

Vitamin D Receptor Gene (FokI) Polymorphism in Sudanese Patients with Chronic Lymphocytic Leukaemia

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Abstract

Epidemiological and laboratory investigations have convincingly shown that vitamin D deficiency is associated with several common diseases, including cancers. It acts via binding to a corresponding intranuclear receptor. The aim of this study was to examine the association of vitamin D receptor (VDR) FokI polymorphism with the risk of CCL, and its impact on the clinical outcome among CCL patients in Sudan. The study included 40 CLL patients, their VDR FokI genotype frequencies and hematological characteristics were determined and compared with 40 age and sex matched normal subjects as control. The frequency of FF genotype (wild type) was higher among controls (87.5%) when compared to CLL patients (67.5%). When odds ratio was calculated for the overall group, we observed a 3-fold increased risk of CLL for those carrying mutant type (heterozygous) Ff genotype (OR 3.370, 95% CI: 1.070-10.613, P = 0.038). No significant differences were observed in the haematological values between CLL patients with FokI wild type (FF) and those with mutant types (Ff). In conclusion, our results indicate that VDR FokI mutant genotypes (Ff) is associated with increased risk of CLL, but with low impact on the clinical outcome.

Keywords: CLL; Vitamin D receptor gene polymorphism; Sudan.

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Introduction

Chronic lymphocytic leukemia (CLL) is characterized by a clonal proliferation and accumulation of neoplastic lymphocytes in the blood, bone marrow, lymph nodes, and spleen. It is the most common leukemia in adults (1). Historically, CLL was viewed as a tumor caused by the accumulation of long-lived but mainly resting lymphocytes with a very low proliferation index (2). However, it has been shown that CLL contains a small fraction of actively proliferating cells, with approximately 2% of cells newly generated each day (3). The median age of patients at diagnosis is 65 years, with only 10 to 15 percent under 50 years of age. In most series, the men are more affected than women. The course of the disease is variable. Whereas some patients with CLL have a normal life span, others die within few years after diagnosis (4,5). There is a wide range of initial presenting features, most commonly painless lymphadenopathy, followed by splenomegaly and or hepatomegaly. Only 5% of patients present with lymphadenopathy without evidence of leukemic infiltration, and in this situation the disease is known as small lymphocytic lymphoma (SLL). CLL is increasingly diagnosed in asymptomatic patients when a lymphocytosis is found at the time of a routine blood count. The international workshop on chronic lymphocytic leukemia (iwCLL) revised guidelines require a lymphocytosis of greater than 5000/ μ l maintained for more than 3 months with the cells expressing the diagnostic immunophenotype for the diagnosis of CLL (6). During the past few years, important advances have been made in the understanding of the biology, natural history, and treatment of CLL. Many investigations have led to the identification of various prognostic factors that can be used to stratify patients into risk categories (7).

Vitamin D endocrine system regulates a broad variety of independent biological processes including bone metabolism, innate immune response, cell proliferation and differentiation (8). Epidemiological and laboratory investigations

have convincingly shown that vitamin D deficiency is associated with several common diseases, including rickets and other bone diseases, diabetes, cardiovascular diseases, autoimmune diseases, tuberculosis and cancer (8-10). 1,25-Dihydroxyvitamin D₃ (1,25(OH)₂D₃, calcitriol), the biologically most active naturally occurring metabolite of vitamin D, has been shown to regulate the growth and differentiation of various cell types, including cancer cells (11). It acts via binding to a corresponding intranuclear receptor (VDR) (12), that is encoded by a large gene (>100 kb) located on the chromosome 12q12-14 (13). Vitamin D response elements have been identified in numerous genes involved in cellular growth, differentiation, apoptosis, invasion and metastasis of tumour cells (14).

Several polymorphisms have been reported for the VDR, one of them is FokI polymorphism, which can be detected by RFLP using the FokI restriction enzyme (15). The polymorphic FokI site in exon 2 results in different translation initiation region due to thymine (T) to cytosine (C) substitution (ATG-ACG). FokI produce two different alleles designated as (F and f) distinguished basis on the presence or absence of FokI restriction site (16). If the initiating translation starts from this alternative site (thymine variant), it results in the generation of a longer VDR protein of 427 amino acids. This polymorphism is referred to as the f allele (17). However, the f allele exerts less transcriptional activity, with the F variant being 1.7-fold more active (18,19). The aim of this study was to examine the association of VDR FokI polymorphism with the risk of CCL, and its impact on the clinical outcome among CCL patients in Sudan.

Materials and Methods

Following informed consent 80 subjects were enrolled: 40 chronic lymphocytic leukemia patients with established diagnosis (Diagnosis based on the

haematological and clinical features) who were attended Radiation and Isotopes Centre of Khartoum (RICK); and 40 apparently healthy subjects as a control. Two ml of EDTA anti coagulated blood was collected from each subject for haematological and molecular analysis. Laboratory investigations were performed at the department of haematology, faculty of medical laboratory sciences, Alneelain University, Sudan. Blood cell count was performed by automated cell counter (Sysmex KX-21N). DNA was extracted by salting out method.

DNA samples fragment was Amplified using the forward primer: (5'-AGCTGGCCCTGGCACTGACTCTGCTCT-3') and reverse primer: (5'-ATGGAAACACCTTGCTTCTTCTCCCTC-3'). The PCR reactions was performed in a final volume of 20 μ l containing (4 μ l premixed ready to use 5x FIREPol master mix (Solis BioDyne,Russian),12.0 μ lDNAase free DW, 3 μ l genomic DNA and 0.5 μ l from each primer).DNA samples were amplified in (Techno TC-412, UK Thermal Cycler) with cycling parameters as follows: Denaturation at 94 ° C for 5 min, 35 cycles at 94 °C for 30 s, 61 ° C for 30 s and 72 ° C for 1 min and one final cycle of extension at 72 °C for 7 min. The T/C polymorphism in the first of two-start codon (ATG) at the translation initiation site of the VDR gene was detected by RFLP using the restriction endonuclease FokI. The PCR product of the 265bp band was digested with 0.5 unit of Fok I restriction enzyme (New England Biolabs); 7 μ L of the digested reaction mixture was then loaded into 3% agarose gel containing ethidium bromide and visualized on agarose gel electrophoresis under UV determined machine (ingenins/syngene bio imaging). The sizes were determined using 50bp ladder. Digestion of the amplified 265 bp PCR product gave two fragments, of 169 bp and 96 bp respectively. Depending on the digestion pattern, individuals were scored as (ff) when homozygous for the presence of the Fok-I site, (FF) when homozygous for the absence of the FokI site, or (Ff) in case of heterozygosity.

Statistical analysis was performed using statistical package for social science (SPSS) software. Evaluation of patient's data was performed using the t-test. Comparison of frequency distribution between groups was made by means of the X^2 test. All tests are two-sided and P-value less than 0.05 have been considered as statistically significant. Crude odds ratios (OR) were also calculated and given with 95% confidence intervals (CI).

Results

The male: female ratio was 1.8 and the median Age was 64 year, with minimum Age of 40 and maximum of 78 years. All patients were tested for the blood cell counts and VDR gene Polymorphism.

The results of blood Count for CLL cases were as follows: Mean haemoglobin (Hb) level 10.6 ± 1.9 g/dL; mean total white cells (TWBC) count $63.3 \pm 58.0 \times 10^3/L$; mean platelet count $166.4 \pm 61.1 \times 10^3/L$. While for the control group: Mean Hb concentration 14.4 ± 1.2 g /dL; mean TWBC count $7.0 \pm 6.3 \times 10^9/L$; mean platelet count $236.3 \pm 46.9 \times 10^3/L$ (table 1).

Table 1. Comparison of haematological characteristics between CLL patients and control subjects

Parameter	Cases	Controls	P. value
Hbmean \pm SD (g/dl)	10.6 ± 1.9	14.4 ± 1.2	0.000
TWBC mean \pm SD ($\times 10^9/L$)	63.3 ± 58.0	7.0 ± 6.3	0.000
Platelets mean \pm SD ($\times 10^9/L$)	166.4 ± 61.1	236.3 ± 46.9	0.000

Table2 showed the distribution of VDR FokI genotype frequencies between CLL patients and controls. When the VDR FokI (FF) genotype was defined as the reference, the OR for the (Ff) genotype was 3.370 (95% CI: 1.070-10.613, P = 0.038).

Table 2. Comparison of VDR FokI genotype frequencies in CLL and controls

Genotype	CLL cases n (%)	Control n (%)	OR	95%CI	P value
FF	27 (67.5)	35 (87.5)	Referent		
Ff	12 (32.5)	5(12.5)	3.370	1.070-10.613	0.038
ff	0	0	-	-	-

Various haematological values, including Hb level, TWBC count, platelets count, revealed no statistically significant differences between CLL patients with FokI wild type (FF) and those with mutant types (Ff) (data were shown in table 3).

Table 3. Comparison of haematological characteristic between CCL patients with wild type and those with mutant types

Parameter	Wild type (FF)	Mutant types (Ff)	P value
Hbmean \pm SD (g/dl)	10.7 ± 1.8	10.6 ± 2.0	.888
TWBC mean \pm SD ($\times 10^9/L$)	61.9 ± 45.7	64.0 ± 63.9	.916
Platelets mean \pm SD ($\times 10^9/L$)	170.4 ± 77.1	164.4 ± 53.4	.775

Discussion

Genetic polymorphisms of various kinds of genes have been recently proved to have important roles in the genesis of human malignancies. Several studies have reported that individuals with mutant genotypes are at increased risk of leukemia. We examined the association between VDR gene polymorphism (FokI) and the risk of CLL. Our study included 40 CLL patients, their FokI genotype frequencies and hematological characteristics were determined and compared with 40 age and sex matched normal subjects as control. The frequency of the FF genotype (wild type) was higher among controls (87.5%) when compared to CLL patients (67.5%). Similar finding was reported in previous study in Sudan (20)

To determine if there was a statistically significant increase risk of CLL development according to the VDR FokI genotypes, we conducted logistic regression analysis, our study showed a statistically significant association between FokI polymorphisms and the risk of CLL. When odds ratios were calculated for the overall group, we observed a 3-fold increased risk of CLL for those carrying mutant type (heterozygous) Ff genotype (OR 3.370, P = 0.038). Mutant VDR genotypes, through its effect on the activity of the receptor and subsequent downstream vitamin-D mediated effects such as modulation of cellular proliferation and differentiation, may influence the susceptibility to CLL.

When comparing the studied haematological values between CLL patients with FokI wild type (FF) and those with mutant types (Ff), we observed no significant differences. This finding suggested low impact of VDR FokI polymorphism in the clinical outcome of CLL.

Mutant VDR genotypes, through its effect on the activity of the receptor and subsequent downstream vitamin-D mediated effects such as modulation of cellular proliferation and differentiation, may influence the susceptibility to CLL. However, further investigation needs to verify this suggestion and to understand the mechanism.

Conclusion

In conclusion, we examined the association of VDR FokI polymorphism and the risk of CLL. Our results indicate that VDR FokI mutant genotypes (Ff) is associated with increased risk of CLL, but with low impact on the clinical outcome.

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