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Original article

Association of leishmaniasis with *TNF* alpha promoter and *SLC11A1* gene polymorphisms in patients of two endemic areas in Mexico

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Abstract

Some Single Nucleotide Polymorphisms (SNPs) of interleukins and other modulatory molecules of the immune response play an important role in susceptibility to infectious diseases, particularly those involving intracellular parasites. In this study, we evaluated allele, genotype and haplotype associations of two SNPs of the *TNF-α* promoter and seven of the *SLC11A1* gene in 79 patients with localized cutaneous leishmaniasis (CL) and 15 with visceral leishmaniasis (VL), compared with 127 and 89 locality paired controls, respectively, from two endemic areas of Chiapas State, Mexico. None of the *TNF-α* alleles and genotypes was associated either to CL or to VL. Alleles rs2276631-C ($P = 0.02$; OR [95%CI] = 2.11 [1.16−3.86]) and rs2279015-G ($P = 0.005$; OR [95%CI] = 2.42 [1.33−4.41]) of *SLC11A1*, were associated with susceptibility to VL, whereas genotypes rs2276631 C/C ($P = 0.003$; OR [95%CI] = 2.65 [1.41−5.00]) and rs2279015 G/G ($P = 0.018$; OR [95%CI] = 2.05 [1.15−3.64]) were significantly increased in CL and VL patients, respectively. Complete haplotypes involved in susceptibility were CGCCGD*ins* with VL and CGCCAD*ins* with CL. CGCCA was the minimal susceptibility haplotype for CL and CCG for VL. Our data suggest that *SLC11A1* gene polymorphisms might have a relevant role in the pathology of leishmaniasis, directing towards susceptibility outcome of this disease in residents of an endemic area.

Keywords: DNA typing; Genetic susceptibility; Leishmaniasis; Polymorphisms; *SLC11A1*; *TNF-α*

1. Introduction

Human leishmaniasis has different clinical forms; it may be cutaneous (CL), mucocutaneous (MCL), or visceral (VL), usually depending on the parasite species [1−3]. The disease is mainly zoonotic and its distribution is limited to the insect vector distribution in warm climates [2]. In Mexico, most recorded cases are CL, largely distributed at the southeast of the country, but also a focus of VL in the central valley of Chiapas was found [4].

Even if clinical manifestations partially depend on the parasite species, the immune response of the host is an important contributing factor. Parasite destruction in infected hosts is mediated by activation of the Th1 type immune response and the corresponding cytokines released, driving infected macrophages to a microbicidal state. Conversely, inhibitory cytokines generated by the Th2 subset also play an

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important role in the susceptibility to the infection [1,5]. Furthermore, relevance of host genetics in susceptibility to diseases has been established through identification of variants in several genes among populations. This has allowed associating certain susceptibility or resistance genotypes to leishmaniasis. One of the most abundant sources of genetic variation comes from single nucleotide polymorphisms (SNPs). Various polymorphic genes have been associated with susceptibility to VL, like *TNF-α* and *TNF-β* in Brazil [6], *SLC11A1* and *IL-4* in Sudan [7,8]; while susceptibility to develop CL is associated to *CXCR1* and *SLC11A1* in Brazil [9], HLA-DRB1 and HLA-DPA1 in Mexico [10], and HLA-Cw7 in French-Guyana [11], and *TNF-α* and *TNF-β* in Venezuela to MCL [12]; furthermore, association with protection to CL has also been shown in the case of HLA-DR2 and HLA-DP1 in Mexico [10]. *TNF-α* and *SLC11A1* polymorphisms are associated with susceptibility to tuberculosis in Iran [13] and Thailand [14], lepromatous leprosy in Thailand [15], and leishmaniasis in Sudan [7]. The goal of the present work was to evaluate genetic susceptibility of a Mexican population to develop leishmaniasis in two endemic areas, associated to *TNF-α* promoter and *SLC11A1* polymorphisms in patients with VL and CL.

2. Materials and methods

2.1. Patients and controls

Blood samples were obtained from 79 to 15 patients with CL and VL respectively, which were not family related, and from 126 (CL controls) and 89 (VL controls) healthy individuals, without any history of the disease, paired by age and residence with the patients. CL patients and controls were from Pichucalco and VL patients and controls were from Tuxtla Gutierrez, Chiapas State, Mexico. Each patient was diagnosed by immunofluorescence antibody test, leishmanin skin test and Giemsa stained smears. These tests were also performed in the control groups to confirm the absence of the disease. This work complies with the current health laws of Mexico, and was approved by the Ethics and Research Committees of the Hospital General "Dr. Manuel Gea Gonzalez" and the Institute of Epidemiological Diagnosis and Reference (InDRE). All participants or their parents were informed about the objectives of the study and were included only after providing written informed consent.

2.2. DNA extraction and genotyping

DNA was obtained from 10 ml of EDTA-peripheral blood using proteinase K and phenol/chloroform extraction [16]. Polymerase chain reaction (PCR) products and genotyping of the digested products were analyzed by gel electrophoresis in 2% agarose or 6% polyacrylamide respectively and visualized with ethidium bromide.

The *TNF-α* polymorphisms analyzed were SNPs −308G/A and −238G/A. The PCR primers for −308 and for −238 were described elsewhere [17,18]. Alleles of −308 polymorphism were determined by digestion with the *NcoI* enzyme and for −238 polymorphism by dot-blot hybridization and two allele specific digoxigenin-11-ddUTP labeled probes [19] using the chemiluminescence method [20]. The alleles of seven polymorphic regions of the *SLC11A1* gene were determined using the primers and conditions described by Liu et al. [21]. The SNPs of *SLC11A1* analyzed were rs2276631 (274C/T) in exon 3; rs3731864 (577−18G/A = INT5) in intron 5; rs17221959 (823C/T) in exon 8; rs2695342 (A318V) in exon 9; rs2279015 (1465−85G/A) in intron 13; rs17235409 (D543N) in exon 15; and rs17235416 (1729 + 55del4 = 3′UTR) in the 3′ untranslated region.

2.3. Statistical analysis

Allele frequencies (AF) and genotype frequencies (GF) were calculated by direct counting and were compared between patients and controls. Chi-square analysis with Yate's correction, considering $P \leq 0.05$ as the minimum level of significance, was performed; exact Fisher test was used when appropriate. Relative risk was calculated as an odds ratio (OR). Ninety-five percent confidence intervals (95%CI) were obtained by using Cornfield's approximation. Haplotypes and linkage disequilibrium (LD) blocks were generated by confidence interval method using Haploview 4.2 (http://www.broadinstitute.org) [22].

3. Results

3.1. TNF-α allele and genotype distribution

Allele and genotype frequencies of −308 and −238 promoter positions of the *TNF-α* gene in CL and VL patients are shown in Table 1. No statistical differences were found for any allele or genotype in CL or VL. In several comparisons large 95% CI were obtained probably due to sample size.

3.2. SLC11A1 allele distribution

Allele frequency of *SLC11A1* polymorphisms in CL and VL patients and their control groups is displayed in Table 2. The rs17221959-C, rs17235409-D and rs17235416-*ins* alleles were the most frequent in all groups; the polymorphisms rs3731864 and rs2695342 were monomorphic with presence only of rs3731864-G and rs2695342-C alleles. Frequencies of rs2276631, rs2279015 and rs17235416 polymorphisms were variable. Alleles rs2276631-C ($P = 0.02$; OR [95%CI] = 2.11 [1.16−3.86]) and rs2279015-G ($P = 0.005$; OR [95% CI] = 2.42 [1.33−4.41]) were associated with susceptibility to VL, whilst none was associated to CL. All the SNPs were in Hardy−Weinberg equilibrium for VL controls, while for CL controls only rs17235409 ($P = 0.012$) and rs17235416 ($P = 0.008$) were not (data not shown). When both groups where analyzed together, the same markers were out of Hardy−Weinberg equilibrium rs17235409 ($P = 0.035$) and rs17235416 ($P = 0.023$).