The Association of HLA-A Gene Polymorphisms with Chronic Periodontitis in Iraqi patients

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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.

Objective: To investigate the association of human leukocyte antigens class I genotype (HLA-A) and the susceptibility and severity of chronic periodontitis in Iraqi patients.

Patients and Methods: The study groups included 50 patients with chronic periodontitis and 20 healthy controls with clinically healthy periodontium. Periodontal parameters used in this study were plaque index (PI), gingival index (GI), probing pocket depth (PPD), clinical attachment level (CAL) and bleeding on probing (BOP). Five ml of venous blood were collected from each participant. DNA was extracted from blood samples, and HLA-A genotyping was performed by polymerase chain reaction-sequence specific oligonucleotide probes (PCR-SSO).

Results: The present data revealed that the frequencies of HLA-A*33 was significantly higher in patients than in healthy controls (P= 0.0268).

Conclusion: This study suggests that HLA-A*33allele may contribute to the increased susceptibility to chronic periodontitis.

Key words: Chronic periodontitis, Genetic factors, human leukocyte antigens, PCR-SSO.

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Introduction

Periodontitis is an infectious and inflammatory disease involves the deeper periodontium resulting in the clinical attachment loss with the destruction of gingiva, periodontal ligament, cementum and alveolar bone [1]. The primary hallmark of periodontitis, is the destruction of periodontal tissue, that is lead to the host immune inflammatory response [2]. The pathophysiology of periodontitis includes interactions between genetic predisposition dependent on many genes and environmental factors. Chronic periodontitis (CP) is one of the most common chronic inflammatory diseases characterized by progressive destruction of the supporting tissues of the teeth [3].Investigations on susceptibility factors of periodontitis have focused mainly on genes that modulate

immunoregulation, such as cytokines, chemokines, cell-surface receptors, enzymes and proteins related to antigen recognition [4]. Certain variants of these genes may predispose to periodontitis or influence the progression of the disease [5][6]. Host susceptibility thus may largely be defined in terms of the genetic makeup of an individual or ethnic group [7].

Human leukocyte antigens (HLAs) are the major histocompatibility complex (MHC) in humans. They are membrane bound glycoproteins or proteins that are present on each cell surface and allow the immune system to recognize “self” from “foreign” [8][9]. Thus, they play an important role in immune responsiveness [10]. Numerous studies in this regard revealed inconsistent results [11][12][13] [14][15][16][17].

However, HLA-A*9 allele or antigen has been found to be risk factor for destructive forms of periodontal disease(PD) in a number of studies conducted in different populations[18][19]. In particular, it found to be consistently associated with aggressive periodontitis in Caucasians [12] [20]. In contrast, the HLA-A*2 and HLA-A*28 are found to be potential protective factors against periodontitis among the same population [12]. HLA-A*30 seemed to associate with protection against aggressive periodontitis in Lebanese [15]. Resistance to periodontal disease was shown to be associated with HLA-A*10, HLA-A*2 and HLA-A*1[10]. Therefore, this study was performed to investigate the association of HLA-A with the susceptibility and severity of chronic periodontitis in Iraqi patients.

Patients and Methods

This study was approved by the Ethics Committee of College of Dentistry / Baghdad University. Fifty Iraqi patients with CP (30 males and 20 females) were enrolled in this study, with an age range of 30 to 50 years were attending the Iraqi National Blood Bank and the laboratories of Al-Yarmouk Teaching Hospital in Baghdad from May to October 2015.The diagnosis was done by assessing and recording at least four sites with probing pocket depth (PPD) ≥ 4 mm and clinical attachment level (CAL) of (1-2 mm) or more according to the international classification system for periodontal disease [21]. Patients were classified into three groups according to the severity of CAL as follows [22]:-GroupI: Fifteen patients with mild CP (CAL of 1-2mm), GroupII: Fifteen patients with moderate CP (CAL of 3-4mm), and GroupIII: Twenty patients with severe CP (CAL of ≥5mm).The control group comprised twenty Iraqi healthy volunteers with clinically healthy periodontium (10 males and 10 females) with an age range of (25-45) years,all of whom had no history or clinical signs of gingivitis and/or periodontitis.

Clinical oral examination was performed for all patients by the same examiner. All periodontal variables were recorded considering four surfaces (buccal/labial, lingual/palatal, mesial and distal) for all teeth except the 3rd molars, the examination was done using Michigan O probe with William’s markings at 1,2,3,5,7,8,9 and 10 mm. Periodontal parameters used in this study were PI, GI, PPD, CAL and BOP.

Five ml of venous blood was withdrawn from each subject under aseptic technique and transferred into EDTA tube (1.5 mg / ml) and kept at –70°Cfor the HLA- A genotyping. DNA was extracted from blood samples by using DNA IQ™ Casework Pro Kit for Maxwell® 16 and Casework extraction kit (Promega /USA). HLA-A genotyping was performed by the PCR-SSO according to the manufacturer's instructions. This method depends on reverse hybridization, using the amplification kits (INNO-LiPA HLA-B Multiplex Plus 100 and

PCR-SSO kits INNO-LiPA HLA-A Update Plus 100) (Innogenetics/ Belgium).

Statistical analysis

Data were submitted to both descriptive and inferential statistical analysis using SPSS v.21 program. The results were presented in terms of percentage frequencies. Chi-square test was used to compare the distribution of different alleles between patients and controls. The risk association with individual allele was calculated as odds ratio (OR) with 95% confidence interval (CI), P values of $0.05 \geq P > 0.01$ were considered significant (S) and $P \leq 0.01$ were considered highly significance (HS).

Results

The demographic variables and clinical parameters (PIL and GI) of the 70 subjects enrolled in this study are illustrated in table-1.

Table (1): Demographic variables and clinical parameters (PLI and GI) in the study and control groups.

			Control No.=20	Chronic Periodontitis No.=50	P-value
Age	Range		25-45	30-50	0.1243
	Mean		34.05	36.08	
	±SD		6.228	4.323	
Gender	Male	No.	10	30	0.5
		%	50%	60%	
	Female	No.	10	20	
		%	50%	40%	
PLI	Mean		0.444	1.819	< 0.0001**
	± SD		0.429	0.366	
GI	Mean		0.372	1.634	< 0.0001**
	± SD		0.358	0.383	

: High significant , **NS: no significant

The number and percentage distribution of different sites according to the presence or absence of BOP of the chronic periodontitis subgroups are shown in table 2.

Table (2): The number and percentage distribution of sites according to the presence or absence of BOP of the CP subgroups**.**

CP subgroups		BOP		Chi	df	p-value
		Score 0	Score 1			
Mild No. =1524	No.	788	736	388.9	5	< 0.0001**
	%	51.71 %	48.29%			
Moderate No. =1476	No.	604	872			
	%	40.92%	59.08 %			
Severe No. = 1832	No.	361	1471			
	%	19.71%	80.30%			

Considering the PIL, GI, PPD and CAL among the three subgroups of chronic periodontitis, a highly significant difference was observed p-value <0.0001, as seen in (table-3).

Table (3): The mean values and SD of the PLI, GI, PPD and CAL of the CP subgroups.

		Mild No. = 15	Moderate No. = 15	Severe No. = 20	F-test	p-value
PLI	Mean	1.551	1.790	2.043	11.03	0.0001**
	± SD	0.375	0.321	0.236		
GI	Mean	1.433	1.569	1.833	5.98	0.0049**
	± SD	0.377	0.328	0.344		
PPD	Mean	1.519	3.313	4.744	387	<0.0001**
	± SD	0.317	0.283	0.390		
CAL	Mean	1.8134	3.680	5.941	669.7	<0.0001**
	± SD	0.164	0.2	0.474		

HLA-A genotyping revealed a significant difference in the frequency of HLA-A*33 allele between patients (22%) and controls (0.0%) Table-4.

Table (4): HLA-A allele frequencies in patients with CP and healthy controls.

		Chronic Periodontitis (50)		Control (20)					P (Fisher's exact)
HLA-A alleles	Total numbers (%)	No.	%	No.	%	OR	EF	PF	
01	4	3	6	1	5	1.213	0.0105	**	NS
02	29 (20.71%)	19	38	10	50	0.613	**	0.1935	NS
03	11 (7.86%)	6	12	5	25	0.409	**	0.1478	NS
11	12 (8.57%)	8	16	4	20	0.762	**	0.0476	NS
23	7 (5%)	7	14	0	0.0	7.069	0.1202	**	NS
24	24 (17.14%)	17	34	7	35	0.957	**	0.0150	NS
26	7 (5%)	5	10	2	10	1.000	**	**	NS
29	3 (2.14%)	3	6	0	0.0	3.021	0.0401	**	NS
30	7 (5%)	5	10	2	10	1.000	**	**	NS
31	8 (5.71%)	4	8	4	20	0.348	**	0.1303	NS
33	11 (7.86%)	11	22	0	0.0	11.937	0.2016	**	0.0268*
34	2 (1.43%)	2	4	0	0.0	2.113	0.0211	**	NS
68	14 (10%)	9	18	5	25	0.659	**	0.0852	NS
69	1 (0.71%)	1	2	0	0.0	1.242	0.0039	**	NS
Total	140/ (100%)	100		40					

**Can not be calculated, equal to zero or not applicable

*: significant

Furthermore, this study also investigates the frequencies of HLA-A alleles in each subgroup of CP patients and healthy control. The comparison between severe CP patients and healthy control showed significant difference in the frequency of HLA-A*03 allele which had higher frequency in the control group (OR=0.069 and P=0.047), as well as a significant increase was also observed in the frequency of HLA-A*23 allele in severe CP patients with frequency (25%), (OR=14.548 and P=0.047), as shown in (table-5). Interestingly, when CP patients were divided into three subgroup according to the severity, the prevalence of HLA-A*33 allele frequency in the group with moderate CP remained significantly higher than those of control group (26.67%), (OR=16.044 and P=0.026), (table-6). However, there was no significant difference in all HLA-A alleles between mild CP patients and healthy controls as described in (table-7).

Table (5): HLA-A alleles frequencies in patients with severe CP and healthy controls.

	Severe(20)		Control(20)					
HLA-A alleles	No.	%	No.	%	OR	EF	PF	P (Fisher's exact)
01	0	0	1	5	0.317	**	**	1.000
02	10	50	10	50	1.000	**	**	1.000
03	0	0	5	25	0.069	**	**	0.047*
11	5	25	4	20	1.333	0.0625	**	1.000
23	5	25	0	0	14.548	0.2328	**	0.047*
24	9	45	7	35	1.520	0.1540	**	0.748
26	3	15	2	10	1.588	0.0555	**	1.000
29	1	5	0	0	3.154	0.0342	**	1.000
30	1	5	2	10	0.474	**	0.0526	1.000
31	0	0	4	20	0.089	**	**	0.106
33	4	20	0	0	11.182	0.1821	**	0.106
34	1	5	0	0	3.154	0.0342	**	1.000
68	1	5	5	25	0.158	**	0.2104	0.182
Total	40		40					

Table (6): HLA-A allele frequencies in patients with moderate CP and healthy controls.

	Moderate (15)		Control (20)					
HLA-A alleles	No.	%	No.	%	OR	EF	PF	P (Fisher's exact)
01	1	6.67	1	5	1.357	0.0175	**	1.000
02	5	33.33	10	50	0.500	**	0.2498	0.492
03	2	13.33	5	25	0.462	**	0.2150	0.672
11	1	6.67	4	20	0.286	**	0.1428	0.365
23	1	6.67	0	0	4.241	0.0510	**	0.429
24	5	33.33	7	35	0.929	**	0.0248	1.000
26	2	13.33	2	10	1.385	0.0371	**	1.000
29	2	13.33	0	0	7.593	0.1157	**	0.177
30	1	6.67	2	10	0.643	**	0.0357	1.000
31	0	0	4	20	0.118	**	**	0.119
33	4	26.67	0	0	16.044	0.2501	**	0.026*
34	1	6.67	0	0	4.241	0.0510	**	0.429
68	4	26.67	5	25	1.091	0.0222	**	1.000
69	1	6.67	0	0	4.241	0.0510	**	0.429
Total	30		40					

Table (7): HLA-A allele frequencies in patients with mild CP and healthy controls.

	Mild (15)		Control (20)					
HLA-A alleles	No.	%	No.	%	OR	EF	PF	P (Fisher's exact)
01	2	13.33	1	5	2.923	0.0877	**	0.565
02	4	26.67	10	50	0.364	**	0.3179	0.296
03	4	26.67	5	25	1.091	0.0222	**	1.000
11	2	13.33	4	20	0.615	**	0.0770	0.680
23	1	6.67	0	0	4.241	0.0510	**	0.429
24	3	20	7	35	0.464	**	0.1597	0.458
26	0	0	2	10	0.239	**	**	0.496
30	3	20	2	10	2.250	0.1111	**	0.631
31	4	26.67	4	20	1.455	0.0834	**	0.700
33	3	20	0	0	11.480	0.1826	**	0.070
68	4	26.67	5	25	1.091	0.0222	**	1.000
Total	30		40					

Discussion

Genetic backgrounds play a key role in susceptibility to and protection against a spectrum of PD. Due to the extensive polymorphism of HLA gene, inter-individual differences in immune response against bacterial antigens can be assumed. The HLA have inconsistent results in aetiopathogenesis of PD in several studies [5][19][28][29]. While many studies have shown associations of HLA polymorphisms with aggressive periodontitis in different populations [11][30]. To the best of our knowledge, this is the first study of HLA class I association with CP in an Iraqi population.

In this study, HLA-A*33 was significantly higher in overall patients and those with moderate CP than controls. while HLA-A*23 allele was significantly higher in severe CP patients as compared to controls. Different results regarding this association was reported in different population. In Libya, Ben-Darif and colleagues observed that HLA-A*30 allele was significantly increased in the frequency among Libyan CP patients as compared to healthy control, but there was non-significant difference of HLA-A*33 in patients with CP as compared to healthy control [31]. On the other hand, other studies [13][17] found significant higher frequencies of HLA-A*11 and HLA-A*29 alleles in CP patients as compared to healthy control. This disparity among several results might be attributed to the effect of ethnicity and racial background on the frequency of HLA alleles, as well as differences in methodology and sample size.

A possible role of HLA in the immune response and development of PD may be related to their ability to bind some processed peptides from bacterial antigens and expressing them on the surface of antigen presenting cells (peptide-HLA class II) or target cells (peptide-HLA class I) in order to present them to T cells (CD4 or CD8) [32]. The binding capacity of the bacterial peptide depends on HLA allotypic structure of their paratope. Failure in this link capacity can compromise the immune response and eventually predispose for different diseases. Furthermore, 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 [33].

Non-significant differences were observed between patients and controls according to gender and age, but there was a predominance of CP among males than in females. Increased prevalence of PD among

males may be due to their less interest and to the ignorance of their oral hygiene rather than females [23]. A highly significant difference was observed between the control group and CP group regarding PLI and GI. This finding is in agreement with the results of studies done by [16][24][25]. Considering the CP subgroups, highly-significant difference was found in all periodontal parameters among the three CP subgroups. These results are consistent with the results of other Iraqi study conducted by [26]. These results are explained by the fact that the microbial biofilm is considered the primary and the major etiological factor responsible for initiation of PD and it is expected to be accumulating more in CP patients [27]. That leads to dilation of arterioles, capillary and venules of dentogingival plexus resulting in increase in permeability of microvascular bed which lead to more gingival inflammation [1].

In conclusion these results give additional evidence that class I (HLA-A) polymorphisms are associated with susceptibility to against CP. Significant higher frequency of HLA-A*33 allele (in total CP and moderate CP patients) and HLA-A*23 allele in severe CP patients as compared to controls emphasize the role of this allele in disease susceptibility or as a risk factor for CP. Further investigations, with larger and diverse populations are required to clarify such association.

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