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Research Article

Association of Vitamin D Receptor (VDR) Start Codon Fok-I Polymorphism with Chronic Myeloid Leukemia

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Abstract

Several studies have reported the contribution of VDR Fok-I polymorphisms in various types of cancer. The aim of this study was to investigate the possible correlation between vitamin D receptor (VDR) gene Fok-I polymorphism and chronic myeloid leukemia.

A total of 77 subjects were enrolled in this study, 40 with CML and 37 healthy volunteers (control group). Venous blood sample was collected from each subject in ethylene diamine tetra acetic acid. Genomic DNA was extracted by salting out method and analyzed for detection of VDR Fok-I polymorphism by polymerase chain reaction-restriction fragment length polymorphism.

The result showed that the genotype F/F was the most frequent (85%) in patients with CML, followed by the genotypes F/f (10%) and f/f (5%) consequently. Similarly in the control group the genotype F/F also was the most frequent (86.5%) followed by the genotype F/f (13.5%); no f/f genotype was detected among the control group. There was statistically significant correlation between CML and the f/f genotype (*P.value*:0.000) but not with the genotypes F/F (*P.value*:0.852) and F/f (*P.value*:0.895). No statistically significant correlation between the VDR Fok-I polymorphism and gender *(P.value:0.61)*. Comparison of age in CML patients with VDR Fok-I genotypes showed no statistically significant difference (*P.value*:0.654). We concluded that the VDR f allele might play a secondary role in CML pathogenesis, since all patients had Philadelphia chromosome which is a well established cause of CML.

Keywords: vitamin D receptor, Fok-I polymorphism, chronic myeloid leukemia, Sudanese.

INTRODUCTION

Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder of the haempoetic stem cells. CML accounts for most cases of myeloproliferative disorder and 20% of all leukemia, with an annual incidence of about ten in 1.000,000 people[1]. CML is a malignancy that is consistently associated with acquired genetic abnormality, the Philadelphia chromosome, which results of rearrangement between BCR-ABL fusion genes [2]. The expression of these genes is influenced by alteration in chromatin structure through change in DNA methylation (chromatin remodeling) mediated by multi protein complex[3]. Vitamin D is a potent regulator of cell growth and differentiation, which has an effect on cell death, tumor invasion, and angiogenesis that makes it a candidate compound for cancer regulation [4]. The nuclear functions of vitamin D require binding to the vitamin D receptor (VDR) [5]. Vitamin D Receptor (VDR) is a receptor that belongs to the super family of nuclear receptor [6]. VDR can form a homodimer or form a heterodimer with a retinoid X receptor after being bound by activated

vitamin D, those dimers can then bind to vitamin D response elements (VDREs) in the promoters of target genes, eventually leading to target gene transcription[7,8]. The gene encoding for VDR is mapped to chromosome 12cen-q12 [9, 10].

Several single nucleotide polymorphisms (SNPs) has been identified in VDR sequence gene, one of them Fok-I which represents an independent polymorphism site[11]. It is located in VDR start codon, affecting the structure and function of encoded protein. VDR polymorphism Fok-I define the presence of T>C transition polymorphism (ATG-ACG) in exon 2 of the VDR gene [12]. Fok-I produce two different alleles designated as (F and f) distinguished basis on the presence or absence of Fok -I restriction site [13].

The allelic variation of this polymorphism code for structurally different receptor protein with different length, long form (f allele, T) and short form (F allele, C) due to translation initiation site [12].

The presence of VDR in a variety of cell lines, beside the increased evidence of VDR involvement in cell differentiation, inhibition of cellular proliferation and angiogenesis in many tumor types, suggest that vitamin D plays a role in cancer [14, 15].

The effect of vitamin D on the treatment of cancer was first identified in myeloid leukemic cells [16].

It has been postulated that, VDR gene Fok-I polymorphism represents a strong positional candidate susceptibility gene for different diseases like Prostate cancer, Uolithiasis, inflammatory bowel disease and Osteoporosis [17].

The pleitropic affect of Vitamin D, VDR and their involvement in normal and malignant cells suggests that the VDR polymorphism may have a role in CML pathogenesis.

The aim of this study was to investigate the association between the VDR Fok-I polymorphism and CML among Ph positive Sudanese patients.

MATERIALS AND METHODS

Patients and samples

This is a case control study conducted at radiation and isotopes center of Khartoum (RICK), Khartoum, Sudan, in the period from February to May 2014.

A total of 40 Sudanese patients with Ph positive CML were enrolled in this study and 37 healthy volunteers were also recruited to participate in this study as a control group.

Three milliliter (ml) of venous blood was collected from all the subjects in ethylene diamine tetra acetic acid (EDTA).

Molecular analysis

Genomic DNA was extracted from EDTA blood sample by salting out method. VDR Fok-I polymorphism start codon exon 2 genotype was determined by polymerase chain reaction (PCR-TECHNE, TC412, UK) and restriction fragment length polymorphism (RFLP). Two micro liter (µl) of DNA was amplified in a total volume of 25 µL containing 1µl of each of the forward primer (5'AGCTGGCCCTGGCACTGACTCTGCTCT -3') and reverse primer: (5'-ATGGAAACACCTTGCTTCTTCTCCCTC -3'), 5 µl master mix (Maxime PCR pre mix kit (I-TAQ), INTRON, KOREA) and 16 µl sterile distilled water.

Thermo cycling conditions for Fok-I allele included initial denaturation at 94^{o}C for 5 mints; then, 35 cycles each consisting of: 94^{o}C for 30 second, 61^{o}C for 30 second and 72^{o}C for 1 minutes; final extension at 72^{o}C for 7 minutes.

The PCR product of the 265 bp band was digested with 1.0 unit of Fok-I restriction enzyme (BIOLABS, NEW ENGLAND). The digested reaction mixture was then loaded into 3% agarose gel containing ethidium bromide and the fragments sizes were determined using 50 bp DNA ladder (SOLIS BIODYNE, ESTONIA) and identified under UV transilluminator on documentation system (SYNGENE, JAPAN)

Digestion of the amplified 265 bp PCR product gave two fragments of 169 bp and 96 bp respectively, if the product was excisable. Depending on the digestion pattern, individuals expressed (f/f) when homozygous for the presence of the Fok-I site**,** (F/F) when homozygous for the absence of the Fok-I site, or (F/f) in case of heterozygosity.

Statistical analysis

Data of this study was collected by structured interview questionnaire and analyzed using statistical package for social sciences (SPSS). Frequency of VDR Fok-I polymorphism and other qualitative variables were determined; age of the patients was compared by independent 2-sample test; correlation between the VDR Fok-I polymorphism and gender was tested by Chi-square test. 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 study was approved by RICK and faculty of medical laboratory sciences, Al Neelain University, and informed consent was obtained from each patient before sample collection.

RESULTS

A total of 40 Sudanese patients diagnosed with ph'-positive CML at RICK were enrolled in this study;

their ages ranged between 8-75 years (Mean ±SD: 41.4±1.5); 27(67.5%) of them were males and 13(32.5%) were females. Further 37 healthy individuals were included as a control group, 30(81.1%) were males and 7(18.9%) were females.

Genotyping of VDR Fok-I alleles was performed by PCR-RFLP.

In patients the genotype F/F was the most frequent, followed by F/f and f/f genotypes consequently; similarly in the control group F/F genotype was the most frequent, followed by F/f, but f/f genotype was not detected among the control group. There was statistically significant correlation between CML and the VDR start codon f/f genotype but not with the genotypes F/F and F/f (Table 1).

In CML patients, there was no statistically significant correlation between the VDR Fok-I polymorphism and gender (Table 2).

Comparison of age in CML patients with VDR Fok-I genotypes showed no statistically significant difference (Table 3).

The allelic frequency of the F allele (0.90) in the patients and (0.93) in control group, while the frequency of f allele was (0.10) in the patients and (0.07) in control group.

No significant deviation from the Hardy–Weinberg equilibrium was observed in patients with CML (X2= 0.237, df=1, P=0.626) and control group (X2= 0.420, df=2, P=0.8).

DISCUSSION

Many researches have suggested the effect of VDR gene polymorphisms in the development of several types of carcinoma[18, 19, 20].

This study is a case- control study conducted to examine the association of VDR polymorphism Fok-I with CML.

Our results showed that F/F genotype was the most common among patients with CML followed by F/f and f/f consequently. In control group F/F was the most common genotype, followed by F/f, while no f/f genotype was detected. This was inconsistent with Alessandra *et al* who found that F/f genotype was the most common in patients with lumbar spine pathologies and control followed by F/F and f/f [21]. Our findings also disagreed with Lei li *et al* who reported F/f genotype was the most common followed by f/f and F/F in Chinese patient with pancreatic cancer[22].

There was statistically significant correlation between CML and the VDR start codon f/f genotype but not with the genotypes F/F and F/f. This findings was consistent with the finding of a study conducted on patients with meningioma which reported that, VDR Fok-I f/f genotype was significantly increased in patients compared to controls [23]. Our results also agreed with study conducted within US population, in which the f/f carriers showed a statistically significant increase in relative risk of breast cancer compared with women with the F/F genotype[24]. Similar to our result, a study in Tunisia population found the Fok-I f allele to be associated with an increased risk of T-cell lymphoma [25]. Our findings was also supported by Jie Wang *et al* who reported that f/f genotype is statistically significant as a risk factor of breast cancer[26]

In contrast, our findings disagreed with study concerning the association of VDR polymorphism with chronic lymphocytic leukemia patients and showed no significant difference in allelic distribution between CLL patient and healthy control population[3]. Also disagreed with another study done on Indian population with epithelial ovarian cancer and concluded that the low blood levels of vitamin D and VDR receptor polymorphism Fok-I is not considered to be a risk factor for the development of ovarian cancer[27]. Furthermore, Oakley Girvan *et al* reported that the FF genotype associated with increased prostate cancer risk among young African-Americans[28].

These variations could be related to the differences in the types of cancers studied, as none of them was conducted on CML patients.

In this study no statistically significant difference was found in mean age in patients with VDR Fok-I genotypes. There was also no statistically significant correlation between the VDR receptor Fok-I polymorphism genotypes and gender, this disagrees with the finding of Kaabachi *et al* who reported significant association with Fok-I polymorphism when stratified patients according to gender and age[29].

In the present study, F allele frequency was 0.90 in CML patients, and 0.93 in control group while, the frequency of the f allele was 0.10 in the patients and 0.07 in control group; no deviation from Hardy-Weinberg equilibrium was observed in all patients and control groups. Mohapatra et al finding was inconsistent with ours as he reported a significant difference in the distribution of VDR genotypes in Indian patient with ovarian cancer[27].

This variation in results might be due to the different type of cancer studied and ethnic variation.

CONCLUSION

There was statistically significant correlation between CML and the f/f genotype (P.value:0.000), this suggests that the VDR f allele might play a secondary role in CML pathogenesis, since all patients had Philadelphia chromosome which is a well established cause of CML.

Table 1
Correlation between VDR Fok-I polymorphism and CML

Group / Genotype	Patient N (%)	Control N (%)	*P.value*
F/F	34(85%)	32(86.5)	0.852
F/f	4(10%)	5(13.5)	o.895
f/f	2(5%)	0(0%)	0.000

Table 2
Correlation between VDR Fok-I polymorphism and gender

Genotype	Male	Female	*P.value*
F/F	24	10	0.611
F/f	4	2	
f/f	1	1	

Table 3
Comparison of age in patients with VDR Fok-I genotypes

Genotype	Age		*P.value*
	Mean	SD	
F/F	34(41.2)	15.5	0.654
F/f	4(48.2)	28.4	
F/f	2(49)	32.5	

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