Molecular Biology Reports (2019) 46:6413–6420
https://doi.org/10.1007/s11033-019-05088-9

ORIGINAL ARTICLE

The effects of serum levels, and alterations in the genes of binding protein and receptor of vitamin D on gastric cancer

Şermin Durak[1] · Arezoo Gheybi[1] · Şeyda Demirkol[1,3] · Soykan Arıkan[2] · Ş. Ümit Zeybek[1] · Filiz Akyüz[4] · İlhan Yaylım[1] · Özlem Küçükhüseyin[1]

Received: 10 July 2019 / Accepted: 18 September 2019 / Published online: 23 September 2019

Abstract

Due to many biological cell functions of vitamin D including regulation of cell survival, proliferation and differentiation, the metabolism of itself gains importance in the development of several types of cancer. This case–control study was designed to evaluate the risk of gastric cancer development in terms of *VDR* rs2228570 & rs731236, and *VDBP* rs7041 polymorphisms, and serum levels of vitamin D. The study consists of 77 gastric cancer patients and 84 healthy individuals. *VDR* and *VDBP* gene polymorphisms and vitamin D levels were determined by using PCR–RFLP and HPLC methods. The distribution of *VDR* or *VDBP* gene variants were not different in study groups. The serum level of 25-hydroxyvitamin D was significantly lower in gastric cancer patients versus controls ($16 \pm 6 \rightarrow 11 \pm 6$ ng/ml) in which male patients have higher levels than females. Although the whole study population lacks normal levels of 25-hydroxyvitamin D, it was found that the risk of the development of gastric cancer was approximately fourfold higher in cases with severe vitamin D (< 10 ng/ml) deficiency. Our results indicate that *VDR* rs731236 & rs2228570 or *VDBP* rs7041 polymorphisms were not risk factors for the development of gastric cancer individually, however, lower serum levels of vitamin D may be a contributory risk for both predisposition and development of gastric cancer.

Keywords Gastric cancer · VDR · VDBP · Vitamin D · Polymorphism

Introduction

Gastric cancer is the fourth most common cancer and the second in cancer-related deaths worldwide [1]. Between 60 and 80 years of age in the world, one in every 36 men and one in 84 women have gastric cancer [2, 3] Every year, more than 950,000 new diagnoses are made and 720,000 patients die from gastric cancer in Europe [1], and more than 50% of new cases occur in developing countries. In Turkey, gastric cancer is the fifth most common type of cancer in males with the incidence of 14%, and the sixth most common type in women with 7% [4]. In addition to the major etiological

✉ Özlem Küçükhüseyin
ozlemkh@istanbul.edu.tr; ozlem.kh@gmail.com

Şermin Durak
sermingad@gmail.com

Arezoo Gheybi
arezoo.gh_62@yahoo.com

Şeyda Demirkol
seyda.erc@gmail.com

Soykan Arıkan
soykanarikan@yahoo.com

Ş. Ümit Zeybek
uzeybek@istanbul.edu.tr

Filiz Akyüz
filiz.akyuz@istanbul.edu.tr

İlhan Yaylım
ilhanyaylim@gmail.com; iyaylim@istanbul.edu.tr

1 Department of Molecular Medicine, Aziz Sancar Institute of Experimental Medicine, Istanbul University, Vakif Gureba Ave., Capa, 34393 Istanbul, Turkey

2 General Surgery Clinics, Istanbul Educating and Training Hospital, Istanbul, Turkey

3 Vocational School of Health Services, Biruni University, Istanbul, Turkey

4 Department of Gastroenterology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey

factors as *Helicobacter pylori* infection, *Epstein Barr* virus, smoking and alcohol consumption, exposure to radiation, pernicious anemia, physical inactivity, excess weight, irregular nutrition and genetic factors, it was shown that geographical and ethnic differences play significant roles in the distribution of gastric cancer in the world as the incidence was higher in black populations, low socioeconomic groups and developing countries [5, 6].

Vitamin D is a steroid hormone in the class of fat-soluble vitamins involved in a diversity of biological processes such as cell proliferation, bone metabolism and cell differentiation [7]. For the function, vitamin D which was carried by vitamin D binding protein (VDBP), connects with Vitamin D Response Elements (VDRE) on DNA by making a triple complex with Vitamin D Receptor (VDR) and Retinoic acid X-receptor. The genomic effects of vitamin D as the regulation of cell apoptosis, differentiation, proliferation, DNA repair, oxidative stress and cellular metabolism, was driven through transcription factors. On the other hand, direct effects which were also known as non-genomic effects as altering the transmembranal transmission of calcium and chlorine were shown by interacting with membrane-bound VDR and activating intracellular secondary messengers [6, 8–11].

VDR protein with 427 amino acid residues is a member of the nuclear receptor family (NR1) and translated by *VDR* gene, located on chromosome 12q13. Although several polymorphisms have been identified in the *VDR* gene FokI (rs2228570), TaqI (rs731236), BsmI (rs544410) and ApaI (rs7975232) are the most common polymorphisms that affect binding of vitamin D to VDR. Among them, FokI polymorphism on exon 2 leads thymine (T) to Cytosine (C) transformation which results in the formation of two different translation initiation region affecting the transcriptional activity of VDR protein. On the other hand, TaqI polymorphism on exon 9 also leads to T to C transformation, however, this change cause a synonymous alternation affecting the mRNA levels of VDR protein [9, 12].

VDBP gene is localized on chromosome 4q11–q13 and expresses VDBP protein, a 52–59 kDa glycoprotein, possessing significant biological functions including fatty acid transport, binding extracellular actin scavenger system, chemotaxis and macrophage activation. Recently, important roles of VDBP-macrophage activation factor (MAF) transformation has been shown in anti-angiogenic mechanism which effects tumor growth by inhibition of cancer cell migration and proliferation [13]. *VDBP* genes have two common single-nucleotide polymorphisms (SNP); rs7041 and rs4588, which affect carrying capacity of VDBP (or binding affinity of vitamin D) as well as serum levels of vitamin D [14–16].

Previous studies suggested the antitumoral role of 1,25-dihydroxy Vitamin D [1,25$(OH)_2D_3$] treatment on gastric cancer cell line by inducing apoptosis [8]. Moreover, Baek et al. [9] reported both the synergistic effect of vitamin D with anti-cancer drugs including paclitaxel, adriamycin, and vinblastine, and the regulatory role of vitamin D as an antagonistic effect on hedgehog signaling by reducing the mRNA expression of target hedgehog genes [9]. On the other hand, as functional vitamin D receptor (VDR) elements have been identified in the promoter region of phosphatase and tensin homolog (*PTEN*, an important inhibitor of cell growth,) gene, Vitamin D regulated *PTEN* expression was reported as a nuclear transcription factor [9]. In the literature, the results of studies examining the relationship between gastric cancer and vitamin D metabolism are quite conflicting [7, 9–18], Therefore, in the present study, the evaluation of vitamin D levels, and the distribution of *VDR* rs2228570 & rs731236 and for the first time *VDBP* rs7041 polymorphisms in Turkish gastric cancer patients in accordance with clinical parameters, and the determination of their roles in the development of gastric cancer were aimed.

Materials and methods

Participants

The present case–control study was carried out in two groups. The control group was selected from 84 healthy individuals with no signs of malignancy and preferably no family history of cancer, and the patient group was consisted of 77 patients diagnosed with gastric cancer followed by Istanbul University Faculty of Medicine Department of Internal Sciences and Istanbul Training and Research Hospital General Surgery Clinic.

All participants in the study provided their written consent prior to the study. This study confirmed with the Helsinki Declaration and, the study protocol was approved by both the Ethical Committee (Decree No. 476413) and the Research Fund of Istanbul University (Project No: TYL-2018-29205).

Serum vitamin D level measurement by HPLC

A high-pressure liquid chromatography (HPLC) system (Spectra System, Thermo Scientific, USA) equipped with a UV detector was used for chromatographic analysis of vitamin D levels. The separation of the analytes was performed on RP C18 analytical column 250×4.6 mm, 5 µm particle size (Knauer, Berlin, Germany). The detection was carried out commercially available vitamin D measurement kit [Reagent kit for HPLC analysis of 25-(OH)Vitamin D3/D2 in serum/plasma, Chromogen Grafelfing, Germany] and bi-level controls [25-(OH)Vitamin D3/D2 Bi-level control, Chromogen Grafelfing, Germany]. All the instrumental

controls, data collection, and quantitation were performed according to the manufacturer's instructions. The HPLC protocol was as follows : flow rate = 0.7 ml/min; column temperature = approximately 25 °C; wavelength = 265 nm.

Polymorphism genotyping

The genomic DNA was obtained by using DNA isolation kit (Jena Bioscience, Jena, Germany), and the polymorphisms were determined by using polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP) method as previously reported [12, 14, 16]. Genotyping was performed by using FokI, TaqI and HaeIII endonuclease restriction enzymes. The fragments were then visualized under UV light after ethidium bromide staining.

Statistical analysis

Statistical analysis was performed using the SPSS software package (revision 21.0; SPSS Inc., Chicago, IL, USA). Statistical significance was accepted as $p < 0.05$. Estimation of relative risk was determined by calculating odds ratio (OR) and confidence intervals. Student's t-test was used to determine the difference in quantitative biochemical parameters as mean ± standard deviation. Chi-Square (χ^2) test was used to compare the qualitative data as percentage (%) including genotype and allelic comparison, and the demographic distribution of cancer prognosis. Allele frequencies were calculated by gene counting method.

Results

The present case–control study includes 77 gastric cancer patients (42% females and 58% males) and 84 healthy individuals (55% females and 45% males), and the study groups have a similar distribution of sex and age ($p > 0.05$). Among the patient group, the frequency of cases with advanced tumor grade (III + IV) was higher than early-stage patients (I + II) (84% → 16%). The demographic and clinical data of the study groups were shown in Table 1.

Table 1 Demographic and clinical data of patient and control groups

Characteristic data	Patients (n = 77)	Control (n = 84)
Age (years)	56.7 ± 1.74	51.42 ± 2.41
Gender (n, %)		
Female	33 (42%)	46 (55%)
Male	44 (58%)	38 (45%)
Tumor grade (n, %)		
I	2 (2%)	–
II	11 (14%)	–
III	33 (43%)	–
IV	31 (41%)	–
Lymph node (n, %)		
N0	8 (10%)	–
N1	30 (39%)	–
N2	30 (39%)	–
N3	9 (12%)	–
Distant Metastasis (n, %)		
Present	24 (31%)	–
Absent	53 (69%)	–
Necrosis (n, %)		
Present	5 (6%)	–
Absent	72 (94%)	–
Perineural invasion (n, %)		
Present	13 (17%)	–
Absent	64 (83%)	–
Angiolymphatic invasion (n, %)		
Present	5 (6%)	–
Absent	72 (94%)	–
Tumor localisation (n, %)		
Gastric cardia	10 (13%)	–
Antrum and corpus	51 (67%)	–
Other	16 (20%)	–
Tumor histology (n, %)		
Adenocarcinoma	50 (65%)	–
Signet ring cell carcinoma	27 (35%)	–

The difference between the groups was analyzed by student's t test for mean values (mean ± SD), and Chi square (×2) test for values with percentage (%)

The distribution of genotype and allele frequencies of *VDR* FokI rs 2228570 and TaqI rs731236 and *VDBP* rs7041 polymorphisms in gastric cancer patients and control groups were shown in Table 2. No significant associations were found between the development of gastric cancer and *VDR* or *VDBP* polymorphisms ($p > 0.05$). Moreover, the clinical parameters were also not associated with *VDR* rs2228570 and *VDBP* rs7041 genotypes and alleles ($p > 0.05$). However, unlike to *VDR* rs2228570 and *VDBP* rs7041, higher frequency of *VDR* rs731236 *TT and Tt* genotypypes were found in advanced stage patients (grade III + IV) than those with early-stage (grade I + II) ($p = 0.03$). However, while early stage patients were lack of tt genotype the comparison of tt genotype according to cancer stage cannot evaluated. Therefore, the frequency of t allel and TT genotype was compared and higher t allele frequency was found in advanced stage patients than TT genotype ($p = 0.06$) (data not shown).

The comparison of 25-hydroxyvitamin D [25(OH) D] serum level among the study groups was shown in Fig. 1. The means of 25(OH)D serum levels in gastric cancer patients versus controls were 11 ± 6 → 16 ± 6 ng/ml ($p = 0.002$), respectively. In addition, in male patients, serum levels of 25(OH)D were found higher than female patients

Table 2 Genotype and allele frequencies of *VDR* FokI rs2228570 and TaqI rs731236, and *VDBP* rs7041 polymorphisms in gastric cancer patients and control groups

Genotypes and alleles	Patient (n, %)	Control (n, %)	*p* value
VDR rs: 731286			
TT	20 (26%)	30 (35%)	**0.29**
Tt	38 (49%)	40 (48%)	
tt	19 (25%)	14 (17%)	
T allele	78 (51%)	100 (60%)	**0.11**
t allele	76 (49%)	68 (40%)	
VDR rs: 2228570			
FF	37 (48%)	42 (50%)	**0.29**
Ff	35 (45%)	31 (37%)	
ff	5 (7%)	11 (13%)	
F allele	110 (71%)	116 (69%)	**0.65**
f allele	44 (29%)	52 (31%)	
VDBP rs: 7041			
TT	12 (15%)	9 (11%)	**0.14**
TG	32 (42%)	48 (57%)	
GG	33 (43%)	27 (32%)	
T allele	56 (36%)	58 (34%)	**0.59**
G allele	98 (64%)	110 (66%)	

The difference between the groups was analyzed by Chi square (x^2) test. Bold values of $p < 0.05$ indicate statistical significance

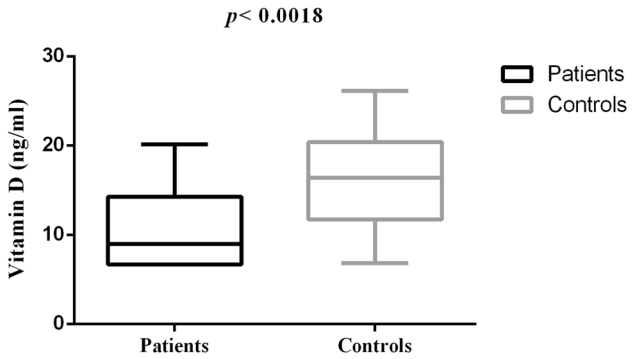

Fig. 1 Serum levels of 25(OH)D in the study groups. The difference between the groups was analyzed by unpaired student's t test

(14 ± 6 → T8 ± 2 ng/ml ($p = 0.001$). However, when examined in terms of clinical parameters as lymph node metastasis, invasion and necrosis status, 25(OH)D levels show no significant difference ($p > 0.05$). Similarly, no significant difference was found between clinical parameters and gender ($p > 0.05$).

Vitamin D status was classified as > 30 ng/ml as sufficient, 21–29 ng/mL as inadequate, < 20 ng/ml as deficient in normal subjects based on Endocrine Society consensus for vitamin D status [19]. In the present study, in both patient and control groups the means of serum 25(OH) D levels were found below 30 ng/ml, and when analyzed after being categorized no significant association was found with cancer prognosis. Table 3 shows the incidence of severe vitamin D deficiency which was indicated as < 10 ng/ml among the study groups. Accordingly, the odds ratio of severe vitamin D deficiency for the development of gastric cancer was ~ 4 (controls: 29% → patients 71%, %95 CI 1.3–13.1, $p = 0.01$). On the other hand, when severe vitamin D deficiency was analyzed according to gender, it was found that in 21% female and 79% male possess higher 25(OH)D levels than 10 ng/ml, and the status is 73% → 27% for vitamin D < 10 ng/ml, respectively, in patients group ($p = 0.01$) (data not shown).

Table 3 The incidence of vitamin D deficiency in the patient and control groups

Vitamin D level status	< 10 ng/ml (n, %)	> 10 ng/ml (n, %)	*p* value
Control	18 (21%)	66 (79%)	**0.03**
Patient	40 (52%)	37 (48%)	
Total	59 (36%)	102 (64%)	

The difference between the groups was analyzed by Chi square (x^2) test. Bold values of $p < 0.05$ indicate statistical significance

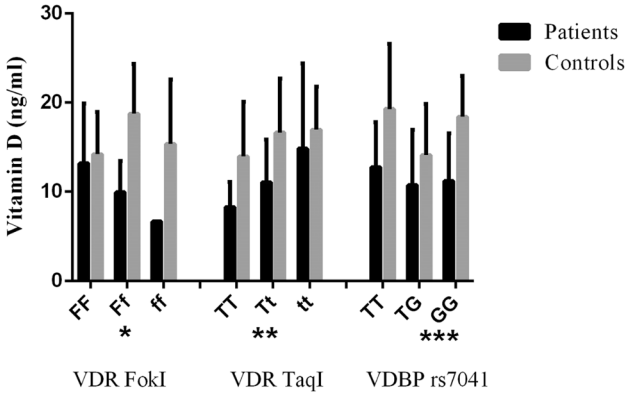

Fig. 2 The comparison of serum levels of 25(OH)D in terms of *VDR* gene rs2228570, rs731236 and *VDBP* gene rs7041 genotypes among the study groups. The difference between and within the groups was analyzed by student's t and two way Annova tests. * = 0.0001, *p* value of *Ff* genotype between patients and controls; ** = 0.005, *p* value of *Tt* genotype between patients and controls; *** = 0.002, *p* value of *GG* genotype between patients and controls

The comparison of serum levels of 25(OH)D in terms of *VDR* gene rs2228570, rs731236 and *VDBP* gene rs7041 genotypes among the study groups were shown in Fig. 2. Accordingly, some important differences were found in serum 25(OH)D levels among the study groups in terms of individual genotype. However, in patient group, serum 25(OH)D levels (Table 4) and demographic parameters as nodal status, invasion, and metastasis (data not shown) did

Table 4 The serum vitamin D levels among *VDR* gene rs2228570, rs731236 and *VDBP* gene rs7041 variants in patients group

Genotypes/alleles	Patient group	*p* value
VDR rs2228570 FokI		
F allele	12 ± 6	0.23
ff genotype	7 ± 0	
f allele	9 ± 3	0.08
FF genotype	13 ± 7	
VDR rs731236 TaqI		
T allele	11 ± 5	0.17
tt genotype	15 ± 10	
t allele	12 ± 6	0.33
TT genotype	8 ± 3	
VDBP rs7041		
T allele	11 ± 6	0.96
GG genotype	11 ± 5	
G allele	11 ± 6	0.52
TT genotype	13 ± 5	

The difference between the groups was analyzed by student's t test

Table 5 The risk of development of gastric cancer in terms of severe vitamin D deficiency and *VDR* and *VDBP* alleles

Gastric cancer patients/controls	OR	95% CI	*p* value
Vit D < 10 ng	4.1	1.3–13	**0.003**
VDR rs2228570			
F allele	1.8	0.6–5.9	0.39
f allele	1.2	0.6–2.2	0.52
Vit D < 10 ng and *F* allele	3.8	1.2–12.5	**0.03**
Vit D < 10 ng and *f* allele	5.3	1.1–26	**0.03**
VDR rs731236			
T allele	0.6	0.3–1.4	0.32
t allele	1.8	0.9–3.7	0.11
Vit D < 10 ng and *T* allele	2.3	0.7–7.2	0.18
Vit D < 10 ng and *t* allele	6.0	1.6–22.9	**0.005**
DBP rs7041			
T allele	0.6	0.3–1.2	0.18
G allele	0.7	0.3–1.8	0.48
Vit D < 10 ng and *T* allele	1.6	0.5–5.1	0.56
Vit D < 10 ng and *G* allele	2.7	0.9–8.3	0.11

The difference between the groups was analyzed by Chi square (x^2) test. Bold values of $p < 0.05$ indicate statistical significance

not show any difference according to genotype and allele distribution.

Table 5 shows the risk of the development of gastric cancer in terms of possessing both serum vitamin D levels < 10 and *VDR* gene rs2228570, rs731236 or *VDBP* gene rs7041 alleles. As given, the risk of the development of gastric cancer was 4.1-fold higher ($p = 0.003$) in cases with severe vitamin D deficiency (serum levels < 10 ng/ml). Carrying *VDR* rs2228570 *F* allele or *f* allele approximately has similar risks ratios. However, with severe vitamin D deficiency, the risks were folded as follows: for *F* allele 1.8 → 3.8 ($p = 0.03$) and for *f* allele 1.2 → 5.3 ($p = 0.03$). Similar effects have been observed in *VDR* rs731236 and *VDBP* rs7041 (Table 5).

Discussion

Cancer is one of the leading causes of death in the world [20], and among several cancer types, gastric cancer has remarkable priority with high morbidity and mortality rates [18]. Although *H. pylori* infection is one of the important risk factor for gastric cancer, lifestyle factors such as inadequate nutritional behaviors and unbalanced diets are common contributors to the development of gastric cancer. Among nutritional factors, vitamin D status has received great interest in recent years [21]. Moreover, in cell culture studies it was shown that vitamin D plays a key role in tumorigenesis, apoptosis, and inflammation and stimulates gastric cancer cell growth by regulating the cycle [8].

Vyas et al. [22] showed a higher prevalence of vitamin D insufficiency (20–29 ng/ml) in gastric cancer patients vs the control group (83% → 63%, OR: 8.8, 95% CI 5–2, $p < 0.001$) and suggested vitamin D associated predisposition to gastric adenocarcinoma. Besides, Eom et al. [23] interestingly, reported any association between vitamin D intake and gastric cancer, however, the serum levels of 25(OH)D was significantly higher in gastric cancer patients than controls. Similar to these studies, in our study serum levels of 25(OH)D was significantly higher in healthy individuals than gastric cancer patients (16 ± 6 → 11 ± 6 ng/ml, $p = 0.002$), which suggests the association of lower serum levels of vitamin D and gastric cancer as an independent risk factor. However, our results showed that both in controls and patient groups serum levels of 25(OH)D were between 20 and 10 ng/ml. Apart from the patient group, control group data indicates lower levels of 25(OH)D in the Turkish population. Furthermore, based on Endocrine Society consensus for vitamin D status [19] Turkish population possess inadequate or deficient vitamin D levels. On the other hand, the distribution in patients group was shown that severe 25(OH)D deficiency (serum level < 10 ng/ml) gains importance in the development of gastric cancer as the risk was fourfold increased in patients than controls. Our finding is consistent with Singh et al. study as they suggested inadequate or deficient serum levels with the prognosis of premalignant phenotypes as intestinal metaplasia to gastric adenocarcinoma [24].

Vitamin D shows its genomic and direct effect through VDR. It has been shown that numerous variations occurred

in the *VDR* gene have correlations between various cancer types including melanoma, colon, breast, ovarian and prostate cancers [25]. In the present study, we investigate *FokI* rs2228570 and *TaqI* rs731236 polymorphisms and compare genotype distributions and allele frequencies between gastric cancer patients and control groups. For both polymorphisms of the *VDR* gene, we found no significant association with the development of gastric cancer ($p > 0.05$). However, the order of the frequency of *TaqI polymorphism was Tt > tt > TT in* advanced stage and it was TT = Tt in early stage which lacks of tt genotype. On the other hand, higher frequency of t allele in the advanced stage than those with early stage suggested the association of t allele to a poorer gastric cancer prognosis. In the literature, there are many conflicting results which reports the association of VDR polymorphisms and gastric cancer. In contrast to our results of rs2228570, Fang et al. reported a positive correlation of FokI with gastric cancer [26]. On the other hand, our results of rs731236 were consistent with the Iranian population as reported in the study by Parsamanesh et al. [27], however, they reported the association of tt genotype with the development of gastric cancer while our results suggest such association with the prognosis.

VDBP is another major protein involved in vitamin D pathway. Vitamin D and its metabolites are transported in plasma by binding to VDBP in a large proportion so that VDBP regulates vitamin D levels in cells and tissues [28]. The most commonly studied polymorphisms in the *VDBP* gene were rs7041 in codon 416 and rs4588 in codon 420 and several studies implied the effects of these *VDBP* gene polymorphisms on serum levels of vitamin D [29–32]. However, in literature some conflicting results still remain as Zhou et al. [14] examined *VDBP* gene rs7041 polymorphisms in gastrointestinal cancers in the Chinese population and reported no association between *VDBP* rs7041 and the development and progression of gastrointestinal cancers. Thus, in the present study we analyze *VDBP* rs7041 polymorphism in gastric cancer, and similar to Zhou et al. [14], no association was found between *VDBP* rs7041 and gastric cancer development. However, while the polymorphisms of the critical genes of vitamin D pathway, *VDR* rs2228570 and rs731236 and *VDBP* rs7041, were not individually associated with gastric cancer development, interesting results were obtained unity with severe deficiency of vitamin D (serum level < 10 ng/ml) or gender.

It was widely known that male gender is an individual risk factor for the development of gastric cancer [33]. Likewise, in our study group male gender was found higher in patients group but the statistical significance was not obtained. On the other hand, the findings of higher serum levels of vitamin D and lower incidence of severe vitamin D deficiency in male patients than female patients revealed the importance of vitamin D intake and metabolism in the development of cancer rather than gender. As well known that lifestyle behaviors including exposure sunlight, low dietary of vitamin D, body mass index (BMI), physical inactivity, different clothing habits, widespread use of sun protection especially in women and increased consumption of ready-to-eat foods were important factors that affect vitamin D status. In our study in order to eliminate the effects of sunlight exposure on Vitamin D status, the blood samples were taken at the same seasonal periods. On the other hand, although carrying mutant alleles solely have no effects on the development of cancer, with severe Vitamin D deficiency, as < 10 ng, the risk of gastric cancer development was increased, and this might be either as a result of severe vitamin D deficiency or the contributory effect of individual polymorphisms as shown in Tables 4 and 5.

Recent studies revealed the lowering effects of VDBP on serum levels of vitamin D. It was well known that the expression of proteins including binding proteins or receptors were dependent on their ligand status. In the present study, as the serum levels of vitamin D was found lower the downregulation of *VDBP* could have been. Besides, it was shown that the polymorphisms of *VDBP* gain importance in the development of cancer [13–16]. In some reports, the effects of *VDBP* variants (Gc1F, Gc1S, and Gc2) on the binding affinity of vitamin D thus carrying capacity of VDBP, and ultimately on serum levels of vitamin D was shown [13, 14]. Moreover, VDBP has important roles in the immune system as it converted to macrophage activation factor (MAF) via glycosylation [14, 16, 29]. This antitumoral effect of VDBP was shown via gradual purification of Gc protein with immobilized β-galactosidase and sialidase activity which in turn results in increased GcMAF activity, and thus decreased nagalase activity [34]. On the other hand, experimental studies show that VDBP was deglycosylated via serum α-*N*-acetylgalactosaminidase (Nagalase) secreted from cancer cells which results in decreased or lost MAF precursor activity leading to immunosuppression, and migration and proliferation of cancer cells [14, 16, 29, 34]. Eventually, these essential interactions could be considered as an additional curative target for the treatment of various cancers. Indeed, serum nagalase levels and Gc-Maf applications began to use for the diagnosis and treatment of several cancer types in a number of laboratories in Holland and the USA. Correspondingly, in our study, the findings of higher prevalence of severe vitamin D deficiency or lower serum levels of vitamin D in the patient group might be related to the lower expression levels of VDBP protein which leads to insufficient MAF activation that effects cancer prognosis. However, no association was found between *VDBP* rs7041 variants and either the development of gastric cancer or serum levels of vitamin D.

Consequently, our results support the hypothesis that significant associations exist between low serum levels

of vitamin D and the risk of gastric cancer development in the Turkish population. Future studies are needed to be conducted with larger sample-size including the expression levels of *VDBP* and nagalase activity to better understand the role of vitamin D in gastric cancers. The present study was prior in Turkish population which evaluates the distribution of critical polymorphisms of the genes of vitamin D metabolism in gastric cancer and will contribute to the literature in this manner.

Acknowledgements The present study was supported by a grant from the Scientific Research Projects Coordination Unit of Istanbul University (Master of Science Project No: TYL-2018- 29205).

Compliance with ethical standards

Conflict of interests The authors declare no conflict of interests.

References

1. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JWW, Comber H et al (2013) Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. Eur J Cancer 49:1374–1403
2. Siegel RL, Miller KD, Jemal A (2018) Cancer statistics, 2018. CA Cancer J Clin 68:7–30
3. Devesa SS, Fraumeni JF Jr (1999) The rising incidence of gastric cardia cancer. J Natl Cancer Inst 91:747–749
4. Irfan ŞEH, Murat G, Güledal B, Selin D (2014) TÜRKIYE KANSER ISTATISTIKLERI
5. Stock M, Otto F (2005) Gene deregulation in gastric cancer. Gene 360:1–19
6. Tsugane S, Sasazuki S (2007) Diet and the risk of gastric cancer: review of epidemiological evidence. Gastric Cancer 10:75–83
7. Burnand B, Sloutskis D, Gianoli F, Cornuz J, Rickenbach M, Paccaud F et al (1992) Serum 25-hydroxyvitamin D: distribution and determinants in the Swiss population. Am J Clin Nutr 56:537–542
8. Bao A, Li Y, Tong Y, Zheng H, Wu W, Wei C (2013) Tumor-suppressive effects of 1, 25-dihydroxyvitamin D3 in gastric cancer cells. Hepatogastroenterology 60:943–948
9. Baek S, Lee Y-S, Shim H-E, Yoon S, Baek S-Y, Kim B-S et al (2011) Vitamin D3 regulates cell viability in gastric cancer and cholangiocarcinoma. Anat Cell Biol 44:204–209
10. Deeb KK, Trump DL, Johnson CS (2007) Vitamin D signalling pathways in cancer: potential for anticancer therapeutics. Nat Rev Cancer 7:684
11. Speeckaert M, Huang G, Delanghe JR, Taes YE (2006) Biological and clinical aspects of the vitamin D binding protein (Gc-globulin) and its polymorphism. Clin Chim Acta 372:33–42
12. Atoum MF, Tchoporyan MN (2014) Association between circulating vitamin D, the Taq1 vitamin D receptor gene polymorphism and colorectal cancer risk among Jordanians. Asian Pac J Cancer Prev 15:7337–7341
13. Ren C, Qiu MZ, Wang DS, Luo HY, Zhang DS, Wang ZQ et al (2012) Prognostic effects of 25-hydroxyvitamin D levels in gastric cancer. J Transl Med 10:16
14. Zhou L, Zhang X, Chen X, Liu L, Lu C, Tang X et al (2012) GC Glu416Asp and Thr420Lys polymorphisms contribute to gastrointestinal cancer susceptibility in a Chinese population. Int J Clin Exp Med 5:72
15. Khayatzadeh S, Feizi A, Saneei P, Esmaillzadeh A (2015) Vitamin D intake, serum Vitamin D levels, and risk of gastric cancer: a systematic review and meta-analysis. J Res Med Sci 20:790
16. Medlej-Hashim M, Jounblat R, Hamade A, Ibrahim JN, Rizk F, Azzi G et al (2015) Hypovitaminosis D in a young lebanese population: effect of GC gene polymorphisms on vitamin D and vitamin D binding protein levels. Ann Hum Genet 79:394–401
17. Pan L, Matloob AF, Du J, Pan H, Dong Z, Zhao J et al (2010) Vitamin D stimulates apoptosis in gastric cancer cells in synergy with trichostatin A/sodium butyrate-induced and 5-aza-2′-deoxycytidine-induced PTEN upregulation. FEBS J 277:989–999
18. Du C, Yang S, Zhao X, Dong H (2017) Pathogenic roles of alterations in vitamin D and vitamin D receptor in gastric tumorigenesis. Oncotarget 8:29474
19. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM, Endocrine Society (2011) Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 96(7):1911–1930
20. Knudson AG (2002) Cancer genetics. Am J Med Genet Part A 111:96–102
21. Guggenheim DE, Shah MA (2013) Gastric cancer epidemiology and risk factors. J Surg Oncol 107:230–236
22. Vyas N, Companioni RC, Tiba M, Alkhawam H, Catalano C, Sogomonian R et al (2016) Association between serum vitamin D levels and gastric cancer: a retrospective chart analysis. World J Gastrointest Oncol 8:688
23. Eom SY, Yim DH, Kim DH, Yun HY, Song YJ (2018) Dietary vitamin D intake and vitamin D related genetic polymorphisms are not associated with gastric cancer in a hospital-based case-control study in Korea. J Biomed Res. 32(4):257–263
24. Singh K, Gandhi S, Batool R (2018) A case-control study of the association between vitamin D levels and gastric incomplete intestinal metaplasia. Nutrients 10(5):629
25. Rai V, Abdo J, Agrawal S, Agrawal DK (2017) Vitamin D receptor polymorphism and cancer: an update. Anticancer Res 37:3991–4003
26. Fang F, Gao J, Han F, Wang HJ (2016) Correlation of gastric cancer and effects of vitamin D receptor gene FokI polymorphism on C-reactive protein and D-dimer in Uygur nationality patients. Zhonghua Yi Xue Za Zhi 96(32):2541–2544
27. Parsamanesh N, Moossavi M, Tavakkoli T, Javdani H, Fakharian T, Moossavi SZ, Naseri M (2019) Positive correlation between vitamin D receptor gene TaqI variant and gastric cancer predisposition in a sample of Iranian population. J Cell Physiol. https://doi.org/10.1002/jcp.28145
28. Haddad JG, Matsuoka LY, Hollis BW, Hu YZ, Wortsman J (1993) Human plasma transport of vitamin D after its endogenous synthesis. J Clin Investig 91:2552–2555
29. Zhang Y, Wang X, Liu Y, Qu H, Qu S, Wang W et al (2012) The GC, CYP2R1 and DHCR1 genes are associated with vitamin D levels in northeastern Han Chinese children. Swiss Med Wkly 142:w13636
30. Sinotte M, Diorio C, Bérubé S, Pollak M, Brisson J (2008) Genetic polymorphisms of the vitamin D binding protein and plasma concentrations of 25-hydroxyvitamin D in premenopausal women–. Am J Clin Nutr 89:634–640
31. Kitanaka S, Isojima T, Takaki M, Numakura C, Hayasaka K, Igarashi T (2012) Association of vitamin D-related gene polymorphisms with manifestation of vitamin D deficiency in children. Endocr J 59:1007–1014
32. Gozdzik A, Zhu J, Wong BYL, Fu L, Cole DE, Parra EJ (2011) Association of vitamin D binding protein (VDBP) polymorphisms and serum 25 (OH) D concentrations in a sample of young Canadian adults of different ancestry. J Steroid Biochem Mol Biol 127:405–412

33. Venerito M, Link A, Rokkas T, Malfertheiner P (2016) Gastric cancer—clinical and epidemiological aspects. Helicobacter 21:39–44
34. Yamamoto N, Suyama H, Yamamoto N (2008) Immunotherapy for prostate cancer with Gc protein-derived macrophage-activating factor; GcMAF. Transl Oncol 1:65–72