Vitamin D- Receptor (VDR) Gene Start Codon Polymorphism (FOKI) with Polycythemia Vera in Sudanese Patients

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Abstract

Background: It has been hypothesized that VDR polymorphisms may influence both the risk of cancer occurrence and prognosis. Polycythemia Vera (PV) is one of a related group of blood cancers known as “MyeloProliferative Neoplasm's” (MPNs) in which cells in the bone marrow that produce the blood cells develop and function abnormally.

This study is conducted to explore the association of vitamin-Dreceptor (VDR) Polymorphism at the FOK1 site with the occurrence of polycythemia vera.

Materials and methods: A total of 72 subjects were enrolled in this study, 35 with Polycythemia Vera and 37 healthy controls, Genomic DNA was extracted from patients’ blood samples by salting out method, and analyzed for determination of VDR start codon polymorphismFokI using PCR-RFLP (restriction fragment lengths polymorphisms).

Results: The results showed that F/F genotype was the most frequent 23(65.7%) among patients with Polycythemia Vera, followed by the genotype F/f 11 (31.4%) and f/f 1 (2.9%) consequently. Similarly the control group showed F/F genotype was the most frequent 32(86.5%) followed by F/f 5(13.5%), no f/f genotype was detected among the control group also there is No significant association was found between Polycythemia Veraand each of the genotypes F/F OR:- 0.331 (CI:0.102-1.077)and F/fOR:2.640 (CI:0.801-8.701) p value= (0.112)

Conclusion: F/F and F/f genotypes are not consideredas risk factors forpolycythemia Vera.

Keywords: VDR, start codon polymorphism, PV polycythemia Vera

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Introduction

Polycythemia Vera(PV) is a clonal disorder arising in a multipotent hematopoietic progenitor cell that causes the accumulation of morphologically normal red cells, white cells, platelets, and their progenitors in the absence of a definable stimulus and to the exclusion of nonclonal hematopoiesis [1-2]. First described in 1892 [3].polycythemia vera is not a new disease and while uncommon, with an incidence of at least 2 per 100,000 [4-6].

A major advancement in understanding the pathogenesis of PV has been the discovery of an acquired mutation occurring in the negative regulatory pseudo-kinase JH2 domain of Janus kinase 2 (JAK2V617F) [7-10].Because it involves a multipotent hematopoietic progenitor cell, the hallmark of polycythemia Vera is trilineage hematopoietic cell hyperplasia. However, erythrocytosis is its most prominent clinical manifestation[11].PV should be suspected when hemoglobinand/or hematocrit levels are elevated, i.e., hemoglobin level greater than 18 g perdl [180 g per L] in white men and 16 g perdl [160 g per L] in blacks and women; hematocritlevel greater than 52 percent (0.52in white men and 47 percent (0.47) in blacksand women)[12].the disease evolves to post-polycythemicmyelofibrosis or transforms to acute myeloid leukemia[13].Biological and epidemiologic data suggest that vitamin D levels may influence cancerdevelopment. The vitamin D receptor (VDR) is a crucial mediator for the cellular effects ofvitamin D and additionally interacts with other cell-signaling pathways that influence cancerdevelopment[14].Genetic variations may phenotypically appear as inter-individual variationsin limiting rates of vitamin D synthesis in the skin, hydroxylation in the liver and in thekidney, transport, metabolism, degradation that would ultimately influence individual's vitaminD status.

The VDR is an intracellular hormone receptor that specifically binds the biologically activeform of vitamin D, 1,25-dihydroxyvitamin D or calcitriol (1,25(OH)2D) and interacts withspecific nucleotide sequences (response elements) of target genes to produce a variety ofbiologic effects. The VDR gene is located on chromosome 12-q-14 and several singlenucleotide polymorphisms (SNPs) have been identified that may influence cancer risk [15]. One of the known DNA sequence variants is a thymine/cytosine (T/C) polymorphism in the first of two potential start (ATG) codons separated by three codons. This polymorphism results in two alleles that can be distinguished by RFLP using the endonuclease FokI[16].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 [17].VDR expression has been described in many types of cancer cells including cells derived from tumors of the breast, prostate, pancreas, colon, bladder, cervix,thyroid, pituitary, skin (squamous cell carcinoma, basal cell carcinoma, and melanoma),glioma, neuroblastoma, leukaemia and lymphoma cells. [18-21].Because of the important role of vitamin D and its receptor in blood cells growth and differentiation, we hypothesized that the FokI polymorphism in the VDR gene might be associated with risk of polycythemia Vera . Therefore, the present study is an attempt to explore whether an association between the VDR FokI polymorphism with primary polycythemia exists or not

Materials and methods

Subjects

It is a case control study, in which 35 Sudanese patients who have been diagnosed with Polycythemia Vera (JAK2 mutation+ve) and complete blood pictures) and 37 healthy volunteers wererecruited.Three milliliter (ml) of venous blood were collected and poured into K_3EDTA anticoagulant.

Molecular analysis

Genomic DNA was isolated from peripheral blood leucocytes usingsalting out method,Reaction mixture of 20µlwasprepared for each sample.It consists of twoµlof genomic DNA, 1µl of each

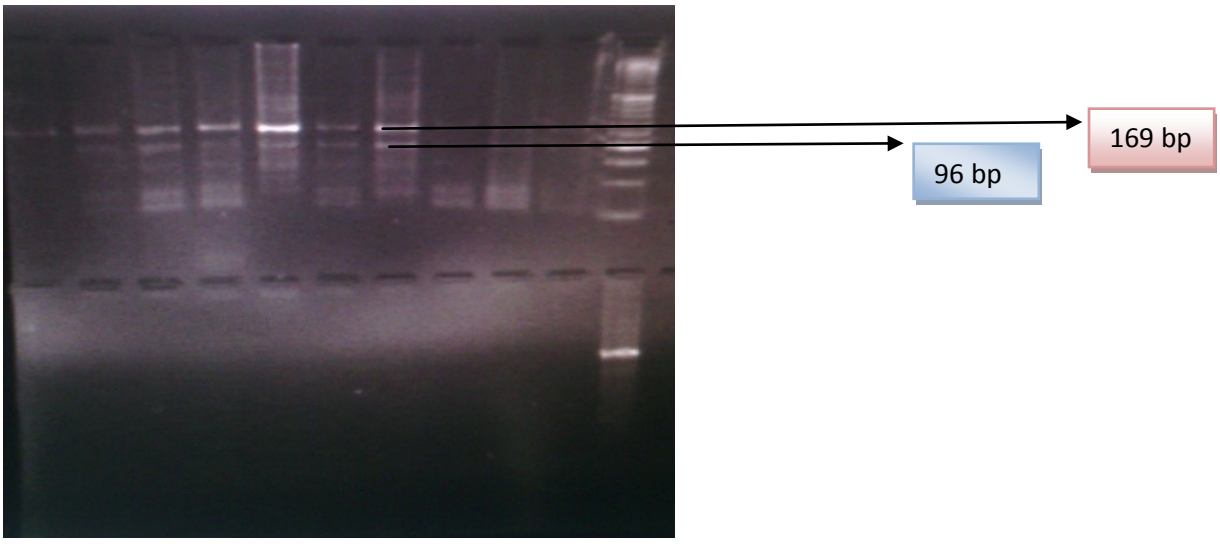
forward and reverse primers,5µl master mix (*i-taq, iNtRON, Korea*) and 11 µl sterile distilled water.Forward:5'-AGCTGGCCCTGGCACTGACTCTGCTCT-3'

Reverse:5'-ATGGAAACACCTTGCTTCTTCTCCCTC-3'

DNA samples were amplified in PCR machine,(*TechneTC-412,UK*), with cycling conditions included initial denaturation at 94 ^{o} C for 5 min, then 35 cycles at 94^{o}C for 30 seconds, 61 ^{o} C for 30 seconds and 72 ^{o} C for 1 min, and final cycle of extension at 72^{o}C for 7 min. The VDR start codon T/C polymorphism was detected by PCR-restriction fragment length polymorphism using the restriction endonuclease Fok1.

The PCR product of the 265bp band was digested with 1.0 unit ofFokIrestriction enzyme (*New EnglandBiolabs, England*) at 37 ^{o} C for 4 hours; 10µl of the digestion mixture was loaded into 3% (agarose gel) containing ethidium bromide and visualized using gel documentation system. The sizes were determined using 50-bp ladder*(SOLIS BIODYNE, ESTONIA*).

Digestion of the amplified 265 bp PCR product results in two fragments, one169 bp and the other 96 bp,as shown in figures(1). Depending on the digestion pattern, study subjects were nominated as (f/f) when homozygous for the presence of the FokI site**,**(F/F) when homozygous for the absence of the FokI site, or (F/f) in case of heterozygosity



Figures (1):The pattern of the digestion fragments.

Statistical analysis

Data of this study was collected from patients' medical filesand samplesthenanalyzed using Statistical Package for Social Sciences (SPSS). Frequencies of F/F, F/f, andf/f genotypes were calculated, and correlation between genotypes and study groups was investigated by exact fisher and Chi-square tests.The allelic frequencies was calculated and the Hardy–Weinberg equilibrium was tested by a goodness-of-fit X2 test to compare the observed genotypic frequencies in normal individuals to the expected genotypic frequencies calculated from the observed allelic frequencies.

Ethical considerations

This studywas approved by RICKand faculty of medical laboratory sciences, Al Neelain University, Khartoum, Sudan, and informed consent was obtained from each patient before sample collection.

Results

The ages of study subjectsranged between 14-70years ,statistical analysis showed that themean age was 37.5 years(Mean±SD: 37.5±12.5); 32 (91.4%) ofpatients were males and only 3(8.6%) werefemales. Further 37 healthy individuals were included as controlgroup, 30(81.1%) of them were males and7 (18.9%) were females.Genotyping of VDR start codon FOkIpolymorphismwasperformed by PCR-RFLP.).Our result showed that the genotype F/F was the most frequent 23 (65.7%)among patients, followed by the genotype F/f 11 (31.4%) and f/f 1(2.9%) consequently. Also in healthy controls the F/F genotype was the most frequent32 (86.5%) , followed by the genotype F/f5 (13.5%), while the genotype f/f was completely absent.In polycythemia patient, there wasstatistically significant Correlation between VDR FokI polymorphisms F/F and F/f genotype with male gender p.value = (0.013) (Table1).

Table 1: Correlation between VDR FokI polymorphism and gender

Gender	F/F	F/f	f/f	p.value
Male	22 (68.8%)	10 (31.2%)	0 (0%)	0.013
Female	1 (33.3%)	1(33.3%)	1(33.4%)	1.0

There was no statistically significant correlation between polycythemia Veraand the genotypes F/F, F/f and f/fp.value (0.112) (Table2).

Table 2: Correlation between VDR start codon genotypes and polycythemia Vera

Genotype	Patients	Controls	*P.value*
F/F	23 (65.7 %)	32 (86.5%)	0.112
F/f	11 (31.4%)	5 (13.5%)	
f/f	1(2.9 %)	0.0%	

The odd ratio for F/F / non F/F genotype = 0.331 with CI= (0.102-1.077) . While for F/f / non F/f is 2.640 with CI=(0.801-8.701).The frequency of the F allele was (0.86) in the patients and (0.92) in the control group while the frequency of the f allele was (0.2) in the patients and (0.08) in the control group.However no significant deviation from the Hardy–Weinberg equilibrium was found in both patients (X2= 0.285, df =2 and P=0.867), and control group (X2= 0.420, df=2, and P= 0.810).

Discussion

Vitamin D stimulation can influence hematopoietic development as experiments treating both normal hematopoietic stem cell lines and leukemic cell lines with the active form of vitamin D led to increased monocyte/macrophage differentiation and increased numbers of those mature cells [22 -23].Several single nucleotide restriction fragment lengthpolymorphisms have been described in the VDR gene inassociation with neoplastic and non-neoplastic diseases.

Several studies on FOKI polymorphisms in the VDRgene have been performed to investigate their implication with different types of cancer in hematological malignancy Eman Mohammadin etal found that there was asignificant association between ALL and the genotypes F/F (OR: 0.234, CI:0.073-0.748 ,P.value:0.014) and F/f (OR: 3.78, CI :1.179 -12.131, P.value:0.025), and conclude that VDR start codon F/F and F/f genotypesmight confer increased risk for the development ofALL [24].Syed Shafiaa, *et al*reportedthat the FokI polymorphism is involved in the increased susceptibility for the development and progression in multiple myeloma in the

ethinic Kashmiri population furthermore those result suggest that f/f genotype is associated with higher risk for developing multiple myeloma[25].Also Yasmine Ahmed*et al*conclude thatVDR FokI polymorphism is associated with increased risk of AML among SudaneseF/F (OR= 1.420, CI =1.122-1.719, P =0.000) and F/f (OR= 1.580 , CI = 1.281-1.878 , p=0.000) [26].Young Ho et al found that there is a significantassociations between VDR FOKI polymorphism with rheumatoid arthritis[27]. Also in non-hematological malignancy Touvier*et al*hadobserved a significant increase in skin cancer risk and increase in breast cancer risk with FokI f/f compared to /FF genotype[28].In the present study, we examined the prevalence of the VDR FokI polymorphism and polycythemia Vera.Our result showed that F/F are the most frequent among all patients and control groups, followed by F/f and f/f in-patientconsequently, no f/f was detected in control group Polythycemia Vera affect male more than female [29]. our result showed thatthere wasstatistically significant association between VDR FokI polymorphismsF/F and F/f genotype with male in compare to female with exact fisher test p.value = (0.013),The frequency of the F allele was (0.86) in the patients with PV and (0.92) in the control group while the frequency of the f allele was (0.2) in the patients with PV and (0.08) in the control group. However, no significant deviation from the Hardy–Weinberg equilibrium was found in both patients and control.and we found that there was no statistically significant correlation between polycythemia Veraand the genotypes F/F, F/f and f/fp.value (0.112) .

No study performed to explore the relationship between FokI polymorphism and polycytheamiavera ‘Pardanani1, *et al* investigated the clinical correlationof vitamin D insufficiency in myeloproliferative neoplasms when they examined the clinical and prognostic relevance of low plasma levels of 25-hydroxyvitamin D (25[OH]D) in myeloproliferative neoplasms (MPN),and they found that there were no significant correlations between 25(OH)D insufficiency, or severe deficiency, and a variety of clinical or laboratory variables in PV [30] AlsoKwa*et al* found that therewas no statistically significant association between theFokI polymorphisms and Chronic Lymphocytic Leukemia (CLL) in a study conducted to investigate the relationship between them [31]. That agreed with our finding.Variations in these studies could be due to differences in the nature of these diseases. Further research on this issue is needed due to small sample size in our study.

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