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RESEARCH ARTICLE

Polymorphisms of IL-4 Gene in Iraqi Behçet's Disease Patients

Israa Harjan Mohsen[1], Mona N. Al-Terehi[2], Athraa H. Mohsen[3], Ali H. Al-Saadi[2]

1. *University of Babylon, College of Nursing.*

2. *University of Babylon, College of Sciences.*

3. *University of Kufa, College of Sciences.*

Abstract

The present research was carried out to reveal the correlation of IL-4 gene polymorphisms with Iraqi Behçet's Disease patients. The technique that used in the present study is PCR-SSCP technique. DNA was extracted from blood. The results show that there was strong association between IL-4 gene and Behçet's Disease there were two patterns (A and B), polymorphisms show significant differences($p>0.05$) between patients and control where pattern (A) was occurred with percentage (2%) and (39%) while in the control and patients respectively .The pattern (B) was occurred with percentage (98%) and (61%) in the control and patients respectively .The results concluded that IL-4 gene polymorphisms were involved in pathogenesis of Behçet's Disease , our finding require more exploration to use this result as early hint of Behçet's Disease prevalence.

Keywords: *IL-4 .PCR-SSCP technique, Haplotypes, Polymorphisms, Behçet's Disease*

Introduction

Behçet's Disease (BD) is a sporadic inflammatory disease which is lead to systemic vasculitis disorder of uncertain etiology .It is characterized by three symptoms occur together are oral aphthous ulcers, ocular lesions and genital sores [1]. This disease varied in its intensity and leading to complication include disorder in the gastrointestinal tract, cardiovascular system and the central nervous system.

The etiology and pathophysiology of disease are unknown but there are several researchers believed that the Behçet's Disease is autoimmune disease which is correlated with genetic factors that make patients more susceptible to the disease [2]. Several cytokines are participated in the inflammation state and it may have an important function in the etiology of numerous inflammatory disorders including, arthritis, and Behçet's Disease.

The study of [3] found that several types of interleukins and interferon-g are increased in the patients of Behçet's Disease. Some interleukins may lead to immunity imbalance in inflammation diseases where several studies showed that IL-2 and IL-4 act on the immune response balance regulation and by this participated in the cause's pathogenesis of some disorders such as Behçet's Disease and lupus [4, 5]. So this study is aimed to evaluate the role of IL-4 gene in patients of Behçet's Disease in Iraqi population.

Materials and Methods

- All samples are gathered from Behçet's Disease patients in Marjan Teaching hospital after obtained written agreement correspond to ethical agreement of Iraq ministry of health. Sample of blood are gathered (2 ml) from patients and healthy people as control group.
- The blood used to extraction of DNA by Favorgene extraction kit and using the nanodrope for detection the DNA concentration and purity [6].
- IL-4 primer was (5'-TA AA CT TG GG AG AACATGGT-3' for the upstream primer and 5'-TG GG GA AA GA TAGAGTAATA-3' for downstream [7].
- PCR Technique conditions: first step is denaturation of DNA for 5min at 94˚C;

second step is 35 cycles (30 s at 94˚C, 30 s at 37˚C, 30 sat 72˚C, and finally 10 min at 72˚C). Electrophoresis were used to detect the PCR products using agarose gel (1.5%agarose, 70 V, 20 mA for 45 min) by staining with ethidium bromide, the size of PCR product were (195) bp for IL-4 gene. Odd ratio at CI 95% and p value (<0.05) used to analysis the results statically.

- SSCP technique include the denaturation of PCR products by SSCP dye (EDTA, form amid and bromophynol blue) 1/1V: V in water bath for 5 min at 95˚C then its embedded in ice for 2min.
- SSCP product electrophoresis: 10 µl of the products were electrophoresis (sample+ dye) were loaded 8% acrylamide/bis gel containing 7%glycerol, and 1X TBE buffer. Buffer temperature 10˚C, Runtime 1.5 h and 100V. Then gel was staining using ethedium bromide for 15 min.
- The frequencies of haplotype were established by the bands variation between patients and control group.
- Qi square and odd ratio at p value <0.05 are used in the statics analysis of results.

Results and Discussion

The DNA results indicated that it has (50-200) ng and purity (1.7-2.2) as show in Figure (1).

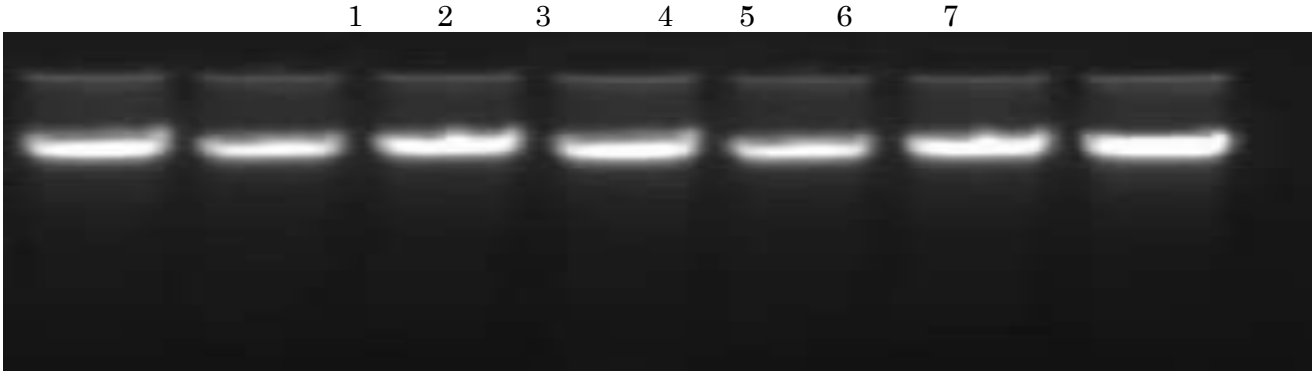

Figure 1: The pattern of Electrophoresis for gnomic DNA, (1-5) for patients group ;(6-7) for control group

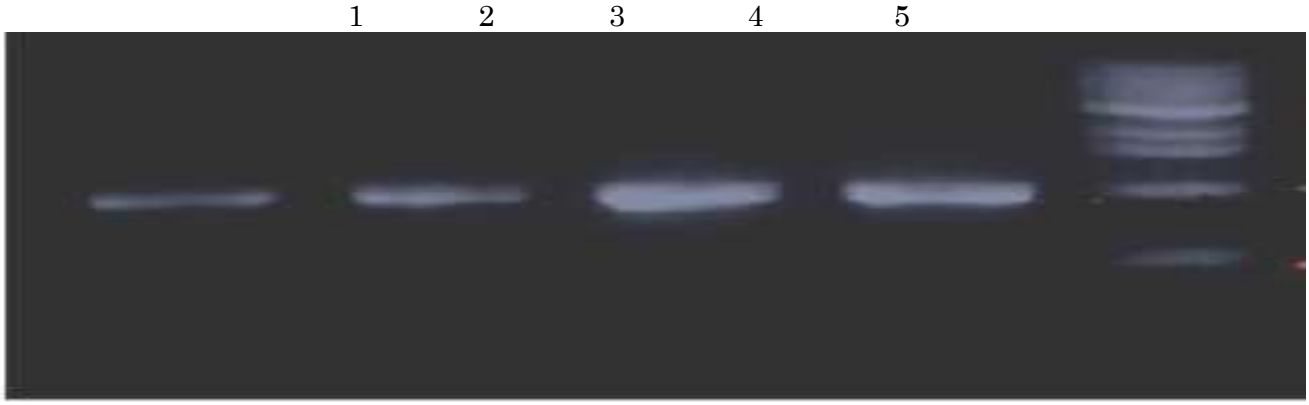

Figure 2: The pattern of Electrophoresis for IL-4 gene PCR products, lane (1-4) for IL-4, lane 5 DNA marker (100 bp)

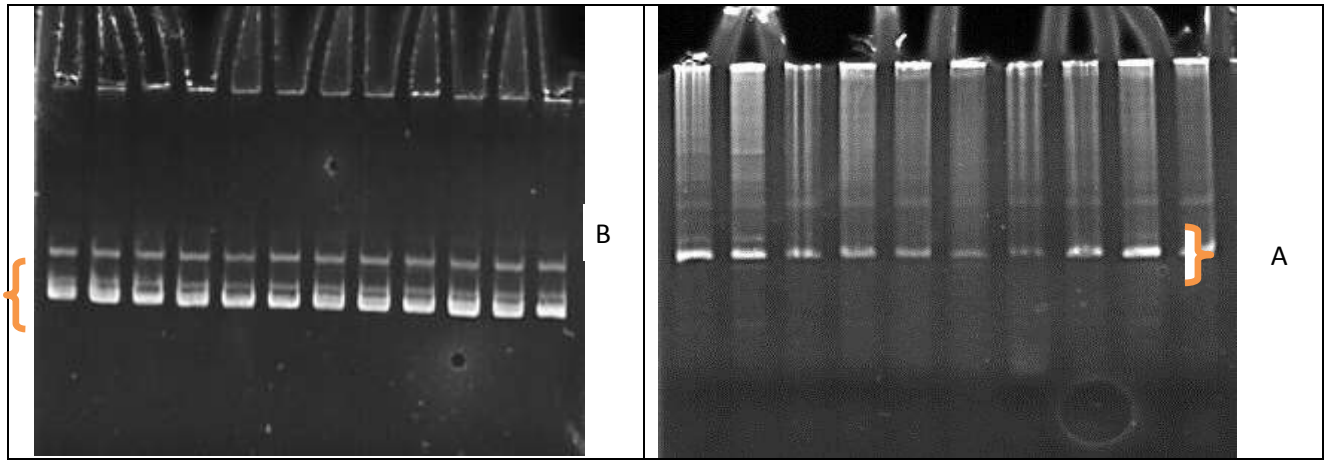

Figure 3: The electrophoresis pattern of PCR-SSCP technique for IL-4 gene, (Pattern A: 2 bands) and (Pattern B: 3 bands) for both patients and control

Table 1: The genotype of IL-4 gene for patients and control groups

Pattern name	Patients%	Control%	OD ratio	*CI 95%*	P-value
A	39%	2%	31.32	7.30-134.42	**< 0.0001**
B	61%	98%	0.031	0.007-0.31	**< 0.0001**

Pattern A: have 2 bands Pattern B: have 3 bands

The results of genetic polymorphisms for IL-4 gene displayed in Table 1 and Figure 3 explained the variant of haplotypes in the groups of the patients and control. (A and B) patterns polymorphisms indicated significant differences **(**P< 0.05) between the patients and control group where pattern (A) was occurred with percentage (2%) and (39%) while in the control and patients respectively .The pattern (B) was occurred with percentage (98%) and (61%) in the control and patients respectively .Behchet disease defined as an inflammatory disease which is the main marker in it is the elevation of cytokines. The sources that participate in the inflammatory reactions enhancement in behchet disease is the overexpression of cytokines and this may be correlated with genetic susceptibility in behchet disease [8]. IL-4 represent one of the main cytokines that play important role in inflammation reactions where it is participate in the T-helper development and B cells activation and differentiation ,also the IL-4 has many anti-inflammatory effects such as inhibition of nitric oxide synthase induction , inhibit the superoxide release from macrophages, and chemotaxis of B-cell and T-cell [9].The study of [10] found that the genotyping polymorphism of IL-4 different between patient and control where the G alleles of IL-4 -1098 and T alleles of IL-4 -590 was more frequented in patients with behchet disease than a control group. While the study of [11] investigate an increase in the frequency of haplotype TTC and decrease in the haplotype TTT of IL-4 gene in Behchet disease when compared with the control group. In the other hand the study of [12] found the association between the homozygote type P1P1genotype of the *IL-4* gene 70 bp VNTR polymorphism in the development of Behchet disease while the mutant P2P2 genotype associated strongly with deep venous thrombosis in the patients with Behchet disease and this, in turn, is associated with ocular involvement which represents the main symptom in Behchet disease. Our results suggest that the polymorphisms in IL-4 gene may be associated with susceptibility to Behchet disease and risk of the disease.

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