Study Polymorphisms of IL-4 Gene Using PCR-SSCP Technique in Iraqi Systemic lupus erythematous Patients

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Abstract
The present study was carried out to detect the association of IL-4 haplotypes polymorphisms with Systemic lupus erythematous(SLE)in Iraqi patients, PCR-SSCP technique used in present study, blood was used to DNA extraction, the results show that there was strong association between IL-4 and SLE in tow haplotypes from three The present study concluded that there was association between IL-4 polymorphisms with SLE, our finding need more investigation to use this polymorphism as early indication of SLE incidence.

Keywords: Cytokines, PCR-SSCP technique, Haplotypes, Polymorphisms

INTRODUCTION
Systemic lupus erythematos (SLE) is chronic autoimmune disease which is cause inflammation in several organs in the body including the integumentary, musculoskeletal, nervous, and cardiovascular systems. The symptoms developed with the progress of the disease which are including butterfly rash , arthralgia , joint swelling and fatigue(Lišněská et al.,2014; Kuhn et al.,2015).
It is believed that SLE may developed from the combination between environmental and genetic factors. Silica dusts , petroleum and ultraviolet light have been found to be play role in the occurrence of the disease (Yuen and Cunningham ,2014).Other studies indicated that immune complex defective may results from the genetic sensitivity where some genetic mutations in integrin-a5M, Fc-gamma receptor, PRDM1-ATG5, HLA-DR2, HLA-DR3, and TNFAIP3 have relation with the as hematologic symptoms ,neuropsychiatric symptoms and which are play crucial role in the pathoge nesis of SLE (Mangan et al.,2016 ;Mohammadoo-Khorasani et al.,2014).
The pathogenesis of the disease may result from the action of some interleukins which are lead to the imbalance in the immunity of the disease (Lourenço and Cava, 2009).Some studies indicated that IL-4 has an important role in the regulation the immunity of the disease (Lourenço and Cava, 2009).Some studies indicated that IL-4 has an important role in the regulation the immunity of the disease (Lourenço and Cava, 2009).Some studies indicated that IL-4 has an important role in the regulation the immunity of the disease (Lourenço and Cava, 2009).Some studies indicated that IL-4 has an important role in the regulation the immunity of the disease (Lourenço and Cava, 2009).Some studies indicated that IL-4 has an important role in the regulation the immunity of the disease (Lourenço and Cava, 2009).Some studies indicated that IL-4 has an important role in the regulation the immunity of the disease (Lourenço and Cava, 2009).Some studies indicated that IL-4 has an important role in the regulation the immunity of the disease (Lourenço and Cava, 2009).Some studies indicated that IL-4 has an important role in the regulation the immunity of the disease (Lourenço and Cava, 2009).Some studies indicated that IL-4 has an important role in the regulation the immunity of the disease (Lourenço and Cava, 2009).Some studies indicated that IL-4 has an important role in the regulation the immunity of the disease (Lourenço and Cava, 2009).Some studies indicated that IL-4 has an important role in the regulation the immunity of the disease (Lourenço and Cava, 2009).Some studies indicated that IL-4 has an important role in the regulation the immunity of the disease (Lourenço and Cava, 2009).Some studies indicated that IL-4 has an important role in the regulation the immunity of the disease (Lourenço and Cava, 2009).Some studies indicated that IL-4 has an important role in the regulation the immunity of the disease (Lourenço and Cava, 2009).Some studies indicated that IL-4 has an important role in the regulation the immunity of the disease (Lourenço and Cava, 2009).Some studies indicated that IL-4 has an important role in the regulation the immunity of the disease (Lourenço and Cava, 2009).Some studies indicated that IL-4 has an important role in the regulation the immunity of the disease (Lourenço and Cava, 2009).Some studies indicated that IL-4 has an important role in the regulation the immunity of the disease (Lourenço and Cava, 2009).Some studies indicated that IL-4 has an important role in the regulation the immunity of the disease (Lourenço and Cava, 2009).Some studies indicated that IL-4 has an important role in the regulation the immunity of the disease (Lourenço and Cava, 2009).

RESULTS AND DISCUSSION
The results of study show DNA extraction from whole blood (figure 1)

Figure 1: Electrophoresis pattern of gnomic DNA in study groups, lane 1-5 DNA from patients lane 6-10 DNA from control.
The results of present study included gene polymorphism of IL-4 in Lupus patients in comparison with healthy .The PCR products of IL-4 was 200bp as show in Figure 2.

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

Materials and Methods
1- Sample and data collection; about 2 ml of whole blood was collected from patients of Systemic lupus erythematous in Marjan hospital. All subjects in this study were taken written consent before participation in this study according to ethical approval of Iraq ministry of health, while control collected from healthy.
2- DNA extraction; DNA was extracted from whole blood using Favor gene extraction kit and concentration and purity were detected using nanodrope (Al-Terehi et al.,2016).
3- IL-4 primer was (5’-TAAACTTGGGAGAACATGGT-3’ for the upstream primer and 5’-TGGGGAAAGATAGAGTAATA-3’ for downstream. (Akina et al.,2016).
4- PCR conditions and size products IL-4 denaturation for 5 min at 94˚C, then 35 cycles (30 s at 94˚C, 30 s at 37˚C, 30 s at 72˚C, and finally 10 min at 72˚C). PCR products were determined by electrophoresis pattern in agarose gel (1.5% agarose, 70 V, 20 mA for 45 min) with ethidium bromide staining, the PCR size product were (195) bp for IL-4 .Statistics, the results were statically analysis using odd ratio at CI 95% nd p value <0.05.
5- SSCP technique, PCR products were denaturation using SSCP dye (EDTA, formamid and bromophynol blue) 1/1 V/V in water bath for 5 min at 95˚C then its child in ice for 2 min.
6- SSCP electrophoresis, the products were electrophoresis as a following About 10 μl of the samples (sample+ dye) were loaded into wells of 8% acrylamide/bis gel containing 7% glycerol, and 1X TBE buffer. In more details; for recipe a 20 × 20 0.1 cm gel format. 8 ml of 40% acrylamide/bis (stoke solution 37.5:1) mixed with 8 ml of 5X TBE, 2.8 ml,100% glycerol, then 40 μl TEMED and 400 μl of 10% ammonium per sulfate were added with 20.8 ml of dH2O After gel was casting sample were loaded and Run under the following conditions. Buffer 5.5 X TBE, Buffer temperature 10˚C, Run time 1.5 h and 100V. Then gel was staining using ethidium bromide for 15 min.
7- Haplotype frequency were determination by variety of bands between patients and control.
8- The statics analysis implemented using Qi square and odd ration at p value <0.05.
The results of IL-4 of gene polymorphism which show in Table 1 and Figure 3 clarified the variation of haplotypes in patients and control, there were three pattern (A, B, and C), polymorphisms show significant differences(p<0.05) between patients and control where haplotype A was appeared in control with (75%) while in patients (16%) and haplotype B was appeared in control with (25%) while in patients (72%) and haplotype C appeared in patients only in percentage reached to (12%) while they are absent in control group. Lupus is autoimmune disease and have many complication that lead to several systemic damage such as lupus nephritis where the study of (Singh 2003) found that IL-4 lead to the deposition of extracellular matrix in the glomeruli and lead to autoantibody production via its direct B-cell effects and lead to tissue damage by its effected directly on target organ and by this it may play crucial role in the development of lupus. Several studies have been reported the association of IL-4 gene polymorphisms in several disease such as rheumatoid arthritis, liver disease, and breast cancer. The present study concluded that there were three haplotype in IL-4 gene polymorphisms associated with SLE in Iraqi patients.

SLE disease risk may result from the association of two risk genotype on IL-4 such as IL4 -590C and STAT6 2892C , IL4 -33C and STAT6 2892C , as well as IL4 9241G and STAT6 2892C (Yu et al.,2010) while the study of (Mohammadoo-Khorasani et al.,2016) found that gene polymorphisms in the IL-4 gene increase the susceptibility to SLE. Our finding need more investigation to detect the mutations in cytokines that may be related with the etiology of SLE.

**CONCLUSION:**

The present study concluded that there were three haplotype in IL-4 are associated strongly with SLE in Iraqi patients.

**REFERENCES**