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Role of HLA DRB1*15 and HLA DRB1*16 alleles in the genetic susceptibility to develop systemic lupus erythematosus (SLE) after *Chikungunya* and *Zika* viruses infection in México

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ABSTRACT

Systemic lupus erythematosus (SLE) is a clinically and genetically heterogeneous disease particularly prevalent in Mexico. Althoughits etiology is unknown, genetic factors strongly influence its presenceas well as triggering factors, such as viral infections, including *Cytomegalovirus* and *Epstein-Barr* virus. Here,the study presents the appearance of de novoSLE (patients who did not present SLE before de virus infection, corroborated by serological analysis and negative for antinuclear antibodies) cases in Mexicans who live near the southern border of Mexico, who presented clinical symptoms of arthritic, hematological, mucocutaneous and renal SLE, after *Zika* and/or *Chikungunya* virus infection. Low resolution class Ⅱ HLA typing was performed, which found a significantly increased frequency *of* HLA DRB1*02 (15 and 16)when compared to a group of 99 healthy individuals (*P*=0.001, OR=4.5, IC95% 1.8~11.0). All the patients were diagnosed with SLE 1 to 3 years after being confirmed with the *Zika*, and/or *Chikungunya* infection. At the point of acute viral infection, none of the patients presented clinical signs or symptoms of autoimmunity or were negative for antinuclear antibodies. In genetically susceptible individuals, *Zika* and *Chikungunya* viral infection can trigger SLE.

Keywords: systemic lupus erythematosus, HLA antigens, *Chikungunya* virus, *Zika* virus, genetic susceptibility

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune systemic disorder particularly prevalent in Mexico with multiple clinical manifestations, in which tissue and cellular damage is mediated by autoantibodies and immune complexes[1-4]. Global incidence has been reported as between 1 to 10 per 100,000 per-

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son-year, while its prevalence is calculated between 20~70 cases per 100,000 person, with regional variations around the world[5]. The pathogenic mechanisms of SLE includes genetic and environmental factors, of which Class Ⅱ main histocompatibility complex (MHC) gene polymorphisms show increased consistent genetic susceptibility in certain populations and have been strongly associated with SLE and its phenotypes, mainly HLA-DR3 and HLA-DQ2[6–8]. In addition, viral infections have been shown to be highly associated with the onset and/or exacerbations of SLE, including *Epstein–Barr* virus*, parvovirus B19* retrovirus and *cytomegalovirus*[9]. The role of other viruses such as *Zika, Chikungunya* and *Dengue* arbov iruses has not been clearly established, but their role could take more relevance specially in geographical areas where there is anactive transmission of these viruses. The reference medical center, Hospital Regional de Alta Especialidad Ciudad Salud, located in Tapachula, near the south border of México and Guatemala where the transmission of *Zika* and *Chikungunya* viruses began in 2014, paid close attention to autoimmune rheumatic diseases through its outpatient. During 2016 and 2017, we recorded an increase in the number of cases of SLE compared to previous years, in a situation that coincided with the outbreak of *Zika* and *Chikungunya*.Therefore, the study performed a retrospective case–control study to evaluate the association of Class Ⅱ MHC genes with the presence of SLE in subjects with previously confirmed *Zika* and/or *Chikungunya* infections.

MATERIALS AND METHODS

Patients

Patients with *de novo* SLE diagnosed between 2015 and 2017, with a background of previously confirmed *Zika* or *Chikungunya* infection (by qRT-PCR and serology) during the active transmission of those arbovirus in Tapachula, Chiapas, were enrolled. The information was gathered from clinical records concerning their arboviral infection including, the time elapsed between the confirmed infection and onset of SLE symptoms,the SLE-onset related symptoms, the SLEDAI at diagnosis, and the autoantibodies profile at SLE diagnosis[10].

Autoantibody profile

In order to establish whether SLE related antibodies found in each subject enrolled were present at the moment of arboviral infection, the study determined autoantibody profiles by immunoenzymatic assay (Euroline ANA profile 3, Euroimmun ®) from stored sera collected to perform molecular diagnoses during the outbreak[11].

HLA typing

To evaluate genetic SLE susceptibility, low resolution HLA typing was performed by Sequence Specific Primers (SSP, TBG technologies) for HLA DRB1 and DQB1 after PCR amplification; allele and haplotype frequencies were determined and compared with the group of 99 healthy individuals (198 alleles) [12].

Statistical Analysis

The study performed non-parametric statistics (Chi Squared test and Fisher exact test) by using the Epi Info program (version 10.0) and the StatCalc Subprogram based on two by two tables. $P < 0.05$ was set of standards statistical significance.

RESULTS

Patients

Between 2015 and 2017, 21 new cases of SLE was registered in the autoimmunity clinic. Of those, 16 patients had a demonstrated arboviral infection. All subjects corresponded to female; averageage at onset of SLE 25.5 years (17~60); all cases were referred 1 to 3 years due to having persistent symptoms such as fever, asthenia, cutaneous manifestations, arthralgias and/or arthritis, anemia and/or leukopenia and hematuria, proteinuria or active urine sediment.

Diagnostic approach

In all cases,the diagnostic approach corresponded to a suspicion of SLE due to the clinical picture. All subjects were tested for antinuclear antibodies by indirect immunofluorescent test and specific antibodies by ELISA and Western blot analysis (Euroimmune®). SLE was confirmed based on 2012 SLICC/ACR classification criteria[13]. Treatment was started according organic damage. The main characteristics of the subjects, including treatment and outcome, were shown in ***Table1***.

Clinical outcome after virus infections

The mean time elapsed between arboviral infection and SLE-onset signs and symptoms was (1.80 ±0.68) years. The common SLE-related signs and symptoms were arthritis in 8 cases (50%), lymphopenia in 7 cases (43.7%), proteinuria in 5 cases (31.2%), mucocutaneous manifestations in 6 cases (37.5%), and neuropathy in 2 cases (12.5%). The evolution time of SLE-related signs and symptoms before confir–

mation of diagnosis was (3.30±1.14)months. SLE–DAI at diagnosis was 12.50±4.89, and the common phenotypes were: hematologic in 8 cases (50%), articular in 8 cases (50%), renal in 5 cases (31.2%), mucocutaneous in 3 cases (18.7%), and neurologic in 2 cases. The main data of each case was shown in ***Table 1***.

HLA gene frequencies

Table 2 showed gene frequencies HLA DRB1 alleles and 16 SLE patients´ (32 alleles) from Tapachula, Chiapas and for comparison, it also showed the group of 99 ethnically matched healthy controls (198 alleles). As it is readily seen that HLA DRB1*04 was the most common allele in patients and controls, confirming that the ethnicity background in patients and healthy controls was the same. Interestingly, there was a significantly increased frequency in HLA DRB1*15 in patients as compared to controls (P =0.05, OR=2.6, 95% CI:1.2~5.4), likewise, HLA DRB1*16 also increased in the patient group as compared to the controls (P =0.03, OR= 3.5, 95% CI:1.6~7.9). The gene frequencies of the remaining alleles in the patient groups were similar to those present in the control group.

Table 1 **Gene frequencies HLA-DRB1 in SLE patients from Tapachula Chiapas**

Alleles	Patients(N=32)		Control(N=198)		P value	OR	95%CI
	n	gf	n	gf			
DRB1*04	9	0.281	47	0.237	0.75	1.2	0.6~2.5
DRB1*15	6	0.188	13	0.066	0.05	2.6	1.2~5.4
DRB1*08	5	0.156	33	0.167	0.9	0.9	0.4~2.3
DRB1*16	4	0.125	5	0.025	0.03	3.5	1.6~7.9
DRB1*03	2	0.063	11	0.056	1	1.1	0.2~5.4
DRB1*14	2	0.063	21	0.106	0.7	0.6	0.1~2.5
DRB1*10	2	0.063	3	0.015	0.3	4.3	0.7~27.0
DRB1*11	1	0.031	20	0.101	0.3	0.3	0.04~2.2
DRB1*01	1	0.031	10	0.051	0.98	0.6	0.1~4.9

Table 2 **Gene frequencies HLA-DQB1 in SLE patients from Tapachula Chiapas**

Alleles	Patients(N=32)		Control(N=198)		P value	OR	95%CI
	n	gf	n	gf			
DQB1*03	14	0.438	92	0.465	0.9	0.9	0.4~1.9
DQB1*05	7	0.219	12	0.061	0.008	3.1	1.6~6.2
DQB1*04	5	0.156	33	0.167	0.9	0.9	0.4~2.3
DQB1*06	5	0.156	15	0.076	0.2	2.3	0.8~6.7
DQB1*02	1	0.031	33	0.167	0.08	0.2	0.02~1.2

Since HLA DRB1*15 and HLA DRB1*16 are subspecificities of HLA DRB1*02, the study also analyzed the frequency of HLA DRB1*02 in patients and controls and found an even stronger statistical significance (P=0.001, OR=4.5, 95% CI: 1.8–11.0).

It is clear HLA DQB1*03 is the most frequent allele in patients and controls (P>0.05) and it is also true for the Mexican population (mestizo and indigenous). The HLA DQB1*05 was found to be significantly increased in the patient group when compared to controls (P=0.008, OR=3.1, 95% CI: 1.6~6.2). The remaining alleles were equally distributed in patients and controls.

DISCUSSION

This paper reported the genetic susceptibility for developing SLE after being infected with the *Chikungunya* and/or *Zika* virus in an endemic region in the south border of Mexico next to Central America.

The study reported the relevance of HLA DRB1*15 and HLA DRB1*16 as risk factors for SLE. It is important to note that gene frequencies of these two alleles are prevalent in oriental populations and in indigenous individuals from Mexico[14]. The relationship between Orientals and the Amerindian populations dated back to ancient individuals that populated America around thirty thousand years ago. However,it is important to note that SLE patients from the southern border differ from SLE patients in urban cities. Their genetic admixture was strongly influenced by Caucasian (white European) individuals where HLA DRB1*03 was the relevant allele [15–16]. This suggests that the mechanism of SLE autoimmunity after *Chikungunya* infection might trigger a different genetic pathway when compared to those positive for HLA DRB1*03.

In conclusion, this paper suggests that the viral infections, particularly in endemic regions including *Zika* and *Chikungunya* viruses, trigger the development of autoimmunity (SLE) in genetically susceptible individuals. HLA typing is a useful marker for

detecting genetic susceptibility in endemic regions with viral infections. Therefore, this paper suggests that HLA typing will also be useful in determining prognosis and individualized treatment.

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