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Research Article

GENETIC ANALYSIS OF HLA-DR ALLELES AND THEIR ASSOCIATION WITH ANTINUCLEAR ANTIBODIES IN MOROCCAN PATIENTS WITH LUPUS NEPHRITIS

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A B S T R A C T

Systemic lupus erythematosus is a chronic autoimmune disease characterized by the production of auto-antibodies in particular antinuclear antibodies. The association between ANA and the Human leukocyte antigen (HLA) genes in lupus nephritis patients has never been studied on a Moroccan population. The aim of this work was to describe the antinuclear antibodies profile in patients with lupus nephritis and to determine the association between HLA alleles and antinuclear antibodies in lupus nephritis patients. Antinuclear antibodies anti-deoxyribonucleic acid (anti-DNA), anti-histone proteins, anti-nucleosome, anti-SS-A, anti-SS-B, anti-Smith antigen (Sm), anti-RNP (ribonucleoprotein) and anti-ribosomal P-proteins were measured using standard clinical laboratory protocols. HLA typing of class II (DRB1) was tested by ''Polymerase chain reaction sequence specific primers'' (PCR-SSP) according to micro generic HLA DNA typing trays in 75 patients with LN. DRB1*07 was higher in lupus nephritis patients with anti-SS-A than in those without ($p = 0.04$). Patients with anti-SS-B had significantly increased frequencies of DRB1*07 versus patients without anti-SS-B.
In the Moroccan population we demonstrated the positive association of HLA-DRB1 with antinuclear antibodies in lupus nephritis patients.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by the production of autoantibodies in particular antinuclear antibodies. These autoantibodies are considered markers of diagnosis of SLE. Worldwide studies have been reported that specific ANA such as anti-DNA , anti-histone proteins, anti-nucleosome , anti-SS-A, anti-SS-B, anti-Sm, anti-RNP, anti-ribosomal P-proteins, are associated with different clinical manifestation of SLE (Sulcebe, 1992; Thompson, 1993; Vlachoyiannopoulos, 1993).Lupus nephritis (LN) complicates SLE in 74% of patients and is a disease with a poor prognosis (Li *et al*., 2013).

Some HLA alleles have been associated with LN (Ramos *et al*., 2010). However, few studies have investigated the HLA association with antibodies production in LN. The association between ANA and the HLA genes in LN patients has never been studied on a Moroccan population. The aim of this work was to describe the ANA profile in patients with LN and to determine the association between these alleles and ANA in LN patients.

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MATERIALS AND METHODS

Patients

Patients recruited from the Departments of Nephrology, Rabat Ibn Sina, University Hospital in the period from January 2013 to March 2014. Unrelated Moroccan SLE patients diagnosed according to the American College of Rheumatology (ACR) Criteria (Hahn *et al*., 2012) were studied. Anti-DNA, anti-Sm, anti-SS-A, anti-SS-B, anti-RNP, anti-Ribosomal P-proteins, anti-nucleosomes, anti-histones antibodies were measured using standard clinical laboratory protocols. Lupus disease activity measured at visit, by the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) (Bombardier *et al*., 1992). The written informative consent was obtained

from all patients and the study was approved by the ethical committee of the Rabat Medicine University.

DNA Extraction and HLA Typing

HLA-DRB1 alleles was tested by ‘‘Polymerase chain reaction sequence specific primers’’ (PCR-SSP). DNA was extracted from the buffy coat fraction of blood samples using a commercial kit (Qiagen) and tested according to micro generic HLA DNA typing trays (One Lambda).

Statistical Analysis

Data entry was done using Excel. Descriptive analysis was performed for demographic and antibodies profiles. The Chi-square test was used to study the association between the allelic phenotype frequencies in SLE patients and antibodies profiles. A p-value of less than 0.05 was considered as statistically significant. The statistical analysis was performed by SPSS, version 13.0, software.

RESULT

LN Patients

This study included 75 unrelated Moroccan LN. Five males and 70 females were recruited with mean age was 35.61 ± 8.50 years. The ANA profile showed in LN patients a positive anti-DNA in 78.7%, anti-Sm in 21.6%, anti-SS-A in 29.3% and anti -SS-B in 17.3%. Demographic and ANA profile of the patients are recapitulated in Table1.

HLA-DRB1 frequencies in LN patients according to the ANA profile

Table 2 shows the HLA-DR alleles frequencies observed in Moroccan LN patients according to the ANA profile. The phenotypic frequency of DRB1*07 was higher in LN patients with anti-SS-A than in those without (50.0 vs 26.4, p = 0.04). Patients with anti-SS-B had significantly increased frequencies of DRB1*07 versus patients without anti-SS-B (p = 0.018).

On the other hand, a higher frequency of the DRB1*15 alleles was observed in LN patients with than in those without anti-DNA, anti-Sm, anti-SS-A and anti-SS-B respectively.

Table1 Demographic and ANA profile among patients with LN

Variables	LN (n=75)
Gender Female	70
Age (years, mean ± SD)	35.61 ±8.50
Age at disease onset (years, mean ± SD)	27.35 ± 7.52
Disease duration (months, Mean ± SD)	99.14 ± 71.01
DNA	59 (78.7)
Sm (%)	16 (21.6)
SS-A (%)	22 (29.3)
SS-B (%)	13 (17.3)
RNP (%)	4 (94.7)
Ribosomal P-proteins (%)	3 (4)
Nucleosomes (%)	2(2.7)
Histones (%)	1 (1.3)

ANA: antinuclear antibodies.
LN: Lupus nephritis; n: number of individuals.
SLEDAI: Systemic Lupus Erythematosus Disease Activity Index.

DISCUSSION

This is the first study shows an association between HLA-DRB1 and ANA in LN patients in the Moroccan population. The distribution of HLA-DRB1 alleles in patients suffering from LN showed that the frequency of HLA-DRB1*07 was significantly increased in patients with anti-SS-A and anti-SS-B respectively.

In our study, the frequency of anti-DNA antibodies was 78.7% in LN patients. Another study also reported that anti-DNA antibodies are prevalent in Sudan patients with LN (Ahmed *et al*., 2017).

Otherwise, several studies have demonstrated a link between anti-DNA and nephritis (Vargas *et al*., 1997). Furthermore, African and Caribbean SLE patients with anti-DNA antibodies appear to have a higher risk of renal involvement (Alba *et al*., 2003).

Anti-SS-A and anti-SS-B antibodies were reported in SLE patients (Fukuda, 2009; Cozzani, 2014; Tarr, 2015). The frequency of anti-SS-A and -SS-B antibodies in our study was 29.3% and 17.3%, respectively. A similar result has been reported in Brazilian, European, Canadian and American populations (Bader-Meunier, 2005; Gomes, 2016; Tarr, 2015; Reichlin, 1999; Jurencák, 200915; Novak *et al*., 2017).

Several studies have shown that certain HLA-DR alleles are associated with SLE (Bhallil *et al*., 2017). However, few studies have examined the associations of the presence of anti-SS-A and anti-SS-B antibodies and HLA-DR in SLE (Ahearn, 1982; Hartung, 1992; Ehrfeld, 1992). In our study we have found a positive association of the DRB1*07 with anti-SS-A and -SS-B antibodies in LN patients. Furthermore, Schur *et al* showed an increase in the frequency of DR7 in anti-DNA positive patients (Schur *et al*., 1982).

In Malay patients with SLE a significant association of the HLA-DRB1*15 with anti-SS-A and anti-SS-B antibodies has been observed (Azizah *et al*., 2001). We found a higher frequency of HLA-DRB1*15 in patients with anti-SS-A and anti-SS-B antibodies but did not turn out be significant after statistical analysis. In the Moroccan population, HLA-DRB1*15 are the susceptibility genes in patients with LN (Bhallil *et al*., 2017). Thus, in our opinion, in Moroccan population, HLA-DRB1*15 predisposes to LN but is not associated with the anti-SS-A and anti-SS-B antibodies response.

The disease heterogeneity can explain the discordant result between our study and others about association between HLA-DRB1*3 and ANA. In our study, HLA-DRB1*03 allele was decreased in patients with anti-SSA and anti-SS-B antibodies. However, an association between this allele and anti-SS-A and anti-SS-B in SLE patients has been reported (Provost *et al*., 1993). Furthermore, HLA-DR3 is associated with anti-SS-A in patients with Sjogren’s syndrome (Manthorpe, 1982; Harley, 1986; Whittingham, 1983) and healthy subjects (Venables *et al*., 1988). Indeed, HLA-DR3 alleles are related to the anti-SS-A antibody response and not to the clinical disease expression (Provost, 1993; Venables, 1988).

Anti-Sm antibody is an important factor in the development of nephritis (Alba *et al*., 2003). In Moroccan population HLA-DRB1*04 is protective genes against LN. In our study, none of the patients with HLA-DRB1*04 has anti-Sm antibodies. It suggested that HLA-DRB1*04 is protective gene against anti-Sm antibody production.

Table 2 HLA-DRB1 frequencies in LN patients according to the ANA profile

ANA	DNA n=59	Sm n=16	SS-A n=22	SS-B n=13	RNP n=4	ribosomal P-proteins n=3	Nucleosomes n=2	Histones n=1
DRB1* Alleles	Yes/NO (%)	Yes/NO (%)	Yes/NO (%)	Yes/NO (%)	Yes/NO (%)	Yes/NO (%)	Yes/NO (%)	Yes/NO (%)
1	5.1/12.5	6.2/6.9	0.0/9.4	0.0/8.1	0.0/7.0	0.0/6.9	0.0/6.8	0.0/6.8
3	39.0/43.8	25.0/43.1	27.3/45.3	38.5/40.3	25.0/40.8	33.3/40.3	0.0/41.1	0.0/40.5
4	18.6/0.0	0.0/17.2	4.5/18.9	7.7/16.1	0.0/15.5	0.0/15.3	0.0/15.1	0.0/14.9
7	33.9/31.2	37.5/32.8	50.0/26.4*	61.5/27.4*	75.0/31.0	33.3/33.3	50.0/32.9	100.0/32.4
8	15.3/0.0	6.2/13.8	9.1/13.2	7.7/12.9	0.0/12.7	0.0/12.5	0.0/12.3	0.0/12.2
9	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
10	8.5/0.0	0.0/8.6	4.5/7.5	0.0/8.1	25/5.6	0.0/6.9	0.0/6.8	0.0/6.8
11	18.8/10.2	12.5/12.1	9.1/13.2	7.7/12.9	0.0/12.7	33.3/11.1	0.0/12.3	0.0/12.2
12	0.0/6.2	0.0/1.7	0.0/1.9	0.0/1.6	0.0/1.4	0.0/1.4	0.0/1.4	0.0/1.4
13	25.0/16.9	31.2/15.5	31.8/13.2	30.8/16.1	50.0/16.9	33.3/18.1	0.0/19.2	0.0/18.9
14	3.4/12.5	6.2/5.2	4.5/5.7	0.0/6.5	0.0/5.6	0.0/5.6	0.0/5.5	0.0/5.4
15	42.4/37.5	62.5/36.2	45.5/39.6	46.2/40.3	25.0/42.3	66.7/40.3	39.7/100.0	100.0/40.5
16	1.7/6.2	6.2/1.7	4.5/1.9	0.0/3.2	0.0/2.8	2.7/2.8	50.0/1.4	0.0/2.7
Blanks	5.1/12.5	6.2/6.9	9.1/5.7	0.0/8.1	0.0/7.0	0.0/6.9	0.0/6.8	0.0/6.8

LN: Lupus nephritis; (*): $p < 0.05$: p significant ; Yes/ No: presence/absence the various antinuclear antibodies; n: number of individuals.

In the Moroccan population anti-DNA is the most prevalent antibodies in LN. Results from our study also identified that anti-SS-A and anti-SS-B antibodies were associated with HLA-DRB1*07 in LN.

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Declaration of Interest

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

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