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Taq1 Polymorphism (rs731236) of Vitamin D Receptor Gene in Children with Acute Lymphoblastic Leukemia

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ABSTRACT

Background: Acute lymphoblastic leukemia (ALL) is the most common childhood cancer. Several studies have shown that ALL occurs as a result of genetic abnormalities. 1, 25-Dihydroxyvitamin D3 as a secosteroid hormone plays an important role in different metabolic pathways. The normal function of vitamin D occurs via binding to a ligand-activated transcription factor i.e. vitamin D receptor (VDR). Therefore genetic variation in VDR may lead to various disorders, including cancer. We aimed to investigate rs731236 polymorphism of VDR gene in children with ALL.
Methods: Genomic DNA was extracted from 50 children under 15 years of age with ALL and 50 age-matched healthy children's whole blood and genetic variation of each participant was detected using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) with taqI restriction enzyme. Statistical analysis was performed using SPSS v.22 following $\chi 2$ test.
Results: The VDR gene polymorphisms were genotyped in a total of 50 individuals with ALL, comparing 50 normal children with a mean age of 5.2±3.4 years. No deviation was observed from Hardy-Weinberg equilibrium (HWE) in the genotypic distribution of the rs731236 ($\chi 2$=0.25, P>0.05). Genotype frequency of TT (Dominant Homozygote), Tt (Heterozygote), and tt (Recessive Homozygote), was 25, 15 and 10 in the case group and 21, 27 and 2 in the control group, respectively which showed a significant difference between two groups (P=0.011). tt genotype showed a strong protective effect against ALL over Tt (OR=9, CI 95%: 1.74-46.59).
Conclusion: We have determined the frequency of Taq1 (rs731236) polymorphism in the VDR gene in children with ALL in Zanjan population. We concluded that genotype variation in VDR gene may have an effect on incidence of ALL.

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Introduction

Acute lymphoblastic leukemia (ALL) is the most common leukemia in children which accounts for more than 80% of leukemia in children under the age of 15.[1,2] ALL occurs as a developmental arrest in lymphocyte maturation in bone marrow also known as the hematopoietic precursor.[3] Results of Genome-Wide Association Studies (GWAS) have determined that variation in several genes is associated with increased susceptibility to ALL.[4]

1, 25-dihydroxyvitamin D (calcitriol), the active form of Vitamin D is a steroid hormone that plays a major role in several metabolic pathways in addition to calcium homeostasis such as cell growth, differentiation, and

immune response. Vitamin D-endocrine system has antiproliferative and differentiating effects on various human tissues and tumors. Therefore, it can mediate angiogenesis, cell death, and tumor invasion and could act as a candidate for cancer regulation. Normal function of vitamin D occurs via binding to specific steroid hormone family of nuclear receptors known as vitamin D Receptors (VDR). VDR modulates transcription of several target hormone responsive genes by forming a heterodimer with retinoid-X receptor.[5,6] The VDR is encoded by a large gene located on chromosome 12q13.11 comprising of 11 exons (eight protein-coding exons and an extensive promoter region which results in generating multiple tissue-specific transcripts.[7,8] VDR gene includes more than 60 allelic variations some of which are epidemiologically involved in the etiology of several types of cancers such as breast, prostate, skin, colorectal, ovary, bladder, and renal cell carcinoma.[9]

Taq1(rs731236)(T>C, chromosome position:48238757) is one of the most common known of these single nucleotide polymorphisms (SNPs) which is a synonymous polymorphism located on codon 352 (isoleucine) of exon 9 near the 3' un-translational region of VDR gene on band 13 of long arm of chromosome 12.[10] There is controversy about the association of VDR Taq1 SNP and cancer risk, so this study aimed to evaluate the association between Taq1 polymorphism and ALL in Iranian children.

Materials and Methods

Participants

The study population consisted of 100 unrelated individuals under the age of 15, including 50 participants with ALL (32 boys and 18 girls) and 50 participants as normal control (30 boys and 20 girls) who referred to Mousavi Hospital, Zanjan, Iran. Blood samples were collected after signing the informed consent by parents. The study was approved by the Ethics Committee of Zanjan University of Medical Sciences.

DNA Extraction

Two ml blood specimens were collected from each person in EDTA-containing tubes; genomic DNAs were extracted from whole blood by DNP™kit (CinaClon, Iran). DNA yield and purity was determined by 1% (w/v) agarose gel electrophoresis and the absorbance at 260/280 nm.

Genotyping

Genotyping was performed by the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP).

Polymerase Chain Reaction (PCR)

The following primers were used to amplify the VDR gene for Taq1 polymorphism: Forward primer: 5′-CAGAGCATGGACAGGGAGCAAG-3′; and Reverse Primer: 5′-GCAACTCCTCATGGGCTGAGGTCTCA-3′. For detection of the Taq1 RFLP, 50–100 ng genomic DNA was amplified with 2X PCR Master Mix (CinaClon, Iran), 0.5mM of each primer, and deionized water in a 25 µL reaction volume. .DNA samples were amplified with cycling parameters as follows: Initial denaturation at 95 °C for 5 minutes followed by 35 cycles of 94 °C for 30 seconds, 64°C for 30 seconds, followed by 72 °C for 30 seconds, and a final extension at 72 ° C for 10 minutes. PCR products were analyzed by 1% (w/v) agarose gel electrophoresis and visualized with a gel documentation and analysis system (UVdoc, England), after staining by SYBR® Safe(ThermoScientific, USA). PCR product size was 490 bp for rs731236.

Restriction Fragment Length Polymorphism (RFLP)

Five µL of each PCR products were digested with TaqI restriction enzyme (Thermo Fisher Scientific, USA) at 65°C overnight. Digested restriction fragments were separated on 2% (w/v) agarose gel and bands were visualized on UV doc, after staining with SimplySafe. Genotypes were determined as TT (490 bp), Tt (490, 290 and 200 bp), or tt (290 and 200 bp) for Taq-I polymorphism.

Statistical Analysis

Statistical analysis was performed using SPSS 22.0 (Chicago, IL, USA). The genotype frequencies of Taq1 SNP were estimated by allele counting for all participants, and the Hardy–Weinberg equilibrium (HWE) was appraised using Chi-square test. Differences in the frequency of the genotypes between the ALL patients and normal controls were tested using Chi- square tests. Odd ratios (ORs) and 95% confidence intervals (95% CI) were calculated to estimate the risk of ALL. Values of $P \leq 0.05$ were considered statistically significant.

Results

The analysis was carried out on 50 ALL patients (32 male and 18 female) with the mean age of 4.5±3.3 years along with 50 normal controls (30 boys and 20 girls) with the mean age of 5.8±3.5 years and overall mean age of 5.2±3.4 years.

The allelic frequency of population was found to be in agreement with Hardy-Weinberg equilibrium (HWE) due to high p-values of Chi-square test (χ^2=0.25, P>0.05). The frequency of different genotypes in each group have been outlined in Table 1. Genotype frequency for TT, Tt and tt was 25 (50%), 15 (30%) and 10 (20%) in the case group and 21(42%), 27(54%) and 2 (4%) in the control group, respectively. We observed the T allele in 69% and 65%, and the t allele in 31% and 35% of ALL patients and control group, respectively. The results of the Chi-Square test showed that there is a significant difference between ALL group and control in term of allelic distribution (χ^2=9.11, P=0.011).

The risk of ALL associated with VDR genotypes are shown in Table 2. We found that the frequency of Taq1 genotype in our ALL patients were not similar to those in the control group. A decreased frequency of Taq1 tt genotype was observed in ALL patients compared with the control subjects who were carrier for Tt or TT genotype. It was statistically significant only just for tt over Tt genotype (OR = 9, 95% CI: 1.74-46.59). It explains the protective effect of tt genotype against ALL to be occurred (P<0.005).

Table 1: Genotype and Allelic Frequency of all participants

Participants	Genotypes			Total
	TT	Tt	Tt	
Control group	25	15	10	50
ALL patients	21	27	2	50
Total	46	42	12	100

Table 2: The risk of ALL associated with VDR Taq1 genotypes.

Genotypes	Odd Ratio	95% CI
Tt over TT	0.47	0.2-1.1
tt over Tt	9	1.74-46.59
tt over TT	4.2	0.83-21.34

Discussion

VDR mediates the function of Vitamin D as an important factor in the regulation of Calcium homeostasis as well as immunomodulation, cell division and differentiation. Many studies have reported that vitamin D has an antiproliferative effect on many cancer types through promoting apoptosis in a variety of malignant cells such as glioma, neuroblastoma, leukemia, lymphoma, breast and colon cancer.[11-13] The regulatory effects of vitamin D in different target tissues are mediated via VDR in which some of its polymorphisms have been suggested to be associated with multiple types of cancers. Therefore, several SNPs in *VDR* gene have been investigated for any probable association with a variety of cancers.[10,14]

In the present study, Taq1 (rs731236) polymorphism of *VDR* gene was investigated for probable association with ALL occurrence in children. Taq1 polymorphism showed a significant association with ALL occurrence. It seems that in our studied population, mutant homozygote genotype of tt has a protective effect over heterozygote (Tt), but this conclusion for t allele over T allele is not reliable due to OR and 95%CI values.

Some studies have shown no significant association between the Taq1 polymorphism and breast cancer susceptibility.[15-21] However, Sillanpää et al. reported that T allele containing genotypes (Tt and TT) could result in decreasing risk of breast cancer. It could not be reliable because the control group was not in Hardy-Weinberg equilibrium for Taq1 genotypes.[21] Another study described Taq1 potential to increase risk of breast cancer to approximately 1.5-folds.[22] Taylor et al. showed that tt genotype of Taq1 polymorphism could lower the risk of prostate cancer and individuals with tt genotype also showed higher levels of 1, 25$(OH)_2D_3$.[23] These results were confirmed in European patients by Correa-Cerro et al.[24] In contrast, Kibel et al. and Cheteri et al. showed no association between TaqI polymorphism and death by prostate cancer in US patients.[25,26] In Europe, a recent report by Gsur et al. did not find an increased risk of prostate cancer associated with TaqI polymorphism.[27]

Evidence showed that Taq1 tt variant was associated with reduced risk of colon cancer (OR=0.5).[28] Also, reports from a Turkish study indicated that colorectal cancer patients with TT genotype had lower serum levels of 25(OH)D as compared to patients with Tt/tt genotypes (P=0.016).[29]

Taq1 t allele was observed to be more frequent, but not statistically significant among Caucasian and Japanese women with ovarian cancer.[30]

There was no report indicating the taq1 effects on ALL incidence, but a recent study by M. Tantawy et al. demonstrated the frequency of Taq1 genotype to be TT (23%), Tt (54%) and tt (23%) among 40 Egyptian pediatric patients with ALL. They showed that the Tt genotype in Egyptian pediatric ALL was significantly correlated with high BMD as compared to other TaqI genotypes (P=0.0420).[31]

In 78 unrelated normal Syrian individuals, the TaqI genotype distribution was 36%, 58% and 6% for TT, Tt and tt, respectively.[32] Inconsistency in frequency of different genotypes may be explained in part by differences in population size or ethnical characteristics.

Conclusion

We investigated the association between Taq1 (rs731236) polymorphism of VDR gene with pediatric ALL. Our study revealed that there might be a positive correlation between decreased risk of pediatric ALL and the tt genotype. However, further studies with different markers on a larger number of cases are recommended to confirm these findings.

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Conflict of Interest: None declared.

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