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Vitamin D receptor gene polymorphisms in pediatric patients with leukemia-lymphoma: Does it have an impact on malignancy?

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ABSTRACT

Objective: Genetic variations have been identified in specific regions of the Vitamin D receptor (VDR) gene and many studies were investigating whether these variations are associated with malignancy. Studies in the VDR on children are scarce. In this study, we aimed to investigate the VDR gene polymorphisms in pediatric patients with the diagnosis of leukemia and lymphoma.

Patients and Methods: Of the 99 participants included in this cross-sectional study, 59 were control, 40 were patients. Fok-I, Bsm-I and Taq-I polymorphism of the VDR gene were investigated in both groups.

Results: While no significant difference was found in the genotype distribution of the three polymorphisms between the patient and control groups, significant results were obtained in Bsm-I and Taq-I allele frequencies (Odds ratio=0.489; CI95%=0.275-0.871 and 0.519; CI95%=0.280-0.964) ($p<0.05$).

Conclusions: In this study, we found that the frequency of allele "A" for Bsm-I and allele "C" for Taq-I was lower in the patient group. Contrary to most publications in the literature, polymorphisms were not found to be risk factors in our study.

Keywords: Childhood cancers, Leukemia, Lymphoma, VDR, Polymorphism

1. INTRODUCTION

Approximately two million new cases of malignancies are reported each year in the United States, of which sixteeen thousand are reported to be in children [1]. There are many studies about the causative factors of cancer. One of the suspected causative factors is vitamin D. Vitamin D is a hormone that acts by binding to the intracellular specific receptor and plays a role in cell proliferation, inflammation, hormone receptor. In some studies, it is suggested that low vitamin D levels may be associated with some autoimmune and allergic diseases, metabolic syndrome, infectious diseases and cancer [2]. Rather than the plasma level, it has been seen that genetic variations detected in the vitamin D receptor (VDR) gene cause cancer and have effects on mortality [3].

Vitamin D receptor is a nuclear receptor and is associated with intracellular signaling pathways. The VDR protein is encoded by the VDR gene located on chromosome 12q12–q14. Polymorphic numerous variations are detected on the VDR gene and these variations are thought to increase the cancer risk [3]. To date, more than sixty polymorphisms have been found in the promoter region, around exon 2-9, and at the 3' end [4]. For example; Fok-I with start codon polymorphism at the 5' end of exon 2 has been shown to encode a shorter VDR protein and show less function [5]. Bsm-I polymorphism at the 3' end of exon 8 does not make any change on either the translated protein or the transcribed mRNA; Taq-I in exon 9 causes silent codon change (ATT→ATC) to add isoleucine to the 352nd position; both of them are involved in VDR gene regulation and mRNA stability [6].

In some studies, it has been stated that VDR Fok-I, Bsm-I, Taq-I polymorphisms may be associated with prostate, breast, kidney and colon cancers (4). In some studies, however, no association was found. There are not many studies on VDR gene polymorphism in children and our study aimed to investigate the relationship between VDR gene polymorphisms and lymphoproliferative malignancies in children.

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2. PATIENTS and METHODS

Design

This is a cross-sectional study, which was approved by Marmara University School of Medicine Clinical Researches Ethics Committee (Protocol number: 09.01.2015-09.2014.0336/70737436-050.06.04). This study was supported by Marmara University Scientific Research Fund (SAG-C-TUP-080.415.0097).

Participants

In this study, the patient group consisted of 30 acute lymphoblastic leukemia (9 low-risk, 16 standard risk, 3 high-risk) three acute myeloid leukemia, four Hodgkin's lymphoma and three non-Hodgkin's lymphoma patients who were at the course of treatment and followed up in the pediatric hematology and oncology outpatient clinic. Only one of the patients was cured during this time period when the study was conducted. The control group consisted of 59 patients who applied to pediatric outpatient clinics with complaints such as upper respiratory tract infection and had no family history of cancer or any chronic disease. The study was conducted following the principles of the Declaration of Helsinki. Written informed consent was obtained from all the participants.

Procedures and Instruments

Fok-I, Bsm-I and Taq-I polymorphism of VDR gene were investigated in both patient and control groups. 4 cc peripheral blood samples were obtained from the participants and taken into a 0.5 M Ethylenediaminetetraacetic acid (EDTA) tube (BD Medical, NJ, USA). Deoxyribonucleic acid (DNA) was obtained by several serial procedures. The isolated DNAs were stored at – 20°C. Polymerase Chain Reaction (PCR) was used to amplify polymorphisms sites of the VDR gene. PCR was carried by total volume of 25 µl reaction containing 12.8 µl distilled water, 2.5 µl 10x buffer solution, 0.7 µl FP, 0.7 µl RP, 0.1 µl Taq polymerase, 2 µl template, 3.7 µl deoxynucleotide triphosphates [dATG, dGTP, dCTP, dTTP] and 2.5 µl of MgCl2.

Primers for Fok-I (rs2228570) polymorphism region;

VDRFF(23b) 5' – AGGATGCCAGCTGGCCCTGGCAC – 3'

VDRFR(26b) 5'-TGGCTGTGAGCGCCGCATGTTCCATG – 3'

- Primers for Bsm-I (rs1544410) polymorphism region;

VDRBF 5' – GCAACCTGAAGGGAGACGTAGC – 3'

VDRBR 5' – TCCTTGAGCCTCCAGTCCAGG – 3'

- Primers for Taq-I (rs731236) polymorphism region;

VDRTF 5' – AGAGCATGGACAGGGAGCAAGGC – 3'

VDRTR 5' – TAGCTTCATGCTGCACTCAGGCTGG – 3'

The gene region was amplified by PCR using these primers. Fok-I polymorphism was found 265 base pairs (bp), Bsm-I polymorphism was found 825 bp, and Taq-I polymorphism was found 740 bp. Using restriction endonuclease enzymes, the digested PCR products were run on 2% agarose gel. It was carried out for 30-50 minutes at 90-100V current and the results were examined in the gel imaging system. Digestion of Fok-I gives T/T (263 bp, 80 bp for homozygote wild type), T/C (343 bp, 263 bp, 80 bp for heterozygote) and C/C (343 bp for homozygote mutant). The digestion of Bsm-I gives G/G (331 bp, 200 bp for homozygote wild type), G/A (531 bp, 331 bp, 200 bp for heterozygote) and A/A (531 bp for homozygote mutant). The digestion of Taq-I gives T/T (479 bp for homozygote wild type), C/T (479 bp, 290 bp, 189 bp for heterozygote) and C/C (290 bp, 189 bp for homozygote mutant).

Statistical Analysis

All data were recorded electronically and SPSS 20.0 statistics program (SPSS Inc, Chicago, USA) was used. Chi-square ($\chi 2$) and Fisher's exact tests were used to compare categorical descriptive data in the study. Whether the measurement data showed normal distribution or not was determined by the Kolmogorov-Smirnov test. A value of $p < 0.05$ was considered statistically significant. The risk probability of alleles was evaluated using odds ratio (OR) and 95% confidence interval (CI).

3. RESULTS

This study consisted of 99 participants. DNA could not be obtained from six patients because four were lymphopenic and two patients died during outpatient follow-up. The patient group consisted of 40 people. 59 participants were included in the control group. 47 (47.5%) participants were male. The mean age of patients was 8.27±4.8 and the mean age of controls was 9.66±4.1. There was no statistical significance in age and gender distribution between groups (p=0.057, p=0.107, respectively).

Genotypic and phenotypic characteristics of participants

Vitamin D receptor gene genotypes and allele frequencies were examined in this study. The distributions of the genotype of Fok-I, Bsm-I, Taq-I polymorphisms in both control and patients are shown in Table I. There was no significant difference in genotype distribution of the three polymorphisms between the patient and control groups (p>0.05).

The allele frequencies of Fok-I, Bsm-I and Taq-I polymorphisms are shown in Table II. There was a significant difference between groups in Bsm-I and Taq-I allele frequencies, but not in Fok-I. Frequency of Bsm-I G allele was 57.5% (n=46) within patient group and 40% (n=47) within control group and frequency of Bsm-I A allele was 42.5% (n=34) within patient group and 60% (n=71) within control group (Odds ratio=0.489; CI 95%=0.275-0.871) (p<0.05). Frequency of Taq-I T allele was 73.8% (n=59) within patient group and 59.3% (n=70) within control group. Frequency of Taq-I C allele was 26.2% (n=21) within patient group and 40.7% (n=48) within control group (Odds ratio=0.519; CI 95%=0.280-0.964) (p<0.05).

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Table I. *Genotype distribution of VDR gene polymorphisms in patient and controls*

Polymorphism	Patient	Control	P value	Odd's ratio (95% CI)
Fok-I genotype				
T/T	2.5% (n=1)	8.4% (n=5)	0.396*	3.61 (0.405-32.160)
T/C	45% (n=18)	44% (n=26)		
C/C	52.5% (n=21)	47.6% (n=28)		
Bsm-I genotype				
G/G	35% (n=14)	18.7% (n=11)	0.109**	0.425 (0.169 – 1.07)
G/A	45% (n=18)	42.3% (n=25)		
A/A	20% (n=8)	39% (n=23)		
Taq-I genotype				
T/T	55% (n=22)	37.3%(n=22)	0.125**	0.486 (0.215-1.101)
T/C	37.5% (n=15)	44% (n=26)		
C/C	7.5% (n=3)	18.7% (n=11)		

**Fisher's Exact Test p value, ** The two-sided p values*

Table II. *VDR allele frequencies in patients and controls*

Polymorphism region	Patient	Control	P value	Odd's ratio (95% CI)
Fok-I				
T allele	25% (n=20)	30.5% (n=36)	0.398*	1.31 (0.694-2.498)
C allele	75% (n=60)	69.5% (n=82)		
Bsm-I				
G allele	57.5% (n=46)	40% (n=47)	0.015*	0.489 (0.275-0.871)
A allele	42.5% (n=34)	60% (n=71)		
Taq-I				
T allele	73.8% (n=59)	59.3% (n=70)	0.037*	0.519 (0.280-0.964)
C allele	26.2% (n=21)	40.7% (n=48)		

** The two-sided p values*

4. DISCUSSION

Mortality due to malignancy has an important place in childhood. In the literature, it has been reported that Fok-I, Bsm-I, Taq-I polymorphisms are frequently associated with cancer [3,4]. Many studies have shown that Fok-I C/C, Bsm-I A/A, Taq-I C/C genotypes are associated with higher cancer risk [5]. In a meta-analysis involving the prostate, breast, skin, ovarian, colorectal cancers and non-Hodgkin lymphoma, it was stated that a significantly increased cancer risk was observed in C/C genotypes compared to T/T genotypes, while the risk was slightly increased in T/C genotypes in Fok-I; whereas in patients with Bsm-I G/G or G/A genotypes, the risk has been reported to be low [5]. On the contrary, Beysel et al., found that the Fok-I T/T genotype was associated with advanced stage in thyroid papillary carcinoma [7]. Yu et al., reported that Bsm-I A allele has a negative association with cancer risk compared to the G allele in lung cancer [8]. In our study, the majority of both groups (93%) were genotypically T/C or C/C in Fok-I and the distribution of patients and controls with the T/T genotype was 2.5% and 8.4%, respectively, which was not statistically significant. Also, Taq-I C allele was seen in lower frequency in the patient group. Similar to the study of Yu et al., in our study, the Bsm-I A allele was significantly more frequent in the control group and we thought that it might be negatively associated with cancer [8].

In a study by Oh and Barrett-Connor, it was reported that 35% of the population had the Bsm-I homozygous mutant genotype and the risk of colon cancer was two times higher than the homozygous wild genotype [9]. Rasool et al., also reported that cancer risk was 2.7 times more in homozygous mutant genotype than wild genotype [10]. Some studies have revealed an increased risk of breast cancer in the mutant Bsm-I genotype [11,12]. In the meta-analysis by Zhang and Song they found an association between breast cancer and the Fok-I polymorphism, they reported that poly-A, Bsm-I, Taq-I and

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Apa-I polymorphisms had no effect [13]. Similarly, in the meta-analysis by Luo et al., it was shown that Apa-I polymorphism did not have a determining role in breast cancer [14]. In the meta-analysis by Tang et al. many polymorphisms were investigated and breast cancer risk was found to be increased in the Fok-I homozygous mutant genotype (OR: 1.16, 95% CI: 1.04–1.30) [15]. Contrary to these studies, Yang et al., reported in a meta-analysis that Fok-I, Bsm-I, Taq-I and Apa-I polymorphisms had no association with breast cancer in Caucasian women [16].

Although, there are many studies in adults, there are limited data on VDR gene polymorphisms in childhood [17,18]. Purdue et al., did not find any association between Fok-I, Bsm-I and Taq-I polymorphisms and non-Hodgkin's lymphoma, and reported that Bsm-I gene polymorphism may lead to an increased risk of diffuse large B cell lymphoma [19]. Tekgündüz et al., did not find any association between Cdx2, Fok-I, Bsm-I, Apa-I, Taq-I polymorphisms and malignancy in patients diagnosed with childhood Hodgkin's lymphoma [20]. Also, Yılmaz et al., found no association between the Taq-I, Fok-I and Bsm-I polymorphisms and pediatric brain cancers [18]. In another study involving pediatric patients with solid cancer, it was stated that in Fok-I CT and CC genotypes were weakly associated with a reduced risk of malignancy formation [21]. In our study, no statistically significant association was observed between VDR Fok-I, Bsm-I, Taq-I genotypes and malignancy. However, there was a significant difference between Bsm-I and Taq-I allele frequencies. We found out that carrying the mutant Bsm-I A allele and the mutant Taq-I C allele has a negative association with cancer risk compared to carrying the Bsm-I G allele and Taq-I T allele.

The small number of participants in the research is the biggest limitation. Our other limitation is; in this cross-sectional study, the control group was defined as those who had no complaints or symptoms suggestive of malignancy at that time and those with no family history of cancer. This should not mean that malignancy will not be seen in this group at all. Perhaps this was the reason why there was no significant difference between the groups. Long-term follow-up of the patients in the control group, re-evaluation of the results of this study, and comparison of the groups will be meaningful.

Conclusion

In conclusion, the association between VDR polymorphisms and malignancy is still controversial, and no association was found between Fok-I and malignancy in our study. Contrary to most publications in the literature, the incidence of mutant Bsm-I A and Taq-I C allele polymorphisms was lower in our study in the patient group, and polymorphisms were not found to be risk factors. It is thought that more meaningful results can be obtained in a study with long-term follow-up with a larger number of participants, perhaps including all childhood malignancies.

Compliance with Ethical Standards

Ethical Approval: The study protocol was approved by the Marmara University School of Medicine, Clinical Researches Ethics Committee (Protocol number: 09.01.2015-09.2014.0336/70737436-050.06.04).

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Conflict of Interest Statement: None of the authors has any conflict of interest regarding the data and conclusions reported in this study.

Authors' Contributions: SGK: Writing – reviewing and editing, data curation, AGT: Investigation, project administration, methodology, AA: Formal analysis,conceptualization, supervision, BY: Data curation. All authors read and approved the final version of the article.

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