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Association of HLA Class II Alleles (DRB1 and DQB1) in Iraqi Women with Endometriosis

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Abstract: Endometriosis is a chronic gynecological disease manifested by the occurrence of ectopic foci of endometrial tissue in the pelvic cavity and/or ovary. Etiopathogenesis of the disease is still poorly understood but there is a growing bulk of evidence that genetic factors and immunological abnormalities play a role in this disease. This study was performed to investigate the association of human leukocyte antigens class II genotypes (HLA-DR and DQ) with the susceptibility to endometriosis. Fifty female patients with endometriosis their age range (19 – 46) years and 30 females as control their ages were matched with the patients were enrolled in this study. Blood was collected from patients and controls, DNA was extracted from blood samples, and then HLA- genotyping was performed by polymerase chain reaction-sequence specific oligonucleotide probes (PCR-SSO). The present findings showed that DRB1*0307, DRB1*0701 and DQB1*0301 alleles were found with highly significant frequencies among patients (22 %; 34% and 24 %), in comparison with healthy control (0.0%) for three alleles, ($P < 0.001$). On the other hand, it was observed that HLA-DRB1*0323 in high frequency among healthy individuals (23.3%) rather than in the patients (0.0%); with ($P < 0.001$).
This study demonstrates that HLA- DRB1*0307, DRB1*0701 and DQB1*0301 alleles may contribute to the increased susceptibility to endometriosis, whereas HLA-DRB1*0323 allele may confer protective effects against it, suggesting HLA-based different etiopathogenesis.

Key words: Endometriosis, HLA, Genotyping.

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Introduction

Endometriosis is a common disorder defined as the growth of endometrial tissue outside the uterine cavity that causing diverse conditions, including infertility, pelvic pain, dysmenorrhea and constipation (1). This disease was first identified and described in 1860 by the Austrian pathologist von Rokitansky, regardless of the fact that it is quite common among women, it is

frequently misdiagnosed (2). The exact incidence of endometriosis in the population is not clear, but reports estimates normally range from 10% to 15% for the general female population; in women with pain, infertility or both, the frequency increases to 35-60%.

Though the aetiopathogenesis of the disease is still poorly understood, there is evidence showing that genetic, endocrine, immunological, and environmental factors play an important role in the genesis and development of edometriosis (3,4). It is well known that genetic contribution plays a significant role on susceptibility to endometriosis, but the genetic markers associated with endometriosis have not been fully elucidated.The major histocompatibility complex (MHC) has been considered candidate markers for endometriosis because they are involved in regulating immune responses. MHC contains over 200 genes in a 4-Mb region of chromosome 6. Approximately 40% of these genes have immunological functions; most notably the genes that encode the human leukocyte antigens (HLA). The HLA proteins are cell surface antigens that present peptides to T-cell receptors, distinguishing self from non-self peptides. However; more than 40 diseases have been associated with various HLAs (5).

HLA markers have been investigated in several studies determining individual susceptibility factors for endometriosis. In some studies that used serological typing methods no deviations in HLA class I or class II allele distribution were found among endometriosis patients compared to controls (6,7,8). However, other study in a Japanese population an increased frequency of class I, HLA-B54 and HLA-Cw7 has been reported (9). Meanwhile, studies employing molecular typing methods have been reported that endometriosis patients may be also associated with various HLA-class I and II alleles (10,9,11). This study was performed to investigate the association of human leukocyte antigens class II genotypes (HLA-DR and DQ) with the susceptibility to endometriosis.

Materials and Methods

Fifty female patients with endometriosis their age range (19 – 46) years and 30 females as control their ages were matched with the patients were enrolled in this study. They were from attendants to Kamal Al-Samari hospital and Baghdad medical city teaching hospital from June 2014 to January 2015. The diagnosis was made by the consultant medical staff, which was based on clinical and ultrasonic examinations. They were newly diagnosed and all of the cases had received no treatment with no complain of chronic or systemic diseases.

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 (DRB1 and DQB1). The DNA was extracted by using the genome DNA extraction kit (Qiagene/ Germany). All DNA was stored at -20°C until tested. HLA-DR and 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 fur-ther 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 (12).

Results and Discussion

This study was performed on 50 women patients with endometriosis and 30 healthy women. The demographic characteristics of patients group and controls group included in this study are presented in table (1). There are no statistical significant differences in age was existed between two study groups. The mean age of patients was 29.7 ± 0.94 years and for healthy controls was 29.6±1.40 year, (Table 1). Concerning the staging of endometriosis, stage 1 (minimal endometriosis) was found in 6 (12 %), stage 2 (mild endometriosis) consist of 13 (26 %), stage 3 (moderate endometriosis) was found in 26 (52%), while the rest 5 (10 %) were represented stage 4 (sever endometriosis). Regarding the family history of disease the current results showed that 3 (6%) of patients had positive family history of endometriosis, while 47 (94%) showed negative family history, as clearly observed in (Table 2).

Table -1: Ages distribution of the studied groups

Age		Study groups		P-value
		Endometriosis Patients n=50	Healthy control n=30	
Age (years)	Range	(19-46)	(20-41)	
	Mean	29.7	29.6	0.947 NS
	SE	0.94	1.40	
	Median	30	29	

SE= Standard error; NS=Non significant ($p>0.05$).

Table -2: Distribution of patients according staging of disease and family history

Clinical Features		Endometriosis Patients n=50
Staging	Stage 1	6 (12 %)
	Stage 2	13 (26 %)
	Stage 3	26 (52%)
	Stage 4	5 (10 %)
Family History	Positive	3 (6%)
	Negative	47 (94%)

n=Normal

Among HLA-DRB1 alleles the present findings noticed that DRB1*0307 and DRB1*0701 alleles were found with highly significant frequencies among patients (22 % and 34%), in comparison with healthy control (0.0%) for both alleles, (P <0.001). On the other hand, it was observed that HLA-DRB1*0323 in high frequency among healthy individuals (23.3%) rather than in the patients (0.0%); with (P <0.001), table (3). Regarding HLA-DQB1 alleles that have significant risk effect in a disease, there was DQB1*0301 which noticed in high frequency in patients (24 %) with significant differences in comparison with healthy control (0.0%) with (P <0.001), (Table 4).

Table -3: HLA-DR genotypes in endometriosis patients and healthy control

	Patients		Controls						
DR-Allele	N	%	N	%	OR	Inverse OR	EF	PF	P (Fisher's exact)
*0101	2	4%	2	6.6%	1.70	0.029	**	0.017	NS
*0301	2	4%	3	10%	2.65	0.064	0.00	0.00	NS
*0307	11	22%	0	0.00%	0.03	31.6	**	**	<0.001
*0309	2	4%	2	6.6%	1.70	0.029	**	0.017	NS
*0313	1	2%	2	6.6%	1.34	**	0.008	**	NS
*0317	1	2%	1	3.3%	0.66	1.5	**	0.009	NS
*0322	3	6%	1	3.3%	0.21	4.8	**	0.060	NS
*0323	0	0.0%	9	23.3%	11.00	0.156	**	**	<0.001
*0402	5	10%	3	16.6%	1.078	0.927	0.36	-0.57	NS
*0403	2	4%	0	0.0%	3.596	0.278	1.44	3.25	NS
*0405	2	4%	0	0.00%	3.596	0.278	1.44	3.25	NS
*0414	0	0.00%	1	3.3%	0.213	4.692	0.00	0.00	NS
*0701	17	34%	0	0.00%	5.0	0.00	0.400	0.00	<0.001
*0703	3	6%	3	10.0%	0.636	1.571	-1.71	0.63	NS
*0707	3	6%	1	3.3%	0.21	4.8	**	0.060	NS
*0708	2	4%	**5**	16.6%	1.596	0.627	1.87	2.15	NS
*0901	0	0.00%	1	3.3%	0.213	4.692	0.00	0.00	NS

	Patients		Controls						
DR-Allele	N	%	N	%	OR	Inverse OR	EF	PF	P (Fisher's exact)
*0902	2	4%	1	3.3%	1.140	0.877	0.25	-0.33	NS
*1001	1	2%	1	3.3%	0.66	1.5	**	0.009	NS
*1101	3	6%	2	6.6%	0.43	2.4	**	0.043	NS
*1102	1	2%	1	3.3%	0.66	1.5	**	0.009	NS
*1104	2	4%	2	6.6%	1.70	0.029	**	0.017	NS
*1106	1	2%	**3**	10%	2.05	**	0.026	**	NS
*1107	2	4%	1	3.3%	1.140	0.877	0.25	-0.33	NS
*1109	5	010%	2	6.6%	1.596	0.627	1.87	2.15	NS
*1112	1	2%	1	3.3%	0.66	1.5	**	0.009	NS
*1122	5	10%	1	3.3%	2.804	0.357	3.22	1.45	NS
*1301	2	4%	1	3.3%	1.140	0.877	0.25	-0.33	NS
*1303	1	2%	1	3.3%	0.66	1.5	**	0.009	NS
*1310	4	8%	2	6.6%	1.257	0.796	0.82	-4.46	NS
*1311	2	4%	1	3.3%	1.140	0.877	0.25	-0.33	NS
*1359	2	4%	0	0.00%	3.596	0.278	1.44	3.25	NS
*1410	2	4%	1	3.3%	1.140	0.877	0.25	-0.33	NS
*1501	2	4%	1	3.3%	1.140	0.877	0.25	-0.33	NS
*1505	3	6%	1	3.3%	0.21	4.8	**	0.060	NS
*1507	3	6%	**3**	10%	0.636	1.571	-1.71	0.63	NS
Total	50	100	30	100					

n=Normal
OR: odd ratio
EF: etiological fraction
PF: preventive fraction.

Table -4: HLA-DQ genotypes in endometriosis patients and healthy control

	Patients		Controls			Inverse			P (Fisher's
DQ-Allele	N	%	N	%	OR	OR	EF	PF	exact)
*0201	7	14%	6	20%	0.479	2.090	-4.36	0.81	NS
*0202	9	18%	6	20%	1.30	0.048	-2.83	0.74	NS
*0301	12	24%	0	0.0%	18.11	0.055	8.50	1.13	<0.001
*0302	7	14%	3	10%	1.078	0.927	0.36	0.57	NS
*0303	5	10%	4	13.3%	1.30	**	0.048	**	NS
*0304	3	6%	3	10%	0.636	1.571	-1.71	0.63	NS
*0305	4	8%	4	13.3%	2.5	0.109	**	**	NS
*0313	11	22%	9	30%	0.712	1.405	-2.83	0.74	NS
*0501	5	10%	3	10%	1.078	0.927	0.36	-0.57	NS
*0502	9	18%	8	26.6%	5.218	0.192	2.43	1.70	NS
*0503	11	22%	7	23.3%	0.661	1.513	-0.51	0.34	NS
*0601	3	6%	2	6.6%	0.43	2.4	**	0.043	NS
*0602	3	6%	1	3.3%	0.21	4.8	**	0.060	NS
*0603	4	8%	2	6.6%	1.257	0.796	0.82	-4.46	NS
*0604	3	6%	1	3.3%	0.21	4.8	**	0.060	NS
*0609	1	2%	1	3.3%	0.66	1.5	**	0.009	NS
*0618	3	6%	0	0.0%	3.596	0.278	1.44	3.25	NS
Total	50	100	30	100					

N:Normal
OR: odd ratio
EF: etiological fraction
PF: preventive fraction.

Endometriosis is classified into one of four stages (I-minimal, II-mild, III-moderate, and IV-severe). The most common stages in this study are the moderate and mild and this agreed with result reported by (13).The projection of genetic susceptibility represented by family history was observed in 6% of patients with endometriosis and this percentage which is in agreement with a study reported by (14) who found 5.9% of patients had positive family history. A study conducted in twins revealed that the incidence of this diasese in monozygotic twins was twice that in dizygotic twins (15). Moreover, it has been shown that the severity of endometriosis is higher among patients with a positive family history (16).

The HLA is known to play a role in the aetiopathogenesis of a number of diseases including endometriosis (17,18). This study was found higher frequencies of HLA-DRB1*0307, HLADRB1**0701 and HLA-DQB1*0301 in endometriosis patients as compared to healthy controls. Different results regarding this association was reported, results differ in different population. In Japan study conducted by Ishii and colleagues reported that high frequency of HLA-DQB1*0301 allele was observed among Japanese females with endometriosis as compared to healthy control, such study confirms the present findings, in which HLA-DQB1*0301 allele was significantly increased, as well as Ishii and colleagues suggested that the HLA systems may be involved in the aetiology of endometriosis. (19) showed that at HLA-DRB1*15 allele positive frequency of endometriosis group was significant higher as compared with those of the normal control group, they mention that the occurrence of endometriosis may be associated with the presence of HLA-DRB1 allele and HLA-DRB1*15 may be one of some factors for the pathogenesis of endometriosis (20). In another molecular typing study the allelic types of HLA-DQA1 and HLA-DRB1 were detected by PCR-SSP in 51 cases of endometriosis, 45 cases of adenomyosis, and 44 normal individuals as the control. It has been reported that the frequencies of HLA-DQA1*0401were significantly increased in the endometriosis group and the adenomyosis group, and the frequencies of HLA-DQA1*0301were significantly decreased in these two groups. There was no significant difference between the frequencies of HLA-DQA1 and HLA-DRB1 of endometriosis and adenomyosis. Thus the results indicate that HLA-DQA1*0301 and *0401 alleles are associated with both endometriosis and adenomyosis, and there is perhaps common mechanism involved in both endometriosis and adenomyosis based on HLA-DQA1 and HLA-DRB1 alleles (21). In Iraq study depend on serological typing methods (microcytotoxicity tests) showed that there were eight of HLA antigens appear a significant increase frequency in patients (HLA-A28, B7, B12, B27, Cw6, Cw7, DR1 and DR11), while two antigens (A19 and B5) showed a significant decreased frequency (22).

In contrast to the present findings, Whang and colleagues (2008) stated an increased frequency of HLA-B39 in Korean patients with endometriosis when compared with control subjects, but the difference was not statistically significant after correction for the multiple comparisons that were made (23). Furthermore, investigation in Japanese patients reported that there is no significant variations of HLA-class

II alleles between endometriosis patients and controls (24). Roszkowski and associates also found that there were no statistically significant differences in the distribution of HLA-DRB1 alleles in polish patients with ovarian endometriosis as compared with control populations, so they concluded that ovarian endometriosis is not associated with particular HLA-DRB1 allele(s). This may indicate that aetiology of this form of endometriosis may be not primarily associated with class II HLA-mediated autoimmune reactions (25).

It is important to recollect that the decrease frequency of HLA typing could be considered as a protective factor for endometriosis. The involvement of HLA molecules in conferring human beings a resistance against development of diseases that have different natures (i.e. autoimmune and infectious diseases) has been demonstrated. Such demonstration has been based on the basis of either the function of HLA molecules on antigen presentation or the HLA region contain immune suppressor genes that are in linkage disequilibrium with HLA genes that code for the antigens involved in a disease-resistance (26,27,28).

The present study revealed that there was significant lower frequency of HLA-DRB1*0323 allele in endometriosis patients when compared to controls therefore, suggesting that this allele may confer protective effects against this disease.

However, the contradictions showed between different studies might be due to the influence of ethnicity and racial background on the distribution of HLA alleles. In addition, differences in methodology, sample size and patient selection could also have served as a source of bias. This study concluded that HLA- DRB1*0307, DRB1*0701 and DQB1*0301 alleles may contribute to the increased susceptibility to endometriosis, whereas HLA-DRB1*0323 allele may confer protective effects against it, suggesting HLA-based different etiopathogenesis.

Recommendations

Further investigations with larger populations are required to clarify the association between HLA-class I and II genotyping in endometriosis.

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