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IL-1β (+3954C>T) Polymorphism in Sickle Cell Disease: A Call for Larger-Scale Studies

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KEYWORDS

Polymorphism, Sickle Cell Disease

ABSTRACT:

Aim: The present study is aimed to evaluate the association of IL-1 β +3954 polymorphisms in Indian patients with SCD and also to determine whether this polymorphism plays a role in the progression of SCD. Since there are few studies on IL-1B+3954 polymorphisms and SCD phenotypes this may be helpful in understanding disease progression with regard to cytokines.

Methods: The study included 150 SCD patients and 150 control. Genomic DNA extraction and PCR-RFLP techniques were used for genotyping IL-1 β +3954 polymorphisms Statistical analysis was performed using SPSS.

Results: Contrasting results were obtained in this study. Among Brazilian SCD patients the IL-1 β +3954 polymorphisms were involved in disease progression where as in our study and from studies from Egypt no association was found.

Conclusion: The IL-1β (+3954C>T) genetic polymorphism was not significantly linked to SCD phenotypes in this study. To fully understand its potential involvement in SCD, larger patient cohorts should be investigated.

Introduction:

There are several hemoglobinopathies resulting from globin gene abnormalities; however, only those linked to sickle cell disease (SCD) are common worldwide. A widespread genetic hemoglobinopathy, SCD typically affects people of African American, Arabian and Indian origin. In India in particular, hemoglobinopathies account for the majority of hereditary illnesses and are therefore very important to the general public [1]. Alterations in the globin gene expression in structural region cause changes in the amino acid composition, which in turn causes sickle cell disorders [2]. The two most common conditions in this group are sickle cell disease (HbSS) and sickle cell trait (HbSA). A point mutation in β-globin's sixth position, which results in the substitution of a valine residue for the glutamate residue, is the cause of hemoglobin-S. This results from a nucleotide mutation in codon 6 of the globin gene, which is found on the short arm of chromosome 11, from adenine to thymine (GAG to GTG). Autosomal recessive inheritance is the mode of inheritance [3]. James B. Herrick of Chicago reported on "peculiar elongated and sickle-shaped corpuscles in a case of severe anemia" in the November 1910 issue of "Archives of Internal Medicine." This article introduced the disease that became known as sickle cell anemia to modern medicine [4]. In 1952, Lehmann and Cutbush reported the first incidence of Hb S from southern Indian tribal people [5]. There are five different haplotypes of the Hb S gene that are known to be prevalent in particular regions. The African haplotypes are represented by Benin, Senegal, Burundi and Cameroon, while the Saudi Arabia/Indian haplotype makes up the fifth. As we can see, while the haplotype is the same across India, each state has a very different clinical severity of SCD [6]. Hemolysis, increased risk of infections and periodic painful vaso-occlusive crises (VOCs) are characteristics of SCD which finally lead to chronic organ damage. Multiple studies have determined the factors linked to

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this variation in order foresee the patients clinical outcome [7].

A significant pro-inflammatory cytokine, Interleukin-1 β (IL-1 β) is vital for the host's defensive mechanisms against infection and damage. It is located in 70–110 kb region of chromosome 2q13–21, which comprises 7 exons and 6 introns. They have a significant impact on the transcription of genes and the resulting functional changes [8]. A number of studies have indicated the role of cytokines in the pathogenesis of SCD, with many reports showing considerably raised plasma levels of interleukin-1β in those with the disease [9]. Vaso-occlusive crisis (VOC) in SCD is believed to be primarily caused by the production of cytokines in response to infection, endothelial cell activation, and other harmful substances [10]. The vaso-occlusion phenomena of SCD has been suggested to be mediated by +3954 cytokine genes of IL-1β through inflammation, cellular adhesion, signalling and transport mechanisms [11] .

Objectives:

The present study is aimed to evaluate the association of IL-1 β +3954 polymorphisms in Indian patients with SCD and also to determine whether this polymorphism plays a role in the progression of SCD. Since there are few studies on IL-1B+3954 polymorphisms and SCD phenotypes this may be helpful in understanding disease progression with regard to cytokines.

Materials and Methods:

In this case-control study on SCD a total of 300 subjects were tested (patients = 150 and Controls =150). Information was collected through a detailed questionnaire. Written informed consent was obtained from all the subjects. The study was approved by the Institutional Ethics Committee, Andhra University, Vishakhapatnam (IEC No:29). Inclusion Criteria for patients is that all SCD patients should be in a steady state, which is defined as the period of time where the patient was not experiencing an acute painful crisis for at least four consecutive weeks after the last crisis and the exclusion criteria is those who had infection or inflammatory condition at time of sampling or those who had been transfused within 3 months prior to the study time were excluded. The Inclusion Criteria for controls is an unrelated, fit, gender and age matched individuals living in same geographic region as of patients and exclusion criteria is people with chronic conditions. 2ml of venous blood was collected in EDTA vials and the Genomic DNA was solated with the Machery Nagel kit (NucleoSpin Blood method) as per manufacturers instruction. The concentration of DNA was estimated using a spectrophotometer at the wavelength of 260nm and the quality was analyzed through a agarose gel electrophoresis, 0.8% (0.8g in 100 ml TEB 1X) and checked usiing a UV transilluminator. The primer sets and restriction enzymes (RE) used throughout the experimental procedures in this study are shown in table no:1. PCR amplification was carried out at a total volume of 25µl.The details of the reaction master mixture and amplification conditions used are as follows, PCR Buffer 1.0µl, $MgCl_2$ 1.5µl, DNTP 0.3µl, forward 0.5µl, reverse 0.5µl, Taq 0.2µl, DNA 0.2µl and molecular water 22.15µl.The cycling conditions are as follows, Initial denaturation for 2 minutes at 95° C, denaturation for 35 cycles at 95°C for 1 min, annealing at 57 °C for 1 min, extension at 72 °C for 1 min and final extension at 72 °C for 5 min [12,13]. PCR products were digested with the corresponding (RE) from New England Biolabs (USA) according to the manufacturer's protocols. Amplified products and restriction fragments were run on 2% agarose gel, stained with ethidium bromide. when observed on a 2% agarose gel , the homozygous genotype (CC) yielded two fragments with lengths of 97 bp and 85 bp, the homozygous genotype (TT) produced a fragment with a length of 182 bp, and the heterozygous genotype (CT) produced three fragments with lengths of 182 bp, 97 bp, and 85 bp . The significance of the difference of alleles and genotype frequencies between the patients and controls was tested using the chi-square test and Yate's Correction. A P value of <0.05 is considered statistical significant.

Table : 1 Primer Sequences of IL-1β (+3954 C>T) Polymorphisms

Primer	Sequence	Restriction Enzyme
Forward	F:5'-TC AGG TGT CCT CGA AGA AAT CAA A-3'	Taq$^{\alpha}$I

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Reverse	R:5'-GCT TTT TTG CTG TGA GTC CCG-3'	

Results :

The allele and genotypic frequencies and distribution of IL-1β (+3954 C>T) polymorphism did not differ significantly between SCD patients and the control group with P value 0.80618 and 0.947337 respectively which is > 0.05. The frequency of CC,CT and TT genotypes in SCD patients is 27.33,50.0 and 22.66 respectively where as in controls the frequency of CC,CT and TT genotypes is 28.66,50.0 and 21.33 respectively which are statistically insignificant (Table 2 and 3).

Table: 2 Allelic distribution of IL-1β (+3954 C>T) Polymorphisms in SCD patients and Controls.

Allele	**Control (n=150)**		**Patients (n=150)**		**Chi Square (X^2) Statistic after Yate's Correction**	**P-value**
	Count	**Frequency(%)**	**Count**	**Frequency (%)**		
C	161	53.66	157	52.39	0.06021678	0.80618
T	139	46.33	143	47.66		

Table: 3 Genotypic distribution of IL-1β (+3954 C>T) Polymorphisms in SCD patients and Controls

Genotype	**Control (n=150)**		**Patients (n=150)**		**Chi Square (X^2) Statistic**	**P-value**
	Count	Frequency(%)	Count	Frequency (%)		
CC	43	28.66	41	27.33	0.1082251	0.947337
CT	75	50.0	75	50.0		
TT	32	21.33	34	22.66		

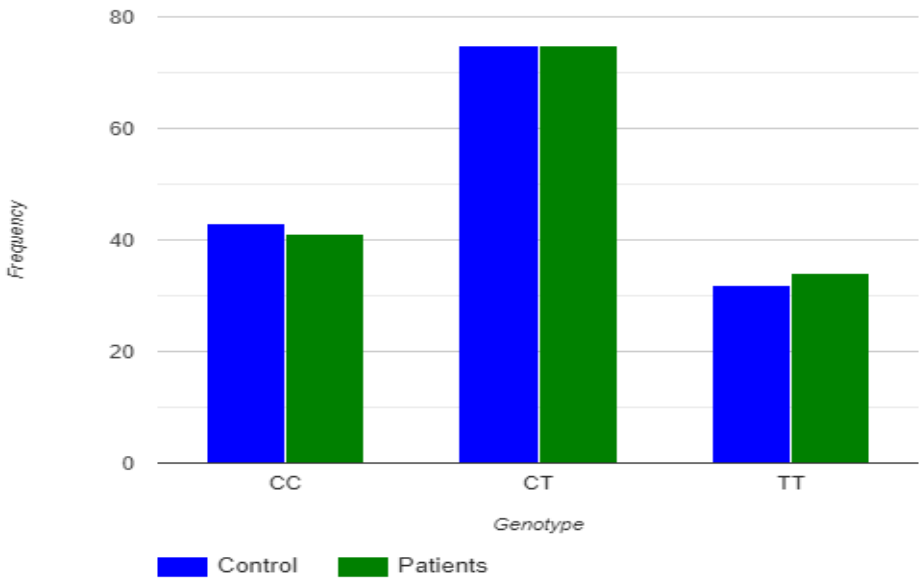

Fig:1 Graphical representation of IL-1β (+3954 C>T) genotypic frequencies

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Discussion:

Cytokines help to maintain a delicate equilibrium between pro-inflammatory and anti-inflammatory processes [14]. Clinical and subclinical microvascular occlusion, along with hemolysis, are key factors in stimulating the production of cytokines and acute phase reactants in SCD. They also contribute to the pathophysiology of vaso-occlusion crises in SCD [15]. Among the cytokines that stimulate inflammation, IL-1β is considered to be one of the most significant.

In a similar study by Vicari et al [16] in Brazil it was observed that IL-1β (+3954C>T) polymorphisms are associated with SCD complications among Brazilian patients and may act as genetic predictors of SCD clinical heterogeneity. This is completely contrast to the results that we have obtained. In a recent study by Khorshied et al. [17] in Egyptian SCD patients it was found that IL-1β (+3954C>T) genotypes has no influence on them. Similar result was obtained by Elammary et al who also worked on Egyptian SCD patients and stated that IL-1β (+3954C>T) polymorphism is not associated with SCD [12]. In other studies involving IL-1β (+3954C>T) polymorphism it was found that these polymorphisms are involved in determination of the disease outcome in periodontal diseases which is chronic inflammatory[18].

Conclusion:

The IL-1β (+3954C>T) genetic polymorphism was not significantly linked to SCD phenotypes in this study. To fully understand its potential involvement in SCD, larger patient cohorts should be investigated.

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Conflict of interest: The authors declare no conflict of interest.

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