Relationship of Vitamin D Receptor Gene Polymorphisms with Adverse Drug Responses in Patients with Colorectal Cancer

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Introduction

Vitamin D receptor (VDR), located on chromosome 12 at q12-14, provides instructions for making a proteins which allows the body to respond to vitamin D. VDR is highly expressed in small intestine and colon, VDR has important regulatory actions for proliferation and differentiation, intestinal barrier function and host defense in the gut [1-2]. Previous studies have shown that people with colorectal cancer (CRC) have lower serum 25-hydroxyvitamin D ($25(OH)_2D_3$) levels than do controls, and vitamin D has been shown to reduce cell proliferation and to support differentiation in human colon cancer cells [3-4]. Also calcitriol aid the anti-tumor activities of multiple chemotherapeutic agents including DNA-damaging agents such as cisplatin, carboplatin and doxorubicin; antimetabolites; like 5-fluorouracil, cytarabine, hydroxyurea, cytarabine and gemcitabine; and microtubule-disturbing agents such as; paclitaxel and docetaxel. Calcitriol take outs anti-tumor effects mainly through induction of cancer cell apoptosis [5]. Colorectal cancer (CRC) is a significant public health problem. There are nearly one million new cases of colorectal cancer diagnosed world-wide each year and half a million deaths . Furthermore CRC is a heterogeneous disease that is caused by the interaction of genetic and environmental factors [6].
We believe that CRC is associated with Vitamin D and Vitamin D receptor (VDR) gene variations and may have an effect over different adverse drug reactions and focuced on patients under Bevacizumab, 5-Fluorouracil, Capecitabine and Oxaliplatin therapy to explore the relationship of VDR polymorphisms with adverse drug reactions. The aim of our study was to investigate whether genotype frequencies of vitamin D receptor gene BsmI (rs1544410), ApaI (rs7975232) and TaqI (rs7312369) polymorphisms are risk factors for CRC cases.

Methods

Sample Collections

In this study, blood samples taken from 36 volunteer colorectal cancer patients with ongoing treatment in Acıbadem Hospitals Group, Istanbul-Turkey.
Blood samples were stored at +4 ° C for a maximum of two days for genomic DNA isolation at Marmara University Molecular Metabolism Research Laboratory.

Genotyping

Genotyping of the 4 polymorphic regions of the VDR gene was determined by the digestion pattern of the amplified DNA fragments using the restriction enzymes for BsmI, ApaI and TaqI. Genomic DNA was amplified by PCR using spesific primers, after that PCR products digested by restriction enzymes and the digested PCR products was separated on %3 agarose gel, after that analyzed using UV transilluminator(BsmI(Fig.1.1) TaqI (Fig.2.) and ApaI (Fig.3.)).

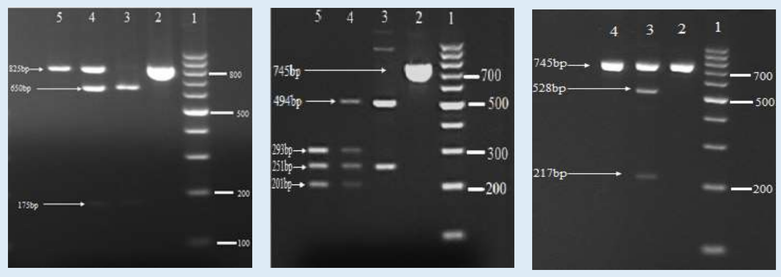

Fig.1. Fig.2. Fig.3.

Fig.1. The BsmI digestion profiles of the 825bp PCR product of the VDR gene. Lane 1, Vivantis 100bp DNA Ladder(100-1000bp). Lane 2, undigested PCR products. Lane 3, AA genotype (homozygous wild type). Lane 4, AG genotype (heterozygous type) and Lane 5 GG genotype (homozygous mutant type).
Fig.2. The TaqI digestion profiles of the 745bp PCR product of the VDR gene. Lane 1, Vivantis 100bp DNA Ladder (100-1000bp). Lane 2, undigested PCR products. Lane 3, TT genotype (homozygous wild type). Lane 4, TC genotype(heterozygous type). Lane 5, CC genotype (homozygous mutant type).
Fig.3. The ApaI digestion profiles of the 745bp PCR product of the VDR gene. Lane 1, Vivantis 100bp DNA Ladder(100-1000bp). Lane 2, undigested PCR products. Lane 3, GT genotype (heterozygous type). Line 4 CC genotype (homozygous mutant type).

Results

Colorectal cancer (CRC) patients had wild type, heterozygous and homozygous mutant genotype frequencies respectively as 22.2%, 55.6%, 22.2% for BsmI. 47.2%, 38.9% and 13.9% for TaqI and 0%, 75%, 25% for ApaI (Table.1.).

Of the 22 patients who declared an epicrisis report, 22.3% had neutropenia, 40.9% had pain-cough-weakness, 18.4% had lesions and 18.4% had no treatment-related complaints.
In the neutropenia case; the homozygous wild, heterozygous and mutant genotype distributions of BsmI polymorphism in patients with respectively as 20%, 40% and 40%; 0%, 60% and 40% for ApaI; polymorphism were calculated respectively as 60%, 0%, and 40% for TaqI.

Pain-cough-weakness complains in CRC patients,had homozygous wild type, heterozygous and homozygous mutant VDR genotype frequencies respectively as 22.1%, 44.6%, 33.3% for BsmI ; 0%, 77.7%, %22.3 for ApaI and 33.4%, 55.5%, 11.1% for TaqI. The other epicrisis report subject is that lesions. VDR genes BsmI, ApaI and TaqI polymorphisms frequency for lesions were homozygous wild, heterozygous and homozygous mutant type respectively as 0%, 50%, 50%; 0%, 100%, 0% and 50%, 50%, 0% (Table.2.). There was not using Oxaliplatin and capecitabine in treatment, but also 3 patients had ApaI heterozygous type that using 5-FU and bevacizumab in colorectal cancer treatment.
Table.1. The distribution of VDR gene variations in colorectal cancer patients.

VDR Gene Polymorphisms	Homozygous Wild n(%)	Heterozygous n(%)	Homozygous Polymorphic n(%)
BsmI (rs1544410)	8(22.2)	20(55.6)	8(22.2)
ApaI(rs7975232)	0(0)	27(75)	9(25)
TaqI(rs731236)	17(47.2)	14(38.9)	5(13.9)

Table.2. The relationship between adverse drug reactions relationship with VDR gene (BsmI, ApaI, TaqI) variants.

VDR Gene Variants/ Adverse Drug Reactions	Neutropenia n(%)	Pain-Cough-Weakness n(%)	Lesions n(%)
BsmI homozygous wild type	1(20)	2(22.1)	0(0)
heterozygous	2(40)	4(44.6)	2(50)
homozygous polymorphic	2(40)	3(33.3)	2(50)
ApaI homozygous wild type	0(0)	0(0)	0(0)
heterozygous	3(60)	7(77.7)	4(100)
homozygous polymorphic	2(40)	2(22.3)	0(0)
TaqI homozygous wild type	3(60)	3(33.4)	2(50)
heterozygous	0(0)	5(55.5)	2(50)
homozygous polymorphic	2(40)	1(11.1)	0(0)

Also 5-FU and bevacizumab using separately in treatment related to adverse drug responses relationship with VDR gene variation datas had shown in Table.3. and Table.4.

Table.3. Comparison of using 5-FU treatment-drug reactions relationship with VDR gene variants.

VDR Gene Variants/ Adverse Drug Reactions	Neutropenia n(%)	Pain-Cough-Weakness n(%)	Lesions n(%)
BsmI homozygous wild type	0(0)	0(0)	1(100)
heterozygous	0(0)	1(100)	0(0)
homozygous polymorphic	1(100)	0(0)	0(0)
ApaI homozygous wild type	0(0)	0(0)	0(0)
heterozygous	0(0)	1(100)	1(100)
homozygous polymorphic	1(100)	0(0)	0(0)
TaqI homozygous wild type	0(0)	0(0)	1(100)
heterozygous	0(0)	1(100)	0(0)
homozygous polymorphic	1(100)	0(0)	0(0)

Number of patients using 5-FU; 6, patients that reported epicrisis number; 3 (neutropenia, pain-cough-weakness, lesions numbers respectively as 1;1;1). The polymorphism of ApaI genotypes of homozygous wild type had not found.

Table.4. Comparison of using Bevacizumab treatment-drug reactions relationship with VDR gene variants.

VDR Gene Variants/ Adverse Drug Reactions	Neutropenia n(%)	Pain-Cough-Weakness n(%)	Lesions n(%)
BsmI homozygous wild type	1(50)	0(0)	1(50)
heterozygous	1(50)	0(0)	1(50)
homozygous polymorphic	0(0)	0(0)	0(0)
ApaI homozygous wild type	0(0)	0(0)	0(0)
heterozygous	2(100)	0(0)	2(100)
homozygous polymorphic	0(0)	0(0)	0(0)
TaqI homozygous wild type	2(100)	0(0)	1(50)
heterozygous	0(0)	0(0)	1(50)
homozygous polymorphic	0(0)	0(0)	0(0)

Number of patients using Bevacizumab;4, patients that reported epicrisis report number; 4 (neutropenia, pain-cough-weakness, lesions numbers respectively as 2;0;2).

Conclusion

According to our knowledge we were not able to find any study in the literature, related to vitamin D receptor and adverse drug reactions (ADRs) in colorectal cancer.

Results had shown that ApaI heterozygous type polymorphism directly related to lesions, BsmI and TaqI gene polymorphisms may not relationship with lesion problem. Despite all these consequences, it was seen that adverse drug reactions caused by drugs used in CRC treatment and VDR gene polymorphisms may be related. However, these results may not be definite outcome between adverse drug reactions and VDR gene polymorphims. There is 1 patient that using 5-FU combine with bevacizumab in colorectal cancer treatments with neutropenia problem; BsmI, ApaI and TaqI gene variantions was all of same homozygous mutant type. Also there is 2 patients that using Bevacizumab in CRC treatment and has neutropenia promlem, ApaI gene variation %100 same to heterozygous type. Although drug reaction due to treatment for patiens, there was not oxaliplatin and capecitabine relationship with advers drug reaction in epicrisis reports. According to the study results, neutropenia was general problem for chemotheraphy adverse effects, but BsmI, ApaI and TaqI genes may be protective for adverse drug reactions or homozygous mutant type of this genes, may provide appropriate substructure.

Pain-cough-weakness complains may be indirectly to heterozygous type of BsmI, TaqI but ApaI may be directly related to complains for CRC patiens.

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