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FREQUENCY OF HLA-DRB1 ALLELES IN IRAQI PATIENTS WITH CHRONIC PERIODONTITIS

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Abstract-

Background: There is growing evidence that genetic aspects play a role in the onset and severity of periodontitis. However; numerous studies have pointed to the contribution of the human leukocyte antigens (HLA)/alleles as a potential genetic factor in aetiopathogenesis of periodontitis.

Aims of Study: This study was performed to investigate the association of human leukocyte antigens class II genetypes (HLA-DRB1) and the susceptibility to chronic periodontitis in Iraqi patients.

Materials and Methods: Thirty subjects with chronic periodontitis and 20 apparently healthy volunteers participated in this study. Blood samples were collected from patients and controls, DNA was extracted from blood samples, and then HLA-RDB1 genotyping was performed by polymerase chain reaction-specific oligonucleotide probes (PCR-SSO) method.

Results: The present data revealed that the frequencies of HLA-DRB1*03 and HLA-DRB1*11 alleles were significantly higher in patients than in healthy controls (P= 0.004 and P=0.006 respectively), on the other hand low frequency of HLA-DRB1*04 allele was found in patients when compared with healthy control (P= 0.020). However, there is no association between the frequency of these specific HLA-alleles and patients with positive family history.

Conclusion: This study demonstrates that HLA-DRB1*03 and HLA-DRB1*11 alleles may contribute to the increased susceptibility to chronic periodontitis. While HLA-DRB1*04 allele may confer protective effects against this disease.

Keywords- Chronic periodontitis, genetic factors, human leukocyte antigens, PCR

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Introduction

Periodontitis (PD) has a multifactorial etiology, and the characteristic tissue destruction is mediated mainly by the aberrant immune response of the host to periodontopathic bacteria. The role of oral microflora in the etiology of various inflammatory periodontal diseases has been well established, and specificity may vary between bacterial etiologies and different forms of periodontal disease [1,2]. However; genetic and environmental factors are known to cause different patterns of colonization by periodontal pathogens. Host response to these periodontal pathogens determines the expression of the disease [3]. Patients are not equally susceptible to PD, and individual susceptibility to the microbial challenge in PD is determined in part by a genetic predisposition [4,5]. However; there is substantial evidence to support that PD as an inheritable disease [6].

It is well known that genetic contribution plays a significant role on susceptibility to PD, but the genetic markers associated with PD have not been fully elucidated. The major histocompatibility complex (MHC) or the genes encoding the HLAs have been considered candidate markers for PD because they are involved in regulating immune responses [7]. More than 40 diseases, most of which are autoimmune in nature, have been associated with various HLAs [8]. HLA class II molecules are especially relevant to the immune response in the event of foreign antigen recognition. Genetic polymorphism of the class II genes directly influences individual's immune reactions and degree of responsiveness. HLA markers have been investigated in several studies determining individual susceptibility factors for PD. The most striking positive associations with PD were found for HLA-A*24 [9, 10] and HLADR4 [11,12]. In addition, HLA-A*9 and HLA-B*15 alleles or antigens have been found to be risk factors for destructive forms of PD in a number of studies conducted in different populations [10, 13], whereas this finding could not be demonstrated in various other studies using different sets of

populations [14,15]. Hence, the purpose of the present study was to assess the association of HLA-DRB1 and the susceptibility to chronic periodontitis (CP) in Iraqi patients.

Materials and Methods

A total of 30 Iraqi patients with CP (21 male and 9 female) were included in this study, their age range from 24 to 64 years. They were from attendants seeking treatment in the department of periodontics, College of Dentistry, Baghdad University from November 2012 to January 2013. Diagnosis was made by specialized dentists in the College. All the cases had received no treatment with no complain of chronic or systemic diseases. Apparently healthy volunteers their ages and sexes were matched to patients consisted of 20 individuals who were considered as control (16 males and 4 females). All of them didn't have medical history or clinic evidence of any chronic or acute diseases; their age ranged between (20-51) years.

Two ml of venous blood were withdrawn from each subject under aseptic technique, then transferred into two EDTA tube (1.5 mg/ml), kept at -20ºC for the genotyping of HLA class II (DR). The DNA was extracted by using the genome DNA extraction kit (Kiagene / Germany). All DNA was stored at -20°C until tested. HLA-DR genotyping were performed by the PCR-SSO according to the manufacturer's instruction, this method depends on reverse hybridization, using the PCR-SSO kit (Histo Type / DNA-SSO Kits-Innogenetics-Line Probe Assay, INNO-LiPA, Belgium). HLA- genotyping was carried out in the HLA-Laboratory of Forensic Medicine Institute/ Baghdad

Statistical Analysis

The results were presented in terms of percentage frequencies, and alleles showing variations between patients and controls were further presented in terms of odds ratio (OR). The significance of these differences was assessed by fisher's exact probability (P). P values of $p<0.05$ were considered statistically significant.

Results

The demographic characteristics of patients group and controls group included in this study are presented in [Table-1]. No statistically significant differences ($p>0.05$) in age or gender existed between two study groups. The mean age of patients was 40.15 ± 10.53 years, and there was male's predominance among patients. Furthermore, (26.6%) of patients had positive family history of CP, while (73.4%) showed negative family history. The differences in clinical periodontal parameters in patients and healthy controls are summarized in [Table-2].

HLA-DRB1 genotyping of patients in comparison with healthy control evoked significant differences in some alleles between both groups. Among DRB1 alleles the present findings noticed that DRB1*03 and DRB1*11 alleles were found with highly significant frequencies among patients (56.6 % and 30.00%), in comparison with healthy control (16.00% and 5.00%), (p =0.004; 0.006 respectively). On the other hand, it was observed that HLA-DRB1*04 in high frequency among healthy individuals (35.00%) rather than in the patients (6.67%); with (p=0.020), as clearly shown in [Table-3].

In regarding to correlation between specific HLA-alleles of HLA-DR (DRB1*03, DRB1*04 and DRB1*11) and patients with positive family history, the present study revealed that there is no significant association between the frequency of these specific HLA- alleles and patients with positive family history [Table-4].

Table 1- Demographic Characteristic in CP Patients and Healthy Control

Parameters		Study groups: CP Patients n=30	Study groups: Healthy control n=20	P-value
Age (years)	Range	(24-64)	(20-51)	
	Mean±SD	40.15±10.53	37.38±9.10	0.254NS
Gender type	Female	9 (30 %)	4 (25%)	0.128NS
	Male	21 (69 %)	16 (75%)	0.672NS
Family history	Positive	8 (26.6 %)	0 (0.0%)	0.001**
	Negative	22 (73.4%)	20 (100%)	0.802NS

*NS= not significant (p>0.05); ** = highly significant difference (p≤0.001).*

Table 2- Clinical Periodontal Parameters in CP Patients and Healthy Control

Clinical periodontal Parameters	Study groups: CP Patients n=30	Study groups: Healthy control n=25	P-value
Plaque index	1.50±0.62	0.85±0.37	<0.001**
Gingival Index	1.26±0.49	0.74±0.29	<0.001**
Proping Pocket Depth (mm)	2.10±0.66	1.17±0.62	<0.001**
Clinical Attachment Loss	3.08±3.74	0.00±0.00	<0.001**
Bleeding on Probing (BOP)	28.85±30.57	6.12±8.08	<0.001**

Table 3- HLA-DRB1 allele frequencies in patients with CP and healthy controls

Alleles	CP Patients N	CP Patients %	Healthy Controls N	Healthy Controls %	OR	P (Fisher's exact)
DRB1*01	1	3.33%	1	5.00%	0.661	NS
DRB1*02	1	3.33%	2	10.00%	1.7	NS
DRB1*03	17	56.60%	4	20.00%	5	<0.004
DRB1*04	2	6.67%	7	35.00%	0.158	<0.020
DRB1*06	2	6.66%	2	10.00%	0.649	NS
DRB1*07	3	10.00%	3	15.00%	0.636	NS
DRB1*08	4	13.33%	5	25.00%	0.479	NS
DRB1*10	5	20.00%	4	20.00%	1.3	NS
DRB1*11	11	30.00%	1	5.00%	18.11	<0.006
DRB1*13	7	26.66%	4	20.00%	1.5	NS
DRB1*14	7	23.33%	3	15.00%	1.078	NS
DRB1*16	5	20.00%	4	20.00%	1.3	NS
Total	30	100	20	100		

Table 4- The risk of having positive family history of disease by HLA genotype among cases with CP.

Allele	Family History of CP: Positive N	Positive %	Negative N	Negative %	P (Fisher's exact)
DRB1*03	6	75%	11	50%	0.137 NS
DRB1*04	0	0.00%	2	9%	0.250 NS
DRB1*11	2	25%	9	40%	0.089 NS
Total	8	100%	22	100%	

Discussion

Genetic backgrounds play a key role in susceptibility to and protection against a spectrum of periodontal diseases. Like other infectious diseases, the HLA have been found to be associated with PD. Although there have been many case-control studies on HLA asso-

ciation and periodontal diseases, but most of these studies have investigated class I, but not class II. To our knowledge, this is the first study of HLA association with CP in an Iraqi population.

The present study was found higher frequency of HLA-DRB1*03 and HLA-DRB1*11 in CP patients in healthy controls. The oddis ratio (OR) for these alleles were (OR=5; OR=18), this mean that the individuals with HLA- HLA-DRB1*03 have five times greater chance of acquiring CP than those of the same population who lack it, and individuals with HLA-DRB1*11 alleles have eighteen times greater chance of acquiring CP.

Different results regarding this association was reported, results differ in different population. In Japan study conducted by Ohyama and colleagues [16], observed that high frequencies of HLA-DRB1*05 was found in Japanese patients with PD as compared to healthy control. On the other hand, Takashiba, et al.,[17] reported that patients Germans with early onset PD expressing high frequency of HLA-DRB1*15 genotype, and those patients may have an accelerated T-cell response to *P. gingivalis* and thus increased susceptibility to early onset periodontitis, i.e. the different types of the HLA marker could directly affect the capability to bind certain bacterial antigens or indirectly have an influence on other immune mechanisms. Moreover; Zhang, et al., showed that there was a significant over-representation of DRB1*15 among Chinese population with severe CP [9]. Whereas Wang and Pan demonstrated that the frequency of DRB1*15 was higher in Chinese population with generalized aggressive PD [18]. In contrast to the present findings Reichert and associates [14] reported that the HLA-DQB1*03 was significantly lower inpatients with CP than that in control. As well, the results of Amirzagar and co-workers also different from this results, they observed significant higher frequency of HLA-DRB1*11 for the Iranian healthy population [19].

Another interesting finding of this study was the insignificant higher frequency of HLA- DRB1*13 in CP patients which account for 26.6% versus 16.0% in healthy controls. Correspondingly, Machulla and colleagues [20], observed that high frequency of HLA-DRB1*13 was found in patients with PD and they concluded that the increased frequency of this may be associated with a higher risk for disease. Inversely, this result is at variance with other studies reported by [7, 21], Chakhtoura, et al., reported that HLA-DRB1*13 alleles seemed to be associated with protection against aggressive periodontitis in Lebanese patients [7].

It is very important to remember that the reduced frequency of HLA typing could be considered as a protective factor for PD. Consistent with the findings of previous reports [16, 20], present study revealed that there was significant lower frequency of HLA-DRB1*04 allele in CP patients when compared to controls. However; Klouda, et al. [22] did not find any associations between this disease and HLA-DR. On the contrary, Stien and associates on a study of the German population, revealed a higher frequency of DRB1*04 in patients with CP [15]. Whilst Mousavi Jazi, et al., found that the HLA-DRB1*04 allele, were significantly higher in patients with aggressive periodontitis compared with control subjects [21]. Therefore, it is speculated that susceptibility/resistance to chronic and aggressive periodontitis may also be influenced by particular HLA marker combinations and other genetic factors [23]. A family history of CP is evident in some patients. However; there is still a shortage of family studies of CP and there is insufficient data in literature which reflects the associations between specific HLA-alleles and this disease. The present work did not find association between specific HLA-alleles of HLA-DR (DRB1*03, DRB1*04 and DRB1*11) and patients with positive family history, this might in part, resulted from the limited number of investigated patients in this study

It is postulated that HLA molecules might be involved in initiating the inflammatory response via pathways other than those involving antigen presentation to immune cells [24]. HLA-DR molecules expressed on human gingival fibroblasts are capable of inducing signaling pathways for the production of cytokines, including monocyte chemoattractant protein 1, IL-6, IL-8 and regulated on activation, normal T-cell expressed and secreted RANTES. These cytokines may have putative roles in sustained inflammation, which is characteristic of PD [24,25].

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 as some studies recruited a control group from blood donors with undetermined dental involvement, whereas in other studies (including the present study), healthy controls were included only after oral examination. In conclusion, our study demonstrates that HLA-DRB1*03 and HLA-DRB1*11 alleles may contribute to the increased susceptibility to chronic periodontitis, while HLA-DRB1*04 allele may confer protective effects against this disease.

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