

## Interleukin-4 Intron 3 VNTR Polymorphism Gene in Leukemic Patients

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Received date: May 10, 2016, Accepted date: May 28, 2016, Publication date: May 31, 2016

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### Abstract

**Background:** Leukemia is a group of chronic malignant disorders of White blood cells and its precursor. Interleukin-4 (IL-4) is inflammatory cytokine that determines the activation and differentiation of B-cells, mast cells, erythroid progenitors. Several studies have investigated the association between IL-4 intron 3 variable number of tandem repeats (VNTR) polymorphism and cancer risk in humans; however, this association is not investigated among patients with leukemia.

**Material and Methods:** The present study aimed to investigate genotype and allele frequencies of IL-4 gene intron 3(VNTR) polymorphism in patients with leukemia compared to healthy control. The study included 231 patients with leukemia and 163 healthy controls. Genomic DNA was isolated from 3 ml of anticoagulated venous blood samples by modified salting out method. IL-4 intron 3 VNTR polymorphism determined by using polymerase chain reaction (PCR) with specific primers. The data were analysed using SPSS software program version 21. P value, Odds ratio (OR) and corresponding 95% confidence interval (CI) were used to estimate the strength of the association.

**Results:** The allele frequency was showed in 25.9% (60/231) leukemic patients while 74.1% (171/231) showed absence of allele compared with the presence of allele in all control group with significance differences of P value=0.00 and risk factor of 4.617 times for leukemia. The frequencies of P1P1, P2P2, and P1P2 genotypes of intron 3 VNTR polymorphism in leukemic patients were significantly different from control group P value=0.00. The result showed, P1P1 and P1P2 allele were highly risk for developing leukemia than P2P2 (OR: P1P1 1.24, 95% CI: 0.675-2.279; OR P1P2: 1.24, 95% CI: 0.568-2.7; OR P2P2:0.72, 95% CI: 0.398-1.3).

**Conclusion:** IL-4 intron 3 VNTR polymorphism could influences in the risk of leukemia; this could be used as early prognostic marker in the course of the disease.

**Keywords:** Interleukin 4 intron 3 VNTR polymorphism; Leukemia, PCR

### Introduction

Leukemia is a group of chronic malignant disorders of WBCs and WBC precursor. It is characterized by replacement of bone marrow by malignant cells results in unregulated proliferation of immature WBCs entering the circulatory system. These leukemic cells may also enter the liver, spleen, or lymph nodes, causing enlargement of these organs [1]. Leukemia is classified into lymphocytic, or myelocytic according to the type of cell which was derived from. It contains four main subtypes; acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML) and chronic lymphocytic leukemia (CLL) with different clinical profiles [2]. Internationally, an estimated 300,000 new cases of leukemia (2.8% of all new cancer cases) are diagnosed each year globally [3]. In Sudan, Leukemia represent the second frequently cancer with rate incidence of (10.0 per 100,000) [4].

Pathogenesis and progression of leukemia influences by intrinsic and extrinsic factors. The intrinsic factors include genetic alterations of cellular pathways, and the extrinsic factors include chemokines and cytokines [5].

Interleukin-4 (IL-4) is important inflammatory mediator cytokines, produced by activated Th2 type CD4+ T cells. IL-4 induces development of Th2 subset of lymphocytes, which is responsible for surveillance and clearance of tumor cells by activation of granulocytes and eosinophils, as well as inhibition of angiogenesis [6]. IL-4 structure consists of four exons and located on chromosome 5q31.1 [7].

The IL-4 gene contains a 70-bp variable number of tandem repeat (VNTR) polymorphism in intron 3 that is associated with IL-4 production. It was proposed that an increased responsiveness of the 2R allele to transcriptional activation might lead to the over expression of IL-4 [8]. The frequent allelic form and the first published sequence in intron 3 consists of three 70 base-pair (bp) repeats (3R); a rarer allele with two repeats (2R) has also been described and there is a third much rarer allele with four repeats (4R) [9].

A number of studies have been reported the association of IL-4 intron 3 VNTR polymorphism with different disease such as, rheumatoid arthritis (RA), immune thrombocytopenic purpura, end-stage renal disease (ESRD), systemic lupus erythromatosus (SLE), bladder cancer, severe malaria (SM), type-2 diabetes, vitiligo, periodontitis and multiple sclerosis (MS) [10-18]. To our knowledge there is no published data about the relationship of IL-4 gene intron 3 VNTR polymorphism and leukemia. The study aimed to analyze the association of IL-4 intron 3 VNTR polymorphism with Leukemia susceptibility and the risk factor of alleles in Sudanese leukemic patients.

## Materials and Methods

This was case-control study included 231 patients aging between 1 and 62-year-old with provisional diagnosis of Leukemia and 163 matched apparently healthy individuals as control. All patients were referred to Radiation and Isotopes Center Khartoum (RICK) (the referring center of cancer in Sudan), during February 2013 and June 2015. Demographic and clinical data have been collected in structured questionnaire from each patient. A total of 3 ml of venous blood samples were collected in EDTA anticoagulant tube from all patients and the matching control. The ethical board at the Ministry of Health, Khartoum-Sudan, granted the ethical approval for the study.

### DNA-extraction

Genomic DNAs were extracted from peripheral blood samples collected from patients and control using modified salting out method as described early [19], and stored at a final concentration of 200 mg/ml in a freezer at -20°C until used for genotype.

### PCR technique

The VNTR 70 bp repeat polymorphism of intron 3 was determined using a genetic molecular technique, polymerase chain reaction (PCR) after a protocol described previously [10]. The PCR reaction was done in 25 µl, total volume containing: 100 ng genomic DNA, 2 µL from each primer forward and reverse (Table 1), 12.5 µl 10 × PCR buffer - 1 U/µl of Taq DNA polymerase, 2.0 mm MgCl<sub>2</sub>, 0.2 mm dNTPs mix, Free nuclease water was added in order to reach the total volume. according to the following protocol: initial denaturation at 95°C for 5 minutes, 30 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds, extension at 72°C for 1 minutes, and final extension at 72°C for 10 minutes. Finally, the PCR products were separated by electrophoresis on a 2% MetaPhor® Agarose gel. After ethidium bromides staining the amplification products were visualized

in UV Transiluminator. PCR product was of 183 bp for P1 allele and 253 bp for P2 allele. In order to ensure the accuracy of our technique, each PCR reaction contain an internal controls for each genotype.

IL-4 70 bp Fwd	5' TAGGCTGAAAGGGGAAAGC3'
IL-4 70 bp Rev	5'CTG TTC ACC TCA ACT GCT CC3'

**Table 1:** Primers sequences-70 bp intron 3 polymorphism [10].

### Statistical analysis

Data were analysed using SPSS statistical package version 21. The  $\chi^2$  test was used to evaluate the Hardy-Weinberg equilibrium for the distribution of the genotypes of the patients and the controls. Odds ratio (OR) and 95% confidence interval (CI) were used for the assessment of risk factors. P value less than 0.05 were considered significant.

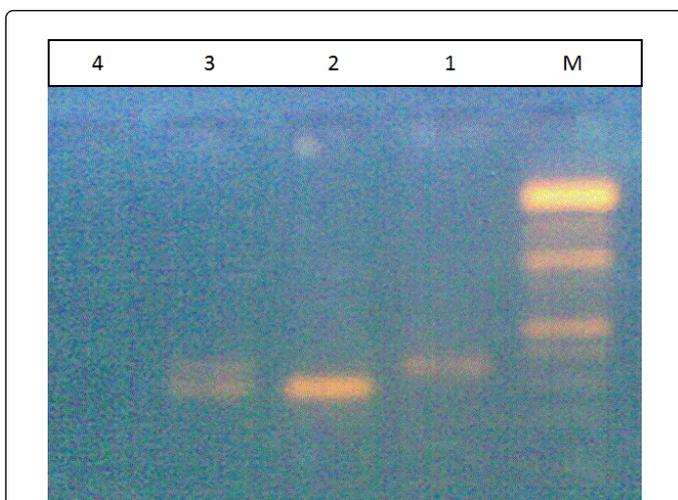
### Results

The study was included 231 leukemic patients (59.3% males, 40.7% females), their ages ranged (1-62 years) and 163 matching apparently healthy controls (73.1% males, 26.9% females). The genotypic and allelic frequencies of the IL-4 intron 3 VNTR polymorphism genes were presented in (Figure 1). A total of 171 (74.1%) of the leukemic patients showed absent allele, whereas 60 (25.9%) patients showed presence of allele compared to the presence of allele in all control group. There was a statistical significant difference between allele loss in leukemic patients and the control group with (P value=0.000 OR: allele loss 4.617, 95% CI: 0.825-3.210). When fishers test applied to see the frequency distribution for the presence and absence of allele among different subgroups of the leukemic patients, the result showed significant differences with P value=0.000 (Table 2).

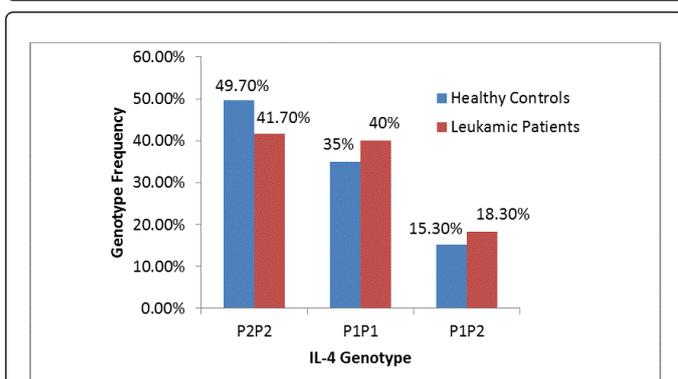
The three genotypes of intron 3 VNTR of IL-4 gene were classified as: P1P1 (183 bp two 70 bp repeat allele), P2P2 (253 bp three 70 bp repeat allele) and P1P2 (both 183 and 253 bp fragments). The frequencies of P1P1, P2P2, and P1P2 genotypes of intron 3 VNTR polymorphism in leukemic patients were 40%, 41.7%, and 18.3% and in the controls were 35%, 49.7%, and 15.3%, respectively. The distribution of genotype and allele frequencies of IL-4 gene intron 3 VNTR polymorphism was statistically different between leukemic patients and control group (p=0.000, OR: P 1/1 1.24, 95% CI: 0.675–2.279, OR P2/2: 0.72, 95% CI: 0.398–1.3, OR P 1/2: 1.24, 95% CI: 0.568–2.7) Figure 2.

Leukemia Subgroups	Patients Frequency	Absence Allele	Presence allele	P Value
ALL	7	2	5	0
AML	113	101	12	
CLL	13	8	5	
CML	98	60	38	
Total	231	171	60	

**Table 2:** Frequency distribution for the presence and absence of allele among different subgroups of leukemia patients.



**Figure 1:** Detection of the VNTR gene polymorphism of the IL-4 gene on 2% agarose gel electrophoresis. M=100-bp marker. Lanes 1 show the P2P2 allele (253bp). Lanes 2 the P1P1 (183bp) genotypes. Lane 3 shows the P1P2 allele (183 and 253 bp). Lanes 4 deleted IL4 genotypes.



**Figure 2:** The frequency of IL-4 genotypes distribution in Leukemic patients (n=60) and healthy controls (n=163).

## Discussion

Leukemia is a group of disorder characterized by accumulation of malignant white cells in the bone marrow and blood [20]. Inflammation has been associated with variant malignancies. Elevated levels of inflammatory cytokines have been linked to inflammatory exacerbation. As a critical anti-inflammatory cytokine, IL-4 has implicated in the pathogenesis of various cancers. For instance, elevated plasma levels of IL-4 were correlated with risk of acute myeloid leukemia [21]. More specifically, several polymorphisms of the gene encoding IL-4 have been proved to be risk factors for cancer. Intron 3 VNTR polymorphism also plays an important role in alteration of IL-4 production which involved in the pathogenesis of malignancy [22].

In this study, we analyzed gene polymorphism of IL-4 intron 3 and its combined risk on leukemia in Sudanese patients. The study provides an evidence, for the first time, that there is complete absent of intron 3

IL-4 VNTR polymorphism gene in (74.1%) of the patients in compare to present of allele in all control groups with significant differences P value=0.00. In addition, we also tried to find out the combined effect of deleted intron 3 IL-4 VNTR genes on Leukemia, and observed that absent of intron 3 IL-4 VNTR polymorphism genotype showed 4.617 times more risk to develop Leukemia. In spite of statistical difference have been observed for the presence and absence of the alleles among the different subgroups of leukemia patients, but we cannot assume it is real because of the big variation in number of patients with different types of leukemia. There are no similar studies that have shown this association, which could allow us to compare our results. It was imperative to investigate if the absent of allele in the IL-4 and genes could specifically influence the leukemia susceptibility.

Previously, intron 3 VNTR polymorphism of IL-4 has been associated with several common diseases, some studies have shown evidences supporting that the P1 allele induced higher expression of IL-4 than the P2 allele and that, the P1 allele may be a susceptibility factor in some diseases [23,24]. The present study in accordance with other results, the frequency of P1 allele, which was supposed to lead to over expression of IL-4, was found higher in leukemic patients than control group. On the other hand the P1P1 and P1P2 were found to have 1.24 times more risk factor to grow up into leukemia compared to P2P2 (OR 0.72).

This is the first study that evaluates the associations between IL-4 gene VNTR polymorphism and Leukemia. And thus, it is hardly possible to provide a clear explanation as to why the P1 allele increases the risk for susceptibility to Leukemia. As a result further studies on the role of IL-4 gene VNTR polymorphism in relation to Leukemia are warranted.

## Conclusion

The present study suggests that, there is strongly association of IL-4 gene intron 3 VNTR polymorphism with susceptibility of leukemia in Sudanese population. Accordingly detection of such polymorphism could be a genetic marker for the risk of developing Leukemia.

## Conflicts of Interest

The authors declare that they have no competing interests.

## Authors' Contributions

Abeer and Prof Imad conceived and designed the Study; Abeer, Ream and Abdelrahim performed the experiments; Ream and Elshibli analyzed the data; Ream and Abdelrahim wrote the paper. All authors read and approved the final manuscript.

## Acknowledgment

We acknowledge the staff at the department of pediatrics hematology at Gaffer Ibnaof hospital for children and Khartoum center for radiation treatment and nuclear medicine for their helps.

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