HLA-DQA1 and HLA-DQB1 genotyping among lichen planus patients in Basrah province

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ABSTRACT

Background: **Lichen planus is an inflammatory, pruritic disease of the skin and mucus membranes, which can be either generalized or localized. Many studies indicated that human leukocyte antigens might have a role in lichen planus (LP). As far as my knowledge, no previous study had done in Iraq about HLA-DQA1 and -DQB1 alleles frequencies in patients with lichen planus.**

Aim: **The aim of the present study is to investigate the additional genetic contribution to lichen planus susceptibility lying on the HLA region, by focusing on the possible differential contribution of the different DQA1 and DQB1 carrying haplotypes.**

Method: **The present study was carried out in College of Medicine during the period between (2012-2014). 50 patients with lichen planus attending Basrah General Hospital and private clinic, and 50 healthy controls were included in the study, with age group from (13-67) years. 100 DNA samples were purified from the blood samples of patients and controls, and then followed by PCR amplification of HLA-DQA1 and DQB1 genes in Cell Research Unit, Biology Department, College of Science, University of Basrah. The sequencing-PCR was done in Korea, Bioneer sequencing laboratories.**

Results & Conclusions: **Results indicated statistically significant decreased frequencies of HLA-DQA1*010201 (P<0.005), DQA1*0201 (P < 0.05), and -DQB1*030201 (P < 0.005) alleles in lichen planus patients, which indicated that these alleles might be a protective factors. Results also indicated statistically significant increased frequencies of DQA1*010401 (P<0.005), DQA1*040101 (P<0.005) DQA1*050101 (P<0.005), DQB1*030101 (P<0.005) and DQB1*050101 (P<0.005) alleles in lichen planus patients, which indicated that these alleles might be risk factors and increase the ability of infection. The present study indicates that genetic constitution through HLA-DQ locus determines the mechanism of disease as well as clinical and pathologic outcomes. More studies are necessary to test genetic dependencies on the basis of larger samples which would increase statistical power. An accurate definition of disease susceptible alleles will improve our understanding of antigen presentation mechanisms prevailing in the etiology of the disease. This knowledge is necessary for the design of improved immune intervention strategies to halt lichen planus progression in patients at risk of developing the disease or those who are already suffering from it.**

Key words: HLA-DQA1 & HLA-DQB1, Lichen planus

دراسة نظائر مستضدات HLA–DQA1 و HLA –DQB1 في المرضى المصابين بمرض الحزاز في محافظة البصرة

الخلفية: مرض الحزاز يسبب التهاب الجلد والأغشية المخاطية، والذي يكون إما عام أو موضعي. بينت العديد من الدراسات إن مستضدات الخلايا البيضاء لها دور مهم في مرض الحزاز. حسب معرفتي ليس هناك دراسة في العراق عن ترددات اليلات HLA–DQA1 و DQB1– في المرضى المصابين بالحزاز.

الهدف: الهدف من الدراسة الحالية هو التحقق من علاقة جينية إضافية لقابلية الإصابة بمرض الحزاز والتي تكون موجودة في منطقة HLA، مع التركيز على علاقات لأنماط أخرى من DQA1 و DQB1.

الطريقة: أجريت هذه الدراسة في كلية الطب، جامعة البصرة خلال الفترة مابين (2012–2014). 50 مريض من المراجعين لمستشفى البصرة العام والعيادة الخاصة، و 50 من الأصحاء تم شمولهم في هذه الدراسة، من الفئة العمرية مابين (13–67) سنة. 100 نموذج DNA تم عزلها من نماذج دم المرضى والأصحاء، بعد ذلك تم تضخيم جينات HLA–DQA1 و HLA–DQB1 باستعمال PCR في وحدة أبحاث الخلية، فرع علوم الحياة، كلية العلوم، جامعة البصرة. تمت دراسة تتابع نظائر الجينات في مختبرات Bioneer في كوريا.

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النتائج والإستنتاجات: وضحت النتائج وجود نسبة تكرار واطئة ذات مغزى ايجابي لنظائر الجينات **HLA–DQA1*010201** (**P<0.005**)، **HLA–DQA1*0201** (**P<0.05**)، **HLA–DQB1*030201** (**P<0.005**) في المرضى المصابين بمرض الحزاز، مما يبين إن هذه النظائر قد تكون عوامل حماية من المرض. بينت النتائج نسبة تكرار عالية ذات مغزى ايجابي لنظائر الجينات **HLA–DQA1*010401** (**P<0.005**)، **DQA1*040101–** (**P<0.005**)، **DQA1*050101** (**P<0.005**)، **DQB1*030101** (**P<0.005**) و **DQB1*050101–** (**P < 0.005**) في المرضى المصابين بمرض الحزاز مما يبين إن هذه النظائر قد تكون عوامل خطورة تزيد من قابلية الإصابة. وضحت الدراسة الحالية إن الدور الوراثي من خلال **HLA–DQ locus** يحدد آلية المرض إضافة إلى النتائج السريرية والمرضية. المزيد من الدراسات ضرورية لاختبار التبعات الوراثية على أساس نماذج أكبر والتي قد تدعم النتائج إحصائيا. إن التعريف الدقيق للاليلات ذات الصلة بالمرض سيحسن فهمنا لآليات تمثيل المستضدات المسببة للمرض. هذه المعرفة ضرورية لتصميم استراتيجيات مثبتة للتدخل المناعي الذي يعمل على إيقاف تقدم مرض الحزاز في المرضى المعرضين لخطر تطور المرض أو أولئك الذين يعانون بالفعل منه.

INTRODUCTION

Lichen planus (LP) is a disease that affects the upper layers of the skin as an itching rash that can sometimes become severe; it is characterized by purple, polygonal. Pruritic and popular eruption and also involve the mucus membranes and the nails.[1] The disease is considered to be rare in children,[2] and is more common in the African American population.[3] The lesions are diagnosed clinically by their "lichen-like" appearance and can be classified by the site they involve, or by their morphology.[4] It can occur in many different forms; annular, linear, hypertrophic, atrophic, bullous, ulcerative and pigmented Wickham's striae.[5,6] Histopathology, reveals hyperkeratosis, hypergranulosis, basal cell degeneration, and eosinophilic colloid bodies in the lower epidermis and superficial dermis.[7] Many studies have suggested a role for local factors and infectious agents, such as an infection with *Borrelia burgdorferi*, human papillomavirus, or hepatitis C virus.[8,9,10] Current opinion suggests an autoimmune pathogenesis.[11,12] Data demonstrate an immune phenotype in lichen planus, characterized by increased levels of Th1-specific cytokines.[13] Patients with widespread lichen planus may respond to narrow-band or broadband UV-B therapy.[14] Low-dose treatment with laser radiation can be very effective in treating symptomatic and erosive oral lichen planus OLP.[15] LP can be triggered by genetic malfunction and/or environmental factors.[16,17] Human leukocyte antigens (HLA) genes of the Major histocompatibility complex (MHC), located on the short arm of human chromosome 6, encode peptides involved in host immune response, are important in tissue transplantation and are associated with a variety of infectious, autoimmune, and inflammatory diseases. Moreover, the HLA loci display an unprecedented degree of diversity and the distribution of HLA alleles among different populations is considerably variable.[18] Gene polymorphisms of different HLA markers as well as the inflammatory cytokines and chemokines have been associated with LP.[19]

MATERIALS AND METHODS

The present study was carried out in college of Medicine during the period between (2012 – 2014). A total of 50 patients with lichen planus (31 males and 19 females) attending Basrah General Hospital and private clinic and a total of 50 healthy controls (23 males and 27 females), with age groups from (13-67) years were included in the present study. Diagnosis was confirmed by the clinical examiner prior to collecting blood samples and written informed consent was obtained from all participants. 100 DNA samples were purified from the blood samples, 50 DNA samples from patients and 50

DNA samples from controls were isolated by using Wizard Genomic DNA purification Kit, Promega Corporation, USA protocol, [20] and then subjected to HLA-DQA1 and HLA-DQB1 genotyping. Out of 100 amplified DNA samples from patients and controls, 24 samples from patients and 50 samples from control group showed results for sequencing (Table-1).

Table 1. Frequencies of DNA samples extracted and typed for HLA-DQA1 and -DQB1 from patients with lichen planus and controls

Typed for HLA-DQ	DNA samples extracted from lichen planus patients	DNA samples extracted from controls	Total 100
	N (%) 50	N (%) 50	
DQA1 + DQB1	24 (48%)	50 (100%)	95

PCR Amplification of HLA-DQA1 and HLA-DQB1 Genes:

The PCR Amplification of HLA-DQA1 gene was done in Cell Research Unit, Biology Department, College of Science, University of Basrah. The protocol based on the protocol done in a previous study.[21] The minimum concentration of the amplified DNA samples was 40 ng/ul and the maximum concentration of the amplified DNA samples was 50 ng/ul. The primer sequences used were as follows: exon 2 of HLA-DQA1 gene (BioNeer Corporation, USA) forward: 5-ATCTTCACTCAGCTGACCA-3; and reverse: 5-GCTGACCCAGTGTCACGGGAG-3. For amplification of HLA-DQB1 gene, the primer sequences used were as follows: exon2 of DQB1 gene (BioNeer Corporation,USA) forward:5-TCCCCGCAGGATTTCGTG-3; and reverse: 5-GGCGACGACGCTCACCTC-3. The GoTaq Green Master Mix 2X (Promega Corporation, USA) preparation was according to the following equation: 2X Mix 92%, 4% of each upstream and downstream primer. For each tube; 25 µl Master Mix was added, 5 µl DNA, and 20 µl autoclaved D.W. Each PCR reaction was performed in a final volume 50 µl. (Table-2) showed the PCR program for amplification of HLA-DQA1 and-DQB1 gene.

Table 2. PCR program for amplification of HLA-DQA1 and –DQB1 gene

Steps	Temperature	Time	No. of cycles
Denaturation 1	96 ºC	5 min	1
Denaturation 2	96 ºC	1 min	44
Annealing	53 ºC (for DQA1) 57 ºC (for DQB1)	1 min	
Extension 1	72 ºC	1 min	
Extension 2	72 ºC	10 min	1

DNA Sequencing –PCR:

The sequencing- PCR of the amplified HLA-DQA1 and HLA-DQB1 genes was done according to sequence based typing in {Korea, Bioneer sequencing laboratories}{49-3, Munpyeong-Dong, Daedeok-Gu, Daejeon 306-220,Korea}. (Figures 1 and 2). The typing of HLA alleles done manually by using HLA typing program online.

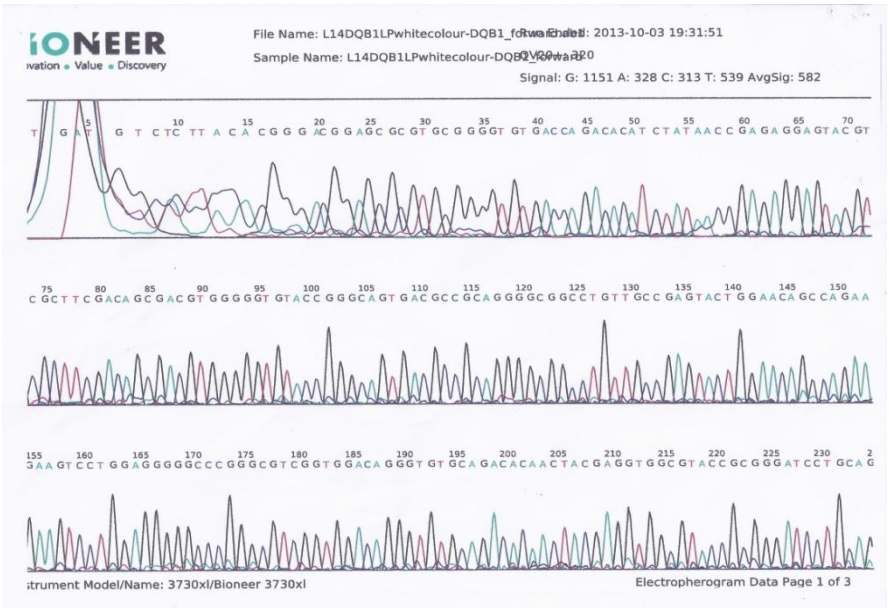

Fig 1. Sequences of the amplified HLA-DQB1 gene

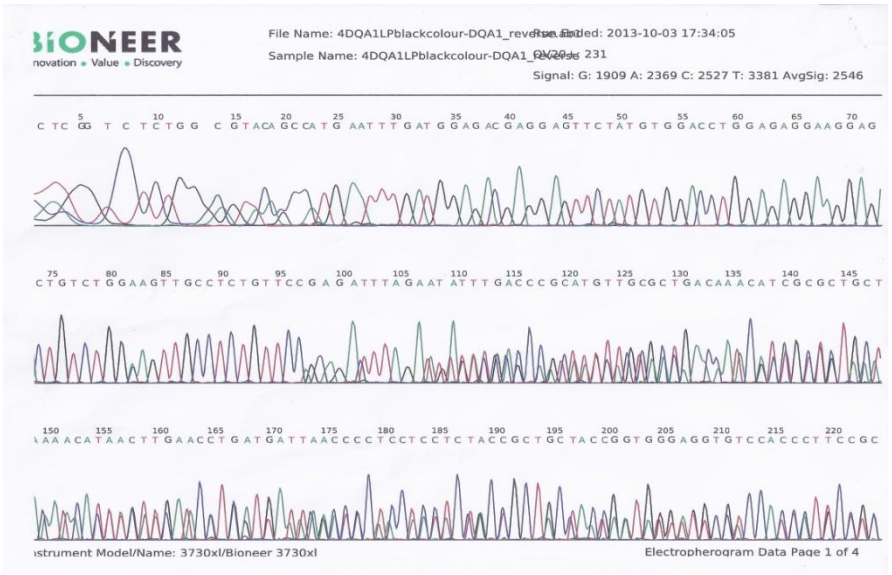

Fig 2. Sequences of the amplified HLA-DQA1 gene

Statistical Analysis:

For qualitative variables, frequency data were summarized as percentage. Statistical significant of differences between two groups was tested by Pearson Chi-square (χ^2) with Yates' continuity correction. Risk was estimated using Odds ratio (OR) and 95% confidence interval (95% CI). P-value was determined by Fisher's exact test, P-value of (< 0.05) was considered statistically significant. Data were analyzed using SPSS program for window (Version 15).

RESULTS

Distribution of lichen planus patients & controls according to Gender:

(Table-3), showed that out of 50 patients with lichen planus, 31(62%) were males and 19(38%) were females. For control group, out of 50 healthy controls, 23(46%) were males and 27(54%) were females. The results showed no significant difference between males and females frequencies when compared with control group **(**(χ^2) = 0.36; P = NS; OR= 0.79; 95% CI= 0.36– 1.73).

Table 3. Distribution of patients with lichen planus and control according to gender

Gender	Lichen planus patients	Controls	Total
	N (%) 50	N (%) 50	N= 100
Male	31 (62%)	23 (46%)	54
Female	19 (38%)	27 (54%)	46

(χ^2 = 0.36; P = NS; OR= 0.79; 95% CI= 0.36– 1.73)

Distribution of patients with lichen planus and controls according to age group:

(Table-4), showed that out of 50 patients with lichen planus, 32(64%) were from age group (13 > 40) and 18(36%) were from age group (> 40). For controls group, out of 50, 34(68%) were from age group (13 > 40) and 16(32%) were from age group (> 40). These results showed no significant difference in frequencies of age groups when compared with controls (χ^2)= 0.18; P= NS; OR= 1.20; 95% CI= 0.52-2.74).

Table 4. Distribution of patients with lichen planus and controls according to age group

Age group	lichen planus patients	Controls	Total
	N (%) 50	N (%) 50	N= 100
13 > 40	32(64 %)	34(68 %)	66
> 40	18(36 %)	16(32 %)	34

(χ^2 = 0.18; P = NS; OR= 1.20; 95% CI= 0.52-2.74))

Distribution of patients with Lichen planus according to gender and type of lesion:

Results in (Table-5), showed that out of 38 patients with papular lesions, 21 (55.26%) were males and 17 (44.74%) were females. Out of 3 patients with Wickham's straiae, 3 (100%) were females. Also results in table.4 showed that out of 3 patients with papular + Wickham's straiae lesions, 3 (100%) were males. Out of 6 papular + hyperatrophy lesions, 2 (33.33%) were males and 4 (66.67%) were females. Results indicated that there was no significant difference between males and females according to type of lesions.

Table 5. Distribution of patients with Lichen planus according to gender and type of lesion

Gender	Type of lesion				Total N= 50	χ^2	P
	Papular N= 38	Wickham's straiae N= 3	Papular + Wickham's straiae N= 3	Papular + hyperatrophy N= 6			
Male	21 (55.26%)	0 (0%)	3 (100%)	2 (33.33%)	26	7.02	NS
Female	17 (44.74%)	3 (100%)	0 (0%)	4 (66.67%)	24		

Distribution of patients with Lichen planus according to age group and type of lesion

Results in (Table-6), showed that out of 38 patients with papular lesions, 27 (71.05%) were from age group (13 > 40) and 11(28.95%) were from age group > 40. Out of 3 patients with Wickham's straiae, **1 (33.33%)** was from age group (13 > 40) and 2 (66.67%) were from age group > 40. Also results in table.5 showed that out of 3 patients with papular + Wickham's straiae lesions, 2 (66.67%) were from age group (13 > 40) and 1 (33.33%) was from age group > 40. Out of 6 papular + hyperatrophy lesions, 2 (33.33%) were from age group (13 > 40) and 4 (66.67%) were from age group > 40. Results indicated that there was no significant difference between patients according to age groups and type of lesions.

Table 6. Distribution of patients with Lichen planus according to age group and type of lesion

Age group	Type of lesion				Total n= 50	X^2	P
	Papular N= 38	Wickham's straiae N= 3	Papular + Wickham's straiae N= 3	Papular + hyperatrophy N= 6			
13 > 40	27 (71.05%)	1 (33.33%)	2 (66.67%)	2 (33.33%)	26	**4.50**	**NS**
> 40	11 (28.95%)	2 (66.67%)	1 (33.33%)	4 (66.67%)	24		

Distribution of patients with Lichen planus according to gender and site of lesion

Results in (Table-7) indicated that out of 32 patients with skin lesion, 15(46.87%) were males and 17 (53.13%) were females. Out of 3 patients with mouth lesion, 2(66.67%) were males and 1 (33.33%) was female. Also results showed that out of 6 patients with genitalia lesion, 5(83.33%) were males and 1(16.67%) was females. For patients with skin, mouth and genitalia lesions, out of 6, 3(50%) were males and 3(50%) were females. For patients with skin, genitalia lesions, out of 3, 1(33.33%) was male and 2(66.67%) were females. Results did not showed significant difference between male and females according to the sits of lesions.

Table. 7 Distribution of patients with Lichen planus according to gender and site of lesion

Gender	Site of lesion					Total N= 50	X^2	P
	Skin N= 32	Mouth N= 3	Genitalia N= 6	Skin + mouth + genitalia N= 6	Skin + genitalia N= 3			
Male	15 (46.87%)	2 (66.67%)	5 (83.33%)	3 (50%)	1 (33.33%)	26	**3.38**	**NS**
Female	17 (53.13%)	1 (33.33%)	1 (16.67%)	3 (50%)	2 (66.67%)	24		

Distribution of patients with Lichen planus according to age group and site of lesion:

Results in (Table-8) indicated that out of 32 patients with skin lesion, 19 (59.38%) were from (13 > 40) age group and 13 (40.62%) were from (> 40) age group. Out of 3 patients with mouth lesion, 3 (100%) was from (13 > 40) age group. Also results showed that out of 6 patients with genitalia lesion, 3 (50%) were from (13 > 40) age group and 3 (50%) were from (> 40) age group. For patients with skin, mouth and genitalia lesions, out of 6, 4 (66.67%) were from (13 > 40) age group and 2 (33.33%) were from (> 40) age group. For patients with skin, genitalia lesions, out of 3, 3 (100%) were from (13 > 40) age group. Results did not showed significant difference between patients from the two age groups according to the sits of lesions.

Table 8. Distribution of patients with Lichen planus according to age group and site of lesion.

Age group	Site of lesion					Total N = 50	X^2	P
	Skin N= 32	Mouth N= 3	Genitalia N= 6	Skin + mouth + genitalia N= 6	Skin + genitalia N= 3			
13 > 40	19 (59.38%)	3 (100%)	3 (50%)	4 (66.67%)	3 (100%)	32	4.20	NS
> 40	13 (40.62%)	0 (0%)	3 (50%)	2 (33.33%)	0 (0%)	18		

HLA-DQA1 genotype frequency of lichen planus patients and controls:

Genotype frequencies of HLA-DQA1 alleles were studied in 50 patients with lichen planus and compared with 50 controls. Out of 50 amplified DNA samples subjected to HLA-DQA1 genotyping, only 24 samples showed results and for controls group, 50 amplified DNA samples showed results. Table.9, indicated that HLA-DQA1*010201 allele was absent in lichen planus patients and present in 22 (44%) out of 50 controls, also HLA-DQA1*0201 allele was absent in patients and present in 8 (16%) out of 50 controls. The decreased frequencies of HLA-DQA1*010201 and HLA-DQA1*0201 alleles in lichen planus patients were statistically significant ((χ^2) = 25.77, P < 0.005, OR= 0.56, 95% CI= 0.44-0.72) and ((χ^2) = 4.31, P < 0.05, OR= 0.84, 95% CI= 0.74-0.95) respectively as compared with controls. Also results showed that HLA-DQA1*010401 allele was present in 14 (58.33%) out of 24 lichen planus patients and in 5 (10%) out of 50 controls. Results indicated that HLA-DQA1*040101 was present in 6 (25%) patients and absent in controls. Also HLA-DQA1*050101 allele was present in 9 (37.5%) patients and absent in controls. The increased frequencies of HLA-DQA1*010401, HLA-DQA1*040101 and HLA-DQA1*050101 alleles in lichen planus patients were statistically significant ((χ^2) = 19.85, P < 0.005, OR = 0.08, 95% CI= 0.02-0.27), ((χ^2) = 13.60, P < 0.005, OR = 1.33, 95% CI = 1.06-1.68) and (χ^2 = 5.35, P < 0.005, OR= 1.60, 95% CI= 1.17-2.18), as compared with controls.

Table 9. HLA-DQA1 genotype frequency of lichen planus patients and controls.

DQA1 allele	LP patients (n=24)		Controls (n=50)		X^2	P	Odds ratio	95% CI
	No.	%	No.	%				
010101/010102/010401/010402/0105	14	58.33	5	10	19.85	< 0.005	0.08	0.02-0.27
010201/010202/010203/010204	0	0.00	22	44	25.77	< 0.005	0.56	0.44-0.72
0103	4	16.67	3	6	2.15	NS	0.32	0.07-1.56
0201	0	0.00	8	16	4.31	< 0.05	0.84	0.74-0.95
030101/0302/0303	2	8.33	12	24	2.60	NS	3.47	0.71-16.97
040101/040102/0402/0404	6	25	0	0	13.60	< 0.005	1.33	1.06-1.68
050101/0503/0505/0506/0507/0508/0509	9	37.5	0	0	5.35	< 0.005	1.60	1.17-2.18

HLA-DQB1 genotype frequency of lichen planus patients and controls

Results in (Table-10) indicated that HLA-DQB1*030201 allele was absent in lichen planus patients and present in 14 (28%) out of 50 controls. The decreased frequency of HLA-DQB1*030201 allele in lichen planus patients was statistically significant ((χ^2) = 14.78, P <

0.005, OR= 0.72, 95% CI= 0.61-0.86) as compared with controls. Also results indicated that HLA-DQB1*030101 allele was present in 10 (41.67%) out of 24 lichen planus patients and in 5 (10%) out of 50 controls. Results indicated that HLA-DQB1*050101 allele was present in 12 (50%) out of 24 lichen planus patients and in 3 (6%) out of 50 controls. The increased frequencies of HLA-DQB1*030101 and HLA-DQB1*050101 alleles in lichen planus patients were statistically significant as compared with controls ((χ^2) = 10.06, P < 0.005, OR = 0.16, 95% CI= 0.05-0.53) and ((χ^2) = 19.43, P < 0.005, OR= 0.06, 95% CI= 0.02-0.26) and respectively.

Table 10. HLA-DQB1 genotype frequency of lichen planus patients and controls

DQB1 allele	LP patients (N=24)		Controls (N=50)		X^2	P	OR	95% CI
	No	%	No	%				
020101/0202/0204	2	8.33	12	24	2.60	NS	3.47	0.71-16.97
030101/030104/0309/0321/0322/0324/030302	10	41.67	5	10	10.06	< 0.005	0.16	0.05-0.53
030201	0	0.00	14	28	14.78	< 0.005	0.72	0.61-0.86
030302	0	0.00	6	12	3.13	NS	0.88	0.79-0.98
0402	0	0.00	1	2	0.49	NS	0.98	0.94-1.02
050101	12	50	3	6	19.43	< 0.005	0.06	0.02-0.26
050201	0	0.00	1	2	0.49	NS	0.98	0.94-1.02
050301	0	0.00	0	0.00	N/A	N/A	N/A	N/A
060101/060103	0	0.00	0	0.00	N/A	N/A	N/A	N/A
060201	0	0.00	0	0.00	N/A	N/A	N/A	N/A
060301/061401	0	0.00	0	0.00	N/A	N/A	N/A	N/A
060401/0634	0	0.00	0	0.00	N/A	N/A	N/A	N/A
060801	0	0.00	0	0.00	N/A	N/A	N/A	N/A
0609	0	0.00	0	0.00	N/A	N/A	N/A	N/A

DISCUSSION

The overall prevalence of lichen planus in the general population is about 0.1-4%.[4] Our results indicated that the most common type of lesion was papular form. In a study done by Boorghani *et al,* in 2010, results showed that the most common type of lesions was reticular form with the characteristic feature of (Wickham's striae).[22] Also our results showed that the skin alone was the common site of involvement. These results do not agree with results of a study done by Abdallat and Maaita on Jordanian patients in 2007. Their results showed that both skin and mucus membranes were the common sites of involvement.[23] The present study showed no significant difference between males and females frequencies when compared with control group. These findings do not agree with results of a study done by Yu *et al,* in 2003, they indicated that lichen planus occurs more commonly in females.[24] A study on Indian cohort showed that female to male ratio was 1:1.5.[1] Female to male ratio was 2:1.[3] Our

study indicated that there was no significant difference in frequencies of age groups when compared with controls. These results agree with the results of Yu and *et al,* in 2003,[24] and also agree with a study done on Jordanian patients.[23] Also Results indicated that there was no significant difference between males and females and age groups according to type and site of lesions. The present study indicate that genetic constitution through HLA-DQ locus determines the mechanism of disease as well as clinical and pathologic outcomes. In other words, immunogenetic background among different ethnicities is manifested as resistance or susceptibility to the development of lichen planus. An accurate definition of disease susceptible alleles will improve our understanding of antigen presentation mechanisms. Much more work deserves this field, with several open questions as which is the causal variant lying on the HLA-DQ region and which are their specific functional implications. As far as my knowledge, no previous study have done in Iraq to investigate HLA-DQA1 and HLA-DQB1 alleles association with lichen planus, also most studies in other countries were done on MHC class I. [24]. Many studies indicate that there is a regulation in antigen presentation, cell-mediated immune response, humoral immune response, and inflammatory response in lichen planus.[13] Since HLA antigens play a major role in antigen presentation and cell-mediated immune response, it is important to study the frequency of HLA alleles in patients with lichen planus and controls to indicate that if these alleles might be a protective factors or risk factors for the disease. The use of DNA-based genetic typing has enabled the identification of susceptible and protective major histocompatibility complex (MHC) class II alleles and haplotypes.[18] The application of this approach has also progressed to locate MHC class I alleles that contribute to the clinicopathology of lichen planus.[24] The present study showed that the decreased frequencies of HLA-DQA1*010201and HLA-DQA1*0201 alleles in lichen planus patients were statistically significant as compared with controls ($P < 0.005$) respectively which indicate that these alleles might be protective factors. Also results showed statistically significant increased frequencies of HLA-DQA1*010401, HLA-DQA1*040101 and HLA-DQA1*050101 alleles in lichen planus patients as compared with controls ($P < 0.005$), ($P < 0.005$) and ($P < 0.005$) which indicate that these alleles might be risk factors. The present study showed statistically significant decreased frequency of HLA-DQB1*030201 allele as compared with controls ($P < 0.005$). Also results showed statistically significant increased frequencies of HLA-DQB1*030101 and HLA-DQB1*050101 alleles in lichen planus patients as compared with controls ($P < 0.005$) and ($P < 0.005$) respectively. Our results indicated a decreased frequency of HLA-DQB1*020101 allele but statistically not significant, this finding does not agree with a study done by Setterfield *et al.* in 2006 on patients with vulvovaginal syndrome: a severe subgroup of lichen planus, which indicated that HLA-DQB1*0201 allele was present in 80% of patients versus 41.8% of controls. [19] The mechanisms underlying MHC association in autoimmune disease are not clearly understood. One long-held view suggests a breakdown in immunological tolerance to self-antigens through aberrant class II presentation of self or foreign peptides to autoreactive T lymphocytes. Thus, it seems likely that specific MHC class II alleles determine the targeting of particular autoantigens resulting in disease-specific associations. Peptide cross-binding phenomena were observed for gene products of several MHC alleles.[13] Recent studies have shown a widespread involvement of genes from the MHC gene region in the clinicopathology of lichen planus.[25,26] These genes are shown to be involved in contributing to progression from the preclinical stage of the disease to clinical disease and also to the occurrence of

autoimmunity.[13] Findings of the present study showed that MHC Class II alleles may also be negatively associated with lichen planus. These findings are useful for the development of future strategies in designing tolerogenic approaches for the prevention or even reversal of lichen planus. The analysis points out that much of the conflicting results of previous association studies originate from inadequate sample sizes and accentuate the value of future investigations of larger samples for identifying linkage in multigenic diseases. A study done on Mexican population found that HLA-DRB1*0101 allele was associated significantly in LP patients compared with healthy controls.[25] Another study done in Kuwait found that There were no significant differences in the antigens of the HLA-ABC loci but there was a significant increase in HLA-DR1 and HLA-DRH and a significant decrease in HLA DR5.[26] Specific increase of HLA-A*68, followed by HLA-A*69, HLA-A*02, HLA-B*13, HLA-B*35, and HLA-DRB1*01, as well as a marked decrease in DRB1*11 was found in Turkish patients with erosive oral lichen planus.[27]

CONCLUSIONS

Our study indicated statistically significant decreased frequencies of HLA-DQA1*010201, DQA1*0201, and DQB1*030201 alleles in lichen planus patients, which indicated that these alleles might be a protective factors. Also a statistically significant increased frequencies of DQA1*010401, DQA1*040101 DQA1*050101, DQB1*030101 and DQB1*050101 alleles were indicated in lichen planus patients, which indicated that these alleles might be risk factors and increase the ability of infection. More studies are necessary to test genetic dependencies on the basis of larger samples which would increase statistical power. An accurate definition of disease susceptible alleles will improve our understanding of antigen presentation mechanisms prevailing in the etiology of the disease. This knowledge is necessary for the design of improved immune intervention strategies to halt lichen planus progression in patients at risk of developing the disease or those who are already suffering from it.

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