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Genotyping of HLA of Class I and II Alleles by PCR-SSO in Gingivitis

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Abstract

Background: Gingivitis is a frequent inflammatory process of the gum tissue that is mainly caused by the accumulation of plaque. Genetic constitution of hosts seems to play a crucial role in periodontal disease.

Aims: This study was performed to investigate the association HLA- class I (A and B) and II (DR) alleles with gingivitis and to identify genes that confer susceptibility or resistance to develop the gingivitis.

Materials and Methods: Thirty patients with gingivitis their age range (22-50) years and 18 healthy controls (20-50) years were enrolled in this study. Plaque index (PI) and gingival index (GI) were employed as clinical parameters. Blood was collected from patients and controls, DNA was extracted from blood samples, and then HLA-class I and class II genotyping was performed by polymerase chain reaction-sequence specific oligonucleotide probes (PCR-SSO).

Results: The current findings revealed that the frequency of HLA*23 allele was significantly decreased in patients when compared to controls (0.0% vs. 16.6%; OR=0.1; *P*=0.047). While the frequency of HLA-DRB1*03 allele was significantly higher in patients than controls (50.0% vs. 22.2%; OR= 3.5; *P*=0.041). Furthermore, HLA-B locus testing did not reveal significant association with the disease however, the frequency of HLA-B*51 allele was higher in patients as compared to controls (30.0 vs. 16.6%; OR=2.6), but statistically not significant (*P*>0.05).

Conclusions: HLA-DRB1*03 allele might contribute to the increased susceptibility to gingivitis and HLA-A*23 could be a protective marker against the disease.

Keywords: *periodontal disease, gingivitis , HLA-class I and II, PCR-SSO.*

INTRODUCTION

Gingivitis is a non-destructive disease that occurs around the teeth. It is a common and mild form of gum disease (periodontal disease) that causes irritation, redness and inflammation of gingiva (1). The most common form of gingivitis, and the most common form of periodontal disease overall, is in response to bacterial biofilms (also called plaque) that is attached to tooth surfaces, termed plaque-induced gingivitis (2). However, without treatment, gingivitis can progress to periodontitis, in which the inflammation of the gums results in tissue destruction and bone resorption around the teeth. Periodontitis can ultimately lead to tooth loss (3, 4).

Generally, it has been assumed that poor dental hygiene is the only cause of gingivitis. However, that's not the case. Interestingly, genetics tends to play a role in the susceptibility (5). Studies reported that the risk of contracting gingivitis is higher when someone within the nuclear family, like the parents, also developed gingivitis. According to a 2000 study, genetic factors may play a critical role in about half of the cases of periodontal disease. Up to 30% of the population may have some genetic susceptibility to periodontal disease (6). However, genetic tests can now be used to determine susceptibility, and early intervention can help keep the oral cavity healthy.

The major histocompatibility complex (MHC), also known in humans as the human leukocyte antigen (HLA) region, located on chromosome 6 and intimately involved in regulation of the immune response, is the most polymorphic gene complex found in the human genome. HLA class I (A, B, and C) molecules are expressed on all nucleated cells and platelets, and are recognized by CD^{8+} cytotoxic T cells. While HLA class II (DR, DQ, and DP) molecules are expressed by antigen-presenting cells. Their function is to present exogenous antigens to CD^{4+} helper T cells (7, 8). Several HLA types are associated with an increased risk of various diseases. HLA and disease associations have been widely studied across the populations worldwide and are found to be important in prediction of disease susceptibility, resistance or protective (9). The HLA have been considered candidate markers for periodontal disease because they are involved in regulating immune responses. While many studies have shown associations of HLA polymorphisms with aggressive and chronic periodontitis in different populations (10–16), little is known about the role of classical HLA-alleles in determining resistance or susceptibility to gingivitis. This prompted us to investigate the association HLA-class I (A and B) and II (DR) alleles with gingivitis and to identify genes that confer susceptibility or resistance to develop the gingivitis.

MATERIALS AND METHODS

Thirty patients with gingivitis (15 males and 15 females), age range 22-50 years were enrolled in this study. They were selected among people referring to periodontics departments in College of Dentistry, Baghdad University from February 2017 till April 2017, who were volunteers to participate in this study. Diagnosis was made by specialized dentists. The control group included 18 healthy individuals, their ages and genders were matched with the patients (9 males and 9 females), age range 20-45 years. Plaque index (PI) and gingival index (GI) were employed as clinical parameters in this study.

Three ml of Whole blood were withdrawn from each subject under aseptic technique, then transferred into EDTA tube, kept at -20°C for the DNA extraction then study genotyping of HLA class I and II. The DNA was extracted by using the genome DNA extraction kit (Qiagene/Germany). All DNA was stored at -20°C until tested. HLA-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). 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 present study revealed that there is no significant difference in age and sex between patients and controls (*P*>0.05). The mean age of patients was (36.6 ± 1.3) years while in control was (33.2 ± 1.5) years. The mean values of PI and GI in patients (1.2 ± 0.2 and 1.3 ± 0.3) are significantly higher than that of healthy control (0.5 ± 0.1 and 0.5 ±0 .2), as shown in table (1).

The current findings revealed that within the HLA-A locus the frequency of HLA*23 allele was significantly decreased in patients when compared to controls (0.0% vs. 16.6%; OR=0.1; *P* =0.047), table (2). While within the HLA-DR locus the frequency of HLA-DRB1*03 allele was significantly higher in patients than controls (50.0% vs. 22.2%; OR= 3.5; *P* =0.041)table (4). On the other hand, HLA-B locus testing did not reveal significant association with the disease however, the frequency of HLA-B*51 allele was higher in patients as compared to controls (30.0 vs. 16.6%; OR=2.6), but statistically not significant (*P* >0.05), table (3).

Table -1: Demographic and clinical characteristics two studied groups

	Patients (30)	**Controls (18)**	***P-Value***
Demographic characteristics			NS
Age range	(22-50)	(20-50)	
Mean ± SE	36.6 ± 1.3	33.2 ± 1.5	
Gender			
Female	15 (50%)	15 (50%)	
Male	9 (50%)	9 (50%)	
Clinical characteristics			
Plaque index (PI)	1.2 ± 0.2	0.5 ± 0.1	P<0.05*
Gingival index (GI)	1.3 ± 0.3	0.5 ±0 .2	p<0.05*

Table-2: HLA-A genotypes in gingivitis patients and healthy control.

	Gingivitis patients (30)		**Controls (18)**		**Odds ratio (OR)**	**P (Fisher's exact)**
HLA-A* Alleles	**N**	**%**	**N**	**%**		
HLA-A*01	6	20.0	2	11.1	2.0	NS
HLA-A*02	3	10.0	1	5.5	1.9	NS
HLA-A*03	9	30.0	2	11.1	3.4	NS
HLA-A*11	6	20.0	3	16.6	1.3	NS
HLA-A*23	0	0.0	3	16.6	0.1	p<0.047*
HLA-A*30	3	10.0	4	22.2	0.4	NS
Blank	33		21			

Table-3: HLA-B genotypes in gingivitis patients and healthy control.

	Gingivitis patients (30)		**Controls (18)**		**Odds ratio (OR)**	**P (Fisher's exact)**
HLA-B*-Alleles	**N**	**%**	**N**	**%**		
HLA-B*07	3	10.0	2	11.1	0.9	NS
HLA-B*15	9	30.0	2	11.1	3.4	NS
HLA-B*27	3	10.0	4	22.2	0.4	NS
HLA-B*35	9	30.0	5	27.7	1.1	NS
HLA-B*39	9	30.0	4	22.2	1.5	NS
HLA-B*41	6	20.0	4	22.2	0.9	NS
HLA-B*44	6	20.0	6	33.3	0.6	NS
HLA-B*51	9	30.0	3	16.6	2.6	NS
HLA-B*52	4	13.3	4	22.2	0.6	NS
Blank	2		2			

Table-2: HLA-DR genotypes in gingivitis patients and healthy control.

	Gingivitis patients (30)		**Controls (18)**		**Odds ratio (OR)**	**P (Fisher's exact)**
HLA-DR*-Alleles	**N**	**%**	**N**	**%**		
HLA-DR*01	6	20.0	3	16.6	1.3	NS
HLA-DR *03	15	50.0	4	22.2	3.5	p<0.041*
HLA-DR *04	9	30.0	3	16.6	2.6	NS
HLA-DR *07	3	10.0	4	22.2	0.4	NS
HLA-DR *09	2	6.6	3	16.6	0.9	NS
HLA-DR *10	3	10.0	3	16.6	0.6	NS
HLA-DR *11	9	30.0	2	11.1	3.4	NS
HLA-DR *13	3	10.0	3	16.6	0.6	NS
HLA-DR *14	3	10.0	1	5.5	1.9	NS
HLA-DR *15	6	20.0	2	11.1	2.0	NS
Blank	1		8			

DISCUSSION

Human leukocyte antigens are important components of host defense against microbial challenge. It is known to play a role in the aetiopathogenesis of a number of diseases including periodontal disease. Numerous studies have shown associations between HLA polymorphisms and periodontitis in different populations (10, 17, 18), but there are no studies available on HLA association with gingivitis. To the best of our knowledge, this is the first study of HLA association with gingivitis in the Iraqi population.

The present study found that the frequency of DRB1*03 allele was significantly higher in patients than controls. The OR for this allele was (3.5), this mean that the individuals with *03 allele have 3.5 times greater chance of acquiring gingivitis than those of the same population who lack it. While the frequency of HLA-A*23 was significantly decreased in patients when compared to controls. Mauramo *et al*., conducted a prospective clinical study examined associations of HLA-A, -B and -DRB1 types with oral diseases in a generally healthy Swiss adult population. The study focused particularly on the two most prevalent oral diseases, caries and periodontal disease. It showed that HLA-B*15, HLA-B*51 and HLA-DRB1*12 were associated with less periodontal disease manifestations. So they suggested that HLA types may contribute to the development of oral diseases in generally healthy Caucasian adults (19). In our study also found that HLA-B*51 was higher in gingivitis but statistically not significant and this could confirm the role of this allele in susceptibility to periodontal disease.

Interestingly, higher frequency of HLA-DRB1*03 allele among gingivitis patients in this study was previously reported in periodontitis (13, 16), this may be indicate that this allele has a crucial role in progression of gingivitis to periodontitis. On the other hand, Stein and associates found that HLA-A*2 was potential protective factors against periodontitis (20). However, Tian and colleagues (21) conducted genome wide association studies on 23 infectious diseases including gingivitis and explore the relationship between individual HLA alleles and susceptibility to infectious diseases, and did not detect associations between HLA loci and gingivitis and they explained the reason for these results may be due to small cohort size. 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 Ag-peptides and thus affect the Ag-specific T-cell response. Hence, these polymorphisms could represent an important susceptibility or resistance factor to periodontal disease (Sippert *et al.,* 2015). In concussion HLA-DRB1*03 allele might contribute to the increased susceptibility to gingivitis and HLA-A*23 could be a protective marker against the disease.

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Conflicts of interest

The authors and planners have disclosed no potential conflicts of interest, financial or otherwise.

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