IL- 4 70 bp VNTR Polymorphism and Sickle Cell Anaemia in Sudan

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Abstract

Objectives The objective of this study is to investigate IL- 4 70 bp VNTR Polymorphism in Sickle Cell Anaemia among Sudanese patients. **Materials and Methods** 94 patients with sickle cell anaemia were enrolled in this cross sectional study, DNA was extracted from blood by salting out method. Conventional PCR was used for Genotyping of interleukin-4 third intron 70 bp VNTR polymorphism. SPSS was used for data analysis. **Results** The 2R3R heterozygous genotype showed a higher frequency 57/94 (60.6%), followed by 3R3R 25/94 (26.6%) and the least 2R2R 12/94 (12.8%). On the other hand, 4R allele was absent in our study population. No significant association was observed between the interleukin-4 third intron 70 bp repeat polymorphism genotypes and complications of sickle cell anaemia among Sudanese patients. **Conclusion** interleukin-4 third intron 70 bp repeat polymorphism genotypes show insignificant association with complications of sickle cell anaemia among Sudanese patients.

Key words: interleukin-4 third intron 70 bp repeat polymorphism, Sickle cell anaemia

Introduction

The interleukin 4 (IL4, IL-4) is a cytokine that induces differentiation of naive helper T cells (Th0 cells) to Th2 cells. Upon activation by IL-4, Th2 cells subsequently produce additional IL-4 in a positive feedback loop. The cell that initially produces IL-4, thus inducing Th0

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differentiation, has not been identified, but recent studies suggest that basophils may be the effector cell.[1]

Interleukin-4 (IL-4) gene is mapped within the cytokine gene cluster on chromosome 5q31.1 [2] and there is a 70 bp variable number of tandem repeat (VNTR) polymorphism in its third intron which could change the expression level of IL-4 gene [3]. This VNTR polymorphism contains three alleles: RP1 allele, with three repeats, RP2 allele, with two repeats, and RP3 allele, with four repeats. The frequency of RP1 allele is higher than RP2 alleles. Likewise, RP3 allele is scarce that has been detected in few populations [4].

Interleukin-4 is the main cytokine of T helper 2 lymphocytes, which has a key role in regulation of humoral immune responses [5].

Sickle cell disease is a chronic inflammatory condition characterized by elevated levels of inflammatory cytokines, which may be regulated by genetic polymorphisms and could be associated with diverse disease presentations and alloimmunization [6].

Sickle cell anaemia is an autosomal inherited disorder of haemoglobin resulting from the homozygous inheritance of the sickle gene [7]. It has variable clinical expression some of which include recurrent haemolysis, vasoocclusive crises and recurrent infections with their attendant sequalae. Few are discovered only by chance on routine haematological examination for other conditions because they run a mild course [8]. Reports have shown that patients with sickle cell anaemia (HbSS), particularly children, have an increased susceptibility to infection leading to increased mortality [9,10]. Opsonophagocytic defect due to an abnormality of the alternative complement pathway, deficiency of specific circulating antibodies, impaired leucocytes function and loss of both humoral and cell mediated immunity [11,12] are some of the mechanisms that have been reported to account for the immunocompromised state in patients with sickle cell disease. By secreting cytokines CD4+ T lymphocyte influence the functions of virtually all other cells of the immune system, including other T cells, B cells, macrophages, and natural killer cells [13]. Two functionally distinct subsets of Helper T cells secrete cytokines which promote the activities ofTH1 subset of CD4+ cells producing IL-2, IFN-γ and TNF-β with IFN- γ thus ultimately activate T cell and macrophages to stimulate cellular immunity and inflammation.

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TH1 cells also secrete IL-3 and GM-CSF to stimulate bone marrow to produce more leukocytes. TH2 subset of CD4+ cells secrete IL-4, IL-5, IL-6 and IL-10 with IL-4 being the prototype, which stimulate antibody production by B cells [14]. The aim of conducting this study is to investigate the frequency and association of IL- 4 70 bp VNTR Polymorphism in Sickle Cell Anaemia,

Materials and Methods

Patients

In this cross sectional study, 94 patients with sickle cell anaemia, attending Jaafar ibn Oaf Hospital and Military Hospital for regular checkup, were involved. Clinical and demographic data were collected in a predesigned questionnaire.

DNA Extraction

Human genomic DNA was extracted from blood samples using a simple salting-out method [15].

Genotyping of interleukin-4 third intron 70 bp VNTR polymorphism

The 70 bp VNTR polymorphism was genotyped by conventional Polymerase Chain Reaction. Forward primer; 5'GAGTCTGGCCAACACAAATCC3' and reverse primer; 5'ACCTCTAGGGTCATGCAGGT3' were used. PCR reaction mixture was prepared by adding 5 µl genomic DNA to a total volume of 20 µl ready master mix (iNtRONbiotechnology, Korea). PCR protocol was: 94 °C for 2 min followed by 35 cycles at 94 °C for 20 s, 58 °C for 10 s, 72 °C for 40 s with a final extension at 72 °C for 3.5 min. The PCR products were estimated with 5 µL 100 bp DNA ladder in 1.5% agarose gels. Gel image was captured by Gel Documentation System (SYNGENE, JAPAN) and analyzed using the Image Analysis Software (GeneSnap 7).

Statistical Analysis

Data analysis was performed using Statistical Package for Social Science (SPSS) version 22. Descriptive statistics was used for description of quantitative and qualitative variables. Chi

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square test was used for Comparison of categorical variables. Statistical significance was defined as a P value less than 0.05.

Ethical Considerations

Ethical approval was obtained from Ministry Health of Khartoum. Informed consent was obtained from all participants in accordance with the requirements and guidelines of the ethical committee.

Results

A total of 94 patients (48 (51.1%) males and 46(48.9%) females) enrolled in this study. At the time of diagnosis, mean of patient ages was 8.95± 6.13., sd. The median age of patients was 7.8 years (range: 6 months – 28 years). Table 3 shows haematological findings in Patients.

Table 1: Haematological Findings in Sickle Cell Anaemia Patients

Variable	Mean ± SD	Median (Range)
Haemoglobin (g/dl)	6.85 ± 1.31	6.7 (4.0 – 12.2)
PCV (%)	21.50 ± 4.01	21.0 (12.6 – 36.2)
RBCs Count ()	2.66 ± 1.13	2.46 (1.38-11.70)
TWBCs Count	15.80 ± 6.96	14.40 (6.0-46.0)
Platelet Count	388.31 ± 200.15	385.50 (4.60-1094.0)

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Table 2: The result of PCR shows three genotypes of interleukin-4 third intron 70 bp VNTR polymorphism classified as:

Allele	**70 bp repeat**
P1P1	183 bp two 70 bp repeat
P2P2	253 bp three 70 bp repeat
P1P2	Both 183 and 253 bp fragments

P1P2 heterozygous genotype showed a higher frequency 57/94 (60.6%), followed by P2P2 25/94 (26.6%) and the least P1P1 12/94 (12.8%). On the other hand, allele with 70 bp repeats was absent in our study population. No significant association was observed between the VNTR 70 bp repeat polymorphism genotypes and complications of sickle cell anaemia among Sudanese patients (P value = 0.07).

interleukin-4 third intron 70 bp VNTR polymorphism P1 allele frequency was 0.6 and P2 allele frequency was 0.4.

Discussion

Nnodim et al. [16] reported a significant higher IFN-γ in SCA patients in Owerri, Nigeria while Musa et al. [17] found no significant difference in SCA patients in Zaria, Nigeria. However, there are no published studies concerning *IL4* SNPs and SCD or its complications.

Although the role of IL4 in SCD is controversial, increased serum IL4 levels were found in steady-state SCD patients compared to normal healthy controls [18]. Remarkably elevated levels of IL4 were noted in a VOC group compared to steady-state SCD patients and healthy controls [19]. IL4 levels correlated well with SCD status in Jamaicans [20]. In addition, Buchs indicated that, 70 bp VNTR polymorphism could change the expression level of IL-4 gene [3]. Our results revealed no significant association between the VNTR 70 bp repeat polymorphism of intron 3

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and sickle cell anaemia in Sudanese patients (P value > 0.05). As these results conflict with the biological plausibility that NO and interleukin levels modulate SCD, they deserve careful interpretation and further exploration.

Conclusion

The present study showed no associations between the IL-4 70 bp VNTR polymorphism and sickle cell anaemia in Sudanese patients. Additional studies on other IL-4 polymorphisms (- 590 C/T, - 34 C/T) might help understanding the role of IL-4 polymorphisms in the heterogeneity of clinical course in sickle cell disease.

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Conflict of interests

The authors have no competing interests.

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