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**RESEARCH ARTICLE**

# HLA Genotyping by PCR-SSO in Iraqi Patients with Psoriasis

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## *Abstract*

**Background:** Psoriasis is an autoimmune disorder. Accumulating evidence suggests that human leukocyte antigens (HLA) are involved in its pathogenesis.
**Aim of the study:** To determine the association of HLA-class I and II alleles with the susceptibility to psoriasis.
**Patients and methods:** Thirty psoriatic patients and sixty healthy controls were genotyped for HLA class I and Class II alleles using polymerase chain reaction sequence-specific oligonucleotides (PCR-SSO).
**Results:**The present study revealed that the frequencies of HLA- C*12, HLA- C*17, HLA- DRB1*07 and HLA-DQB1*02 were significantly higher among patients as compared to control (P=0.0174; P<0.0001; P=0.0136 and P=0.0004 respectively). On the other hand low frequencies of HLA-C*04 and HLA- DQB1*01 alleles were found in patients when compared with control (P=0.0003 and P=0.0009 respectively).
**Conclusion:**These results may provide an additional evidence for association of the HLA region with psoriasis. In particular this study revealed that HLA-C*12, HLA- C*17, HLA- DRB1*07 and HLA-DQB1*02 alleles may be as a risk factors associated with psoriasis while HLA-C*04 and HLA- DQB1*01 alleles as protective factors.



## Introduction:-

Psoriasis is a T-cell mediated immune disorder characterized by keratinocyte hyper-proliferation with genetic predisposition triggered by particular stimulating factors. The accurate etiology of psoriasisis unknown(Raut*et al.*, 2013).

Prevalenceof psoriasis varies according to ethnicity and geographical area, with a peak incidence of approximately 2% of the population (Krueger and Bowcock, 2005). The genetic factors that related with psoriasis have not been fully illustrated, the human leukocyte antigens (HLA) have been considered candidate marker for psoriasis because they are contributed in regulating the immune responses (Henseler, 1997; Elder *et al.*, 2001).

Certain studies have reported that susceptibility to psoriasis is associated with different HLA alleles in different ethnic groups.Psoriasis has demonstrated to be associated with HLA-A*01, A*02, B13, B17, B39, B57, Cw*06, Cw*07, and DR7, DQA1*0201(Kim *et al.*, 2000; Atasoy*et al.*, 2006; Umapathy*et al.*,2011). Particular HLA alleles act as adapter of disease expression, diminishing the threshold for evolving psoriasis in susceptible persons. The aim of this study was to determine if there is association between HLA- alleles and the susceptibility to psoriasis.

## Patients and Methods:-

Thirty Iraqi patients with psoriasis were included in this study. They were from attendants seeking treatment in outpatient clinic- Department of Dermatology and Venereology in Baghdad teaching Hospital from April to September 2015. Diagnosis was made by dermatologist. In addition 60 apparently healthy volunteers their ages and

sexes were matched with patients were enrolled in this work as control group. Two ml of venous blood were withdrawn from each subject under aseptic technique then transferred into EDTA tube, kept at -20ºC for the genotyping of HLA.The samples were collected after obtaining informed consent from patients. The DNA was extracted by Qiagen / Germany kit. HLA genotyping were performed by the PCR-SSO according to the manufacturer's instruction, (Histo type / DNA-SSO Kits-Innogenetics-Line Probe Assay, INNO-LiPA, Belgium). HLAgenotyping was carried out in the HLA-typing laboratory of Al-Karama teaching hospital, Baghdad.

**Statistical Analysis:-**

The results presented as percentage frequencies. Alleles variations were further presented in term of odds ratio (OR). P value of $p<0.05$ was considered statistically significant.

## Results:-

This study was performed on 30 patients with psoriasis and 60 healthy controls. The demographic characteristics of patients and controls included in the current study are presented in table (1). There were no statistical significant differences between two studied groups according to age and gender ($p>0.05$). The mean age of patients was 31.25 ± 3.45 year and for healthy controls was 29.30±1.20 year table (1).

The frequencies and odds ratios (ORs) of HLA-A, HLA-B, HLA-C, HLA-DR and HLA-DQ alleles in patients compared with controls are shown in tables (2, 3, 4, 5 and 6). The current study showed that the frequencies of HLA- C*12, HLA- C*17, HLA- DRB1*07 and HLA-DQB1*02 were significantly higher among patients as compared to control (*P*=0.0174, OR=6.56; *P*< 0.0001, OR=56; *P*=0.0136, OR=3; *P*=0.0004, OR=3.45 respectively). On the other hand low frequencies of HLA-C*4 and HLA- DQB1*01 alleles were found in patients when compared with controls (*P*=0.0003, OR=0.108; *P*=0.0009, OR=0.158 respectively).

**Table -1: Ages and sex distribution of the studied groups**

| | | Study groups | | P-value |
|---|---|---|---|---|
| | | Psoriatic patients n=30 | Healthy control n=60 | |
| **Age** | | | | |
| **Age (years)** | **Range** | (21-47) | (23-44) | |
| | **Mean** | 31.25 | 29.30 | 0.897 NS |
| | **SD** | 3.45 | 1.20 | |
| **Gender type** | **Female** | 15 (50%) | 29 (48.33%) | 0.726 NS |
| | **male** | 15 (50%) | 31 (51.66%) | |

**SD= Standard deviation; NS=Non significant (p>0.05).**

**Table-2: HLA-A alleles frequencies in patients with psoriasis and healthy controls**

| NO. of allele | PATIENTS | % | Healthy | % | p value | OR | CI (95%) |
|---|---|---|---|---|---|---|---|
| A*01 | 4 | 6.67% | 13 | 10.83% | 0.4304 | 0.588 | 0.183 to 1.89 |
| A*02 | 14 | 23.33% | 40 | 33.33% | 0.2269 | 0.609 | 0.300 to 1.24 |
| A*03 | 6 | 10.00% | 10 | 8.33% | 0.783 | 1.22 | 0.422 to 3.54 |
| A*11 | 8 | 13.33% | 12 | 10.00% | 0.6155 | 1.38 | 0.533 to 3.59 |
| A*23 | 1 | 1.67% | 4 | 3.33% | 0.6661 | 0.492 | 0.0537 to 4.50 |
| A*24 | 5 | 8.33% | 6 | 5.00% | 0.51 | 1.73 | 0.505 to 5.91 |
| A*25 | 0 | 0.00% | 2 | 1.67% | 0.5531 | 0.392 | 0.0185 to 8.30 |
| A*26 | 5 | 8.33% | 7 | 5.83% | 0.5377 | 1.47 | 0.445 to 4.84 |
| A*28 | 1 | 1.67% | 3 | 2.50% | 1 | 0.661 | 0.0673 to 6.50 |
| A*29 | 2 | 3.33% | 2 | 1.67% | 0.6016 | 2.03 | 0.279 to 14.8 |
| A*30 | 5 | 8.33% | 7 | 5.83% | 0.5377 | 1.47 | 0.445 to 4.84 |
| A*31 | 2 | 3.33% | 5 | 4.17% | 1 | 0.793 | 0.149 to 4.21 |
| A*32 | 2 | 3.33% | 1 | 0.83% | 0.258% | 4.1 | 0.364 to 46.2 |
| A*33 | 0 | 0.00% | 1 | 0.83% | 1 | 0.658 | 0.0264 to 16.4 |
| A*34 | 0 | 0.00% | 1 | 0.83% | 1 | 0.658 | 0.0264 to 16.4 |
| A*36 | 0 | 0.00% | 1 | 0.83% | 1 | 0.658 | 0.0264 to 16.4 |
| A*66 | 0 | 0.00% | 1 | 0.83% | 1 | 0.658 | 0.0264 to 16.4 |
| A*68 | 3 | 5.00% | 2 | 1.67% | 0.3351 | 3.11 | 0.504 TO 19.1 |
| A*69 | 0 | 0.00% | 1 | 0.83% | 1 | 0.658 | 0.0264 to 16.4 |
| A*74 | 2 | 3.33% | 1 | 0.83% | 0.258 | 4.1 | 0.364 to 46.2 |
| TOTAL | 60 | 100.00% | 120 | 100.00% | | | |

**Table-3: HLA-B alleles frequencies in patients with psoriasis and healthy controls**

| NO. of allele | PATIENTS | % | Healthy | % | p value | OR | CI (95%) |
|---|---|---|---|---|---|---|---|
| B*07 | 4 | 6.67% | 12 | 10.00% | 0.584 | 0.643 | 0.198 to 2.09 |
| B*08 | 4 | 6.67% | 7 | 5.83% | 1 | 1.15 | 0.324 to 4.11 |
| B*13 | 0 | 0.00% | 6 | 5.00% | 0.1805 | 0.146 | 0.00806 to 2.63 |
| B*14 | 2 | 3.33% | 1 | 0.83% | 0.258 | 4.1 | 0.364 to 46.2 |
| B*15 | 3 | 5.00% | 5 | 4.17% | 1 | 1.21 | 0.279 to 5.25 |
| B*17 | 0 | 0.00% | 1 | 0.83% | 1 | 0.658 | 0.0264 to 16.4 |
| B*18 | 0 | 0.00% | 6 | 5.00% | 0.1805 | 0.146 | 0.00806 to 2.63 |
| B*27 | 0 | 0.00% | 4 | 3.33% | 0.3028 | 0.214 | 0.0113 to 4.04 |
| B*35 | 3 | 5.00% | 13 | 10.83% | 0.2698 | 0.433 | 0.119 to 1.58 |
| B*37 | 3 | 5.00% | 5 | 4.17% | 1 | 1.21 | 0.279 to 5.25 |
| B*38 | 3 | 5.00% | 4 | 3.33% | 0.6875 | 1.53 | 0.330 to 7.05 |
| B*40 | 0 | 0.00% | 4 | 3.33% | 0.3028 | 0.214 | 0.0113 to 4.04 |
| B*41 | 0 | 0.00% | 6 | 5.00% | 0.1805 | 0.146 | 0.00806 to 2.63 |
| B*44 | 3 | 5.00% | 2 | 1.67% | 0.3351 | 3.11 | 0.504 to 19.1 |
| B*45 | 1 | 1.67% | 1 | 0.83% | 1 | 2.02 | 0.124 to 32.8 |
| B*48 | 2 | 3.33% | 1 | 0.83% | 0.258 | 4.1 | 0.364 to 46.2 |
| B*49 | 7 | 11.67% | 8 | 6.67% | 0.2642 | 1.85 | 0.637 to 5.37 |
| B*50 | 12 | 20.00% | 12 | 10.00% | 0.1014 | 2.25 | 0.943 to 5.37 |
| B*51 | 6 | 10.00% | 9 | 7.50% | 0.5764 | 1.37 | 0.464 to 4.05 |
| B*52 | 5 | 8.33% | 3 | 2.50% | 0.1191 | 3.55 | 0.818 to 15.4 |
| B*53 | 0 | 0.00% | 1 | 0.83% | 1 | 0.658 | 0.0264 to 16.4 |
| B*55 | 0 | 0.00% | 3 | 2.50% | 0.5518 | 0.277 | 0.0141 to 5.46 |
| B*57 | 0 | 0.00% | 1 | 0.83% | 1 | 0.658 | 0.0264 to 16.4 |
| B*58 | 2 | 3.33% | 3 | 2.50% | 1 | 1.34 | 0.219 to 8.28 |
| B*60 | 0 | 0.00% | 1 | 0.83% | 1 | 0.658 | 0.0264 to 16.4 |
| B*78 | 0 | 0.00% | 1 | 0.83% | 1 | 0.658 | 0.0264 to 16.4 |
| TOTAL | 60 | 100.00% | 120 | 100.00% | | | |

**Table-4: HLA-C alleles frequencies in patients with psoriasis and healthy controls**

| NO. of allele | PATIENTS | % | Healthy | % | p value | OR | CI (95%) |
|---|---|---|---|---|---|---|---|
| C*01 | 0 | 0.00% | 1 | 0.83% | 1 | 0.658 | 0.0264 to 16.4 |
| C*02 | 2 | 3.33% | 8 | 6.67% | 0.4996 | 0.483 | 0.0992 to 2.35 |
| C*03 | 3 | 5.00% | 3 | 2.50% | 0.4019 | 2.05 | 0.401 to 10.5 |
| C*04 | 2 | 3.33% | 29 | 24.17% | 0.0003 | 0.108 | 0.0249 to 0.471 |
| C*05 | 2 | 3.33% | 9 | 7.50% | 0.3408 | 0.425 | 0.0889 to 2.03 |
| C*06 | 9 | 15.00% | 16 | 13.33% | 0.8203 | 1.15 | 0.474 to 2.77 |
| C*07 | 21 | 35.00% | 39 | 32.50% | 0.7403 | 1.12 | 0.582 to 2.15 |
| C*08 | 2 | 3.33% | 8 | 6.67% | 0.4996 | 0.483 | 0.0992 to 2.35 |
| C*12 | 6 | 10.00% | 2 | 1.67% | 0.0174 | 6.56 | 1.28 to 33.6 |
| C*14 | 0 | 0.00% | 2 | 1.67% | 0.5531 | 0.392 | 0.0185 to 8.30 |
| C*16 | 2 | 3.33% | 1 | 0.83% | 0.258 | 4.1 | 0.364 to 46.2 |
| C*17 | 11 | 18.33% | 0 | 0.00% | < 0.0001 | 56 | 3.23 to 969 |
| C*18 | 0 | 0.00% | 2 | 1.67% | 0.5531 | 0.392 | 0.0185 to 8.30 |
| TOTAL | 60 | 100.00% | 120 | 100.00% | | | |

**Table-5: HLA-DRB1-alleles frequencies in patients with psoriasis and healthy controls**

| NO. of allele | PATIENTS | % | Healthy | % | p value | OR | CI (95%) |
|---|---|---|---|---|---|---|---|
| DR*01 | 0 | 0.00% | 3 | 2.50% | 0.5518 | 0.277 | 0.0141 to 5.46 |
| DR*02 | 0 | 0.00% | 5 | 4.17% | 0.171 | 0.174 | 0.00943 to 3.19 |
| DR*03 | 6 | 10.00% | 17 | 14.17% | 0.487 | 0.673 | 0.251 to 1.81 |
| DR*04 | 4 | 6.67% | 14 | 11.67% | 0.43 | 0.541 | 0.170 to 1.72 |
| DR*07 | 15 | 25.00% | 12 | 10.00% | 0.0136 | 3 | 1.30 to 6.92 |
| DR*08 | 0 | 0.00% | 4 | 3.33% | 0.3028 | 0.214 | 0.0113 to 4.04 |
| DR*09 | 0 | 0.00% | 4 | 3.33% | 0.3028 | 0.214 | 0.0113 to 4.04 |
| DR*10 | 0 | 0.00% | 4 | 3.33% | 0.3028 | 0.214 | 0.0113 to 4.04 |
| DR*11 | 12 | 20.00% | 26 | 21.67% | 0.8486 | 0.904 | 0.420 to 1.95 |
| DR*12 | 3 | 5.00% | 4 | 3.33% | 0.6875 | 1.53 | 0.330 to 7.05 |
| DR*13 | 12 | 20.00% | 11 | 9.17% | 0.3914 | 0.522 | 0.140 to 1.95 |
| DR*14 | 0 | 0.00% | 4 | 3.33% | 0.3028 | 0.214 | 0.0113 to 4.04 |
| DR*15 | 6 | 10.00% | 7 | 5.83% | 0.3634 | 1.79 | 0.575 to 5.60 |
| DR*16 | 2 | 3.33% | 2 | 1.67% | 0.6016 | 2.03 | 0.279 to 14.8 |
| DR*17 | 0 | 0.00% | 3 | 2.50% | 0.5518 | 0.277 | 0.0141 to 5.46 |
| TOTAL | 60 | 100.00% | 120 | 100.00% | | | |

**Table-6: HLA-DQB1-alleles frequencies in patients with psoriasis and healthy controls**

| NO. of allele | PATIENTS | % | Healthy | % | p value | OR | CI (95%) |
|---|---|---|---|---|---|---|---|
| DQ*01 | 3 | 5.00% | 30 | 25.00% | 0.0009 | 0.158 | 0.0460 to 0.542 |
| DQ*02 | 27 | 45.00% | 23 | 19.17% | 0.0004 | 3.45 | 1.74 to 6.83 |
| DQ*03 | 13 | 21.67% | 27 | 22.50% | 1 | 0.953 | 0.450 to 2.02 |
| DQ*04 | 3 | 5.00% | 6 | 5.00% | 1 | 1 | 0.241 to 4.15 |
| DQ*05 | 3 | 5.00% | 13 | 10.83% | 0.2698 | 0.433 | 0.119 to 1.58 |
| DQ*06 | 11 | 18.33% | 13 | 10.83% | 0.1706 | 1.85 | 0.773 to 4.42 |
| DQ*07 | 0 | 0.00% | 7 | 5.83% | 0.0972 | 0.125 | 0.00702 to 2.23 |
| DQ*08 | 0 | 0.00% | 1 | 0.83% | 1 | 0.658 | 0.0264 to 16.4 |
| TOTAL | 60 | 100.00% | 120 | 100.00% | | | |

## Discussion:-

The evolution of psoriasis was caused by some environmental factors in susceptible individuals with a particular genetic background. Psoriasis is a T cell-mediated disease associated with some HLA antigens, as is common for most autoimmune diseases (Nepom and Ehrlich 1991; Valdimarsson*et al.*,1995). To our knowledge, this study is the first in Iraq to investigate the association of HLA-alleles with psoriasis by molecular method PCR-SSO. The frequency distribution was constructed to give an insight on which of the HLA-A, B, C, DR and DQ alleles was

more frequent or infrequent in patients and controls for each of the five loci, which were used in this study. The association of several HLA-class I and class II antigens with susceptibility for development of psoriasis has been reported in the different populations, showing different results.

The present study was found significant higher frequencies of HLA-C*12, HLA- C*17, HLA- DRB1*07 and HLA-DQB1*02 in psoriatic patients than that in healthy controls (10.00%, 18.33%, 25.00% and 45.00% vs. 1.67%, 0.00%, 10.00% and 19.17%). Kasitelani and colleagues found highly significant increases in DR07 and Cw*0602 in psoriatic patients compared with controls, and conclude that Cw*0602 and –DR07 antigens were associated with a significant risk of psoriasis in the Croatian population (Kasitelan*et al.,* 2000).In addition the previous Iraqi study done byAlwan(1996) whousing HLA serotyping method found that psoriatic patients express high frequencies ofHLA-A1, B13, Cw7 and DR7 antigens.

Moreover,Lihi*et al.,* 2010reported that HLA alleles (A*03, B*07, B*13, B*27, B*38, B*51, Cw*03, Cw*06, Cw*07, Cw*12, DRB1*15, DQB1*0602) were significantly higher in Canadian psoriatic patients group than healthy controls and these results agree with our result regarding the elevation of Cw*12 allele among psoriatic patients. Henseter and Christophers have defined 2 types of psoriasis, the type I is inherited and associated with Cw6 and DRB1*07, while type II occurs sporadically later in life (Henseter*et al.*, 1985). However, in India study conducted by Umapathy and colleagues 2011revealed significant increase in frequencies of HLA-A2, B8, B17 and B44 among Indian patients when compared with controls.

The HLA system encodes molecules that play key role in exogenous and endogenous antigen presentation to CD4+ and CD8+ Th cells respectively. Antigen presentation and T cell activation act as a key to triggering the autoimmune diseases (Simmonds and Gough,2004).Several hypotheses have been suggested to explain how variation in HLA genes could trigger autoimmunity. The specific HLA alleles responsible for psoriasis disease have proved more difficult to follow. The Psoriasis was associated with HLA-C for several decades ago. A possible explanation of why psoriasis was difficult to analyze is that other HLA alleles are involved in addition to HLA-C. The studies also showed interaction between HLA-C and ERAP1 (endoplasmic reticulum amino-peptidase involved in peptide trimming before HLA class I presentation).The polymorphism in ERAP1 exert an effect only in people with psoriasis who carry the HLA-C risk allele (Strange *etal.*, 2010). Interaction with ERAP1 was also found for ankylosing spondylitis, in which the disease association is restricted to individuals with HLA-B27 (Evans *etal*., 2011).

It is well known that the low frequency of some HLA alleles could be considered as a protective factor for psoriasis. Another interesting finding of this study was the significant decrease frequencies of HLA-C*04 and HLA-DQB1*01 alleles in patients which account for 3.33% and 5.0% versus 24.17% and 25% in healthy controls respectively. Conversely, Umapathy*etal*.,2011 observed that the frequencies of HLA-A28, B5, B12 and B15 were significantly decreased in psoriasis patients as compared to control. ***In conclusion*** these results may provide an evidence for association of the HLA region with psoriasis. In particular our findings revealed that HLA- C*12, HLA- C*17, HLA- DRB1*07 and HLA-DQB1*02 alleles may be as a risk factors associated with psoriasis while HLA-C*04 and HLA- DQB1*01 alleles as protective factors.

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