Israa Harjan Mohsen /J. Pharm. Sci. & Res. Vol. 10(3), 2018, 613-614

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

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

**Israa Harjan Mohsen**
*University of Babylon, College of Sciences, Iraq*

**Abstract**
The present study was carried out to detect the association of IL-4 haplotypes polymorphisms with Systemic lupus erthymatous(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 erythematous (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(Lisnevskaia *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-alpha$_M$, Fc-gamma receptor, *PRDM1-ATG5*, HLA-DR2, HLA-DR3, and TNFAIP$_3$ have relation with the as hematologic symptoms ,neuropsychiatric symptoms and cutaneous symptoms(Ceccarelli *et al.*,2015 ; Tsokos *et al.*,2016).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 balance of immune response represented between Th1 and Th2 which are play crucial role in the pathogenesis of SLE (Mangan *et al.*,2006 ;Mohammadoo-Khorasani *et al.*,2016).

## 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. (Alcina,*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 .Statics, 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 ethedium 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.

## RESULTS AND DISCUSSION

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

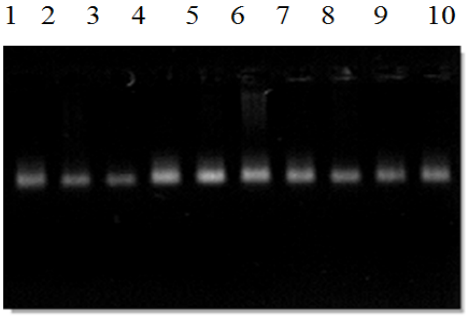


**Figure (1) Electrophoresis pattern of gnomic DNA in study groups, lane 1-5 DNA from patients lane 6-10 DNA from control.**

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.

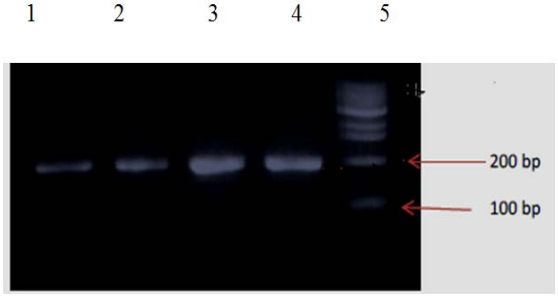


**Figure 2: Electrophoresis pattern of PCR product of IL-4 gene, , lane 1,4 for IL-4, lane 5 DNA marker (100 bp).**

**Table 1**

| PATTEREN | PATIENTS | CONTROL | Odd ratio | 95 % CI | P Value |
|---|---|---|---|---|---|
| A 3 BANDS | 16% | 75% | 15.7500 | 7.8172- 31.7329 | < 0.0001 |
| B 2Bands | 72% | 25% | 7.7143 | 4.1133- 14.4676 | < 0.0001 |
| C 1bands | 12% | 0 | 28.389 | 1.6568-486.4752 | 0.021 |

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.( Kurkó *et al.*,2013;Zheng *et al.*,2013; Al-Terehi *et al.*,2016).In present study the 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-Khorasan 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


1- Kuhn, A.; Bonsmann,J. ; Anders, H.J.; Herzer, P.; Tenbrock, K . and Schneider, M. (2015). The Diagnosis and Treatment of Systemic Lupus Erythematosus. Dtsch Arztebl Int. 2015 Jun; 112(25): 423–432.

2- Lisnevskaia L, Murphy G, Isenberg D. Systemic lupus erythematosus. *Lancet*. 2014;384:1878-1888.

3- Ceccarelli F, Perricone C, Borgiani P, et al. Genetic factors in systemic lupus erythematosus: contribution to disease phenotype. *J Immunol Res*. 2015;2015:745647.

4- Yuen HK, Cunningham MA. Optimal management of fatigue in patients with systemic lupus erythematosus: a systematic review. *Ther Clin Risk Manag*. 2014;10:775-786.

5- Tsokos, G.C.; Lo, M.S.; Reis,P. and Sullivan,K.E.(2016). New insights into the immunopathogenesis of systemic lupus erythematosus. *Nature Reviews Rheumatology* 12, 716–730.

6- Lourenço, E.V. and Cava, A.L.(2009). Cytokines in Systemic Lupus Erythematosus. Curr Mol Med.; 9(3): 242–254.

7- Mohammadoo-Khorasani M[1], Salimi S[1], Tabatabai E[1], Sandoughi M[2], Zakeri Z[2], Farajian-Mashhadi F[3].( 2016). Interleukin-1β (IL-1β) & IL-4 gene polymorphisms in patients with systemic lupus erythematosus (SLE) & their association with susceptibility to SLE. Indian J Med Res. May;143(5):591-6. doi: 10.4103/0971-5916.187107.

8- Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, Hatton RD, Wahl SM, Schoeb TR, Weaver CT.( 2006 )Transforming growth factor-beta induces development of the T(H)17 lineage. Nature. ;441:231–234.

9- Alcina, M. Fedetz, D. Ndagire, O. Fernández, L. Leyva, M. Guerrero, PLoS ONE., 2016, 4, 1 : e4137.

10- Singh RR.2003.IL-4 and many roads to lupuslike autoimmunity.Clin Immunol. 2003 Aug;108(2):73-9.

11- J. Kurkó, T. Besenyei, J. Laki, T. T. Glant, K. Mikecz, and Z. Szekanecz, "Genetics of rheumatoid arthritis—a comprehensive review," Clinical Reviews in Allergy & Immunology, vol. 45, no. 2, pp. 170–179, 2013.

12- Z. Zheng, X. Li, Z. Li, and X. C. Ma, "IL-4− 590C/T polymorphism and susceptibility to liver disease: a meta-analysis and meta-regression," DNA and Cell Biology, vol. 32, pp. 443–450, 2013.

13- Al-Terehi.M., Hasan,A.H, AL-Jboory .M.J. ; Al-Saadi,A.H, Zaidan,H.K, obiad,S.J. Haplotype Polymorphisms in Cytokines Genes Using Pcr-Sscp Technique in Iraqi Breast Cancer Patients.Der Pharma Chemica, 2016, 8(22):27-31.

14- H-H. Yu, P-H. Liu, Y-C. Lin, WJ Chen, J-H. Lee, L-C. Wang, Y-H. Yang, B-L. Chiang.(2010).Interleukin 4 and STAT6 gene polymorphisms are associated with systemic lupus erythematosus in Chinese patients .Volume: 19 issue: 10, page(s): 1219-1228.

15- Al-Terehi M., Ghaleb R, Al-Oubaidy SH., Al-Saadi A.,. Zaidan H. (2016) Study *TNF-α* gene polymorphism in Type 1 Diabetic Patients Using ARMS technique Journal of Chemical and Pharmaceutical Science CPS Volume 9 Issue 3,1107-1111.