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Publication date
2009

Document Version
Final published version

Published in
Uluslararasÿ¸ Hematologi-Onkoloji Dergisi : UHOD = International Journal of Hematology and Oncology

Determination of ApaI and TaqI Polymorphisms of VDR Gene in a Group of Turkish Patients with Colorectal Cancer

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ABSTRACT

Colorectal cancer (CRC) is the leading cause of cancer death among human around the world. The vitamin D receptor gene (VDR) is a member of the nuclear receptor super family, which is expressed in the tissue of gastrointestinal tract, known to modulate the rate of cell proliferation. We aimed to investigate the genotype and allele frequencies and association of the VDR gene: c.1025-49G>T (ApaIG>T) and c.1056T>C (TaqIT>C) polymorphisms with CRC in Turkish patients. Fifty-six patients with CRC and 169 healthy individuals were enrolled to study, and their DNA was isolated. A polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique was used to detect the frequency of c.1025-49G>T and c.1056T>C polymorphisms. The prevalence of the c.1025-49G>T and c.1056T>C alleles and the genotype frequencies in patients with CRC was similar to that in the normal population. The investigated polymorphisms in the VDR gene do not represent a significant risk factor for CRC in our population.

Key Words: VDR gene, Colorectal cancer, Polymorphism, PCR, RFLP

ÖZET

Kolorektal Kanserli Bir Grup Türk Hastasında VDR Geni ApaI ve TaqI Polimorfizmlerinin Belirlenmesi

Kolorektal kanser (KRK), Dünyada insanlar arasında kanserden ölümün önde gelen sebebidir. Vitamin D reseptör (VDR) geni, mide bağırsak dokusunda ifadesini bulan ve hücre bölünme oranını düzenleyen çekirdeğe ait reseptör büyük ailesinin bir üyesidir. Türk hastalarında KRK ile VDR geni: c.1025-49G>T (ApaIG>T) ve c.1056T>C (TaqIT>C) polimorfizmlerinin ilişkisini, genotip ve allel oranlarını araştırmayı amaçladık. KRK'li 56 hasta ve 169 sağlıklı birey çalışmaya dahil edildi ve bunların DNA'sı izole edildi. c.1025-49G>T ve c.1056T>C polimorfizmlerinin belirlenmesinde, Polimeraz zincir reaksiyonu-restriksiyon parça uzunluk polimorfizm (PCR-RFLP) tekniği kullanıldı. KRK'li hastalarda c.1025-49G>T ve c.1056T>C allel ve genotip frekanslarının yaygınlığı, normal popülasyondaki değerlerle benzerdir. VDR geninde araştırılan polimorfizmleri, toplumumuzda KRK için anlamlı bir risk faktörü olduğunu göstermiyor.

Anahtar Kelimeler: VDR geni, Kolorektal kanser, Polimorfizm, PCR, RFLP

INTRODUCTION

Colorectal cancer (CRC) is the third most common tumor and the fourth most common cancer-related cause of death in both genders worldwide. CRC incidence and mortality vary markedly between ethnicities in different populations. The vitamin D receptor gene (VDR) may be an important modulator of the risk of CRC, either independently or in conjunction with vitamin D, and calcium intake.[1]

Polymorphisms in the VDR gene (OMIM 601769) may influence the expression or function of the vitamin D (1,25-dihydroxyvitamin D3) receptor.[2]

The VDR gene TaqI polymorphism is associated with numerous diseases, such as diabetes mellitus type 1 (T1DM)[3], and psoriasis[4]; while not associated with osteoporosis[5], T2DM[6], breast cancer[7], and prostate cancer[8]. In the same way, some studies indicate that the genotypes of ApaI polymorphism is a significant association with prostate cancer[8], Graves' disease[9], and Epithelial Ovarian Cancer[10]; while not association with osteoporosis [5], (T1DM)[3], T2DM [6].

Molecular variants of the VDR gene may be related to the development of colon cancer.[1,11,12] The VDR Tru9I polymorphism may be associated with lower risk for colorectal adenoma, particularly in interaction with various risk factors, but not with calcium or vitamin D in a USA population.[13] The homozygous variant genotypes (CC) are associated with a decreased risk of squamous cell carcinoma of the head and neck (SCCHN) compared to the common TaqI TT genotypes.[14]

Some studies demonstrate that there is no association of ApaI, TaqI, BsmI, FokI, and Tru9I polymorphisms of the VDR gene with patients with colorectal adenoma, compared with healthy controls in a USA and Russian populations.[15-19] Whereas, the other study shows that the ApaI TT genotype of the VDR gene are associated with an increased risk for renal cell carcinoma, while the other genotypes (TaqI CC and BsmI TT) are no associated with this carcinoma.[20]

The purpose of this study was to investigate the association of the VDR gene c.1025-49G>T and c.1056T>C polymorphisms with CRC in a Turkish population.

MATERIAL AND METHODS

Individuals and DNA Isolation

Fifty-six patients with CRC (30 females and 26 males: average age: 56±2), who were diagnosed at the Surgery Unit in Harran University and 169 healthy controls (79 females, mean age 57±3; and 90 males, mean age 56±3), who did not have any disease, were investigated in this study. Blood was taken from these individuals, and genomic DNA was extracted from nuclear cells by using a standard salting out procedure, as described by Miller et al.[21]

PCR-RFLP

The VDR gene NM001017535: c.1025-49G>T (ApaIG>T or g.59979G>T) and c.1056T>C (TaqIT>C or g.60058T>C) polymorphic sites were investigated by a touchdown polymerase chain reaction (PCR) method modified by us, and restriction fragment length polymorphisms (RFLP) technique. The PCR reactions were performed in a 10-ml of reaction volume, including 1xPCR buffer, 2 mM MgCl2, 0.2 mM each deoxynucleotide triphosphate (dNTPs, Fermentas, St. Leon-Rot, Germany), 0.2 μM of each primer (A:5'-CAGAGCATGGACAGGGAGCAAG-3', B:5'-GCAACTCCTCATG GCTGAGGTCTCA-3')[22] (BioBasic Inc, Ontario, Canada), 40 ng of DNA, and 0.3 unit of Taq DNA Polymerase (Fermentas). The touchdown PCR program was performed at 94˚C for 3 min (initial denaturation), 10 cycles, with 94˚C for 30s, 72-62˚C for 30s (decreasing 1˚C per cycle), 72˚C for 30s, and 20 cycles, with 94˚C for 30s, 62˚C for 30s, 72˚C 30s; and 72˚C for 5 min (final extension).

The PCR products (5 µl) of VDR gene were separately restricted in a 20 µl reaction volume for two hours with 1.5 Units of ApaI at 37˚C and TaqI (Fermentas, St. Leon-Rot, Germany) at 65˚C, respectively. The digested PCR product was separated on 2% agarose gel, and analyzed using Alpha-Imager Gel System (AlphaInnotech, San Leandro, California USA). The ApaI product; G allele yielded fragments of a 528-bp, 217-bp (wild type), and T allele yielded 745-bp (mutant) (Figure 1); and TaqI T allele yielded fragments of 494-bp, 251-bp (wild type), and C allele yielded 293, 251, 201-bp (mutant) (Figure 2).

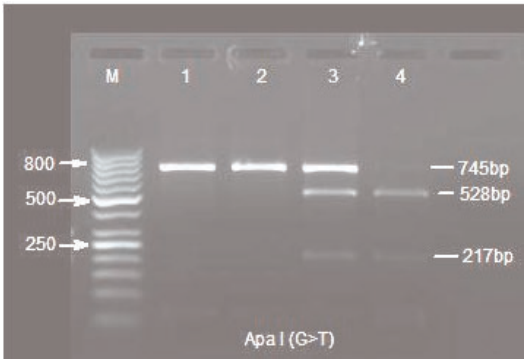

Figure 1. The *Apa*I restriction profile of the *VDR* gene. Lane M: GeneRuler DNA ladder (50-1000bp, Fermentas); lane 1: undigested PCR product; lane 2: TT genotype (homozygous, mutant); lane 3: GT genotype (heterozygous); lane 4: GG genotype (homozygous, wild type).

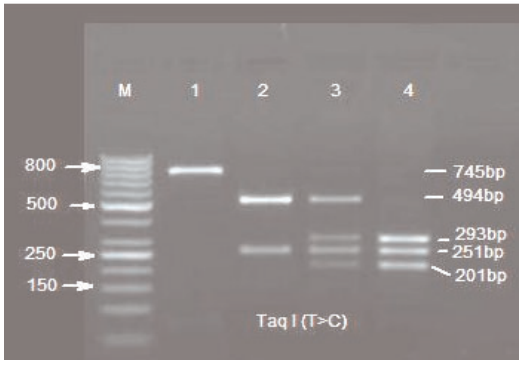

Figure 2. The *Taq*I restriction profile of the *VDR* gene. Lane M: GeneRuler DNA ladder (50-1000bp, Fermentas); lane 1: undigested PCR product; lane 2: TT genotype (homozygous, wild type); lane 3: TC genotype (heterozygous); lane 4: CC genotype (homozygous, mutant).

Statistical Analysis

Genotype and allele frequencies of c.1025-49G>T and c.1056T>C polymorphic sites of the VDR gene were tested for Hardy-Weinberg equilibrium by using chi-square test. Besides, genotype and allele frequencies observed in this study were analyzed with Fisher's exact test by using the SPSS statistics program. Statistical significance was defined as $p < 0.05$. The odds ratio (OR) was calculated to measure the strength of the association observed (Table 1).

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 c.1025-49G>T and c.1056T>C polymorphisms in the VDR gene were investigated by PCR-RFLP analysis in CRC patients and healthy subjects. The frequencies of the alleles and the genotypes were in Hardy-Weinberg equilibrium among the patients and the controls.

There was no significant association between the VDR gene c.1025-49 TT (mutant) and c.1056 CC (mutant) genotypes and CRC patients, compared with healthy control group (Table 1). In addition, we could not reveal any effect of the VDR gene c.1025-49 T (OR: 0.815, 95%CI: 0.529-1.254) and c.1056 C (OR: 0.673, 95%CI: 0.419-1.082) alleles on the risk of CRC (Table 1).

DISCUSSION

Higher serum 25-OH vitamin D levels are associated with decreased colorectal adenoma risk, and vitamin D has been shown to reduce proliferation and increase differentiation in human colon cancer cells.[19] The VDR polymorphisms are important factors in the assessment of colorectal cancer risk, possibly indicating vitamin D and calcium as preventive measures.[13] Vitamin D is a potential agent for the prevention of colorectal cancer possibly through mechanisms mediated by the VDR.[9, 15, 16] These findings led to the question of whether variants in the VDR gene influence an individual's susceptibility to colorectal cancer development. Polymorphisms in the VDR gene may influence the expression or functioning of the vitamin D (1,25-hydroxyvitamin D3) receptor protein.[2]

In our study, there was no the association of the c.1025-49G>T and c.1056T>C polymorphisms of the VDR gene in patients with CRC and healthy controls in a Turkish population. These findings indicated that the VDR gene c.1025-49 T allele was not a risk for CRC patients (55.4%), compared with healthy control (60.4%). Similarly, c.1025-49 TT genotype frequency in CRC patients (30.4%) was

Table 1. Statistical analysis of SNPs in patients with CRC vs. control group

SNP Genotype/Allele	CRC patients (n:56)	Healthy controls (n:169)	X^2	OR (95% CI)	p-values
c.1025-49G>T					
GG	11 (19.6%)	26 (15.4%)	0.555	1.344 (0.616-2.934)	0.533
GT	28 (50.0%)	82 (48.5%)	0.037	1.061 (0.580-1.942)	0.878
TT	17 (30.4%)	61 (36.1%)	0.611	0.772 (0.403-1.479)	0.611
G	50 (44.6%)	184 (39.6%)	0.869	1.228 (0.797-1.890)	0.376
T	62 (55.4%)	204 (60.4%)	0.869	0.815 (0.529-1.254)	0.376
c.1056T>C					
TT	30 (53.6%)	69 (40.8%)	2.772	1.672 (0.910-3.072)	0.120
TC	22 (39.3%)	81 (47.9%)	1.266	0.703 (0.380-1.301)	0.282
CC	4 (7.1%)	19 (11.2%)	0.770	0.607 (0.197-1.868)	0.456
T	82 (73.2%)	219 (64.8%)	2.694	1.485 (0.925-2.386)	0.106
C	30 (26.8%)	119 (35.2%)	2.694	0.673 (0.419-1.082)	0.106

Abbreviations: X^2 = Chi-square, OR = Odds ratio, CI = Confidence interval, SNP = Single Nucleotide Polymorphism

not significant difference from healthy group (36.1%). On the other hand, the present study findings demonstrated that the frequency of c.1056 C allele of VDR gene allele frequency was not significant association between CRC patients (26.8%) and healthy control (35.2%). Furthermore, although the c.1056 CC genotype frequency of the VDR gene in patients with CRC (7.1%) was lower than in healthy subjects (11.2%), this difference was not significant (p=0.456) (Table 1). The VDR multiple polymorphisms have been examined for the association with CRC in several populations. In this manner, our results corresponded with those reported in the literature for various populations. Recent studies demonstrate that there is no association between the VDR c.1056 TT (37.7%), TC (46.9%) and CC (15.3%) polymorphism in 3'UTR of VDR gene and patients with colorectal adenoma, compared with healthy controls (38.9%, 45.6%, and 15.5%, respectively) in a USA population.[15-17] Whereas, the expression of the nuclear vitamin D receptor (VDR), which is involved in regulating cell growth and proliferation, may contribute to the development of CRC.[2] The finding that genetic variation in the vitamin D pathway contributes to risk supports a role for vitamin D in colon cancer etiology.[12] The TT genotype at the c.1025-49 site of the VDR gene may be a risk of incidence and poor prognosis factor for renal cell carcinoma (RCC) in the Japanese population.[20]

Two case-control studies report a decreased risk of colorectal neoplasia in participants with the c.1056 CC genotype compared with those with TC or TT genotype either overall [4] or in conjunction with low calcium or vitamin D intake.[3]

In conclusion, we have not found any evidence that differences in the oncogenic properties of the VDR gene c.1025-49 T and c.1056 C alleles could confer a genetic predisposition to colorectal carcinogenesis. We proposed for detect whether the sequence differences in the VDR gene are susceptibility variants for CRC, additional studies in different populations are required in a large study group.

Acknowledgement

We thank to the staff of the General Surgery Unit of Harran University Hospital for collecting blood samples from patients with CRC.

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