



Egyptian Society of Rheumatic Diseases

The Egyptian Rheumatologist
www.rheumatology.eg.net
www.elsevier.com/locate/ejr


ORIGINAL ARTICLE

HLA-DRB1 alleles in Egyptian rheumatoid arthritis patients: Relations to anti-cyclic citrullinated peptide antibodies, disease activity and severity



Amal F. Soliman^{a,*}, Sami El-Said Egaila^a, Amal Idris Ali^b,
 Naglaa Ibrahim Azab^b, Hoda H. Al-Gohary^a

^a Rheumatology, Rehabilitation and Physical Medicine Department, Faculty of Medicine, Benha University, Egypt

^b Medical Biochemistry Department, Faculty of Medicine, Benha University, Egypt

Received 3 March 2016; accepted 18 March 2016

Available online 14 May 2016

KEYWORDS

DRB1 alleles;
 Rheumatoid arthritis;
 Anti-CCP antibodies;
 RF;
 Severity;
 Activity

Abstract *Background:* Human leukocyte antigen HLA-DRB1 alleles encoding a common amino acid sequence called shared epitope in the third hypervariable region of DRB1 molecule have been identified as risk alleles for rheumatoid arthritis (RA).

Aim of the work: The aim was to study HLA-DRB1 01, 04 and 10 alleles in Egyptian RA patients and determine their relation with anticyclic citrullinated peptide (anti-CCP) antibody level, disease activity, clinical and radiological severity.

Patients and methods: The study involved 40 RA patients and 20 control. Simplified disease activity index (SDAI) was calculated, clinical severity was assessed using the mechanical joint score (MJS) and radiological severity evaluated using the simple erosion narrowing score (SENS). HLA-DRB1 genotyping and anti-CCP antibodies were detected.

Results: The mean patients' age was 41.6 ± 12.7 years and disease duration 8.9 ± 7.7 years. The frequency of HLA DRB1 01, 04 and 10 in patients was 42.5%, 60% and 25% respectively. Of them 04 was significantly higher than in controls ($p = 0.013$) and was associated with anti-CCP positive cases ($p = 0.0008$) while the absence of HLA-DRB1 alleles was significantly associated with negative anti-CCP negative RA ($p = 0.0008$). There were significant associations between HLA-DRB1 01 and 04 with SDAI ($p = 0.0002$ and $p = 0.005$, respectively); between HLA-DRB1 04 and 10 with SENS ($p = 0.002$ and $p = 0.001$ respectively) and between HLA-DRB1 01, 04 and 10 with MJS ($p = 0.02$, $p = 0.03$ and $p = 0.02$, respectively).

* Corresponding author at: Rheumatology, Rehabilitation and Physical Medicine, Faculty of Medicine, Benha University, 13516, Egypt. Mob.: +20 1224622762.

E-mail address: amal_fathy17@yahoo.com (A.F. Soliman).

Peer review under responsibility of Egyptian Society of Rheumatic Diseases.

<http://dx.doi.org/10.1016/j.ejr.2016.03.007>

1110-1164 © 2016 Publishing services provided by Elsevier B.V. on behalf of Egyptian Society of Rheumatic Diseases.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Conclusion: HLA-DRB1 04 is associated with RA in Egyptian patients and is strongly associated in the production of elevated titers of anti-CCP antibodies which contribute to the development, severity and activity of the disease.

© 2016 Publishing services provided by Elsevier B.V. on behalf of Egyptian Society of Rheumatic Diseases. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune chronic inflammatory disease and the genetic factor may be concerned in disease initiation and in the severity of its course [1]. The human leukocyte antigen (HLA) region, also well known as the major histocompatibility complex (MHC), has an impact on the genetic risk and may account for RA susceptibility [2,3] in 50–60% of cases [4]. In particular, HLA-DRB1 alleles encoding a common amino acid sequence called the shared epitope (SE) in the third hyper-variable region have been identified as risk alleles for RA [2]. These alleles are also associated with a more severe disease type [5,6].

Rheumatoid arthritis is characterized by the presence of various autoantibodies [7]. Best well known are the rheumatoid factors (RFs), which are antibodies against the Fc part of the immunoglobulin (IgG) molecules. They are not specific for RA; however, the detection of RF (IgM) is used in RA diagnosis [8]. Yet, anti-cyclic citrullinated peptide (anti-CCP) antibodies are highly specific markers for diagnosis of RA [9], preceding the appearance of the disease [10] and are reported to be good predictors for the development of RA [9]. They are also associated with a more severe disease course [9,11]. These data suggest that these antibodies have a causative role in the pathogenesis of RA [12,13].

The shared epitope hypothesis proposes that the SE plays a role in the presentation of ‘arthritogenic’ antigens to T lymphocytes [3]. Anti-CCP antibodies are directed to antigens containing the non-standard amino acid citrulline [14,15], for example, citrullinated fibrin which is found in the rheumatoid joint [16,17]. Although citrullination or SE deimination of arginine residues of proteins may result in the formation of epitopes that are targets of antibodies, help from T lymphocytes is likely required for long-term B cell responses and antibody isotype switching. Accordingly, in transgenic mice for one of the SE alleles ‘HLA-DRB1*0401’ it was demonstrated that citrullination of arginine residues of peptides at the peptide side-chain position interacting with the SE, significantly increases peptide-MHC affinity. This leads to an increased binding of these citrullinated peptides to MHC class II molecules leading to the activation of CD4-positive T cells [18]. Since not all RA patients have anti-CCP antibodies [14], this suggests that the presence of anti-CCP antibodies is not obligatory for the development of arthritis or that the pathogenic mechanisms underlying anti-CCP-positive and anti-CCP-negative RA are different. It was reported that the phenotype of RA patients with anti-CCP antibodies is similar to that without with respect to clinical presentation but differs in the disease course [19].

Since these data are showing an existing relation between anti-CCP antibodies and SE alleles and anti-CCP is highly specific for RA, a thorough analysis of HLA class II alleles involved in generating autoantibodies to citrullinated antigens

is important to gain a better understanding of this particular immune response [12]. In this study, we aimed to study HLA-DRB1 01, 04 and 10 alleles in Egyptian RA patients and determine their relation with anti-CCP antibody level, disease activity, clinical and radiological severity.

2. Patients and methods

This study included 40 RA patients selected from the Rheumatology, Rehabilitation and Physical Medicine Department, Benha University Hospitals, Benha City, Qalyobia governorate, Egypt. They were diagnosed according to the 2010 EULAR/ACR criteria [20]. 20 age and sex matched healthy volunteers were included as the control group. Patients were enrolled in the study after taking written informed consents from them. The study was approved by the ethics committee of the Faculty of Medicine, Benha University.

All the patients were subjected to full history taking and thorough clinical examination with stress on the locomotor system. Clinical disease severity was assessed using the mechanical joint score (MJS) [21]. A postero-anterior plain radiographic view of both hands was obtained for the assessment of radiological severity using the simple erosion narrowing score (SENS) [22] derived from the Sharp/van Der Heijde method as a simplified and easier scoring method. Disease activity was assessed using the simplified disease activity index (SDAI) [23].

Laboratory investigations were performed on the patients and control including the erythrocyte sedimentation rate (ESR), serum C-reactive protein (CRP) (CRP LATEX TEST KIT; BIOTEC Laboratories Ltd) (positive results ≥ 6 $\mu\text{g/ml}$) and serum rheumatoid factor (RF) by nephelometry (Turbox RF-PAIA KIT; Orion Diagnostica) done on Turbox plus analyzer (positive results ≥ 25 IU/ml). Serum anticyclic citrullinated peptide antibodies (anti-CCP3) were assessed by semi-quantitative enzyme-linked immunosorbent assay (ELISA) (QUANTA Lite™ CCP3 IgG ELISA kit supplied by INOVA Diagnostics, Inc.-USA). Assessment was according to the manufacturer’s instructions. Results ≥ 20 U/ml were considered positive. Blood collected on EDTA containing tubes were stored at -80 °C for later HLA-DRB1 genotyping.

2.1. HLA-DRB1 genotyping

Genomic DNA was extracted from 200 μl of whole blood using QIA amp DNA blood mini kits, catalog number 51104, supplied by Qiagen-Germany according to the manufacturer’s instructions. Two-hundred μl of DNA was eluted. The extracted DNA was then stored at -20 °C until further processing. HLA-DRB1 alleles (DRB1 01, DRB1 04, DRB1 10) were identified by conventional polymerase chain reaction (PCR) using specific primers, common reverse primer CTC

Table 1 Demographic features and laboratory parameters of the RA patients and control as well as the clinical characteristics, medications used disease activity and severity of the RA patients.

Parameter mean \pm SD (range) or n (%)	RA patients (n = 40)	Control (n = 20)
Age (years)	41.6 \pm 12.7 (20–66)	43.3 \pm 13.9 (20–64)
Age at disease onset (years)	32.7 \pm 7.03 (18–50)	–
Disease duration (years)	8.9 \pm 7.7 (1–30)	–
Sex: female:male	36 (90): 4 (10)	17 (85): 3 (15)
Smoking	4 (10)	3 (15)
<i>EA manifestations</i>	4 (10)	–
<i>Disease activity</i>		
SDAI	22.96 \pm 13.6 (7.5–49.3)	–
mild	23 (57.5)	
moderate	10 (25)	
severe	7 (17.5)	
<i>Disease severity</i>		
Clinical: MJS	12.2 \pm 12.1 (0–38)	–
Radiological: SENS	22.8 \pm 24.9 (2–76)	–
<i>Medications</i>		
Methotrexate mg/w (n = 32)	14.9 \pm 7.4	–
Prednisolone mg/d (n = 15)	4.1 \pm 5.6	–
Leflunomide mg/d (n = 8)	4.0 \pm 7.9	–
Chloroquine mg/d (n = 21)	125 \pm 133.6	–
<i>Laboratory parameters</i>		
Anti-CCP positivity		
20 (50)		
0 (0)		
RF positivity	29 (72.5)	0 (0)
CRP positivity	30 (75)	0 (0)
High ESR (mm/1st hr)	31 (77.5)	0 (0)

RA: rheumatoid factor, EA: extra-articular, SDAI: simplified disease activity index, MJS: mechanical joint score, SENS: simple erosion narrowing score, anti-CCP: anti-cyclic citrullinated peptide, RF: rheumatoid factor, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate.

GCC GCT GCA CTG TGA AG forward primer for DRB1 01 CTT GTG GCA GCT TAA GTT TGA AT, forward primer for DRB1 04 GTT TCT TGG AGC AGG TTA AAC and, forward primer for DRB1 10 CAC AGC ACG TTT CTT GGA GG [24]. Amplification was done using Taq PCR Master Mix Kit supplied by Qiagen. The PCR mix for each allele contained 25 μ l of Taq PCR master. Mix 2 \times , 2.5 μ l (0.5 μ M) of forward primer, 2.5 μ l (0.5 μ M) of reverse primer, 10 μ l of the template DNA and 10 μ l of nuclease free water to reach a final volume of 50 μ l. G storm thermal cycler-UK was used for amplification according to the following program: initial denaturation at 94 $^{\circ}$ C for 3 min., 35 cycles of denaturation at 94 $^{\circ}$ C for 30 s., annealing at 53 $^{\circ}$ C for 30 s. and extension at 72 $^{\circ}$ C for 1 min., followed by final extension at 72 $^{\circ}$ C for 10 min. then hold at 4 $^{\circ}$ C. 10 μ l of each amplified DNA & 1000 bp ladder (molecular weight marker) were separated on 2% agarose gel containing 0.3 μ g/ml of ethidium bromide, photographed and analyzed (agarose gel electrophoresis)

Statistical analysis: The collected data were computed and statistically analyzed using SPSS version 17 software for windows (SPSS Inc., Chicago). Suitable statistical techniques were calculated as range, mean, standard deviation, median and frequency (percentage). Chi square and Z tests were used to test the significance for frequency and the corrected Chi square test for frequencies <5. Student's *t* test and ANOVA were used for comparison of 2 groups and >2 groups respectively. Odds

ratio (OR) with 95% confidence interval (95% CI) values were also calculated. Pearson correlation coefficient was estimated to correlate the DRB1 genotypes and anti-CCP antibody level. *P* values <0.05 were considered significant.

3. Results

Forty Egyptian RA patients with a mean age of 41.6 \pm 12.7 years (range: 20–66 years) and 20 control with a mean age of 43.3 \pm 13.9 years (range: 20–64 years) were included in the study. The demographic features and laboratory parameters of the RA patients and control as well as the clinical characteristics of the patients are presented in Table 1.

The frequency of HLA-DRB1 01, 04 and 10 in RA patients and control are shown in Table 2. Only the frequency of 04 allele was significantly higher in RA patients compared to the controls.

The relation of the studied HLA-DRB1 01, 04, 10 alleles with the different demographic and clinical parameters in RA patients is presented in Table 3. The disease duration was significantly (*p* = 0.0014) longer in HLA-DRB1 10 positive cases. The SDAI was significantly higher in RA patients with positive 01 and 04 alleles (*p* = 0.002 and *p* = 0.005, respectively). Clinical disease severity (MJS) was significantly higher in patients with positive 01, 04 and 10 alleles (*p* = 0.022, *p* = 0.03 and

Table 2 Frequency of HLA-DRB1 01, 04 and 10 in RA patients and control.

HLA-DRB1 frequency <i>n</i> (%)	RA patients (<i>n</i> = 40)	Control (<i>n</i> = 20)	<i>p</i>	OR (95% CI)
01	17 (42.5)	6 (30)	0.35	1.7 (0.6–5.4)
04	24 (60)	5 (25)	0.013	4.5 (1.4–14.8)
10	10 (25)	2 (10)	0.18	3 (0.6–15.3)
No alleles	10 (25)	10 (50)	0.06	0.3 (0.1–1.03)

HLA: Human leukocyte antigen, RA: rheumatoid arthritis. Bold value is significant at $p < 0.05$.

$p = 0.02$, respectively). However, radiological disease severity (SENS) was significantly higher in those with a positive 04 or 10 allele ($p = 0.002$ and $p = 0.001$, respectively).

Frequency of HLA-DRB1 genotypes among anti CCP and RF positive and negative RA patients is presented in Table 4. A positive 04 allele was the only HLA-DRB1 significantly associated with anti-CCP positivity ($p = 0.0008$). The presence of ≥ 1 alleles was significantly associated with a positive anti-CCP ($p = 0.0008$). The anti-CCP antibodies were positive in 13 of the 29 RF sero-positive RA patients (44.8%) and in 7 of the 11 RF negative RA patients (63.6%). There was a significant correlation of the serum anti-CCP antibodies level with a ≥ 1 positive allele ($p = 0.003$), a positive 04 and 10 ($p = 0.035$, $p = 0.005$) alleles. Anti-CCP antibodies were also significantly correlated with the disease activity ($p = 0.032$), clinical ($p = 0.034$) and radiological severity scores ($p = 0.033$) (Table 5).

4. Discussion

The HLA-DRB1 alleles encoding the SE were found to be associated with the severity and susceptibility of RA and

include 0101, 0102, 0104, 0401, 0404, 0405, 0408, 0413, 0416 and 1001 alleles [25]. These alleles are subtypes of HLA-DRB1 (01, 04 and 10). The aim of this study was to evaluate the 01, 04 and 10 alleles in the Egyptian RA patients. Our results reported a frequency of 30%, 25% and 10% for HLA DRB1 01, 04 and 10 respectively in the normal subjects of Benha City in Qalyobia governorate. Their frequency in the normal subjects of Assuit governorate was 5%, 10% and 15% respectively [26]. In Asian populations, the frequency was 5.7%, 4.9% and 7.4% and in the European populations it was 25.3%, 26.5% and 1.1%, respectively. However, in both the Asian and European populations the reported frequency was limited to some SE alleles of HLA DRB1 04 [27]. Moreover, in a Kuwaiti normal population, the frequency was 5.7%, 22.8% and 14.3% respectively [28].

On the other hand, the frequency of HLA DRB1 01, 04 and 10 in RA patients in the present study was 42.5%, 60% and 25% respectively. Of them, HLA DRB1 04 was significantly higher in RA patients than in controls. In accordance, it was reported that there are differences in the strength of the association between different SE alleles and RA. The HLA-DRB1 04 alleles represent a considerably stronger susceptibility factor than the other SE alleles [29]. Also, Alsaied et al., [28] reported that only HLA-DRB1 04 was significantly higher in the RA patients. Fathi and associates [26] reported a frequency of 45%, 35% and 0% for HLA-DRB1 01, one or two 04 and 10 in RA patients respectively. Both HLA DRB1 01 and 04 were significantly higher and 10 was significantly lower in RA patients than in the normal control of that study. However HLA-DRB1 10 was more prevalent in Spanish RA patients [30]. On the other hand, in Chinese RA patients, HLA-DRB1 01 was not associated with RA but 04 frequency was significantly higher than in the healthy Chinese individuals [31].

Our study reported a significant association of a positive HLA-DRB1 01 and 04 with disease activity and clinical disease severity however, positive HLA-DRB1 04 and 10 alleles were

Table 3 Relation of the studied HLA-DRB1 01, 04 and 10 status with the different demographic and clinical parameters in the rheumatoid arthritis patients.

Parameter mean \pm SD (range) or <i>n</i> (%)	HLADRB1 in RA patients (<i>n</i> = 40)					
	01		04		10	
	Positive (<i>n</i> = 17)	Negative (<i>n</i> = 23)	Positive (<i>n</i> = 24)	Negative (<i>n</i> = 16)	Positive (<i>n</i> = 10)	Negative (<i>n</i> = 30)
Age	41.8 \pm 14.7	41.4 \pm 11.2	43.5 \pm 12.4	38.7 \pm 12.9	41.8 \pm 15.	39.2 \pm 10.9
Age at onset	31.6 \pm 6.6	33.4 \pm 7.4	33.2 \pm 5.5	31.8 \pm 8.9	34.3 \pm 8.7	32.1 \pm 6.4
Disease duration	10.2 \pm 9.4	7.9 \pm 6.2	10.3 \pm 8.4	6.9 \pm 6.3	14.5 \pm 9.6	7.1 \pm 4.1*
Sex F:M	17:0	19:4	24:0	12:4*	9:1	27:3
<i>Disease activity</i>						
SDAI	31.7 \pm 13.4	16.5 \pm 9.8**	27.7 \pm 13.9	15.8 \pm 9.6*	30.1 \pm 13.1	20.5 \pm 13.2
mild	4 (23.5)	19 (82.6)**	10 (41.7)	13 (81.3)*	3 (30)	20 (66.7)*
moderate	6 (35.3)	4 (17.4)	7 (29.2)	3 (18.8)	4 (40)	6 (20)
severe	7 (41.2)	0 (0)*	7 (29.2)	0 (0)*	3 (30)	4 (13.3)
<i>Disease severity</i>						
MJS	17.2 \pm 13.4	8.5 \pm 9.7*	15.5 \pm 12.5	7.3 \pm 9.8*	20.4 \pm 14	9.5 \pm 10.1*
SENS	31.1 \pm 28.1	43.1 \pm 13.8	38.9 \pm 24.6	13.6 \pm 22.9*	40.8 \pm 31	16.8 \pm 1.4*

HLA: Human leukocyte antigen, RA: rheumatoid arthritis, SDAI: simplified disease activity index, MJS: mechanical joint score, SENS: simple erosion narrowing score.

* Significant.

** Highly significant difference from the corresponding values at $p < 0.05$.

Table 4 Frequency of HLA-DRB1 genotypes among anti CCP and RF positive and negative RA patients.

HLA-DRB1 genotype frequency n (%)		Rheumatoid arthritis patients (n = 40)					
		Anti-CCP			RF		
		Positive (n = 20)	Negative (n = 20)	OR (95CI)	Positive (n = 29)	Negative (n = 11)	OR (95CI)
Allele	01	11 (55)	6 (30)	2.9	13 (44.8)	4 (36.4)	1.4
	04	18 (90)	6 (30)**	21	19 (65.5)	5 (45.5)	2.3
	10	7 (35)	3 (15)	3.1	7 (24.1)	3 (22.3)	0.9
	None	0 (0)	10 (50)**	–	7 (24.1)	3 (22.3)	0.9
≥1 alleles		20 (100)	10 (50)**	–	22 (75.9)	8 (72.7)	1.2
• 1 allele		9 (45)	6 (60)	0.5	10 (34.5)	5 (45.5)	0.5
• 2 alleles		6 (30)	3 (30)	1	7 (24.1)	2 (18.2)	1.4
• 3 alleles		5 (25)	1 (10)	3	5 (17.2)	1 (9.1)	2.1

RA: rheumatoid factor, anti-CCP: anti-cyclic citrullinated peptide, RF: rheumatoid factor.

** Highly significant difference from the corresponding values at $p < 0.05$.

Table 5 Correlation of anti-CCP level with the presence of different HLA DRB1 genotypes alleles, with disease activity, clinical and radiological severity score in RA patients.

R (p)	Anti-CCP
<i>DRB1 genotype</i>	
01	0.21 (0.19)
04	0.43 (0.035)
10	0.53 (0.005)
≥1 allele	0.56 (0.003)
<i>Disease activity</i>	
SDAI	0.45 (0.032)
<i>Disease severity</i>	
Clinical: MJS	0.42 (0.034)
Radiological: SENS	0.41 (0.033)

Anti-CCP: anti-cyclic citrullinated peptide, SDAI: simplified disease activity index, MJS: mechanical joint score, SENS: simple erosion narrowing score. Bold values are significant at $p < 0.05$

significantly associated with radiological severity. Fathi and associates [26] reported the significant association of HLA DRB1 04 alleles with more activity of the disease but not 01. They also reported significant association of HLA-DRB1 01 and 04 with the radiologically severe disease. Also, Lin and associates [31] revealed a significant association of HLA-DRB1 04 with clinically and radiologically severe disease in Chinese populations. However, Kinikli and associates [32] reported no association of HLA-DRB1 01 or 04 with the clinical or radiological severity of the disease in the Turkish population.

In a previous study, anti-CCP Abs were detected in 53% of RA patients [25]. Our study also detected them in 50% of RA Egyptian patients. Moreover our work like previous studies have documented the absence of anti-CCP antibody production among healthy individuals [9,10,31]. Our study reported that carrier of HLA-DRB1 01, 04 and 10 was associated with production of anti-CCP antibodies being significant only with 04. Previous studies found that carrier of one or two HLA-DRB1 SE alleles was significantly associated with production of anti-CCP antibodies in a Dutch RA population [14,25]. Our study revealed that in the Egyptian patients, the presence of one or more of the alleles studied, was significantly associ-

ated with anti-CCP positive RA. Berglin et al. [33], Hughes et al. [34] and Kaltenhauser et al. [25] found that the HLA-DRB1 01 and 04 SE alleles conferred the highest risk for development of anti-CCP antibodies in RA patients.

There was a significant correlation between high serum level of anti-CCP antibodies and HLA-DRB1 (04 and 10) alleles, SDAI score, radiological and clinical severity score. This agrees with Gourrand et al. [7] and Berglin et al. [33] who suggested that measurement of serum anti-CCP levels might be of clinical significance for detection of disease severity and activity. They mentioned that RA patients with positive SE and positive anti-CCP had a significantly higher rate of destruction in the joints than did all other patients. Which means that a higher rate of joint damage was only found when both SE alleles and anti-CCP antibodies were present. A previous study reported that the SE alleles correlated with the presence of anti-CCP Abs, but not with the presence of RF [35]. Also Kinikli and associates [32] reported no association with seropositivity of RA. This coincides with our results. On other hand Ucar et al., found that HLA-DRB1*01 and *04 were determined to be higher in RA patients with +RF [36].

HLA-DRB1 04 was associated with RA in 60% of the patients in our study; therefore, the possibility that other genes may add to the pathogenesis of RA cannot be denied. In addition to genetic factors, some environmental factors have been concerned as predictors of RA. The 2–3-fold higher prevalence of the disease in women, primarily before menopause, has been interpreted as indicating a role for hormonal or reproductive factors [32]. Smoking is a well-established risk factor for the development of RA and it was found that smoking contributes to the development of RA in SE positive and anti-CCP positive patients [15], indicating that the predisposition to RA may be due to an interaction between genetic and environmental factors.

In conclusion, HLA-DRB1 04 was associated with RA Egyptian patients particularly those positive to anti-CCP antibodies. It was strongly associated in the production of elevated titers of anti-CCP antibodies which contribute to the disease development, severity and activity of RA disease. The presence of anti-CCP antibodies and/or HLA-DRB1 04 or 10 is associated with a poor radiological outcome whereas 01 and 04 are associated with higher grade of disease activity and clinical severity. Therefore the identification of susceptible allele in RA patients may assist a physician to take early decision

regarding starting of intensive therapy to prevent joint damage. Further longitudinal studies are recommended with large numbers of patients and controls to assess the predictive role of each allele in RA susceptibility, to study the polymorphic character of HLA genes and to emphasize the relationships of environmental with genetic factors.

Conflict of interest

None.

References

- [1] Gheita TA, Azkalanay GS, Gaber W, Mohey A. Clinical significance of serum TNF α and -308 G/A promoter polymorphism in rheumatoid arthritis. *Egypt Rheumatol* 2015;37:49–54.
- [2] Gregersen PK, Silver J, Winchester RJ. The shared epitope hypothesis: an approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. *Arthritis Rheum* 1987;30:1205–13.
- [3] El-Saadany HM, Amer WH, Khalil HS, Gaber RA, Elshweikh SA. Association of STAT4 polymorphism with susceptibility and severity of rheumatoid arthritis and systemic lupus erythematosus in Egyptian patients. *Egypt Rheumatol* 2016;38:21–7.
- [4] Mokbel A, Rashid L, Al-Harizy R. Decreased level of soluble receptors of advanced glycosylated end products (sRAGE) and glycine82serine (G82S) polymorphism in Egyptian patients with RA. *Egypt Rheumatol* 2011;33(1):53–60.
- [5] Wagner U, Kaltenhauser S, Sauer H, Arnold S, Seidel W, Hantzschel H, et al. HLA markers and prediction of clinical course and outcome in rheumatoid arthritis. *Arthritis Rheum* 1997;40:341–51.
- [6] van Zeben D, Hazes JM, Zwinderman AH, Cats A, Schreuder GM, D'Amato J, et al. Association of HLA-DR4 with a more progressive disease course in patients with rheumatoid arthritis: results of a follow up study. *Arthritis Rheum* 1991;34:822–30.
- [7] Gourraud PA, Boyer JF, Barnette T, Abbal M, Cambon-Thomsen A, Cantagrel A, et al. A new classification of HLA-DRB1 alleles differentiates predisposing and protective alleles for rheumatoid arthritis structural severity. *Arthritis Rheum* 2006;54(2):593–9.
- [8] Vossenaar ER, van Venrooij WJ. Anti-CCP antibodies, a highly specific marker for (early) rheumatoid arthritis. *Clin Appl Immunol Rev* 2004;4:239–62.
- [9] van Gaalen FA, Linn-Rasker SP, van Venrooij WJ, de Jong BA, Breedveld FC, Verweij CL, et al. Autoantibodies to cyclic citrullinated peptides predict progression to rheumatoid arthritis in patients with undifferentiated arthritis: a prospective cohort study. *Arthritis Rheum* 2004;50(3):709–15.
- [10] Rantapaa-Dahlqvist S, de Jong BA, Berglin E, Hallmans G, Wadell G, Stenlund H, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum* 2003;48(10):2741–9.
- [11] Hussein MS, Abdel Ghany SE, Elashkar DS, Rabea MY, Nosair NA. Anti-CCP hs (high sensitive) in Egyptian rheumatoid arthritis patients associated with chronic hepatitis C virus infection. *Egypt Rheumatol* 2016;38(1):15–20.
- [12] Balandraud N, Picard C, Reviron D, Landais C, Toussiot E, Lambert N, et al. HLA-DRB1 genotypes and the risk of developing anti citrullinated protein antibody (ACPA) positive rheumatoid arthritis. *PLoS ONE* 2013;8(5):e64108.
- [13] Kuhn KA, Kulik L, Tomooka B, Braschler KJ, Arend WP, Robinson WH, et al. Antibodies against citrullinated proteins enhance tissue injury in experimental autoimmune arthritis. *J Clin Invest* 2006;116(4):961–73.
- [14] Huizinga TW, Amos CI, van der Helm-van Mil AH, Chen W, van Gaalen FA, Jawaheer D, et al. Refining the complex rheumatoid arthritis phenotype based on specificity of the HLA-DRB1 shared epitope for antibodies to citrullinated proteins. *Arthritis Rheum* 2005;52(11):3433–8.
- [15] Wagner CA, Sokolove J, Lahey LJ, Bengtsson C, Saevarsdottir S, Alfredsson L, et al. Identification of anticitrullinated protein antibody reactivities in a subset of anti-CCP-negative rheumatoid arthritis: association with cigarette smoking and HLA-DRB1 'shared epitope' alleles. *Ann Rheum Dis* 2015;74(3):579–86.
- [16] Masson-Bessiere C, Sebbag M, Durieux JJ, Nogueira L, Vincent C, Girbal-Neuhauser E, et al. In the rheumatoid pannus, antiflaggrin autoantibodies are produced by local plasma cells and constitute a higher proportion of IgG than in synovial fluid and serum. *Clin Exp Immunol* 2000;119(3):544–52.
- [17] Vossenaar ER, Nijenhuis S, Helsen MM, van der Heijden A, Senshu T, van den Berg WB, et al. Citrullination of synovial proteins in murine models of rheumatoid arthritis. *Arthritis Rheum* 2003;48(9):2489–500.
- [18] Hill JA, Southwood S, Sette A, Jevnikar AM, Bell DA, Cairns E. Cutting edge: the conversion of arginine to citrulline allows for a high-affinity peptide interaction with the rheumatoid arthritis associated HLA-DRB1*0401 MHC class II molecule. *J Immunol* 2003;171(2):538–41.
- [19] van der Helm-van Mil AH, Verpoort KN, Breedveld FC, Toes RE, Huizinga TW. Antibodies to citrullinated proteins and differences in clinical progression of rheumatoid arthritis. *Arthritis Res Ther* 2005;7(5):R949–58.
- [20] Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, et al. Rheumatoid arthritis classification criteria: an American college of rheumatology/European league against rheumatism collaborative initiative. *Arthritis Rheum* 2010;62:2569–81.
- [21] Johnson AH, Hassell AB, Jones PW, Matthey DL, Saklatvala J, Dawes PT. The mechanical joint score: a new clinical index of joint damage in rheumatoid arthritis. *Rheumatology* 2002;41(2):189–95.
- [22] van der Heijde D, Dankert T, Nieman F, Rau R, Boers M. Reliability and sensitivity to change of a simplification of the Sharp/van der Heijde radiological assessment in rheumatoid arthritis. *Rheumatology* 1999;38(10):941–7.
- [23] Smolen JS, Breedveld FC, Schiff MH, Kalden JR, Emery P, Eberl G, et al. A simplified disease activity index for rheumatoid arthritis for use in clinical practice. *Rheumatology (Oxford)* 2003;42(2):244–57.
- [24] Schreuder GM, Hurley CK, Marsh SG, Lau M, Maiers M, Kollman C, et al. The HLA dictionary 1999: a summary of HLA-A, -B, -C, -DRB1/3/4/DQB1 alleles and their association with serologically defined HLA-A, -B, -C, -DR and -DQ antigens. *Tissue Antigens* 1999;54:409–37.
- [25] Kaltenhauser S, Pierer M, Arnold S, Kamprad M, Baerwald C, Hantzschel H, et al. Antibodies against cyclic citrullinated peptide are associated with the DRB1 shared epitope and predict joint erosion in rheumatoid arthritis. *Rheumatology (Oxford)* 2007;46(1):100–4.
- [26] Fathi NA, Ezz-Eldin AM, Mosad E, Bakry RM, Hamed HB, Ahmed S. Diagnostic performance and predictive value of rheumatoid factor, anti-cyclic-citrullinated peptide antibodies and HLA-DRB1 locus genes in rheumatoid arthritis. *Int Arch Med* 2008;22(1):20.
- [27] Griffiths B, Situnayake RD, Clark B, Tennant A, Salmon M, Emery P. Racial origin and its effect on disease expression and HLA-DRB1 types in patients with rheumatoid arthritis: a matched cross-sectional study. *Rheumatology (Oxford)* 2000;39(8):857–64.
- [28] Alsaedi K, Alawadhi A, Al-Saeed O, Haider MZ. Human leukocyte antigen DRB1*04 is associated with rheumatoid arthritis in Kuwaiti patients. *Joint Bone Spine* 2006;73(1):62–5.

- [29] Silman AJ, Pearson JE. Epidemiology and genetics of rheumatoid arthritis. *Arthritis Res* 2002;4(3):S265–72.
- [30] Yelamos J, Garcia-Lozano JR, Moreno I, Aguilera I, Gonzales MF, Garcia A, et al. Association of HLA-DR4-Dw15 (DRB1*0405) and DR10 with rheumatoid arthritis in a Spanish population. *Arthritis Rheum*. 1993;36(6):811–4.
- [31] Lin L, Chen Y, Xiao Z, Huang S, Yang Z. The association of HLA-DRB1 alleles with rheumatoid arthritis in the Chinese Shantou population: a follow-up study. *Biochem Cell Biol* 2007;85(2):227–38.
- [32] Kinikli G, Ateş A, Turgay M, Akay G, Kinikli S, Tokgöz G. HLA-DRB1 genes and disease severity in rheumatoid arthritis in Turkey. *Scand J Rheumatol* 2003;32(5):277–80.
- [33] Berglin E, Johansson T, Sundin U, Jidell E, Wadell G, Hallmans G, et al. Radiological outcome in rheumatoid arthritis is predicted by presence of antibodies against cyclic citrullinated peptide before and at disease onset, and by IgA-RF at disease onset. *Ann. Rheum. Dis.* 2006;65(4):453–8.
- [34] Hughes LB, Morrison D, Kelley JM, Padilla MA, Vaughan LK, Westfall AO, et al. The HLA-DRB1 shared epitope is associated with susceptibility to rheumatoid arthritis in African Americans through European genetic admixture. *Arthritis Rheum* 2008;58(2):349–58.
- [35] Kroot EJ, de Jong BA, van Leeuwen MA, Swinkels H, van den Hoogen FH, van't Hof M, et al. The prognostic value of anti-cyclic citrullinated peptide antibody in patients with recent onset rheumatoid arthritis. *Arthritis Rheum* 2000;43(8):1831–5.
- [36] Uçar F, Çapkin E, Karkucak M, Yücel B, Sönmez M, Alver A, et al. Associations of HLA-DRB1 alleles with anti-citrullinated protein antibody-positive and anti-citrullinated protein antibody-negative rheumatoid arthritis in northern east part of Turkey. *Int J Rheum Dis* 2012;15(6):538–45.