



## Analysis of HLA-DQB1 Alleles Frequency in Patients with Chronic Periodontitis

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### ABSTRACT

**Background:** Periodontitis is a pathological inflammatory condition of the periodontal tissues surrounding the teeth. It is a multiple factor disease caused by genetic factors, environmental factors, and periodontal bacteria. Human leukocyte antigen is the most polymorphic genetic system in man. Genes of this region influence susceptibility to certain diseases including periodontitis. **Objectives:** This study was established to shed light on the possible association of HLA class II (HLA-DQB1) alleles with chronic periodontitis. **Materials and Methods:** The study involved 40 Iraqi patients with chronic periodontitis and 40 healthy controls. Periodontal parameters used in this study were plaque index (PI), gingival index (GI), probing pocket depth (PPD) and clinical attachment level. DNA was extracted from a blood sample taken from each subject, then HLA-DQB1 gene was amplified using sequence specific oligonucleotide primed PCR (PCR-SSO). Genotyping of HLA-DQB1 alleles were performed using a reverse a hybridization automatic line probe assay (Auto-LiPA). **Results:** There was an increased frequency of HLA-DQB1\*05:02 allele in patients as compared to controls group (25% and 7.5% respectively) with  $P=0.04$  and odds ratio=4.11. In contrast, the frequency of DQB1\*03:01 allele was higher among controls group as compared to periodontitis patients ( $P=0.04$ , odds ratio=4.11). **Conclusion:** Our data show a correlation between the HLA-DQB1 locus and the occurrence of periodontitis in Iraq, supporting DQB1\*05:02 as a predisposing allele for this disease.

**Keywords:** Periodontal diseases, Human leukocyte antigen-DQ, Alleles, Polymorphism

### INTRODUCTION

Periodontal disease is a major oral health-related problem that affects a large number of patients all over the world. It is an infectious disease of the structures surrounding the tooth, which results from complex interactions between plaque microorganisms and host immune system [1,2]. There is considerable literature regarding the inflammatory nature of the periodontitis, and the relationship between these infections and systemic inflammation with increasingly numerous references in the literature with regard to the genetic predisposition of the host, and how it responds to infection. The host immune response, both innate and adaptive, is the key factor in the genesis of the immune response to the offending pathogenic microorganisms [3,4]. Genetic factors are known to influence inflammatory and immune responses in periodontitis. Researchers have concentrated on the identification of genetic polymorphisms in several aspects of immunity. Allelic variants at multiple gene loci probably influence periodontitis susceptibility [5].

Several genetic studies have shown that the human leukocyte antigen (HLA) region on chromosome 6p21.31 is an important factor associated with different diseases [6]. Many studies in Iraq reported associations between HLA and some diseases and cancers [7-14]. The HLA component of the immune system, encoded by highly polymorphic genes that vary across racial/ethnic groups, has been suggested to be a biologically based risk factor for periodontitis and thus may explain some of its variation by race/ethnicity. Due to the crucial role played by HLA class II molecules in antigenic peptide presentation to T helper cells in both the blood periphery and during thymic selection and education, components of the HLA class II region (DRB1 and DQB1) have been associated with periodontitis [15]. A number of HLA- class II alleles have been reported to be associated with the occurrence of periodontitis, while others were reported to be associated with protection against the disease [16-18]. So, this study was established to shed light on

the possible association of HLA-DQB1 alleles with chronic periodontitis in a sample of Iraqi patients compared with healthy controls.

### MATERIALS AND METHODS

The test group consisted of forty Iraqi patients with chronic periodontitis, with an age range of 22 to 50 years. They were selected among people referring to periodontics departments in College of Dentistry, Baghdad University for diagnosis and treatment of periodontitis from February 2017 till April 2017, who were volunteers to participate in this study. Diagnosis was made by specialized dentists (single examiner conducted the periodontal assessment in order to minimize the variation in the data). The control group included 40 healthy unrelated patients, age and sex matched (age range of 19-45 years). They were from the staff and graduate students of College of Dentistry. Plaque index (PI), gingival index (GI), probing pocket depth (PPD) and clinical attachment level (CAL) were employed as clinical periodontal parameters in this study.

Three ml of blood were withdrawn from each subject under aseptic technique, then transferred into EDTA tube, kept at -20°C for the genotyping of HLA class II. The DNA was extracted by using the genome DNA extraction kit (Qiagene/Germany). All DNA was stored at -20°C until tested. HLA-DQ genotyping were performed by the PCR-SSO according to the manufacturer's instructions, this method depends on reverse hybridization, using the PCR-SSO kit (Histo Type/ DNA-SSO Kits-Innogenetics Line Probe Assay, INNO-LiPA, Belgium).

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) along with the 95% confidence interval (95% CI). The significance of these differences was assessed by Fisher's exact probability (P). P values of <0.05 were considered statistically significant.

### RESULTS

The study involved 80 individuals, 40 of whom were patients with chronic periodontitis, while 40 controls were healthy individuals. At the time of sample collection, the ages of the patients and controls ranged between 19 and 50 years (20% males and 20% females by patients and 55% males and 45% females by controls). No differences were observed between patients and controls according to gender and age; moreover, 25% of patients had positive family history. Periodontal clinical parameters of the samples are presented in Table 1.

**Table 1 Characteristics of patients with chronic periodontitis and control population**

Variables	Control (40)	Periodontitis (40)
Gender (M/F)	22 / 18	20 / 20
Frequency	55% / 45%	50% / 50%
Age (years, mean ± SD)	40.06 ± 4.6	43.8 ± 5.9
Family history	—	10 (25%)
Plaque index	0.67 ± 0.45	2.16 ± 0.64
Gingival index	0.57 ± 0.31	1.98 ± 0.50
Mean probing depth	1.8 ± 0.52	2.9 ± 0.61
Clinical attachment loss	—	3.18 ± 1.04

M: Male; F: Female; SD: Standard Deviation

Allele frequencies of HLA-DQB1 for patients and control are shown in Table 2. The frequency of HLA-DQB1\*05:02 allele was significantly higher in patients compared with controls group (25% and 7.5% respectively) with P=0.04 and odds ratio=4.11. In contrast, the frequency of DQB1\*03:01 allele was significantly increased among controls as compared to patients (P=0.04, odds ratio=4.11). Other alleles like HLA-DQB1\* 04:01 and 06:18 were detected in patients group and not in control group.

**Table 2 Human leukocytes antigens (HLA-DQB1) allele's frequencies in patients with periodontitis and healthy control groups.**

HLA-DQB1* allele	Healthy control N=40	%	Periodontitis patients N=40	%	Odds ratio (95% confidence interval)	P (Fisher's exact)
02:01	7	17.5	7	17.5	1 (0.27 - 2.98)	1
02:02	2	5	5	12.5	0.37 (0.07 - 2.02)	0.25
03:01	10	25	3	7.5	4.11 (1.04 - 16.30)	0.04*
03:02	4	10	5	12.5	0.9 (0.25 - 2.78)	0.31

03:03	8	20	8	20	1 (0.319 - 3.14)	1
03:04	12	30	0	0	NA	NA
04:01	0	0	11	27.5	NA	NA
05:01	8	20	0	0	NA	NA
05:02	10	25	3	7.5	4.11 (1.04 - 16.30)	0.04*
05:03	8	20	8	20	1 (0.32 - 3.14)	1
06:01	2	5	5	12.5	0.37 (0.07 - 2.02)	0.25
06:02	3	7.5	4	10	1.37 (0.29 - 6.56)	0.7
06:09	6	15	7	17.5	0.84 (0.25 - 2.74)	0.77
06:18	0	0	15	37.5	NA	NA

N: number; NA: Not Applicable

## DISCUSSION

Periodontitis disease is a multifactorial in nature. While microbial and other environmental factors are believed to initiate and modulate periodontal disease progression, there now exist strong supporting data that genetic polymorphism play a role in the predisposition to and progression of periodontitis [19]. The HLA system plays an important role in regulating immune response because it takes part in the defence against pathogens [20]. However; very few studies have been conducted in Iraqi population evaluating the role of HLA-genes that play role in the pathogenesis of periodontal disease. Only two prior studies to date have been conducted investigation the association between HLA and chronic periodontitis specifically [21,22].

In previous studies, conflicting results have been presented regarding the relationship between the HLA-DQ genotype and susceptibility to chronic periodontitis in different populations. This conflict might be due to differences in the racial background, choice of controls and disease diagnostic. In the current study, to obviate bias in the results, the controls subjects were selected included only after oral examination. Our study found that a greater frequency of HLADQB1\*05:02 ( $P=0.04$  and odds ratio=4.11) in the patients group compared with controls group, this means that HLADQB1\*05:02 allele considered to be a risk candidate for developing disease, while DQB1\*03:01 allele is a protective allele.

A study done by Mousavi Jazi, et al. [23] revealed that the frequencies of HLA-DQB1\*03:02 and HLA-DQB1\*03:05 alleles were significantly higher in Iranian patients with periodontitis compared with control subjects, whereas the frequency of the HLA-DQB1\*06:03 allele was significantly lower in patients compared with controls. Takashiba et al. [16] reported that German patients with early onset periodontitis expressing high frequency of DQB1\*0602 genotype, and those patients may have an accelerated T-cell response to *Porphyromonas gingivalis* and thus increased susceptibility to this disease. On the other hand, in Japan study conducted by Hideki, et al. [24], observed that DQB1\*05:03, and DQB1\*06:02 were found more frequently “susceptible” in the early onset periodontitis patients than in healthy controls. In contrast, DQB1\*04:01 was found less frequently “resistant” in patients. They concluded that the DQB1 molecule plays a crucial role in the pathogenesis of periodontitis and that the susceptibility of disease may be determined by the binding ability between the peptide and HLA-DQ antigens. In consistent to the present findings Reichert and associates [25] reported that the HLA-DQB1\*03 was significantly lower in patients with periodontitis than that in control. Other study reported that HLA-DRB1\*04/DQB1\*03:02 haplotype had a decreased colonization risk of *Aggregatibacter actinomycetemcomitans* in chronic periodontitis [26]. It is well known that HLA surface molecules have a key role in antigen presentation and activation of T-cells. The polymorphisms of HLA can directly affect the binding capability of antigen peptides and thus affect the antigen-specific T-cell response. Hence, these polymorphisms could represent an important susceptibility or resistance factor to periodontitis [27].

## CONCLUSION

The current study shows a correlation between the HLA-DQB1 locus and the occurrence of chronic periodontitis in Iraq, supporting DQB1\*05:02 as a predisposing allele for this disease.

## CONFLICT OF INTEREST

We declare that we have no conflict of interests.

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