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Determination of HLA-DR Genotyping in a Sample of Iraqi Patients with Oral Lichen Planus

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ABSTRACT

Lichen planus (LP) is a chronic inflammatory mucocutaneous disorder. Oral Lichen Planus (OLP) is often asymptomatic with or without skin involvement. OLP can be a source of severe morbidity and has a potential for malignant transformation. It affects women more than men, mostly during adulthood. This study was performed to investigate the role of human leukocyte antigens class II genotypes (HLA-DR) as a potential genetic factor in the aetiopathogenesis of OLP. Blood samples were collected from Thirty-Two OLP patients and thirty apparently healthy subjects as a control group to assess HLA-DR genotyping using polymerase chain reaction sequence specific oligonucleotide (PCR-SSO) method. The current study revealed that the HLA-DR1 alleles were observed with higher significant frequency in patients representing (17.19%) as compared to healthy control representing (3.33%), with P-value (0.016). High frequency of HLA-DRB1*01 alleles in patients may be considered as a risk factor in OLP.

Keywords: Oral lichen planus, HLA-DR, PCR-SSO.

1. INTRODUCTION

Oral lichen planus (OLP) is a relatively common, chronic, immuno-inflammatory, and potentially premalignant condition which is a rather common disease in the middle-aged and elderly populations [1]. OLP consider the disease of stratified squamous epithelia with unknown etiology [2]. Clinically several subtypes have been described, but the following six clinical types of OLP lesions were more observed, individually or combined: atrophic, bullous, erosive, papular, plaque-like and reticular [3]. The prevalence of OLP varies from 0.5% to 4% of the general population, and the malignant transformation rate is (0–2%) [4]. It tends to be more chronic in nature than the cutaneous disease, affecting women more commonly than men, with age of onset on average around 60 years; meanwhile, it is rare in childhood [5]. The relative risk is (3.7%) in people with mixed oral habits, in non-users of tobacco is lowest (0.3%) and highest (13.7%) among those who smoked and chewed tobacco [6]. The reported OLP malignant transformation rates vary from 0.4% to 12.5%, with an overall average rate of 1.09% cited in a recent meta-analysis and systematic review of 7,806 patients in 16 studies [7]. World Health Organization (WHO) in their Global Oral Health Program designated OLP a premalignant condition [8]. OLP has been suggesting as an ideal model of inflammation induced cancer [9].

The genetic predisposition has been hypothesized in OLP etiology [10]. Genetic factors influencing immune function may contribute to OLP pathogenesis; many studies have focused on correlation between HLA and OLP, demonstrating that an association has been observed with HLA-A3, A11, A26, A28, B3, B5, B7, B8,

DR1, and DRW9 [4, 11-14]. The HLA-DR1 is frequently associated with cutaneous idiopathic LP, but not in OLP and the HLA-DR6 is usually linked to hepatitis C virus-associated OLP. In Italian patients with OLP infected with Hepatitis C Virus (HCV), the HLA-DR6 allele is significantly expressed [15], whereas in Mexican Mestizo population was found HLA-DRB1*01: 01 associated with a genetic susceptibility for LP [16]. Another study suggests that HLADR6 may be responsible for the unfamiliar geographic heterogeneity of the association between HCV and OLP [17].

2. MATERIALS AND METHODS

Thirty-two patients attending the dermatological outpatient clinic at the Medical City Hospital in Baghdad from June 2014 till February 2015 were eligible for this study. All patients were diagnosed by a specialist physician. In addition, 30 apparently healthy volunteers were considered as control group their age and gender matched with patients group. The ethical committee of the college of Medicine / Al-Nahrain University approved this study, and all samples were obtained with informed consent in accordance with the Medical City Hospital in Baghdad declaration. Two ml of blood sample was collected in EDTA tube from each patient and control group for DNA extraction and then stored at -80˚C. Also, three ml of saliva was collected from each patient and control group in a sterile cup after 5 minutes from rinse of the mouth by water which stored in an ice box. The saliva samples were centrifuged at 2,600g for 15 minutes at 4°C. Then 0.1µl of Protease inhibitor cocktail was added to each milliliter of the supernatant to prevent protein degradation. Then all samples were stored at −80°C until the further procedure.

2.1 Determination of HLA-DR typing by polymerase chain reaction sequence specific oligonucleotide (PCR-SSO) probe

2.1.1 DNA extraction

QIAamp DNA Mini Kit (Qiagene, USA) was designed for rapid purification of an average of 6µg of total DNA from 200µl of human blood in EDTA. All DNA samples were profiled for concentration and purity by Nanodrop instrument, the purity of the samples was ranging from 1.72 to 1.93 to be enrolled for the HLA-typing.

2.1.2 HLA-DR amplification

The INNO-LiPA HLA-DRB1 Amplification Plus Kit (Fujirebio, Belgium; Code-Key: FRI92425) for *in vitro* use, designed to amplify nucleic acid for the second exon of the human leukocyte antigen (HLA) DRB1 locus performed by PCR.

2.1.3 INNO-LiPA HLA-DRB1 Plus

Is a line probe assay (Fujirebio, Belgium; Code-Key: FRI66815), designed for the molecular typing of human leukocyte antigen (HLA) DRB1 alleles at the allele group level (DRB1*01 to DRB1*16). The INNO-LiPA was based on the principle of reverse hybridization for HLA typing. Biotinylated DNA material was amplified, chemically denatured, and the separated strands were hybridized with specific oligonucleotide probes immobilized on membrane-based strips as parallel lines. This is followed by a stringent wash step to remove any mismatched amplified material. After the stringent wash, streptavidin conjugated with alkaline phosphatase has been added and bound to any biotinylated hybrid that previously formed. Incubation with a substrate solution containing a chromogen results in a purple/brown precipitate by a wash step the reaction was stopped, and the reactivity pattern of the probes was recorded.

2.2 Statistical analysis

The statistical analysis of this prospective study performed with the statistical package for social sciences (SPSS) version 21.0 and Microsoft Excel 2013. Categorical data formulated as count and percentage. Chi-square test used to describe the association of these data. Alternatively, Fisher exact test was used if there is 25% of a cell less than expected count.

3. RESULTS AND DISCUSSION

The mean age of patients was 48.16+13.56 years, there was no statistically significant difference ($p>0.05$) in gender between both studied groups. Twenty patients were females representing (62.5%); meanwhile, only 12 representing (37.5%) of patients were males. Regarding the smoking habit, there was a significant difference ($p<0.05$) between the studied groups. Among OLP patients, the current data showed that 13 patients representing (40.6%) were smokers and 19 patients representing (59.4%) were non-smokers; meanwhile, only 4 individual representing (13.3%) were smokers among control group, ($p = 0.016$) as in Table (1). The OLP patients were classified into three subgroups according to the clinical presentation, 14 patients were with reticular form representing (44%), 11 patients were with erosive form representing (34%), and only 7 patients were with plaque like form representing (22%) as shown in Figure (1).

3.1 HLA-DR genotyping

HLA-DR1 was observed with higher frequency among 11 patients representing (17.19%) as compared to 2 subjects in control group representing (3.33%), with P-value (0.016). Meanwhile, HLA-DR11 frequency was higher in 16 subjects among the control group representing (26.67%) than that in 4 patients representing (6.25%), ($p=0.003$) as in Table (2). The frequency of HLA-DRB1*01 was (18.18%) in erosive type, Plaque like was (14.29) and (17.86%) in reticular type, ($p=0.947$) as in Table 3.

Table 1: Demographic variables associated with study groups.

Variables			Study groups		Total
			Healthy control	OLP	
Age groups	20-30 years	No.	2	4	6
		%	6.7%	12.5%	9.7%
	31-40 years	No.	11	4	15
		%	36.7%	12.5%	24.2%
	41-50 years	No.	10	10	20
		%	33.3%	31.3%	32.3%
	>50 years	No.	7	14	21
		%	23.3%	43.8%	33.9%
Total		No.	30	32	62
		%	100.0%	100.0%	100.0%
P value		**0.102 NS**			
Gender	Male	No.	12	12	24
		%	40.0%	37.5%	38.7%
	Female	No.	18	20	38
		%	60.0%	62.5%	61.3%
Total		**No.**	30	32	62
		%	100.0%	100.0%	100.0%
P value		**0.523 NS**			
Smoking	No	No.	26	19	45
		%	86.7%	59.4%	72.6%
	Yes	No.	4	13	17
		%	13.3%	40.6%	27.4%
Total		**No.**	30	32	62
		%	100.0%	100.0%	100.0%
P value		**0.016 S**			
Mean age of patients		48.16+13.56 years			
P value		**0.075 NS**			

NS= Non significant; S= Significant

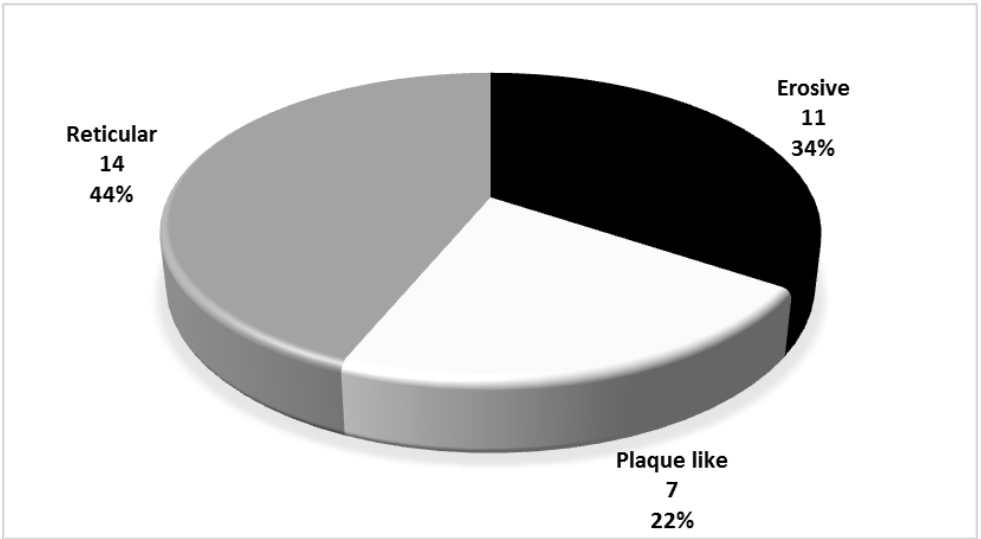

Figure 1: Distribution of OLP patients according clinical type of OLP.

Table 2: HLA-DR frequencies in OLP patients and control group.

	Study groups				P Value	OR	CI
	OLP		Control				
	No.	%	No.	%			
DR1	11	17.19%	2	3.33%	0.016 S	6.02	1.27 to 28.4
DR3	9	14.06%	10	16.67%	0.804 NS	0.818	0.307 to 2.18
DR4	14	21.88%	6	10.00%	0.089 NS	2.52	0.899 to 7.07
DR7	2	3.13%	6	10.00%	0.154 NS	0.29	0.0562 to 1.50
DR8	2	3.13%	3	5.00%	0.672 NS	0.613	0.0988 to 3.80
DR9	1	1.56%	0	0.00%	1 NS	2.86	0.114 to 71.6
DR10	4	6.25%	0	0.00%	0.119 NS	9	0.474 to 171
DR11	4	6.25%	16	26.67%	0.003 S	0.183	0.0573 to 0.587
DR12	1	1.56%	1	1.67%	1 NS	0.937	0.0572 to 15.3
DR13	8	12.50%	7	11.67%	1 NS	1.08	0.367 to 3.19
DR14	1	1.56%	2	3.33%	0.610 NS	0.46	0.0406 to 5.22
DR15	7	10.94%	6	10.00%	1 NS	1.11	0.349 to 3.50
DR16	0	0.00%	1	1.67%	0.484 NS	0.307	0.0123 to 7.70

Table 3: HLA-DR genotypes in three clinical types of oral lichen planus.

HLA-DR genotype	Erosive		Plaque like		Reticular		P value
	No	%	No	%	No	%	
DRB1*01	4	18.18%	2	14.29%	5	17.86%	0.947
DRB1*03	3	13.64%	3	21.43%	3	10.71%	0.641
DRB1*04	4	18.18%	2	14.29%	8	28.57%	0.501
DRB1*07	1	4.55%	1	7.14%	0	0.00%	0.407
DRB1*08	0	0.00%	0	0.00%	2	7.14%	0.265
DRB1*09	1	4.55%	0	0.00%	0	0.00%	0.379
DRB1*10	3	13.64%	0	0.00%	1	3.57%	0.189
DRB1*11	1	4.55%	2	14.29%	1	3.57%	0.369
DRB1*12	1	4.55%	0	0.00%	0	0.00%	0.379
DRB1*13	2	9.09%	2	14.29%	4	14.29%	0.837
DRB1*14	1	4.55%	0	0.00%	0	0.00%	0.379
DRB1*15	1	4.55%	2	14.29%	4	14.29%	0.495
DRB1*16	0	0.00%	0	0.00%	0	0.00%	-

Oral lichen planus most often occurs in persons 30 to 80 years of age, with a greater prevalence in females which is in agreement with Gorouhi et al., (2014) [11]. The present result showed that the OLP is more prevalent in patients with age more than 50 years with a mean age (48.16) and this result is consistent with other study reported by Munde et al., (2013) [18]. Also, the current results denoted a predominance of OLP among females than males which is comparable with other study conducted in India by Patil et al., (2012) [19], who indicated that LP occurs more commonly in females and the ratio of female to male was 2:1. The interpretation of higher incidence in females may be attributed to the hormonal differences (e.g. estrogen) between them and in turn, their effects on the immune responses. Those hormones make women normally tend to mount more robust immune responses and these responses tend to be more TH2 responses, hence may enhance the development of autoimmune phenomena [20].

Different morphological types of OLP were presented in the current study; however, the reticular form was more common in patients with OLP. This high frequency was in agreement with previous reports which suggest that the reticular types were the predominant type in OLP [21-22]. Evidence collected from patients with OLP; primarily with erosive or reticular forms of the disorder indicate mainly a type 1 immune response leading to damage of oral mucosal surface epithelium [23]. This response involves participation of plasmacytoid and myeloid dendritic cells [24], CD4 and CD8 T cells [25], natural killer cells, and mast cells [26], which is dominated by soluble factors characteristic of type 1 responses (interferons, interleukin-12, tumor necrosis factor alpha, and other factors), without apparent contributions by B lymphocytes and antibodies [25].

The high frequency of an HLA-DR1 allele (17.19%) among OLP patients in present study is in agreement with the study by Carrozzo et al. (2005) [16], which indicated that the most OLP worldwide is related with HLA-DR1 particularly the DRB1*0101 allele. A study by Luis-Montoya et al., (2007) [17] done on Mexican population found that HLA-DRB1*0101 allele was associated significantly in LP patients compared with healthy controls. 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 [27]. Specific increase of HLA-A*68, followed by HLAA*69, HLA-A*02, HLA-B*13, HLA-B*35, and HLA-DRB1*01 was found in Turkish patients with erosive oral lichen planus by Gülsüm et al., (2006) [28].

The present study revealed that there was significant lower frequency of HLA-DRB1*11 allele in erosive OLP patients when compared to controls which come in accordance with a Turkish study by Gülsüm et al., (2006) [28], which indicated that the frequency of HLA-DRB1*011, was markedly decreased. It is very important to remember that the reduced frequency of HLA typing could be considered as a protective factor for OLP. A study by Ghaliani et al., (2011) [29] in Iran indicated that the HLA-DR2, DR4, and DR7 were a significant increase in different clinical types of OLP. The discrepancies observed between various studies could be caused, in part, by the influence of ethnicity and racial background on the distribution of HLA alleles. Moreover, differences in methodology, sample size and patient selection could also have served as a source of bias.

4. CONCLUSION

The genetic constitution through HLA-DR locus may determine the mechanism of disease as well as clinical and pathologic outcomes. In the current study high frequency of HLA-DRB1*01 alleles in patients may be considered as a risk factor in OLP. Also, statistically significant decrease frequencies of HLA-DRB1*11 alleles, which may indicates that these alleles might be a protective factors.

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