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 México. Although its etiology is unknown, genetic factors strongly influence its presence as well as triggering factors, such as viral infections, including Cytomegalovirus and Epstein-Barr virus. Here, the study presents the appearance of de novo SLE (patients who did not present SLE before the 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 virus 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 México with multiple clinical manifestations, in which tissue and cellular damage is mediated by autoimmune antibodies and immune complexes. Global incidence has been reported as between 1 to 10 per 100,000 per-
infections.

Class II of SLE includes genetic and environmental factors, of which Class II main histocompatibility complex (MHC) gene polymorphisms show increased consistency 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 arbovirus has not been clearly established, but their role could take more relevance specifically in geographical areas where there is an active 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 these 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; average age 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 (Euroimmun®). SLE was confirmed based on 2012 SLICC/ACR classification criteria [13]. Treatment was started according to organic damage. The main characteristics of the subjects, including treatment and outcome, were shown in Table 1.

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 \((3.30 \pm 1.14)\) months. SLE–DAI at diagnosis was \(12.50 \pm 4.89\), 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

<table>
<thead>
<tr>
<th>Alleles</th>
<th>Patients (N=32)</th>
<th>Control (N=198)</th>
<th>(P) value</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRB1*04</td>
<td>9 0.281</td>
<td>47 0.237</td>
<td>0.75</td>
<td>1.2</td>
<td>0.6–2.5</td>
</tr>
<tr>
<td>DRB1*15</td>
<td>6 0.188</td>
<td>13 0.066</td>
<td>0.05</td>
<td>2.6</td>
<td>1.2–5.4</td>
</tr>
<tr>
<td>DRB1*08</td>
<td>5 0.156</td>
<td>33 0.167</td>
<td>0.9</td>
<td>0.9</td>
<td>0.4–2.3</td>
</tr>
<tr>
<td>DRB1*16</td>
<td>4 0.125</td>
<td>5 0.025</td>
<td>0.03</td>
<td>3.5</td>
<td>1.6–7.9</td>
</tr>
<tr>
<td>DRB1*03</td>
<td>2 0.063</td>
<td>11 0.056</td>
<td>1</td>
<td>1.1</td>
<td>0.2–5.4</td>
</tr>
<tr>
<td>DRB1*14</td>
<td>2 0.063</td>
<td>21 0.106</td>
<td>0.7</td>
<td>0.6</td>
<td>0.1–2.5</td>
</tr>
<tr>
<td>DRB1*10</td>
<td>2 0.063</td>
<td>3 0.015</td>
<td>0.3</td>
<td>4.3</td>
<td>0.7–27.0</td>
</tr>
<tr>
<td>DRB1*11</td>
<td>1 0.031</td>
<td>20 0.101</td>
<td>0.3</td>
<td>0.5</td>
<td>0.04–2.2</td>
</tr>
<tr>
<td>DRB1*01</td>
<td>1 0.031</td>
<td>10 0.051</td>
<td>0.98</td>
<td>0.6</td>
<td>0.1–4.9</td>
</tr>
</tbody>
</table>

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

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

Since HLA DRB1*15 and HLA DRB1*16 are sub-specificities 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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References


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