VDR Gene *Bsm*IG> A Polymorphism and Risk of Colorectal Cancer in Şanlıurfa Province, Turkey

Türkiye'nin Şanlıurfa İlinde *VDR* Geni *Bsm*IG> A Polimorfizmi ve Kolorektal Kanser Riski

Fuat DİLMEÇ, MD,[a]
Feridun AKKAFA, MD,[a]
Abdullah ÖZGÖNÜL, MD,[b]
Ali UZUNKÖY, MD[b]

Departments of
[a]Medical Biology,
[b]General Surgery,
Harran University Faculty of Medicine,
Şanlıurfa

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Yazışma Adresi/*Correspondence:*
Fuat DİLMEÇ, MD
Harran University Faculty of Medicine,
Department of Medical Biology,
Şanlıurfa,
TÜRKİYE/TURKEY
fdilmec@yahoo.com.tr

ABSTRACT Objective: Colorectal cancer (CRC) is responsible for cancer deaths all around the world and its incidence is increasing annually. Polymorphism in the vitamin D receptor gene (*VDR*) may potentially influence the gene expression or function of the vitamin D receptor protein. The purpose of this study was to investigate the genotype and allele frequencies and association of the *VDR* gene c.1024 + 283G> A (g.58980G> A or *Bsm*IG> A) polymorphism with CRC in Sanliurfa population, Turkey. **Material and Methods:** In this study, we examined the allele and genotype incidence of this polymorphism in 56 patients with CRC (30 females and 26 males; average ages 56.1 ± 6.7) from Şanlıurfa province between 2007 and 2008. Controls were randomly selected from 169 healthy individuals (79 females and 90 males; mean age 57.1 ± 6.4) from the same area. Their DNA were isolated from whole blood, and were scanned using polymerase chain reaction-restriction fragment length polymorphism technique to determine the frequencies of c.1024 + 283G> A polymorphisms of *VDR* gene. **Results:** There was no difference in age, gender, diet, or smoking and alcohol habits between the study groups. In addition to that, comparison of patients with or without polymorphism, revealed that their ages were similar (p= 0.942). The prevalence of *VDR* gene c.1024 + 283 A allele in patients with colorectal cancer (33.9% was significantly smaller when compared to the healthy individuals (45.9%) (p= 0.028). **Conclusion:** Our data indicated that the *VDR* gene c.1024 + 283A allele increased the risk for CRC in our population. In the other hand, further studies are necessary to corroborate these findings.

Key Words: Colorectal neoplasms; genetics; receptors; polymerase chain reaction; restriction fragment length polymorphism

ÖZET Amaç: Kolorektal kanser (KRK), dünya çapında insanlar arasında kansere bağlı ölümlerden sorumludur ve sıklığı her yıl artmaktadır. Vitamin D reseptör genindeki (*VDR*) polimorfizm, potansiyel olarak gen ekspresyonu veya vitamin D reseptör proteinin işlevini etkileyebilir. Bu çalışmanın amacı, Türkiye'de Şanlıurfa'da yaşayan insanlarda KRK'i ile *VDR* geni c.1024 + 283G>A (g.58980G>A veya *Bsm*IG>A) polimorfizminin allel ve genotip oranlarını belirlemek ve ilişkilerini araştırmaktır. **Gereç ve Yöntemler:** Bu çalışmada, 2007 ile 2008 yılları arasında, Türkiye'nin Şanlıurfa ilinden alınan KRK'li 56 hastada (30 kadın ve 26 erkek; ortalama yaş 56.1 ± 6.7) bu polimorfizmin allel ve genotip oranlarını inceledik. Kontroller, aynı bölgeden herhangi bir hastalığı olmayan 169 sağlıklı bireyden (79 kadın ve 90 erkek; ortalama yaş 57.1 ± 6.4) rastgele seçildi. DNA'lar tam kandan elde edildi ve *VDR* geni c.1024 + 283G>A polimorfizmlerinin oranlarını belirlemek için polimeraz zincir reaksiyonu-restriksiyon parça uzunluk polimorfizm tekniği kullanıldı. **Bulgular:** Her iki çalışma grubu arasında yaş, cinsiyet, diyet, sigara içimi ve alkol alışkanlıkları arasında herhangi bir fark yoktu. İlave olarak, polimorfik olan ve olmayan hastaların karşılaştırmasında, yaş bakımından herhangi bir anlamlı fark yoktu (P= 0.942). *VDR* geni c.1024+283 A allelinin oranı, sağlıklı bireylerle (%45.9) karşılaştırıldığında KRK'li hastalarda (%33.9) anlamlı şekilde daha azdı (P= 0.028). **Sonuç:** Bulgularımız, bizim toplumumuzda *VDR* geni c.1024+283 A allelinin, KRK riskini artırdığını göstermektedir. Diğer yandan, bu bulguların doğrulanması için ileri çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Kolorektal neoplazma; genetik; reseptörler; polimeraz zincir reaksiyonu, restriksiyon parça uzunluk polimorfizmi

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Colorectal cancer (CRC) is the third most common tumor and the fourth most common cancer-related cause of death in both genders worldwide, including Turkey.[1-3] It has been emphasized that genetic and environmental factors play an important role in the development of the CRC.[4] The vitamin D receptor (VDR) may modulate risk of colorectal cancer either independently or in conjunction with calcium and vitamin D intake to a large extent.[5]

Several polymorphisms of the *VDR* gene have been identified using molecular methods in intron 8 (*Bsm*I and *Apa*I) and one in exon 9 (*Taq*I).[6] It has been indicated that the *Bsm*I polymorphism in the *VDR* gene was linked to the risk of developing CRC.[5,7]

The c.1024 + 283G> A polymorphism is associated with many diseases, such as non-Hodgkin lymphoma (NHL) subtypes, breast cancer, Type 2 diabetes mellitus (T2DM), multiple sclerosis, osteoporosis, and prostate cancer, but not with renal cell carcinoma, prostate cancer or epithelial ovarian cancer.[8-17]

Recent studies demonstrated that the *VDR* gene c.1024 + 283 G allele, the AA genotype is associated with colorectal adenoma risk in the presence of low vitamin D and calcium intake.[18,19] Molecular variants of the *VDR* gene may be related to the development of colon cancer, and AA genotype reduces risk of colorectal cancer in people who do not use aspirin or steroidal anti-inflammatory drugs.[20,21] Another study demonstrated that some variants of *Taq*I and *Fok*I single nucleotide polymorphisms (SNPs) of *VDR* gene may protect against colorectal carcinogenesis in Turkish population.[22]

However, many investigations suggest that there is no significant association between *Bsm*I polymorphism and colorectal adenoma risk.[1,23,24] In addition, it has been demonstrated that there is no association between the *VDR* c.1024 + 283G>A polymorphisms and survival, overall or among adenocarcinoma patients in a USA population.[25] Finally, it has been shown that the frequency of *VDR* gene *Bsm*I polymorphisms varies from a population to another.

We aimed to investigate the association between the *VDR* gene c.1024 + 283G> A polymorphisms and colorectal cancer in the population of Sanliurfa province, Turkey.

MATERIAL AND METHODS

INDIVIDUALS AND DNA EXTRACTION

Fifty-six patients with CRC (30 females and 26 males; average ages 56.1 ± 6.7) from Şanlıurfa province, Turkey were diagnosed as clinical stage III according to the World Health Organization Guidelines by our serior pathologist in the General Surgery Unit in Harran University between 2007 and 2008.[26] The patients were diagnosed as CRC (colon and rectum) with physical examination, abdominal ultrasound and tomography findings. Stage IV patients were excluded. One hundred sixty-nine healthy people (79 females and 90 males; mean ages 57.1 ± 6.4) from the same area acted as controls. We matched the CRC patients and the healthy controls for age and sex. EDTA-blood was collected from these persons, and genomic DNA was extracted from nucleated blood cells using a standard salting out procedure, as described previously.[27]

POLYMERASE CHAIN REACTION-RESTRICTION FRAGMENT LENGTH POLYMORPHISM

The c.1024 + 283G> A (g.58980 or *Bsm*IG> A) polymorphic site of the *VDR* gene (GI: 7421) was amplified by a touchdown polymerase chain reaction (PCR) method modified by us, and examined by restriction fragment length polymorphism (RFLP) technique. The PCR reaction was carried out in a 10-mL reaction volume as follows: 1 x PCR buffer, 2 mM $MgCl_2$, 0.2 mM each deoxynucleotide triphosphate (dNTPs, Fermentas), 40 ng of DNA, 0.2 μM of each primer (in exon 7, A: 5'-CAACCAAGACTACAAGTACCGCGTCAGTGA-3', and in intron 8, B: 5'-AACCAGCGGGAAGAGGTCAAGGG-3') (Bio-Basic Inc, Ontario, Kanada), and 0.3 unit of *Taq* DNA polymerase (Fermentas).[24] The touchdown PCR conditions: initial denaturation at 94°C for 3

min, followed by 12 cycles at 94˚C for 30s, 72-60˚C for 30s (decreasing 1˚C per cycle), 72˚C for 30s, and 20 cycles at 94˚C for 30s, 60˚C for 30s, 72˚C 30s, and a final extension at 72˚C for 5 min.

PCR products (5-µL) were digested in a 20-µL reaction volume for two hours with 1.5 Units of *Bsm*I at 37˚C (Fermentas, St. Leon-Rot, Almanya). The digested PCR product was separated on 2% agarose gel, and was analyzed using Alpha-Imager System (AlphaInnotech, San Leandro, California ABD). The profiles of the *VDR* gene with *Bsm*I; G allele (wild type) yielded fragments of a 646-bp, 176-bp, and A allele (mutant) yielded 822-bp (Figure 1).

STATISTICAL ANALYSIS

Student's t-test was used to determine the differences in the means of the demographic parameters using the SPSS statistics program (Table 1). Genotype and allele frequencies of the c.1024 + 283G> A polymorphic site of the *VDR* gene were tested for Hardy-Weinberg equilibrium using Chi-square test. Genotype (GG, GA, and AA) and allele (G and A) frequencies observed in this study were analyzed with Fisher's exact test using the SPSS statistics program. All of the tests for statistical significance were two-sided. Statistical significance was determined as $p< 0.05$. The odds ratio (OR) was calculated to measure the strength of the association observed (Table 2).

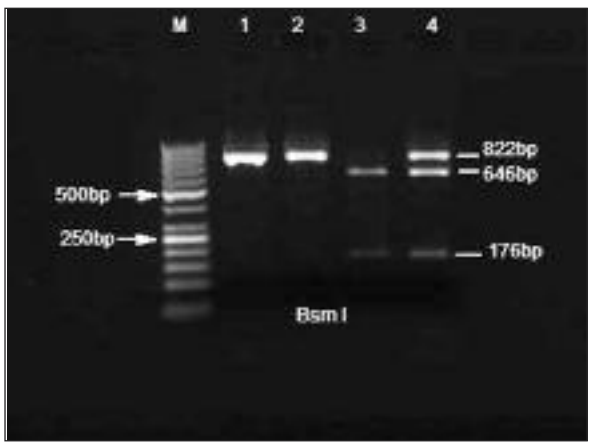

FIGURE 1: The c.1024 + 283G> A RFLP profiles of the *VDR* gene. Lane M: DNA Marker (1000-50bp, Fermentas), lane 1: undigested PCR product, lane 2: AA genotype (homozygous, polymorphic), lane 3: GG genotype (homozygous, wild-type), lane 4: GA genotype (heterozygous).

ETHICS

The institutional review board approved the study, and written informed consents were obtained from all patients. The study complied with the Helsinki Declaration.

RESULTS

The *VDR* c.1024 + 283G> A polymorphisms were investigated in all of the 72 patients with CRC and in 169 healthy controls using PCR-RFLP. The diet, smoking, and alcohol habits were similar between CRC patients and healthy individuals.

The distribution of age and sex of both study groups are shown in Table 1. There were no statistically differences in age or sex of the patients and healthy subjects (p= 0.307). In 56 patients, there were 22 GG (not polymorphic, wild type), 30 GA (heterozygous), and 4 AA (homozygous, polymorphic) genotypes of the c.1024 + 283G> A polymorphisms. However, Student's t-test did not show any significant difference in age with respect to presence of polymorphism (p= 942) (Table 1).

The frequencies of the alleles and the genotypes were in Hardy-Weinberg equilibrium among the patients and the controls. There was a significant association between the *VDR* gene c.1024 + 283 GG genotype (reference genotype) and CRC patients when compared to healthy control group (Table 2). In addition, we observed that the frequency of *VDR* gene c.1024 + 283 A allele was significantly different between the patients with CRC and healthy controls (p= 0.028). In this manner, c.1024 + 283 A allele appears as a risk for CRC (OR= 0.606; 95%CI= 0.388-0.947).

DISCUSSION

The *VDR* gene consists of 11 exons of which exons 2-9 are being transcribed.[28] Vitamin D (1,25-dihydoxyvitamin D_3) receptor is known to mediate the pleiotropic biological actions of 1,25-dihydroxyvitamin D_3 through its ability to modulate the expression of target genes. The regulation of this ligand-activated cellular transcription factor is reported to occur at both transcriptional and post-translational levels.[29]

TABLE 1: Disribution of demographic parameters in both patients and healthy individuals, and in both patients without polymorphic and with polymorphic genotypes, respectively.

Variations	Patients with CRC	Healthy individuals	p
Number of subjects (n)	56	169	
Age (years)	56.1 ± 6.7*	57.1 ± 6.4*	0.307
Female/male	30/26	79/90	-
	Patients with CRC (n= 56)		
	c.1024 + 283G> A GG genotype (non-polymorphic)	c.1024 + 283G> A AA genotype (polymorphic)	
Number of genotypes	22	4	-
Ages (years)	55.9 ± 9.1*	56.3 ± 2.8*	0.942

CRC: Colorectal cancer.

*mean ± standart deviation.

TABLE 2: Statistical analysis of SNP polymorphisms in patients with CRC vs. control group.

SNP genotype/allele *VDR* c.1024 + 283G> A	CRC patients (n= 56)	Healthy controls (n= 169)	X^2	OR (95% CI)	P
GG	22 (39.3%)	42 (24.9%)	4.305	1.957 (1.032-3.710)	Reference
GA	30 (53.6%)	99 (58.6%)	0.431	0.816 (0.444-1.498)	0.536
AA	4 (7.1%)	28 (16.6%)	3.063	0.387 (0.130-1.158)	0.120
G	74 (66.1%)	183 (54.1%)	4.888	1.649 (1.056-2.576)	Reference
A	38 (33.9%)	155 (45.9%)	4.888	0.606 (0.388-0.947)	0.028

Abbreviations: CRC: Colorectal cancer, X^2= Chi-square, OR= Odds ratio, CI= Confidence interval, SNP= Single nucleotide polymorphism.

In our study, although the c.1024 + 283 AA genotype frequency of the *VDR* gene was lower in CRC patients (7.1%) when compared to healthy individuals (16.6%), we observed that this genotype frequency in CRC patients was not significantly different from the healthy controls. In spite of these results, we demonstrated that the frequency of the *VDR* c.1024 + 283 A allele was significantly smaller in patients with CRC compared to the controls (33.9% vs. 45.9%, respectively; OR= 0.606, p= 0.028). The association between the *VDR* gene polymorphisms and cancer development has been shown in several studies. However, the relationship between the *VDR* polymorphisms and CRC is controversial, and has not been confirmed by all studies. Boyapati et al. suggested that the calcium-colorectal adenoma association varied in relation with c.1024 + 283 genotype, so that the participants with at least one G allele were at much lower risk for colorectal adenoma.[18] Kim et al. suggested that the AA genotype of *Bsm*I was inversely associated with colorectal adenoma risk in the presence of low vitamin D and calcium intake. Similarly, those with the lowest tertile of calcium intake and the AA genotype have a reduced risk of colorectal adenoma.[19] Kadiyska et al. and Slattery et al. suggested that molecular variants of the *VDR* gene might be related to the development of colon cancer in United States and Bulgarian populations.[7,20] Slattery demonstrated that *VDR* gene with AA genotypes reduced the risk of colorectal cancer among nonusers of aspirin/steroidal anti-inflammatory drug to a similar extent as that observed among aspirin or steroidal anti-inflammatory drug users regardless of the genotype in the United States population.[21] Our data was congenial with these results. In addition, a recent study demonstrated that *Taq*I TC and *Fok*I TC or *Taq*I TT and *Fok*I TC genotypes of *VDR* gene might protect against colorectal carcinogenesis in Turkish population.[22] It al-

so worths mentioning that our study related to *VDR* gene c.1024 + 283G> A polymorphic site is the first study performed in patients with CRC in a group of Turkish population.

However, these results were not confirmed the other studies. Ingles et al. indicated that there was no significant association between *Bsm*I and colorectal adenoma risk either when African-Americans were excluded, or when whites were analyzed separately.[23] Speer et al. pointed out that the c.1024 + 283G> A allele and genotype frequencies of the *VDR* gene in patients with rectal cancer (A, 42%; G, 58%; and AA, 23%; GA, 38%; GG, 39%) and in controls (A, 43%; G, 57%; and AA, 19%; GA, 48%; GG, 33%) were not statistically different.[24] Flugge et al. indicated that there was no association between any single variant and colorectal cancer.[2] Zhou et al. suggested that there was no association between the *VDR* gene c.1024 + 283G> A polymorphisms and survival, overall or among adenocarcinoma patients in a USA population.[25] Obara et al. showed that the c.1024 + 283G>A polymorphism of the *VDR* gene did not play a significant role in the increased risk and poor prognosis of RCC in Japanese.[14]

The frequency of *VDR* gene polymorphisms in CRC varies from population to population due to the heterogeneous genetic and environmental factors on the disease. On the other hand, the CRC patients with (c.1024 + 283 AA genotype) or without polymorphism (c.1024 + 283 GG genotype) were similar with regard to their age (p> 0.05). The diet, and smoking and alcohol habits were similar between CRC patients and the healthy individuals since they lived in the same region.

Additional studies are needed to verify the correlations with regard to *VDR* polymorphisms in Turkish and other racial-ethnic groups in the world.

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