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Short Communication

Association between HLA-DRB1 alleles and development of antibodies to infliximab in Iraqi patients with rheumatoid arthritis

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ABSTRACT

Background: Many of patients with Rheumatoid arthritis were currently successfully treated with infliximab (anti-tumor necrosis factor); however, about 30% of the patients do not responded to infliximab. One of postulated hypotheses if not responding to is the fast clearance of infliximab due to development of infliximab-anti-infliximab complexes. Objective: To study the association between HLA-DRB1 alleles and development of anti-infliximab antibodies in Iraqi patients with rheumatoid arthritis were treated with infliximab. Patients and Methods: Thirty five Iraqi RA patients (26 females and 9 males) compared with 25 healthy controls were enrolled in this study from beginning of March 2014 till end of September 2014. All patients were diagnosed by full history, complete clinical examination and laboratory test. Anti-infliximab antibodies were measured by using enzyme-linked immunosorbent assay (ELISA) in serum of Iraqi patients with RA treated with infliximab more than 3 months duration, genotyping of the patients was done by using polymerase chain reaction with sequence-specific primers (PCR-SSP). Statistical analysis was done using SPSS version (20) computer software. Results: There was no significant association between 35 patient's mean age ((45.31±11.24) and 25 healthy control's mean age (42.13±10.22). HLA-DRB1*04(51.42%) was the most frequent alleles was identified in patients group while HLA-DRB1*03(28 %) was the most frequent alleles was identified in control group. An association was reported in the study regarding HLA-DRB1 *04 between the patients (51.42%) and control groups (20%) (p<0.05). No association was reported between development of anti-infliximab antibodies in (25) patients and (10) patients without antibodies formation with HLA-alleles (*01,*03, *04,*07 and *11 (p> 0.05). Conclusion: HLA-DRB1*04(DR4) allele was associated with susceptibility of RA in Iraqi population and development of anti-infliximab antibodies not affected by genetic factor (HLA-DRB1 alleles) of patients with Rheumatoid Arthritis.

Keywords: Anti-infliximab antibodies, HLA-DRB1 genotyping, Rheumatoid arthritis, Infliximab, polymerase chain reaction.

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic chronic autoimmune inflammatory polyarthritis that leads to joints destruction and severe disability if not treated early (Wolfe and Hawley, 1998; Smolen et al., 2014). The

Table 1. Distribution of patients and control groups according to their mean age±SD.

Group	Number	Mean age(yr.)	Std.Deviation
Patients	35	45.31	±11.24
*Controls	25	42.13	±10.22
P-Value not significant			

pathological events of RA result from increase of Tumor necrosis factor alpha (TNFα) expression (Feldmann and Maini, 2001). Tumor necrosis factor alpha antagonists such as infliximab (INF) which is a chimeric monoclonal antibody that have been introduced and approved by Ministry of Health-Iraq in 2010 as highly effective agents for the treatment of Iraqi patients with moderate to severe Rheumatoid arthritis that are not responding to conventional treatment. Infliximab is a chimeric (murine-human) antibody with 25% murine sequence that can induce formation of anti-infliximab antibodies (ATIs), however, these (ATIs) leading to lack of efficacy to Infliximab or adverse reaction (Cassinotti and Travis, 2009). The probability of RA inheritance is estimated in about 60% (Kochi et al., 2009; De Vries, 2011) and it found that the most important genetic risk factors are the overall genetic susceptibility for RA is HLA which estimated in around 30%-50% (Bax, 2011; De Vries, 2011; Hoovestol and Mikuls, 2011).

Gregresen and colleagues in 18 years later demonstrates that the main HLA-DRB1 associated with susceptibility to RA include: (DRB1*0101,*0102,*0401,*0402,*0405,*0408,*1001,*1402) (Tézenas et al., 2000; Korendowych et al., 2003).

MATERIALS AND METHODS

METHODS

Five ml(5ml) of blood were aspirated from each individual, 3ml were left to clot at room temperature, then centrifuged at 3000 rpm/min for 5 min, and serum was collected in aliquots to store in (-20ºC) until needed for investigation of anti- infliximab antibodies.

Other two milliliters (2ml) was stored in ethyelenediaminetetraacitic acid (EDTA) tubes in deep freezer at temperature (-70 c) for DNA extraction. Identification of HLA-DRB1 alleles was done by the polymerase chain reaction and subsequent oligonucleotide hybridization (SSP-PCR).

Kits and reagents

Human anti infliximab antibody ELISA kit (*Matriks Biotek*, Germany), QIAamp® DNA Mini Kit (Fujirebio, Belgium), INNO-LiPA HLA-DRB1 Amp Plus kit (Fujirebio, Belgium) and INNO-LiPA HLA-DRB1 Plus kit (Fujirebio, Belgium).

Statistical analysis

Statistical analysis was done using SPSS version (20) computer software. T test was used to analyze the data, and calculation of mean difference, Fisher>s exact and Chi-square test for comparison of proportion, P-value of less than 0.05 was considered as statistically significant-value <0.01 as highly significant and P-value <0.001 as extremely significant.

RESULTS

Patients and control

Thirty five patients with RA on infliximab therapy with mean age (45.31±11.24) randomly selected, were investigated for anti-infliximab antibodies and typing for HLA-DRB1 by using PCR technique and were compared to 25 healthy individuals with mean age (42.13±10.22) from Al-karama teaching hospital (kidney transplantation-unit). There was no statistical difference between patients and control group regarding their mean age, table 1.

Demographic and baseline characteristics of HLA-genotyping RA patients.

The main characteristic of the patients which listed in the table (2) were determined before HLA-DRB1 genotyping. The predominant patients were women (74.3%).

HLA-DRB1 alleles in RA patients

HLA-DRB1 genotypes of RA patients have been demonstrated in figure (1). Among 35 patients with RA randomly selected for HLA genotypes HLA-DRB1*04(51.42%) was the most frequent alleles identified.

The HLA-DRB1 –alleles in control group

HLA-DRB1 genotypes of healthy control have been demonstrated in figure (2). Among 25 healthy control investigated for HLA-DRB1 genotypes, HLA-DRB1*03(28 %) was the most frequent alleles identified.

An association was reported in the study regarding

Table 2. Demographic and baseline characteristic of RA patients were included for HLA-DRB1 genotyping.

Characteristics	RA patients(n=35)
Age,mean±SD years	45.31±11.24
Diseases duration years±SD	9.51±6.59
Female	26(74.3%)
Male	9(25.7%)
Female: male ratio	2.9:1
ESR mean±SD (mm/h)	43.71±25.19
CRP mean±SD (ml/L)	14.76±17.48
Diseases activity(CDAI)±SD	27.11±13.82

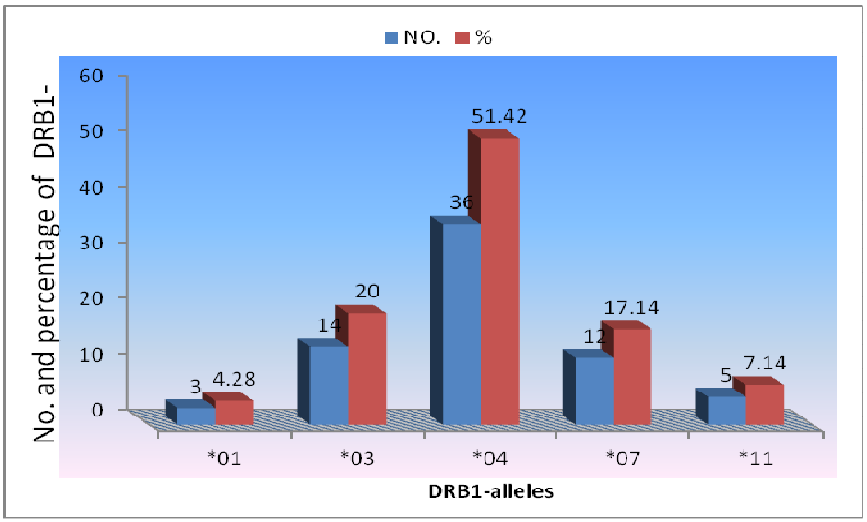

Figure 1. frequencies of HLA-DRB1 alleles in patients group.

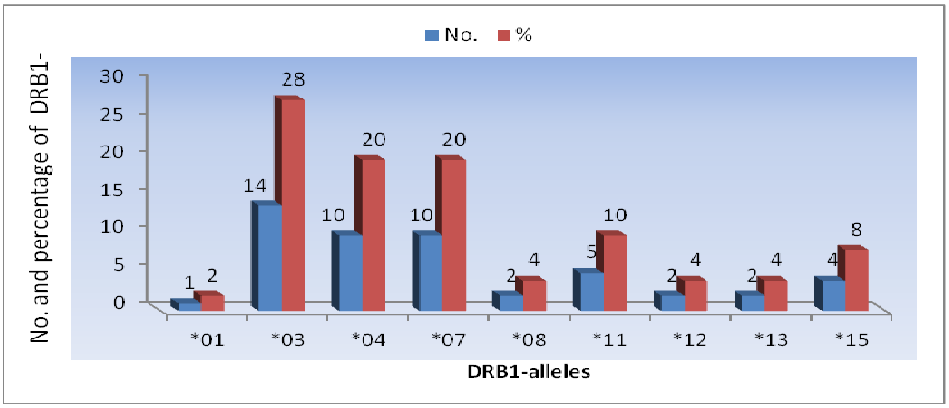

Figure 2. Frequencies of HLA-DRB1 alleles in control group.

HLA-DRB1 *04 between the patients and control groups ($p < 0.05$) as shown in table (3)

Association of anti-infliximab with HLA-genotyping

The table (4) showed that no association between development of anti-infliximab antibodies in (25) patients and (10) patients without antibodies formation with HLA-alleles (*01,*03, *04,*07 and *11 ($p > 0.05$).

Out of 35 patients were investigated for HLA-DRB1 genotyping, twenty five of them were positive for anti-infliximab Ab and 10 patients were negative. Different clinical and laboratory parameters listed in table (4) were

Table 3. Distribution of HLA-DRB1 alleles frequencies in RA patients and controls of Iraqi population.

Genotype HLA-DRB1	RA patients(n=35)		Healthy Control(n=25)		Odd ratio 95% confidence interval	p-value
	n	*AF (%)	n	AF (%)		
*01	3	4.28	1	2	2.25 0.22-22.99	0.49
*03	14	20	14	28	0.52 0.18-1.48	0.22
*04	36	51.42	10	20	107.76 5.93-1955.83	0.001
*07	12	17.14	10	20	0.78 0.27-2.26	0.65
*08	0	0	2	4	na	na
*11	5	7.14	5	10	0.66 0.17-2.60	0.55
*12	0	0	2	4	na	na
*13	0	0	2	4	na	na
*15	0	0	4	8	na	na

AF; alleles frequencies, na: not applicable

Table 4. Association of anti-infliximab antibodies with HLA-DRB1 alleles genotypes in patients with Rheumatoid arthritis.

DRB1	Anti-infliximab Ab		Total	p-value
	Ab +ve	Ab -ve		
*01	0	2	2	
*03	7	0	7	
*04	12	5	17	0.0914
*07	4	2	6	
*11	2	1	3	
total	25	10	35	

used for comparison between positive and negative anti-infliximab Ab patients. No parameters investigated different between two groups (p>0.05).

DISCUSSION

This study to the best of our knowledge is the first study which was conducted in Arab Iraqi population with RA on infliximab therapy by investigating their genotypes (HLA-DRB1-alleles frequencies) and relation to development of antibodies against infliximab.

The distribution of HLA-DRB1 alleles in different races and ethnicities around the world were investigated by different literatures (Tézenas et al., 2000; Kochi et al., 2009).

However, the most frequent HLA-DRB1 allele in RA patients reported by the current study was the appearance of HLA-DRB1*04 in a higher frequencies (51.42%) as compared to healthy control (20%), (OR=107.76, 95% CI=5.93-1955.83, P value=0.001).

Furthermore, the finding of this study was in accordance with previous studies which had been reported that HLA-DRB1*04 was the most frequent allele in patients with RA in many populations mainly in the nearby populations (Mediterranean and Middle Eastern), (Korendowych et al., 2003; Atouf et al., 2008; Dhaouadi et al., 2011; Sandoughi et al., 2011; Uçar et al., 2012; Mourad and Monem, 2013).

Interestingly, and in contrast to current findings, the striking finding in a recent study was done by Al-Timimi *et al.* (2014) in the Kurd population of the same country who reported that HLA-DQB1*06 was the most common allele associated with Kurd RA patients (Al-Timimi et al., 2014), additionally, Al-Swailem *et al.* (2006) was reported an association of HLA-DRB1*08 with RA in Saudi Arabians population (Al-Swailem et al., 2006), the difference between this finding and the findings reported by other studies might be related to geographical and genetic variations (Rincon et al., 2003).

On other hand, the current study was showed no linked between the HLA-DRB1 alleles of patients with RA and development of antibodies to infliximab (p=0.0914).

In relation of genotypes of the current RA patients to disease activity (CDAI, CRP and ESR) and Anti-CCP, we observed that there was no difference between HLA-DRB1 alleles with positive and negative anti-infliximab patients groups (CDAI (p=0.772), CRP (p=0.309), ESR (p=0.305) and Anti-CCP (p=0.134)) respectively**,** this results was in disagreement with other studies, for example, the study which was reported in Japanese patients with RA by Wakitani *et al.* (1997) who had

confirmed a strong association between HLA-DRB1*04 and high disease activity (Wakitani et al.,1997), furthermore, the HLA-DRB1*04 was reported to be associated with increase in severity of RA in Netherlands (Van Gaalen et al., 2004), Northern Europe (Gorman et al., 2004) and Caucasians (Fries et al., 2002; Mewar et al., 2008). However the racial and geographical variation might be contributed to such difference. On other hand, results of other previous studies carried out in Greece (Boki et al., 1993) and Turkey (Kinikli et al., 2003) were complied with the current findings.

CONCLUSIONS

HLA-DRB1*04(DR4) allele was associated with susceptibility of RA in Iraqi population and the development of anti-infliximab antibodies not affected by genetic factor (HLA-DRB1 alleles) of patients with Rheumatoid Arthritis.

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