original article

Association of human leukocyte antigen-DRB1 with anti-cyclic citrullinated peptide autoantibodies in Saudi patients with rheumatoid arthritis

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BACKGROUND: The genetic association between human leukocyte antigen (HLA)-DRB1 alleles and the risk of development of autoantibodies has been investigated, but there are few studies from the Gulf region.
OBJECTIVES: To investigate the association between the HLA-DRB1 shared epitope and the risk for development of autoantibodies in rheumatoid arthritis (RA) patients in a Saudi population.
DESIGN: Analytical cross-sectional study.
SETTING: Tertiary care hospital in Riyadh, Saudi Arabia.
PATIENTS AND METHODS: We enrolled consecutive Saudi RA patients attending the rheumatology clinic between January and April 2015. Previously published data on HLA typing on unmatched healthy controls were used for comparison. HLA typing was performed using sequence-specific oligonucleotide probes (SSOP). Rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP) antibodies, and antinuclear antibodies (ANA) were also measured. Logistic regression analysis was used to study the autoantibodies as possible explanatory variables for the presence of the HLA-DRB1 shared epitope.
MAIN OUTCOME MEASURE(S): The association between the presence of the shared epitope and the risk of developing anti-CCP antibodies, ANA, and RF.
RESULTS: In 76 patients with RA, carrying the shared epitope was associated with a significantly higher risk of having RA [OR=2.65, 95% CI (1.42-4.94), *P*=.0009]. However, only HLA-DRB1*04:05 was significantly associated with RA [OR=3.73, 95% CI (1.61-8.96), Pc=.016]. In the logistic regression analysis, only anti-CCP was significantly associated with the shared epitope [OR=14.51, 95% CI (1.53-137.49), *P*=.02].
CONCLUSIONS: Our analysis indicates that the presence of the HLA-DRB1 shared epitope is strongly associated with the development of anti-CCP antibodies in Saudi patients with RA.
LIMITATIONS: A larger sample size is needed to confirm our finding.

Rheumatoid arthritis (RA) is a chronic inflammatory disease affecting mainly the synovial joints and leading to reduced quality of life.[1] The pathogenesis of RA is complex and is thought to be multifactorial with both genetics and environmental factors involved in disease susceptibility.[2] One of the earliest genetic factors implicated in RA is the *HLA-DRB1* gene.[3] The association with *HLA-DRB1* was found to be restricted to certain alleles namely, *HLA-DRB1*04:01, *04:04, *04:05, *04:08, *01:01, *01:02, *14:02* and **10:01*.[4] These alleles encode a conserved five amino acid sequence in residues 70–74 of the *HLA-DRB*, commonly known as the shared epitope. It has been proposed that all RA-associated *HLA-DRB1* alleles share a conserved motif of amino acid residues (QKRAA/QRRAA/RRRAA) in the hypervariable region (HVR3) of the DRB1 molecule.[5] Indeed, the *HLA-DRB1** shared epitope is classically considered

as a marker of disease susceptibility and severity in Caucasian individuals.[6] Several research studies have implicated the interaction between the shared epitope motif and the environment.[7] Many autoantibodies have been described in RA, but anti-cyclic citrullinated peptide antibodies (anti-CCP) have been proven to be more specific and sensitive in the diagnosis of RA, and therefore appear to be better predictors of the progressive disease.[8] In this study, we investigated the *HLA-DRB1* gene association with RA and the risk for the development of autoantibodies.

PATIENTS AND METHODS

Consecutive Saudi RA patients attending the Rheumatology Clinic at the National Guard Hospital in Riyadh between January and April 2015 were enrolled in this study. Patients with non-Saudi origins were excluded from the study. HLA results on previously described unmatched healthy controls were used for comparison.[9] All patients met the 1987 American College of Rheumatology classification criteria for the diagnosis of rheumatoid arthritis.[10] The HLA typing of the controls were collapsed to four digits to make the comparison at the allele level only. The demographic data on the patients collected from files included age, age at onset, gender, and presence of rheumatoid factor (RF) and anti-CCP. Controls were healthy Saudis of different ages and both genders.[9] Healthy Saudi students and staff at King Saud Bin Abdulaziz University for Health Sciences in Riyadh were invited to participate in this study as controls. This study was approved by the local institutional review board.

HLA-typing

HLA typing was conducted using sequence specific oligonucleotide (SSO) high definition kit LABType SSO HD (One Lambda Inc., Canoga Park, CA, USA). The HLA typing was carried out according to the manufacturer's instructions. Briefly, the HLA typing procedure consisted of DNA extraction, amplification, hybridization, reading on a Luminex machine (LABScan 100, *http://www.onelambda.com/*), and interpretation using HLA Fusion software.

Autoantibodies

The RF was determined by the nephelometry method (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany). An RF value greater than 10 IU/mL was considered positive. Anti-CCP was determined by enzyme-linked immunosorbent assay (ELISA) using anti-CCP ELISA (IgG) test kit (INOVA Diagnostics, San Diego, USA). A value greater than 20 U/mL was considered positive. Antinuclear antibody (ANA) was assayed using the CMIA method (DiaSorin, Saluggia, Italy) and a value greater than the 1.5 index was considered positive.

Statistical analysis

Statistical analysis was conducted using version 12.0 of the software Statistics and Data (Stata) available from StataCorp, College Station, TX, USA. Continuous data were expressed as mean and standard deviation (SD). The data was normally distributed based on the Shapiro-Wilks test. Categorical data were expressed as numbers and percentage. To investigate which of the risk factors was associated independently with the shared epitope, a logistic regression analysis was performed, with shared epitope as the dependent variable and the presence of anti-CCP antibodies, ANA, and RF as possible explanatory variables. Association between different HLA alleles was analyzed using the odds ratio (OR) with 95% confidence interval (CI). The *P* value was corrected by the number of alleles observed (Pc). A Pc value of less than 0.05 was considered statistically significant.

RESULTS

Of 76 consecutive patients enrolled in the study, 90% were females. The age of onset among males was significantly older than females (50.4[8.6] years vs 41.7[11.5] years, *P*=.0245), while the current age was not significantly different between the genders (55.1[10.4] males vs 52.1 [11.1] females, *P*=.3794). Most of the women were married (97%) with a mean of 7 kids per mother. Most of the patients were RF positive and anti-CCP positives (77.6% and 75%, respectively), while a smaller number were ANA positive (26.3%) (**Table 1**). Controls were 158 healthy Saudis of different ages (between 21 and 81 years, mean 39.5 years and median 38 years). Controls were of both genders (male=81 and female=77).

Carrying the shared epitope was associated with a significantly higher risk of developing RA (OR=2.65, 95% CI: 1.42-4.94), *P*=.0009) (**Table 1**). *HLA-DRB1*04:01, *04:05, *15:01* and **16:02* were significantly associated with RA. However, after correcting for multiple testing only *HLA-DRB1**04:05 remained significant (OR=3.73, 95% CI (1.61-8.96), Pc=0.016) (**Table 2**). On the other hand, *HLA-DRB1*03:01* and **07:01* were significantly protective against RA, but after correcting for multiple testing, this significance disappeared. In the logistic regression analysis, only anti-CCP was found to be associated with the shared epitope (OR=14.51, 95% CI (1.53-137.49), *P*=.02) (**Table 3**).

DISCUSSION

In this study, we describe the demographic and HLA alleles in a group of Saudi patients with RA. Interestingly, most of our patients were females (9:1, F: M). Although this finding of a preponderance of females is in agreement with previous studies in Saudi Arabia,[11,12] further investigation is needed to confirm whether this finding is a hallmark of the disease in this region, or whether it is a study bias. This is especially relevant since our studied population consisted mainly of soldiers and their families. One could possibly argue that the low prevalence of the disease in males is due to their work nature and the heavy exercise regimen they undertake.[13] Interestingly, the age of onset in our patients was earlier than that reported in the West.[14] However, the 10-year gap in age of onset between the two genders in the Western population[14] is also true in our study population, with men developing the disease at least 10 years later compared to women.

An observation that might be unique to this population is that most women were married with a large number of children. This is a reflection of the typically large family size in the Saudi population.[15] The target population in our hospital was military personnel and their families. As the mean age of onset of the females in this cohort was 41 years, most of those women were probably married.

The majority of the cases did not suffer from the severe form of the disease as shown by the low prevalence of extra-articular manifestations. RF and anti-CCP antibodies were equally high in this cohort, but not ANA.

Table 1. Characteristics of the rheumatoid arthritis population (n=76).

	N	%
Gender		
Male	8	10.5
Female	68	89.5
Total	**76**	**100.0**
Married	74	97.4
Single	2	2.6
Extra-articular manifestations	5	6.58
RF positive	59	77.6
Anti-CCP positive	57	75.0
ANA positive	20	26.3
Shared epitope status[a]		
Patients SE +ve	34	44.74
Controls SE +ve	37	23.42

[a]OR 2.65, 95% CI 1.42-4.94 (*P*=.0009). RA: rheumatoid arthritis; RF: rheumatoid factor; Anti-CCP: anti-cyclic citrullinated peptide antibodies; ANA: antinuclear antibody

The presence of the shared epitope strongly predicted the presence of anti-CCP antibodies. This association between the shared epitope and anti-CCP is well documented in the literature.[16-18]

The shared epitope positivity was significantly high

Table 2. HLA-DRB1 frequency in rheumatoid arthritis (RA) patients and healthy controls.

	RA patients (n=76)		Healthy controls (n=158)		OR	95% CI	*P* Pc
DRB1*03:01	13	8.6	52	16.5	0.47	0.23-0.92	.021 0.82
DRB1*04:01	7	4.6	2	0.6	7.58	1.41-75.30	.003 0.12
DRB1*04:05	18	11.8	11	3.5	3.73	1.61-8.96	.0004 0.016
DRB1*07:01	21	13.8	84	26.6	0.44	0.25-0.76	.0019 0.074
DRB1*15:01	19	12.5	20	6.3	2.11	1.03-4.32	.024 0.92
DRB1*16:02	4	2.6	1	0.3	8.51	0.83-420.50	.0225 0.88

Statistically significant comparisons shown here. *Pc* is corrected *P* value for multiple allele testing. Data were analyzed using 2×2 table for odds ratios using cci command in Stata. Correction for *P* values were by multiplying the *P* value by the number of alleles. All of the tested alleles are in **Appendix 1**.

in this cohort compared to controls. This was reflected by the increased frequency of *HLA-DRB1*04:01* and *04:05. The latter was only significant after correcting for the multiple allele testing. *HLA-DRB1*04:05* has been shown to be associated with RA in other Asian and Arab populations.[19,20] Previously, Al-Arfaj[12] described HLA association in a cohort of 92 Saudi RA patients. The main finding was the association of *HLA-DR10;* his study, however, lacked a control group. In another study from Saudi Arabia, Al-Swailem and co-workers[11] reported an association with *HLA-DRB1*04:05*; they did not, however, relate this to the autoantibody profile of the patients. Thus, our study is unique in reporting a strong association between carrying the shared epitope status and developing anti-CCP antibodies in Saudi patients with RA.

In summary, we describe consecutive RA patients attending the Rheumatology Clinic at the National Guard Hospital in Riyadh. Most of the patients were married women with large families. Most of them were positive for RF and anti-CCP antibodies. The latter was strongly predicted by the presence of the shared epitope. Since in patients with RA, autoantibody testing is a strong predictor of the severity of the future disease course, where autoantibody tests results can influence treatment decisions,[8] our finding might be useful for early diagnosis of severe RA and for opting for aggressive treatment in patients carrying the shared epitope. A larger sample size is needed to confirm our current finding.

Table 3. Logistic regression analysis of shared epitope status and the risk for developing anti-CCP antibodies, ANA, and RF.

	Odds Ratio	95% CI	*P*
Anti-CCP	14.51	1.53-137.49	.02
ANA	1.87	0.54-6.53	.32
RF	2.71	0.41-17.91	.30

Anti-CCP: anti-cyclic citrullinated peptide antibodies; ANA: antinuclear antibody; RF: rheumatoid factor

Pseudo R squared=0.1869.

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