

Association of Vitamin D Receptor (VDR) Start Codon FokI Polymorphism with Acute Myelogenous Leukemia

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Abstract

Background: *Vitamin D start codon polymorphism has been suggested to be associated with the increase risk of developing different hematological malignancies including AML.*

Objective: *This study aimed to investigate the association between VDR start codon F/f polymorphism and the risk of AML among Sudanese.*

Study Design: *Hospital-based case-control study.*

Materials and Methods: *A total of 36 Sudanese patients with acute myelogenous leukemia were enrolled in this study. In addition 37 age and sex matched healthy volunteers were included as a control group. Five milliliters (ml) of venous blood were collected and poured into K3 ethylene diamine tetra acetic acid (EDTA) and genomic DNA was extracted from all samples using salting out method. A 260 bp fragment of VDR gene was amplified by PCR and digested by restriction enzyme (FokI) to detect the polymorphism.*

Results: *The result showed that the genotype FF was the most frequent (72.2%) in patient with AML, followed by the genotype Ff (27.8%). Also in the healthy control group the genotype FF was the*

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most frequent (86.5%), followed by the genotype Ff (13.5%); the genotype ff was absent in both study groups.

There was statistically significant association between AML and each of the genotypes FF (OR= 1.420, CI =1.122-1.719, P =0.000) and Ff (OR= 1.580, CI = 1.281-1.878, p=0.000).

Conclusion: Both FF and Ff genotypes are associated with increased risk of AML among Sudanese.

Keywords: VDR start codon polymorphism, acute myelogenous leukemia, Sudanese

Introduction

Acute Myeloid leukemia (AML)- also known as acute myelogenous leukemia or acute non lymphocytic leukemia (ANLL) is a cancer of the myelogenous line of blood cells, characterized by the rapid growth of abnormal white blood cells that accumulate in the bone marrow and interfere with the production of normal blood cells. AML is the most common acute leukemia affecting adults, and its incidence increases with age.^[1] Vitamin D plays a central role in maintaining serum calcium and skeletal homeostasis but is also involved in the regulation of other vital cellular processes including cellular differentiation, proliferation, apoptosis, and angiogenesis.^[2] It was reported that vitamin D has differentiation on multi-lineage or myelogenous precursor cells instead of the increase in hematopoietic cells. Furthermore, vitamin D₃ has been shown to be a very potent inhibitor of CD34+ leukemic blasts, while preserving clonogenic activity of normal human CD34+ hematopoietic progenitor cells.^[3,4] Vitamin D receptor (VDR) also known as the calcitriol receptor or as nuclear receptor subfamily1, group I, member 1(NR1I1), is a member of the nuclear receptor family of transcription factors.^[2]

The VDR forms a heterodimer with the retinoid-X receptor and binds to specific gene products. In humans, the

vitamin D receptor is encoded by the VDR gene.^[5] It contains a start codon polymorphism (SCP) which is three codons upstream of a second start site (ATG). The vitamin D receptor (VDR) has a thymine/cytosine (T/C) polymorphism located in the first of two potential start (ATG) codons, that can be detected by a restriction fragment length polymorphism (RFLP) using the endonuclease FokI. The C variant, which lacks the first ATG and restriction site, results in a shorter VDR and is referred to as the *F* allele. The T variant (*f* allele) initiates at the first ATG and hold the restriction site.^[6] Studies concern with the association between of vitamin D receptor (VDR) start codon FokI Polymorphism with different types of cancers showed variable results. A study showed a significant associations with VDR FokI polymorphism and prostate, breast, colon-rectum and skin cancers.^[7] This study aimed to examine the association of vitamin D receptor (VDR) start codon FokI Polymorphism with acute myelogenous Leukemia.

Materials and methods

Study population

This is a case control study carried out in Khartoum state, Sudan. A total of 36 Sudanese patients with acute myelogenous leukemia, attended radiation and isotope center of Khartoum (RICK) in the period from February to May 2014 were enrolled in this study. In addition, 37 healthy volunteers were included as a control group.

Sample collection

Five milliliters (ml) of venous blood were collected from each subject and pour into Ethylene diamine tetra acetic acid (EDTA) blood container.

DNA extraction and molecular analysis

Genomic DNA was extracted from all samples using salting out method. Reaction mixture of 20 µl was prepared for each sample. It consist of 2µl of genomic DNA, 1 µl of each forward primer (5'-AGCTGGCCCTGGCACTGACTCTGCTCT-3') and reverse primer (5'-ATGGAAACACCTTGCTTCTTCTCCCTC-3'), 5 (µl master mix (*i-taq*, *iNtRON*, *Korea*) and 11 µl sterile distilled water.

DNA samples were amplified in Thermal Cycler (*Techne-TC-412*) with cycling conditions as follows: Denaturation at 94 ° C for 5 min, 35 cycles each consist of 94° C for 30 seconds, 61 ° C for 30seconds and 72° C for 1 minute, and one final cycle of extension at 72°C for 7minutes.

The PCR product of 265 bp band was digested with 0.5 unit of Fok I restriction enzyme (*New England Biolabs, England*), it is incubated at 37 ° C for 1 hours; after that, 7µL of the digested reaction mixture was loaded into 3% agrose gel containing ethidium bromide and visualized using gel documentation system (*Syngene, Japan*). 50bp DNA ladder was loaded with each batch of samples. Digestion of the amplified 265 bp PCR product results in two fragments (169 bp and 96 bp). Depending on the digestion pattern, individuals were genotyped as (ff) when homozygous for the presence of the FokI site, (FF) when homozygous for the absence of the FokI site, or (Ff) in case of heterozygosity.

Statistical analysis

Data of this study was collected from patients' medical files, and analyzed using Statistical Package for Social Sciences (SPSS). Frequencies of genotypes were calculated, and correlation of genotypes with study groups was tested by Chi-square. The Hardy–Weinberg equilibrium was tested by a goodness-of-fit X_2 test to compare the observed genotypic

frequencies in normal individuals to the expected genotypic frequencies calculated from the observed allelic frequencies.

Ethical considerations

This study was approved by RICK and faculty of medical laboratory sciences, Al Neelain University, and informed consent was obtained from each participant before sample collection.

Result

A total of 36 Sudanese patients with AML were enrolled in this study; 20 (55.5%) were males and 16(44.5%) were females. Further 37 healthy volunteer were included as a control group. The results revealed the presence of two alleles (F and f) in both patients and control groups, the genotype FF was the most common (Frequencies: 72.2% and 86.5% respectively), followed by the genotype Ff (Frequencies: 27.8% and 13.5% respectively), while the genotype ff was totally absent. There was statistically significant correlation between the VDR start codon genotypes FF and Ff and AML (Table 1).

Table 1: Correlation between VDR start codon FokI polymorphism and AML.

Genotype	Patients	Control	<i>P.value</i>
FF	26(72.2%)	32(86.5%)	0.000
Ff	10(27.8%)	5(13.5%)	0.000

There was no statistically significant correlation between VDR FokI polymorphism and gender (Table2).

Table 2: Correlation between VDR start codon FokI polymorphism and gender

Gender	FF	Ff	<i>P.value</i>
Male	15(75%)	5(25%)	0.132
Female	11(68.8%)	5(31.2%)	

Both FF and Ff genotypes were found to be associated with an increased risk of AML (OR= 1.420, CI =1.122-1.719, P =0,000 for FF and OR= 1.580, CI = 1.281-1.878,P=0.000 for Ff).The frequency of the F allele was 0.86 in the patients and 0.92 In the control, while the frequency of the f allele was 0.14 in the patients and 0.08 in the control group. However no significant deviation from hardy-Weinberg equilibrium was found in both patients ($X^2= 0.859$, $df =2$, $P= 0.8$) and control group ($X^2=0.420$, $df=2$, and $p=0.810$).

Discussion

The vitamin D receptor (VDR) is a crucial mediator for the cellular effects of vitamin D. In fact, it has been hypothesized that polymorphisms in the VDR gene affect cancer risk and the relevance of VDR gene restriction fragment length polymorphisms for various types of cancer has been investigated by a great number of studies.^[7]In this study, we examined the frequency of VDR start codon genotypic variants in Sudanese AML patients with comparison to control group. Our results showed that, in both study groups the FF genotype was the most common followed by the genotype Ff, while the genotype ff was absent in both groups. There was statistically significant correlation between VDR start codon FF and Ff genotypes and AML. The results of many studies concerning with the association of VDR polymorphism in different types of malignancies in different populations showed different results. Gandini, *et al* showed a significant associations with VDR FokI polymorphism and prostate, breast, colon-rectum and skin cancers.^[7] Chuan observed a marginally positive correlation between the FokI gene polymorphism and prostate cancer.^[8] The study by Xu, *et al* reported that, the VDR gene FokI variant was related to an increased risk of ovarian cancer.^[9] All these studies were consistent with our finding. In contrast, a study by Yanga, *et al* did not find any association of the most

controversial FokI genotype with breast cancer risk in Caucasian women.^[10] Furthermore Gandini, *et al* found a significant associations between FokI and ovarian cancer for ff genotype.^[11] These divergent results indicated that, the distribution of VDR start codon genotypes may differ according to ethnicity. Our study showed that, the FF and Ff genotypes were associated with 1.4 and 1.6 fold increase risk for AML respectively. This finding was supported by study done by Huang *et al* who reported that, the VDR start codon FF genotype may increase the risk of early-onset prostate cancer and is associated with more aggressive disease.^[12] Another study of Qing *et al* showed the FF genotype may increase the risk of prostate cancer in individuals of Asian ethnicity.^[8] Another study by Mohapatra *et al* reported that, the Ff genotype is associated with a two fold increase in ovarian Cancer risk.^[13] In the present study the frequency of the F allele was 0.86 in the patients and 0.92 in the controls, while the frequency of the f allele was 0.14 in the patients and 0.08 in the control group. However no significant deviation from Hardy-Weinberg equilibrium was found in both patients ($\chi^2= 0.859$, $df= 2$, $P= 0.8$) and control groups ($\chi^2=0.420$, $df=2$, and $p=0.810$).

Conclusion

VDR FokI polymorphism is associated with increase risk of AML among Sudanese.

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