beyin kanseri (PBC) dahil olmak üzere çeşitli kanser türlerinin gelişimi üzerindeki etkilerine işaret etmektedir. Yağda çözünen vitaminler sınıfında yer alan ve steroid hormon olarak etki gösteren D vitamini biyolojik etkilerini reseptörü (VDR) ile vitamin D bağlayıcı protein (VDBP) aracılığıyla gerçeklestirmektedir. Çalışmamızda, D vitamini düzeyleri ile VDR rs2228570, VDR rs731236, VDBP 7041 polimorfizmlerinin PBC gelişimi üzerindeki etkilerinin araştırılması amaçlanmıştır. Çalışma grubu, beyin kanseri tanısı konmuş 71 hasta ile 84 sağlıklı bireyden oluşturulmuştur. D vitamini düzeyi, yüksek basınçlı sıvı kromatografisi yöntemiyle, VDR FokI (rs2228570), TaqI (rs731236) ve VDBP rs7041 polimorfizmleri polimeraz zincir reaksiyonu ve restriksiyon parça uzunluk polimorfizmi yöntemleriyle belirlenmiştir. Çalışmamızda VDR FokI gen varyantlarına ait dağılım primer beyin kanseri ve alt gruplarında FF>Ff>ff; VDBP rs7041 varyantları TG>GG>TT olarak, VDR TaqI varyantlarının ise primer beyin kanseri ve meningiomada Tt>TT>tt, gliomada TT>Tt>tt olduğu tespit edilmiştir. D Vitamini düzeyleri tüm hasta gruplarında ve kontrol grubunda normal düzeyinin altında ölçülmüş, bu durum Türk toplumundaki vitamin D düzeylerinin literatürle uyumlu bir şekilde düşük seviyede olduğunu göstermiştir. Çalışmamız sonuçları, beyin tümörleri gelişiminde düşük serum D vitamini seviyesinin bireysel bir risk faktörü olabileceğini, ancak VDR rs2228570 ile rs731236 ve VDBP rs7041 polimorfizmlerinin hastalık gelişim riskine etkisinin olmadığını göstermektedir.

Anahtar Kelimeler: Beyin tümörleri, glioma, meningioma, VDR, VDBP, D vitamini

Corresponding Author/Sorumlu Yazar: Özlem Küçükhüseyin **E-mail:** ozlemkh@istanbul.edu.tr
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INTRODUCTION

Brain tumors account for 1.8% of all cancers worldwide and constitute 2.3% of cancer-related deaths (1). Although primary brain tumors possess very heterogeneous pathological mechanisms, the contribution of genetic factors to the development of brain tumors is not completely understood. Recent studies focus on identifying genetic factors in the risk of cancer development due to its importance in choosing appropriate individual cancer treatment strategies (2). Besides, studies investigating the relationship between micronutrient intake and cancer development also attract attention. In fact, vitamin D and its metabolites have been shown to be associated with various types of cancer due to their direct potential to impair proliferative capacity of cancer cells and their anti-cancerogenous and toxic effects on cancer cells (3).

Vitamin D is a member of the fat-soluble vitamin family, and acts as a steroid hormone rather than a vitamin because it is incorporated into the systemic circulation to effect the target tissue and its circulating amount is regulated by feedback mechanisms. Vitamin D is taken either directly through nutrition or is endogenously synthesized in the body, however, active form of vitamin D synthesis occurs in two different tissues as liver and kidneys. Both of the skin synthesized and the dietary forms of vitamin D are converted to 25-Hydroxy Vitamin D (25-(OH) D) in the liver, which is the result of the first hydroxylation. The half-life of 25-(OH)D is 2-3 weeks and its serum level is 1000 times greater than its biologically active form, 1,25-Dihydroxy Vitamin D (1,25-$(OH)_2$D) metabolite. Thus, 25-(OH)D is the best measurement parameter for consideration of serum vitamin D levels. The second hydroxylation of 25-(OH)D metabolites by the 1 alpha hydroxylase enzyme in the kidney leads to the formation of the active form of vitamin D, 1,25-$(OH)_2$D (4).

The biological effect of vitamin D is carried out by binding its membrane-bound receptor (VDR) through vitamin D binding protein (VDBP). The direct effects, also known as non-genomic effects of vitamin D is driven by interacting with VDR. The engagement with VDR activates intracellular secondary messengers which leads normal serum levels of calcium and phosphate, regulation of osteoblastic functions and bone resorption (4,5). On the other hand, transcription factors mediate the genomic effects as cell proliferation, differentiation, and apoptosis as well as DNA repair and oxidative stress (5-7).

VDR required for vitamin D function has been shown to be present in more than 50 tissues including the central nervous system (CNS) cells as microglia, astrocyte, oligodendrocyte cells and peripheral nervous system (PSS) cells as Schwann cell nuclei which affects the metabolism of brain cells (6,8,9). *In vitro* studies in microglia cells confirmed the metabolisation of 25-(OH)D to 1,25-$(OH)_2$D in the brain (10). On the other hand, it is well-known in vitamin D metabolism that, 24-hydroxylase (CYP24A1) inactivates the active form of vitamin D by converting it to 1,24,25-$(OH)_3$D in a dose dependent manner. In fact, it was shown in rat primary glia cells that the expression of CYP24A1 mRNA was increased with the excess amounts of 1,25-$(OH)_2$D, which points the regulation of vitamin D levels in the brain (11).

VDR protein is a member of the nuclear receptor family. Several polymorphic sites have been identified on VDR gene. Among the most common VDR gene polymorphisms on chromosome 12q13 which alters the binding capacity of active vitamin D to VDR, FokI (rs2228570) and TaqI (rs731236) attract attention. rs2228570 polymorphism on exon 2 leads an amino acid substitution [thymine(T)→Cytosine(C)] that alters the transcriptional activity of VDR protein by formation of two different translation initiation region. As for rs731236 polymorphism on exon 9 a T to C alternation occurs and results in a synonymous transformation (isoleucine → isoleucine) that affects the mRNA levels of VDR protein (6,12-15).

VDBP, another important molecule in vitamin D metabolism, is a glycoprotein acting in various biological functions as fatty acid transportation, chemotaxis and macrophage activation (7). Several polymorphic sites have been identified on VDBP gene localized on chromosome 4q11–q13 including rs7041 and rs4588. Both polymorphisms of VDBP was responsible for the alteration of binding affinity and/or carrying capacity of VDBP that affects vitamin D serum levels (16).

Previous studies reported that the disorders of vitamin D metabolism play a central role in the pathogenesis of various diseases by affecting cell homeostasis and growth. Besides, the effects of VDR gene polymorphisms on various types of carcinomas such as breast, prostate and colon have been widely studied (6,12,13). The present study aims to investigate the effects of VDR rs2228570, rs731236, and for the first time VDBP rs7041 polymorphisms and serum levels of vitamin D on the risk of primary brain cancer development in Turkish society

MATERIAL AND METHOD

Participants

The study group comprised of 71 primary brain cancer patients followed by Istanbul University-Cerrahpasa Medical Faculty, Department of Neurosurgery and 84 healthy volunteers with no signs and no family history of any malignancy.

The present study conformed with the Helsinki Declaration. All participants in the study signed their written consent prior to the study. The blood samples were taken only with written informed consent. The study protocol was also approved by the local ethical committee (Decree No: 2017/1480) and the Research Fund of Istanbul University (Project Number: TYL-2018-28203).

Measurement of Serum Vitamin D Levels

High-Performance Liquid Chromatography (HPLC) technique was used to measure the serum vitamin D levels. HPLC system (Spectra System, Thermo Scientific, USA). RP C18 analytical column 250 × 4.6 mm, 5 µm particle size (Knauer, Berlin, Germany) was used to separate the analytes. The detection was performed with a UV detector by using commercial vitamin D measurement kit and bi-level controls [Chromogen Grafelfing, Germany]

according to the manufacturer's instructions (flow rate = 0.7 ml/min; column temperature = 25 °C; wavelength = 265 nm).

Genotyping

Genomic DNA (gDNA) was isolated by a commercial DNA purification kit (Jena Bioscience, Jena, Germany). gDNA was amplified by polymerase chain reaction (PCR) and the genotypes were analyzed with restriction fragment length polymorphism (RFLP) method as previously reported (12-17). Table 1 shows the PCR-primer sequences of rs2228570, rs731236 and rs7041 polymorphisms. The PCR products were 272 base pairs (bp) for VDR FokI, 347 bp for VDR TaqI and 482 bp for VDBP rs7041. Genotyping was performed by RFLP method with *FokI, TaqI* and *HaeIII* endonuclease restriction enzymes. The fragments were then viewed under UV light after staining with ethidium bromide. The fragments of VDR FokI polymorphism were 272 bp for wild type homozygous (FF) genotype and 198+74 bps for mutant (ff) genotype; VDR TaqI were 347 bp for wild type homozygous (TT) genotype and 293+54 bps for mutant (tt) genotype.; VDBP rs7041 were 482 bp for wild type homozygous (TT) genotype and 298+184 bps for mutant (GG) genotype.

Table 1. The PCR primer sequences of VDR and VDBP gene polymorphisms.

SNP	Primer sequence
VDR rs:2228570 (FokI)	5'-GATGCCAGCTGGCCCTGGCACTG-3'
	5'-ATGGAAACACCTTGCTTCTTCTCCCTC-3'
VDR rs:731236 (TaqI)	5'-CAGAGCATGGACAGGGAGCAAG-3'
	5'-GCAACTCCTCATGGGCTGAGGTCTCA-3'
VDBP rs:7041	5'-AAATAATGAGCAAATGAAAGAAGAC-3'
	5'-TCTACTCATTTCTTTGCTGTTATTG-3'

SNP: single nucleotide polymorphism

Statistical Analysis

Statistical analysis was performed using the SPSS software package (revision 21.0; SPSS Inc., Chicago, IL, USA). Chi-Square ($\chi 2$) test and Student's t-test or One-Way Anova test were used to compare the frequency of gene variants and identify the effects on the biochemical activity, respectively. $p<0.05$ was considered as statistical significance. The relative risk determination was estimated by calculating odds ratio (OR) and confidence intervals. Allele frequencies were made according to the gene counting technique. The figures were obtained by using GraphPad Prism 6.0 (San Diego, CA, USA).

RESULTS

The study groups were age and sex matched ($p>0.05$). The control group comprised of 63.1% females and 36.9% males with 49.3% females, and 50.7% males in the patient group. The mean age was 40.14±13.18 in the control group, and 42.00±13.43 in the patient group. The baseline of the patients group was shown in Table 2. Among the glioma and meningioma patients

Table 2. Baseline characteristics of the patients group.

Clinical features	Primary Brain Tumors (n=71)	Glioma Cases (n=40)	Meningioma Cases (n=31)
Tumor Histology (n,%)			
Astrocytoma + Oligoastrocytoma	53 (75.0%)	30 (75.0%)	-
Oligodendroglioma	18 (25.0%)	10 (25.0%)	-
Tumor Grade (n,%)			
Grade I	25 (35.8%)	5 (12.1%)	22 (69.5%)
Grade II	22 (30.4%)	16 (39.4%)	5 (17.4%)
Grade III	14 (19.6%)	9 (24.2%)	4 (13.1%)
Grade IV	10 (14.3%)	10 (24.3%)	-
Tumor Localization (n,%)			
Right Hemisphere	36 (51.1%)	22 (53.3%)	4 (11.7%)
Left Hemisphere	32 (44.7%)	18 (46.7%)	14 (47.1%)
Midline	3 (4.3%)	-	13 (41.2%)
Necrosis Pathology (n,%)			
present	38 (52.9%)	21 (52.9%)	-
absent	33 (47.1%)	19 (47.1%)	-
Vascular Endothelial Proliferation (n,%)			
present	56 (78.9%)	32 (78.9%)	-
absent	15 (21.1%)	8 (21.1%)	-

The difference between the groups was analyzed by Chi square (X^2) test for values with percentage (%).

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group, the frequency of cases with advanced tumor grade (III + IV) was lower than early-stage patients (I + II) (48.5%→51.5% and 13.0%→87.0%, respectively), as well as primary brain cancer (66.1% → 33.9%) (data not shown).

The genotype and allele frequencies of VDR rs2228570 and rs731236 and VDBP rs7041 polymorphisms in primary brain cancer patients, meningioma and glioma cases, and controls were shown in Table 3. No significant relations were found between the risk of primary brain cancer and VDR or VDBP polymorphisms (p>0.05). The difference between VDR rs2228570, rs731236 or VDBP rs7041 variants and clinical parameters of primary brain tumors (Table 4) and glioma or meningioma (data not shown) were also not significant (p>0.05).

Figure 1 shows the comparison of vitamin D (25-hydroxyvitamin D, 25-(OH)D) serum levels among the study groups. The means of serum 25-(OH)D levels in primary brain cancer, glioma and meningioma patients versus controls were 14.33±1.20 ng/ml, (p=0.042), 13.09±1.69 ng/ml (p=0.039), 15.74±1.66 ng/ml (p>0.05) and 18.75±1.81 ng/ml, respectively. However, no significant differences were observed between vitamin D serum levels and clinical parameters as sex, necrosis pathology, vascular endothelial proliferation, sex and advanced or early tumor grades (data not shown, p > 0.05).

The serum levels of 25-(OH)D among VDR rs2228570, rs731236 and VDBP rs7041 variants in primary brain cancer patients and controls were shown in Figure 2. The difference in the levels of vitamin D among VDR and VDBP variants was not significant, except heterozygous VDR FokI genotype, Ff (p=0.047).

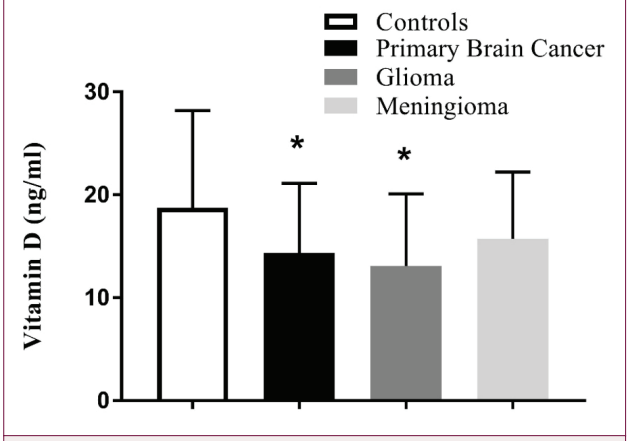


Figure 1. Serum levels of 25-(OH)D in the study groups. Unpaired student's t test was used to estimate the difference between the groups.

Table 3. The distribution of *VDR* rs2228570 and rs731236, and *VDBP* rs7041 genotypes and alleles in the whole study groups.

Genotypes and Alleles (n,%)		Control (n=84)	Primary Brain Tumors (n=71)	Glioma Cases (n=40)	Meningioma Cases (n=31)
VDR rs2228570	FF	41 (48.8%)	37 (52.1%)	20 (50.0%)	17 (54.8%)
(FokI)	Ff	32 (38.1%)	25 (35.2%)	16 (40.0%)	9 (29.0%)
	ff	11 (13.1%)	9 (12.7%)	4 (10.0%)	5 (16.2%)
	F Allele	114 (67.9%)	99 (69.7%)	56 (70.0%)	43 (69.4%)
	f Allele	54 (32,1%)	43 (30.3%)	24 (30.0%)	19 (30.6%)
VDR rs731236	TT	31 (36.9%)	29 (40.8%)	18 (45.0%)	11 (35.5%)
(TaqI)	Tt	40 (47.6%)	32 (45.1%)	16 (40.0%)	16 (51.6%)
	tt	13 (15.5%)	10 (14.1%)	6 (15.0%)	4 (12.9%)
	T Allele	102 (60.7%)	90 (63.4%)	52 (65.0%)	38 (61.3%)
	t Allele	66 (39.3%)	52 (36.6%)	28 (35.0%)	24 (38.7%)
VDBP rs7041	TT	9 (10.7%)	8 (11.3%)	5 (12.5%)	3 (9.7%)
	TG	49 (58.3%)	43 (60.6%)	25 (62.5%)	18 (58.1%)
	GG	26 (31.0%)	20 (28.2%)	10 (25.0%)	10 (32.3%)
	T Allele	67 (39.9%)	59 (41.5%)	35 (43.7%)	24 (38.7%)
	G Allele	101 (60.1%)	83 (58.5%)	45 (56.3%)	38 (61.3%)

Chi-square test was used to compare genotypes in the study group. For determining allele frequencies gene count method was used. n, number of individuals; p<0.05 denoted statistical significance.

Table 4. The distribution of clinical features according to *VDR* rs2228570 and rs731236, and *VDBP* rs7041 variants in primary brain tumor patients group.

Clinical features		*VDR* FokI				*VDR* TaqI				*VDBP*			
		FF Genotype	Ff Genotype	ff Genotype	*p value*	TT Genotype	Tt Genotype	tt Genotype	*p value*	TT Genotype	GT Genotype	GG Genotype	*p value*
Tumor Histology	**A+OA (n=53)**	44.4%	44.4%	11.2%	0.646	37.1%	44.4%	18.5%	0.683	14.8%	59.3%	25.9%	0.921
	OD (n=18)	66.7%	22.2%	11.1%		55.6%	33.3%	11.1%		11.1%	66.7%	22.2%	
Tumor Grade	**I (n=25)**	61.9%	28.6%	9.5%	0.921	38.1%	47.6%	14.3%	0.649	14.3%	52.4%	33.3%	0.916
	II (n=22)	47.1%	35.3%	17.6%		41.2%	52.9%	5.9%		11.8%	52.9%	35.3%	
	III (n=14)	66.3%	36.4%	-		36.4%	54.5%	9.1%		18.2%	72.7%	9.1%	
	IV (n=10)	50.0%	37.5%	12.5%		25.0%	37.5%	37.5%		12.5%	62.5%	25.0%	
Necrosis Pathology	**(+) (n=38)**	44.4%	55.6%	-	0.637	55.6%	22.2%	22.2%	0.218	11.1%	55.6%	33.3%	0.658
	(-) (n=33)	62.5%	37.5%	-		37.5%	62.5%	-		25.0%	62.5%	12.5%	
Vascular endothelial proliferation	**(+) (n=56)**	46.7%	53.3%	-	0.582	46.7%	33.3%	20.0%	0.451	13.3%	66.7%	20.0%	0.773
	(-) (n=15)	75.0%	25.0%	-		25.0%	75.0%	-		25.0%	50.0%	25.0%	

Chi-square test was used to compare variants in the study groups. n, number of individuals; p<0.05 denoted statistical significance; A, Astrocytoma; OA, Oligoastrocytoma; OD: Oligodendroglioma.

Figure 2. The serum levels of 25-(OH)D among VDR rs2228570 FokI, rs731236 TaqI and VDBP rs7041 variants in primary brain cancer patients and controls. Student's t and Anova tests were used to evaluate the difference between and within the groups. *is donated as statistical significance of *Ff* genotype between primary brain cancer patients and controls.

DISCUSSION

Primary brain tumors are one of the most common types among the cancer cases in the world (1,17). In Turkey, it was reported that brain cancer constitutes ~2% of all cancer types in 2017 and said to be in the top 10 cancers (18). Although the exact mechanism of the formation of brain tumors was not fully understood, environmental factors such as exposure to toxic chemicals or ionizing radiation, nutritional behaviors as micronutrient intake including vitamin D and genetic predisposition are some of the risk factors for the risk of primary brain tumor development (4,5,13).

Vitamin D, found in the 1800s, was shown to have important functions such as the regulation of calcium balance, bone metabolism and more recently in cancer progression and development by its genomic effects as the regulation of cell proliferation, differentiation and apoptosis, DNA repair, oxidative stress and cellular metabolism through transcription factors (4-7,19). In 2008, a report showing the evidence of the causal link between vitamin D and cancer was published by the international cancer research center. Vitamin D is considered to be among the steroid hormones that regulate cell functions such as cell proliferation and differentiation. The effect of active form of vitamin D, 1,25-$(OH)_2$D, on cell growth and differentiation of cancer cells makes this molecule a candidate marker in the regulation of tumor cells (20). Several studies reported the vitamin D-inhibition of proliferation and differentiation of malignant cells in various tissues such as breast, colon, skin, lung and brain (3,21-29). It has been conducted that high levels of 25-(OH)D, a vitamin D storage form, are associated with low incidence in various types of cancer (3,21-29). In fact, Shi

et al. showed lower levels of 25-(OH)D (<20 ng/ml) in 71.6% of patients diagnosed with breast cancer (25). Mezawa et al. reported significant relationships between colorectal cancer and vitamin D insufficiency (26). Moreover, Atoum et al. reported a 19.2 fold increased risk with vitamin D insufficiency (<10 ng/ml) in colorectal cancer patients (15). On the other hand, in a meta-analysis on optimal serum 25-(OH) D levels to protect from colorectal cancer, Gorham et al. reported that colorectal patients with optimal 25-(OH)D (33 ng/ml) serum levels possess 50% decreased cancer risk compared to patients with ≤12 ng/mL 25-(OH)D (27). In contrast to these studies, in a case-control study among 200 patients diagnosed with lung cancer and a control group of 400 people conducted by Gromowski et al., the average 25-(OH) D levels of lung cancer patients has been reported as 17.1 ng/ml while it was 17.2 ng/ml in the controls, and the study concluded as serum vitamin D levels do not affect the development of lung cancer (28).

Ogus et al. evaluated the serum vitamin D levels in the Turkish population and reported the average vitamin D levels as 22.80±13.27 ng/ml (22.49±13.88 in women, 23.73±10.57 ng/ml in men). According to their study conducted on 2012, at least 24% of women, 29% of men and 25% of the whole population have optimal serum vitamin D levels (29). In our study, the mean 25-(OH)D level of the control group consisting of 84 people was measured as 18.75±1.81 ng/ml, while it was 14.33±1.20 ng/ml, (p=0.042) in primary brain cancer patients, 13.09±1.69 ng/ml (p=0.039) in glioma patients and 15.74±1.66 ng/ml (p>0.05) in meningioma patients. Holick et al. classified vitamin D levels as deficient (<20 ng/ml), insufficient (21-29 ng/ml) and adequate (> 30 ng/ ml) (30). Accordingly, vitamin D deficiency is observed in the entire study group, since both the control group and the patient group have 25-(OH)D levels below 20 ng/ml. Our results are compatible with Ogus et al. (29). Despite contradictory reports related to Vitamin D and cancer risk in the literature, in our study, when evaluated for primary brain cancer and its subgroups, glioma and meningioma, our results revealed that vitamin D levels may have an independent effect on primary brain cancer and glioma development.

The biological effect of vitamin D was triggered by binding its specific receptor, VDR. While VDR is an important molecule in multiple pathways such as insulin growth factor (IGF), it also involves in inflammation and estrogen-related pathways that may be associated with cancer prognosis (3). In many studies, the effect of VDR gene polymorphisms has been shown in the development of various cancer types including breast, brain, prostate and colorectal cancer (3,6,12-15,18-28). One of the common VDR gene polymorphisms in exon 2 is FokI (rs2228570) polymorphism which leads in an alternative transcription initiation site by the substitution of cytosine (C) thymine (T) and alters the activity of the VDR protein. On the other hand, in TaqI (rs731236) polymorphism of exon 9, another well-known polymorphism, a T→C nucleotide substitution (ATT→ATC) leads to a silent mutation (isoleucine → isoleucine) in codon 352 affecting the secondary structure of the RNA transcript (12-15). Studies have reported that VDRs localized in neuronal and glial cells affect the metabolism of brain cells and alter VDR expression (31,32).

The potential effect of vitamin D on cancer treatment was first described in myeloid leukemia cells (33), and latter synthetic vitamin D analogs have been proposed for use in the treatment of central nervous system tumors. In fact, phase II clinical trials have been associated with the positive effects of vitamin D treatment on glioblastoma cells (33-36). It has been reported that increased vitamin D production in glioma cells after treatment may regulate cell proliferation (31). Many studies have also noted the effect of VDR polymorphisms on various types of cancer.

In the case-control study of Moossavi et al., VDR-FokI (rs2228570) ff genotypes (OR=4.31, 95%CI: 2.99-6.22, p=0.0001,) and f allele (OR=4.83, 95%CI: 0.99-23.8, p=0.035) were found to have a high risk of colorectal cancer (37). Yilmaz et al. reported no association between brain cavity and VDR-FokI variants in pediatric brain cancer patients diagnosed with malignant brain tumor (38). In study of Tang et al. conducted with 5284 cases and 7500 control groups in Europe, a significant increase in breast cancer risk and VDR-FokI polymorphism variants was reported (39). Toptas et al. reported a positive correlation between meningioma patients and VDR-FokI variants in brain cancer patients (p=0.004) in contrast to glioma patients (p>0.05) (3). In the present study, the relationship between polymorphisms in VDR Fok-I and Taq-I and brain cancer risk was evaluated. The distribution of genotype variants of VDR FokI polymorphism in primary brain tumors and its sub-groups, glioma and meningioma were found as FF> Ff> ff, respectively. The order of genotype frequencies in the control group was similar to that of the patient group. No significant association was found between VDR FokI polymorphism and brain cancer development. Besides, the serum levels of 25-(OH)D does not correlate with VDR TaqI genotypes in patients and control groups except Ff genotype which was found to be lower in brain cancer patients. Our results partially comply with the results of previous studies (3,38). Our findings indicate that, in meningioma patients, contrary to the study of Toptas, there is no relationship between the development of meningioma and rs2228570 variants; however, our results in glioma patients are similar to those of Toptas (3) and Yilmaz (38) et al.. Since this different result may be related to the inadequate number of specimens belonging to the meningioma patient group, which is the subtype of primary brain cancers in our study group, it may be useful to re-examine the number of patients by expanding the number of the samples and the experiments in which the situation comprise the primary limitation of our study for detection of the risks in cancer subgroups.

In the study of Toptas et al. which was conducted on patients diagnosed with brain cancer in the Turkish population, VDR-TaqI (rs731236) polymorphism variants were also examined in meningioma and glioma patients. The findings stated that there was no significant relationship (p>0.05) (3). Similarly, the

study of Yilmaz et al. found no association between brain tumors and VDR-TaqI polymorphism variants in pediatric brain cancer patients (38). In colorectal cancer patients, Moossavi et al. also showed any significant difference for VDR-TaqI variants (37). In contrast to these studies, Tang et al. reported a significant relationship between breast cancer and VDR-TaqI, BsmI and ApaI polymorphisms variants (39). In the study of Chen et al. published in 2018, the distribution of VDR-TaqI polymorphism in prostate cancer indicated that there was an association with Asians and especially with Japanese population (40). In our study, while the order of VDR TaqI variants was found as Tt> TT> tt in primary brain cancer and meningioma patients, and TT> Tt> tt in glioma patients vs Tt> TT> tt in controls, no relation was found between VDR TaqI variants and primary brain cancer, glioma and meningioma patients. In addition, the lack of a significant relationship between the 25-(OH) vitamin D level and the VDR TaqI gene variants indicates that there is no relationship between VDR Taq polymorphism and brain cancer.

VDBP, which plays a very important role in vitamin D metabolism, is a glycoprotein that carries vitamin D metabolites in the blood. VDBP, which is high in the blood, is required to carry vitamin D to many tissues and prevent the occurrence of various diseases (7,16). Although VDBP has been shown to have more than 120 variants, two important phenotypic alleles have been widely studied as VDBP rs7041 which causes an amino acid change (aspartic acid → glutamic acid) in codon 416 and VDBP rs4588 causes threonine to lysine change in codon 420 (7,16,14). Zhou et al. reported no association between VDBP rs7041 polymorphism and gastrointestinal, colorectal, gastric, esophageal and hepatocellular cancers (14). In contrast, Baykara et al. indicated a significant relation with lung cancer (41), as Anderson et al. in breast cancer (42). In our study, VDBP rs7041 distribution was found as TG> GG> TT. GG> TT in the whole patients' groups and controls. Our results showed any association between VDBP rs7041 polymorphism and the risk of the development of brain tumors similar to several reports in the literature. On the other hand, the lack of any association between VDBP variants and vitamin D serum levels indicates the existence of no correlation with brain tumors. To our knowledge, our findings of VDBP rs7041 are the first results in brain tumors in Turkish population which fills the gap in this subject.

Prospective and retrospective studies in the literature indicates that 25-(OH)D levels lower than 20 ng/ml increase the mortality rates by 50% in several cancer types (43). Low life expectancy was also indicated in patients diagnosed with cancer due to vitamin D deficiency. Vitamin D, of which the levels were affected by dietary habits, exposure to sunlight, seasonal changes, sex, age, malabsorption disorder, and the use of certain medications that affect absorption, has different survival roles in vital mechanisms through its blood levels via genomic and non-genomic functions as acting as anti-inflammatory, anti-oxidant, as well as playing part in DNA damage repair, apoptosis, autophagic cell death, anti-proliferation and differentiation (4,5,19,20,44).

CONCLUSION

In conclusion, our results showed that polymorphisms in the vitamin D receptor (rs2228570 and rs731236) and binding protein (rs7041) have no effect on the development and prognosis of brain tumors. However, lower serum levels of vitamin D may be an independent risk factor for the development of glioma and primary brain cancer. On the other hand, in line with the literature, vitamin D levels in Turkish society were low as it was measured below normal levels as <20 ng/mL in both patient group and control group.

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