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Anti-cyclic citrullinated peptide antibodies and association with HLA alleles in Moroccan patients with systemic lupus erythematosus

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ABSTRACT: Systemic Lupus Erythematosus (SLE) patients may have arthritis in early stages of the disease. This study aims to investigate the prevalence of anti-CCP in SLE patients from Morocco and its association with HLA class II alleles. Anti-cyclic citrullinated peptide antibodies (Anti-CCP) were measured using ELISA in 88 SLE patients with and without arthritis. Levels ≥ 25 units/ ml defined a positive test of anti-CCP antibodies. Positive anti-CCP was detected in 8 % of SLE patients whose 85.71% % with arthritis and 14.28% without arthritis. The mean titer of anti-CCP antibodies in the SLE group was 83.75 U/ml. HLA class II alleles typing was performed by polymerase chain reaction-sequence-specific primers (PCR-SSP). We found an increase of HLA-DRB1*04 frequency and decrease of DRB1*07 frequency in SLE patients with anti-CCP positive. In the Moroccan population we demonstrated the presence of high titer of anti-CCP in SLE. Results from our study also identified the frequency of HLA-DRB1*04 allele is increased in SLE with anti-CCP positive.

KEYWORDS: Anti-cyclic citrullinated peptide antibodies, Systemic Lupus Erythematosus, Human leukocyte antigen, arthritis, Moroccan population.

1 INTRODUCTION

Systemic lupus Erythematosus (SLE) is an autoimmune disease with multi-organ involvement. In up to 90% of lupus patients are suffering from arthritis [1], [2]. Arthritis is a major cause of morbidity in SLE patients [3], [4].

Anti-cyclic citrullinated peptide (anti-CCP) antibodies are specific and sensitive assay in Rheumatoid arthritis (RA) diagnosis [5]. Furtheremore, in SLE positive anti-CCP is detectable [6], [7].

Most studies have focused on the role of HLA class II (DR, DQ) in lupus. In the Moroccan population the positive association of HLA class II alleles with LN has been reported [8].

The current study evaluates the prevalence of anti-CCP in SLE patients and evaluates the distribution of the HLA class II alleles in SLE patients with anti-CCP positive.

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2 MATERIALS AND METHODS

2.1 PATIENTS AND CONTROLS

We recruited patients from the Departments of Nephrology, Rabat Ibn Sina University Hospital between January 2013 and March 2014. SLE was diagnosed according to the American College of Rheumatology (ACR) criteria [9]. The ethics committee of the Rabat Medicine University approved the study and a written informed consent was obtained from all patients.

2.2 DNA EXTRACTION AND HLA TYPING

DNA was extracted from blood samples of patients using a commercial kit (Qiagen). HLA typing of class II (-DRB1* and -DQB1*) was tested by ''Polymerase chain reaction sequence specific primers'' (PCR-SSP) according to generic HLA DNA typing trays (One Lambda).

2.3 MEASUREMENT OF ANTI-CCP

IgG-class anti-CCP was measured by enzyme-linked immunosorbent assay (Immunoscan CCPlus). Levels ≥ 25 units/ ml defined a positive test as suggested by the manufacturer.

2.4 STATISTICAL ANALYSIS

Calculation was carried out using the commercial SPSS software package for Windows (version 13.0). The frequencies in SLE patients were compared by the Chi-square test or Fisher's two-tailed exact probability test as appropriate. A p-value smaller than 0.05 was considered statistically significant.

3 RESULTS

This study included 88 unrelated Moroccan SLE. Ten males and 78 females were recruited with mean age was 36.43 ± 10.81years.

Positive anti-CCP was detected in 07 out of 88 patients (8%). 85.71% patients with positive anti-CCP were female. In patients with positive anti-CCP, the mean level was 83.75 U/mL.

Arthritis was present in 85 SLE patients (96.60 %). In SLE patients with positive anti-CCP, arthritis was noted in 6 patients with arthritis (85.71%) and 1 patients of the non-arthritis (14.28 %) (Table1).

Table 1. Demographic and clinical characteristics of SLE patients

Variables	SLE (n=88)
Gender Female	 78
Age (years mean ± SD)	36.43 ± 10.81
Age at disease onset (years)	28.10 ± 9.21
Disease duration ((months Mean ± SD)	98.58 ± 69.36
Arthritis	85

SLE: Systemique lupus erythematosus; n: number of individuals.

The generic typing of DRB1* and DQB1* in patients with a positive anti-CCP showed an increase of HLA- DRB1*01, -DRB1*04 and -DQB1*06 allele frequency compared with patients with a negative anti-CCP. However, the value was not significant after statistical analysis (Table2-3).

The frequency of the HLA-DRB1*07 allele increased by 36.7% in patients with a negative anti-CCP compared to 0.0% in patients with a positive anti-CCP, however the value is not significant (Table 2).

On the other hand, DQB1*02 allele is less frequent in patients with a positive anti-CCP compared to the patients with a negative anti-CCP.

Table 2. HLA-DRB1* allele frequencies in Moroccan SLE patients with and without positive Anti CCP

DRB1* Alleles	Allele frequencies (%)		P-value
	Positive Anti CCP (n=7)	Negative Anti CCP (n=60)	
1	28.6	5	0.08
3	28.6	40	0.69
4	28.6	15	0.32
7	0.0	36.7	0.86
8	14.3	11.7	1
9	0.0	0.0	-
10	0.0	6.7	1
11	0.0	11.7	1
12	0.0	1.7	1
13	42.9	18.3	0.15
14	0.0	6.7	1
15	57.1	40.0	0.44
16	0.0	3.3	1
Blanks	0.0	5.0	1

SLE: Systemic Lupus Erythematosus; n: number of individuals.

Table 3. HLA-DQB1* allele frequencies in Moroccan SLE patients with and without positive Anti CCP

DQB1*Alleles	Allele frequencies (%)		P-value
	Positive Anti CCP (n=7)	Negative Anti CCP (n=60)	
2	28.6	65.0	0.09
3	57.1	35.0	0.41
4	0.0	10.0	1
5	28.6	23.3	0.66
6	85.7	48.3	0.10
Blanks	0.0	18.3	0.58

SLE: Systemic Lupus Erythematosus; n: number of individuals.

4 DISCUSSION

A few studies examined the relationship between anti-CCP and SLE. Our data showed that positive anti-CCP was detected in 8 % of patients with SLE. Our result of positive anti-CCP has been observed in Iranian (4.7%) [10]; European (6.8%) [11]; Brazilian (13.76%) [12]; patients enrolled in the University of Florida Center for Autoimmune Disease(17%) [13]; and Chinese (13.8%) [14] SLE patients. We believe, this discordant result of frequency can be explained by the disease heterogeneity.

The analysis of the gender frequencies in SLE patients with a positive anti-CCP shows the female predominance. A similar observation has been reported in the Iranian patients [12]. This result may be explained by the female predominance observed in human SLE.

In Moroccan SLE patients with positive anti-CCP, the mean level was higher than Iranian [10] and Chinese SLE patients [14]. These differences between our findings and others can be explained by difference in patient number and type of ELISA kit used.

Anti-CCP antibodies were more prevalent (85.71%) in SLE patients with arthritis than those without arthritis (14.28) .A similar result have reported by Y-F Qing and R.A. Habeeb, who found that anti-CCP-positive SLE patients were more likely associated with arthritis and that anti-CCP antibodies may play a role in pathogenesis as regards the development of arthritis in SLE patients [15], [16].

In the present study, an increase of DRB1*04 allele frequency in patients with a positive anti-CCP. Furthermore, the frequency of DRB1*04 is increased in Moroccan RA [17]. Thus, it is speculated that DRB1*04 predisposes to arthritis in SLE Moroccan patients.

The decrease of the DRB1*07 allele in SLE patients with a positive anti-CCP and in those in Moroccan RA [17] suggest that DRB1*07 may play a protective role against arthritis in SLE Moroccan patients.

Indeed, we did not found the difference of DRB1*15 frequency between patients with a positive anti-CCP and those with a negative anti-CCP. Contrary to our findings, DRB1*15 predisposes to lupus nephritis for the Moroccan patients population [8].

5 CONCLUSION

Our study shows the presence of anti-CCP in SLE Moroccan patients. Moreover, we found an increase of HLA-DRB1*04 frequency and decrease of DRB1*07 frequency in SLE Moroccan patients with positive anti-CCP. To confirm the study it would be interesting to increase the samples size. Furthermore, a regular follow-up will reveal the real clinical value of anti-CCP in SLE patients.

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DECLARATION OF INTEREST

The authors declare that they have no conflicts of interest concerning this article.

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