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Polymorphism of HLA-DR and HLA-DQ in rheumatoid arthritis patients and clinical response to methotrexate--a hospital-based study

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Original Article

Polymorphism of HLA-DR and HLA-DQ in rheumatoid arthritis patients and clinical response to methotrexate - a hospital-based study

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Abstract

Objective: To investigate the frequency and distribution of DRB1 and DQB1 alleles in Patients with rheumatoid arthritis (RA) and analyze the relationship between clinical response to methotrexate (MTX) and the HLA-DR and HLA-DQ genotypes in these patients.

Methods: In this case-control study, the HLA-DRB1 and HLA-DQB1 polymorphism in 91 RA patients and 91 healthy controls was done using polymerase chain reaction and sequence specific primers.

Results: There was no statistical difference in frequencies of HLA-DRB1*03, DRB1*04, DRB1*07, DRB1*10, DRB1*11, DRB1*12, DRB1*13, DRB1*14, DRB1*15 and DRB1*16 genotypes between patients and controls. However, DRB1*01 was found to be significantly more common (p=0.015) in RA patients compared to controls. HLA-DRB1*15 was more common in patients (43.5%) compared to controls (30.8%) but results were not significant. HLA-DRB1*08 and DRB1*09 were present in negligible number in patients as well as controls while HLA-DRB1*12 was conspicuously absent in controls. Similarly, DQB1*06 was also significantly more common (p = 0.01) among the patients compared to healthy control subjects, while there was no statistical difference in the frequencies of DQB1*02, DQB1*03, DQB1*04 and DQB1*05 among the cases and the controls. RA susceptibility in most patients appeared to be associated with the HLA-DRB1*01/DQB1*06 genotype. Regarding association between HLA-DR or HLA-DQ genotype and clinical response to methotrexate (MTX), the data showed that with the exception of HLA-DRB1*03, there appears to be no association between the particular subtypes of HLA-DR and HLA-DQ. HLA-DRB1*03 was significantly more common among non-responders to MTX alluding to the possibility that another genes responsible for MTX metabolism, might be in linkage disequilibrium with HLA-DRB1*03 in the Pakistani population, thereby making such individuals non-responsive to MTX-therapy.

Conclusion: RA susceptibility in most Pakistani patients is associated with the HLA-DRB1*01/DQB1*06 genotype. HLA-DRB1*03 was found to be significantly more common among non-responders to MTX treatment suggesting that Pakistani patients with this genotype are less likely to benefit from MTX (JPMA 56:452;2006).

Introduction

The occurrence of rheumatoid arthritis (RA) is strongly associated with the expression of particular human leukocyte antigen (HLA) class II alleles in several racial groups bearing the third hypervariable region shared epitope sequence, 67-74:LLEQKRAA.[1-3] In Caucasians, RA-related subtypes of DR1 and DR4 occur more frequently than the subtypes of DR10 or DR14[4.5] A DR4-DQ3 association has been reported in Indian RA patients[6], whereas an increased frequency of DR1 and DR10 has been found to be in RA patients from other population groups.[7-9] All these studies indicate that the association of a particular allele with RA varies among different races and it would be important to study the frequencies of DR and DQ alleles in Pakistani RA patients.

Moreover, association between a particular allele of HLA-DR in RA patients and clinical response to methotrexate (MTX) has also been a subject of great interest among rheumatologists due to the well documented short-term efficacy of this drug in the treatment of refractory RA.[10-13] In one study, HLA-DR2 was found to be associated with a good response to MTX, while another study, reported a lack of association between HLA-DR2 and substantial clinical response to MTX.[11,14]

The present study was undertaken to investigate the frequency and distribution of DRB1 and DQB1 alleles in a hospital based Pakistani population of RA patients and to analyze the relationship between clinical response to MTX and the HLA-DR and HLA-DQ genotypes in these patients.

Patients and Methods

Ninety one RA patients (16 males and 75 females) visiting the Rheumatology Clinic at the Aga Khan University (June 1998 to June 2000) and A.O. Clinic, Karachi, were selected randomly for this study. All the patients fulfilled the diagnostic criteria of the American Rheumatism Association for RA. Their mean SD age was 43.4 3 years. Majority (87%) of patients were rheumatoid

factor positive. They belonged to all the major ethnic groups of the country and all had been receiving MTX for at least 6 months and were assessed clinically every month. Clinical response to MTX was assessed according to the criteria described by O'Dell et al.[15] After 12-24 weeks of treatment with MTX, those showing 50% or more reduction in ESR, Richie index, number of swollen joints and morning stiffness as compared to index at entry were classified as "responders", while those showing little improvement in these parameters were called 'non-responders'.

Equal number of controls were randomly selected from a pool of healthy individuals who had their HLA typing done for organ donation and represented all the major ethnic groups in Pakistan. Their mean SD age was 38.5 ± 8.3 years. Controls were free from any chronic illness as revealed by their clinical examination and biochemical tests. Informed consent was obtained from the participants and the study was approved by the Ethics Review Committee of the Aga Khan University.

Blood samples (5 ml each) of these patients and controls were obtained in tubes containing EDTA as anticoagulant. White blood cells were separated on Ficoll/Hypaque and DNA was extracted according to Miller et al.[16]

Briefly, white blood cells collected in 300 l TE buffer (10 mM Tris. 1mM EDTA, pH 7.4) were homogenized using a plastic hand homogenizer and incubated at 65°C for 30 minutes after adding 20 µl proteinase-K (10 mg/ml) and 80 µl of 10% SDS. Then, 100 µl of 6 M NaCl and 10 µl of N-cetyl, N.N. N-trimethyl ammonium bromide (CTAB) were added and an additional incubation at 65oC was carried out for 20 minutes. This was followed by addition of 720 µl of chloroform: isoamylalcohol (24:1) mixture and centrifuged at 12000 g for 15 minutes. The upper layer was removed and DNA was precipitated from it using isopropanol. Finally, after washing with 70% ethanol, DNA pellet was resuspended in 50 - 100 µl TE buffer, pH 7.4 and stored at -70°C until analysis.

HLA-DRB1 and DQB1 genotyping was performed by the Dynal AllSetTM PCR-SSP low resolution typing kits according to the manufacturer's instructions (Dynal, UK). A typical PCR-SSP reaction consisted of 1 X PCR buffer, 200 µM deoxyribonucleotide triphosphate, 5% glycerol, sequence specific primers, 0.4 unit Taq polymerase and 100 ng extracted DNA in a final volume of 10 µl. After thermal cycling the amplified products were separated by electrophoresis on a 2% agarose gel, stained with ethidium bromide and photographed under UV illumination. A set of 9 primer pairs was used for HLA-DQ1 typing, while a set of 24 primer pairs was used for DRB1 typing, respectively.

Statistical evaluation was performed using Statistica for Windows (version 5.0 statsoft, Inc, USA). Descriptive analysis was carried out for patient's demographic and clinical data, whereas the frequencies of HLA-DRB1 and DQB1 alleles were compared among responders, non-responders and control subjects and calculated using the Chi-square test with Yates correction. For all tests, a two-tailed p-value of < 0.05 was considered to be significant.

Results

HLA class-II typing by PCR was performed on a group of 91 RA patients and 91 healthy subjects. All the patients were diganosed to have an active PA on the basis of clinical and laboratory findings. Table 1 shows patients' clinical and demographic characteristics. The patients group comprised of 75 females and 16 males with a female to male ratio of 4.6:1. The mean age was 43 years with a range between 19-65 years. More than 50% of the patients had moderate disease, whereas 30% demonstrated severe type of arthritis and the rest of the patients were classified under mild disease by ARA criteria. The mean SD duration of illness was 7.03 ± 4.48 years. The rheumatoid factor was positive in 87% of the patients.

The distribution of HLA- DRB1 and HLA-DQB1 genotypes in controls and patients is shown in Table 2. HLA-DRB1*03, HLA-DRB1*07, HLA-DRB1*10, HLA-DRB1*11 and HLA-DRB1*15 were the most common HLA-DRB1 genotypes identified in the control group, whereas HLA-DRB1*01, HLA-DRB1*03, HLA-DRB1*07, HLA-DRB1*10, HLA-DRB1*11, HLA-DRB1*13 and HLA-DRB1*15 were the commonest genotypes in the patient group. HLA-DRB1*01 was significantly more common (P=0.015) in patient group compared to the control group. The frequency of HLA-DRB*04 was similar in both control and patient groups. HLA-DRB1*15 was more common among patients (43.5%) than the control subjects (30.8%), however, the frequency values were not statistically significant. The study demonstrated that HLA- DQB1*06

Table 1. Demographic and clinical characteristics of patients with rheumatoid arthritis.

Variable		Frequency (%)
Gender	Males	16(17.8)
	Females	75 (82.2)
Age (mean SD years)		43.4 ± 11 years
Duration of illness (mean SD years)		7.03 ± 4.48
Severity of the disease	Mild	12 (13)
	Moderate	52 (57)
	Severe	27 (30)
Rheumatoid factor	Positive	79 (87)
	Negative	12 (13)

Table 2. Distribution of HLA-DRB1 and HLA-DQB1 genotypes in patients with rheumatoid arthritis versus the distribution in normal healthy control subjects.

HLA genotype	No. (%) Controls (n=91)	No. (%) Patients (n=91)	p - value
HLA-DRB1			
*01	6 (6.6)	17 (18.5)	0.015*
*03	27 (29.7)	25 (27.5)	NS
*04	9 (9.9)	10 (10.9)	NS
*07	24 (26.3)	16 (17.4)	NS
*08	1 (1.1)	1 1.1)	NS
*09	1 (1.1)	1 (1.1)	NS
*10	17 (18.7)	16 (17.4)	NS
*11	19 (20.9)	13 (14.4)	NS
*12	0 (0)	5 (5.4)	-
*13	11 (12.1)	18 (19.6)	NS
*14	13 (14.3)	10 (10.9)	NS
*15	28 (30.8)	40 (43.5)	NS
*16	7 (7.6)	5 (5.5)	NS
HLA-DQB1			
*02	41 (45)	39 (42.4)	NS
*03	33 (36.3)	29 (31.5)	NS
*04	0 (0)	4 (4.3)	NS
*05	48 (52.7)	35 (38)	NS
*06	30 (33)	47 (51.1)	0.01**

*Yates corrected p-value=0.025; Odds ratio=3.25; 95% C.I. (1.13-9.8)
**Yates corrected p-value=0.016; Odds ratio=2.17; 95% C.I. (1.14-4.14)
NS= Not significant

Table 3. Distribution of HLA-DRB1 and DQB1 genotypes in patients with rheumatoid arthritis who responded to treatment with methotrexate and in those who did not respond to this treatment.

HLA genotype	No. (%) Responders (n=48)	No. (%) Non - responders (n=43)	P-value
HLA-DRB1			
*01	6(12.2)	6 (14)	NS
*03	7(14.3)	18 (37.2)	0.004*
*04	8 (16.3)	2 (4.7)	NS
*07	12(24.5)	4 (9.3)	NS
*08	-	1(2.3)	-
*09	-	1(2.3)	-
*10	10 (20.4)	6 (14)	NS
*11	10 (20.4)	3 (7)	NS
*12	2(4.1)	3 (7)	NS
*13	9 (18.4)	9 (20.9)	NS
*14	7 (14.3)	3(7)	NS
*15	20 (40.8)	20 (46.5)	NS
*16	3 (6.1)	2(4.7)	NS
HLA-DQB1			
*02	20 (40.8)	19 (44.2)	NS
*03	17 (34.7)	12 (27.9)	NS
*04	4 (8.2)	-	-
*05	21 (42.9)	14 (32.6)	NS
*06	23 (46.9)	24 (55.8)	NS

NS = Not significant
*Yates corrected p-value=0.007; Odds ratio=4.22; 95% C.I. (1.4-13.1)

was significantly (P = 0.01) more common in the patient group compared to the controls (51.1% vs 33.0%). The frequency of HLA-DQB1*05 was very high among controls (52.7%) compared to patients (38%), but the difference was not statistically significant. HLA-DRB1*08 and HLA-DRB1*09 were present in a negligible number (1.1%) in both patient and control groups. HLA-DRB1*12 and HLA-DQB1*04 were conspicuously absent in our normal population, while these values present in patients (5.4% and 4.3%, respectively).

The distribution of HLA-DRB1 and DQB1 genotypes in patients who responded to MTX treatment was compared with the genotypes in patients showing no response to MTX (Table 3). HLA-DRB1*03 was found to be significantly (P=0.004) more common among non-responders (37.2%) compared to responders (14.3%). In both the groups, HLA DRB1*15 was the most frequent genotype. It was present in 46.5% of the non-responders and in 40.8% of the responders. However, the difference was not significant. Similarly, HLA-DQB1*06 also appeared to be more common among the non-responders compared to responders (55.8% vs 46.9%), but the difference was not statistically significant.

Discussion

The association of HLA antigens with RA has been well characterized and documented in the literature.[3,4,8,17] We have carried out this study to examine whether variable response to MTX observed in RA patients was associated with HLA-DR or DQ genotypes.

Depending upon geographical location, HLA-DR associations with RA vary from population to population. For example, HLA-DR1 is strongly associated with RA in Greeks[18] generally the frequency of HLA-DR4 is exceptionally high among RA patients of other European Caucasian populations.[8] Similarly, in certain South Asian countries, including India, HLA-DR4 was reported in the majority of RA patients.[19,20] In contrast to these studies, HLA-DR4 frequency was not significantly different between the control and patient groups in our study. However, strong association of RA with HLA-DRB1*01 observed in this study is in line with the findings of Constantinidou et al.[18] who have also reported association of this genotype with RA in Greek population. With the exception of HLA-DRB1*03, patients who demonstrated good response to MTX treatment failed to show any significant association with HLA-DR types at the level of resolution of this study. HLA-DRB1*03 genotype, however, was found to be significantly more common among non-responders, indicating influence of genotype in response to MTX treatment.

Our data showed non-significant but increased frequency of HLA-DRB1*15 in RA patients of Pakistani origin. This is in sharp contrast to the findings of Taneja et al.[19] who have reported a decreased frequency of HLA-DRB1*15 in Indian patients compared to RA- free controls. Similar to our findings, Hameed et al.[21] have also shown an increased frequency of HLA-DR2 in RA patients compared to healthy controls (33.1% vs 19%) but the difference was not statistically significant (P= 0.053). In their study, DR2 was not resolved into DRB1*15 and 16 subtypes. These reports suggest that HLA-DRB1*15 may show different associations with RA in different populations. However, this could only be confirmed by additional studies on larger patient and control series in the populations in question. HLA-DRB1*15 is a relatively common genotype with a frequency varying from 14-46% depending on the ethnic group.[18] The frequency of HLA-DR2 (DR15 and DR16) in our control group was 34.4% this is a smaller to 38.4% and 33.5% reported earlier by Zafar et al.[22] and Hannan et al.[23] respectively, in Pakistani populations. However, Hameed et al.[21] presented lower frequencies (19%) of HLA-DR2 in their control group of Pakistani origin. HLA-DRB1*08 and HLA-DRB1*09 were present in very low frequencies (1%) in Pakistani population. Out of 91 patients, there were only 2 (one in each group) of these genotypes. Our observation of higher frequency of HLA-DRB1*01 in our RA patients is further supported by an increased frequency of the DRB1*01-DQB1*06 haplotype in RA patients compared with healthy controls (P< 0.01).

MTX is commonly used for the treatment of RA patients in Pakistan with majority of patients responding to this treatment.[24] Earlier studies have demonstrated an association between the HLA-DR2 and substantial response to MTX treatment in RA patients.[11,15] However, our results with the exception HLA-DRB1*03 which was more common among non-responders, demonstrated no apparent association between distribution of any HLA-DRB1 and DQB1 genotypes and response to MTX treatment. Similar findings pertaining to DR2 specificity have also been reported by Alarcon et al.[14] who have shown that neither HLA-DR2 nor any other HLA-DR specificity is significantly associated with a substantial clinical response to MTX in patients with RA. Although, we have noticed higher frequencies of HLA-DRB1*15 and HLA-DQB1*06 in non-responders compared to responders, this trend was non-significant and was in accordance with the findings of Alarcon et al.[14] In a recent study by Criswell et al.[25], genetic variation in the HLA-DRB1 and other loci in close proximity has been found to be associated with response to treatment of early RA. Significantly high frequency of distribution of HLA-DBR1*03 among non-responders in our study alludes to the possibility that other loci responsible for MTX metabolism might be in linkage disequilibrium with HLA-DRB1*03 in Pakistani population, making such individuals non-responsive to MTX treatment.

Conclusion

We report a novel finding that RA susceptibility in most Pakistani patients is associated with the HLA-DRB1*01/XOB1*06 genotype. However, the effectiveness of HLA-DRB1*01 and DQB1*06 testing as a genetic marker for RA could be established following confirmation of our findings in a larger study group. HLA-DRB1*03 was found to be significantly more common among non-responders to MTX treatment. Our study shows that Pakistani patients with this genotype are less likely to benefit from MTX therapy.

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