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Tumor necrosis factor-alpha promoter polymorphism in Mexican patients with Chagas' disease

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Abstract

The aim of this study was to investigate whether the promoter's polymorphisms at the TNF alpha (TNF-α) gene were associated with the genetic susceptibility to Chagas' disease. We analyzed the TNF-α (positions −308, and −238) polymorphisms in a sample of 54 serologically positive chagasic individuals and in 169 healthy controls. The patients were divided according to clinical characteristics as asymptomatics ($n = 27$), and chronic chagasic cardiopathy (CCC) patients ($n = 27$). The whole group of patients showed increased frequencies of −308 T2 (A) allele when compared to healthy controls (pC = 0.008, OR = 3.03). When the analysis was carried out separately in asymptomatic and CCC patients, increased frequencies of T2 (A) allele and T1T2 (AG) genotype in the group of patients with CCC were found when compared to asymptomatic individuals (pC = 0.0002 and pC = 0.003, respectively) and healthy controls ($pC = 4 \times 10^{-7}$, OR = 7.02, and pC = 0.0006, OR = 5.29, respectively). The present study demonstrates that Chagas' disease is associated with TNF-α polymorphisms in the Mexican population. The TNF-308 T2 allele could be directly involved in the genetic susceptibility to the chronic phase of the disease.

Keywords: Cardiomyopathy; Chagas' disease; Genetic susceptibility; Tumor necrosis factor-alpha; *Trypanosoma cruzi*

1. Introduction

Chagas' disease is caused by the intracellular protozoan *Trypanosoma cruzi* and contributes significantly to cardiovascular morbidity and mortality in countries of Latin America. The clinical manifestations of Chagas' disease include an acute, and a chronic phase [1]. The acute phase is usually asymptomatic or runs a mild febrile course that subsides within a few weeks. Then, a chronic phase develops. Most individuals may remain asymptomatic for life, having circulating antibodies against *T. cruzi* (latent or indeterminate phase). Nearly 30% of infected individuals develop the conspicuous cardiac and/or digestive manifestations after 10–20 years post-infection. Severe heart disorders, rhythm or conduction abnormalities, or a specific dilated cardiomyopathy generally lead to death. In spite that the pathogenesis of heart damage in chronic Chagas' disease is still controversial, the participation of autoimmune mechanisms has been suggested [2–6]. The heterogeneity in the clinical expression of Chagas' disease suggests the involvement of genetic factors on its pathogenesis. Different studies have reported several markers of genetic susceptibility to chagasic cardiomyopathy [7]. Results have been inconsistent and new genes such as tumor necrosis factor-alpha (TNF-α) need to be analyzed. The TNF-

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α codifies a potent immunomodulator and pro-inflammatory cytokine [8] that mediates diverse pathological processes. High frequency of TNF-α -producing lymphocytes and increased plasma levels of TNF-α have been reported in Chagas' disease patients [9,10]. In addition, Talvani et al. [11] reported a high correlation between the high TNF-α levels and the degree of heart dysfunction. The gene that codifies for TNF-α is located within the major histocompatibility complex (MHC) class III region in human chromosome 6 [12]. This gene contains several polymorphic sites, of which two are the most studied. The first is located at position −238 in relation to the transcription start site, in which the presence of adenine (A) defines the TNF-238 A allele; and, the presence of guanine (G) defines the wild type and more common allele TNF-238 G [13]. The second polymorphism is located at position −308; the presence of G defines the TNF-308 T1 allele and A defines TNF-308 T2 allele [14]. It has been demonstrated that these different allelic forms have functional implications. The TNF-308 T2 allelic form results in a two-fold greater transcription compared to TNF-308 T1 form in PMA-stimulated Jurkat and U937 cells [15]. Thus, the aim of the present study was to investigate the role of TNF-α polymorphisms (−238 and −308 positions) in the genetic susceptibility to *T. cruzi* infection as well as in the development of chagasic heart disease in the Mexican population.

2. Materials and methods

2.1. Studied patients

The study included 54 unrelated Mexican individuals (25 males and 29 females, mean age = 46.25 ± 9.19 years) who had at least two *T. cruzi* positive tests of the next four: ELISA, PCR, IFI or hemoaglutination. The patients were diagnosed at the Instituto Nacional de Cardiología "Ignacio Chávez". The patients were classified according to clinical, electrocardiographic (ECG), and echocardiographic characteristics as asymptomatics and chronic chagasic cardiopathy patients (CCC). The asymptomatic population (50%) consisted of apparently healthy individuals having normal or minor right bundle branch blockade on ECG, without cardiomegaly. The CCC group (50%) included patients with either arrhythmia-related symptoms, five or more extrasystoles per minute, embolic episodes, cardiomegaly, or congestive heart failure. Additionally, one group of 169 not related healthy individuals with neither symptoms, nor previous diagnosis of Chagas' disease, was studied as control group. All studied individuals (patients and controls) were from the Southeast of Mexico and they shared the same environmental and socioeconomic living conditions. All study participants were Mexican Mestizos and each individual was asked about his birthplace as well as that of his parents, maternal and paternal grandparents. We considered Mexican Mestizos only those individuals who, for two generations, including their own, had been born in Mexico. A Mexican Mestizo is defined as someone born in Mexico who is a descendant of the original autochthonous inhabitants of the region and of individuals, mainly Spaniards of Caucasian and/or Black origin, who came to America during the 16th century. The Institutional Ethics and Research Committees approved this study and all patients signed an informed consent.

2.2. DNA extraction

Genomic DNA from whole blood containing EDTA was isolated by a standard technique [16].

2.3. TNF-238 and TNF-308 polymorphisms

Genotyping for the −238G/A polymorphism was performed using a PCR fragment amplified with modified primers (forward primer 5′-AAACAGACCACAGACCTGGTC-3′ and reverse primer 5′-CTCACACTCCCCATCCTCCCGGATC-3′) that include a restriction site for the *Bam*HI enzyme as previously described [17]. The TNF-308 polymorphism was analyzed using the forward primer 5′-GAGCAATAGGTTTTGAGCG*C*CAT-3′ and the reverse primer 5′-GGGACACACAAGCATCAAG-3′ to create a restriction site for the *Nco*I enzyme [18]. The PCR products were analyzed by phototyping in 2% agarose gels, stained with ethidium bromide.

2.4. Statistical analysis

Allele and genotype frequencies of TNF-α polymorphisms were obtained by direct counting. Also, Hardy–Weinberg equilibrium was tested using the chi-square test. The differences between groups were determined using Mantel–Haenzel χ^2 analysis, which was combined with the 2×2 contingency tables using the EPIINFO statistical program (Version 5.0; USD Incorporated 1990, Stone Mountain, GA, USA). Fisher's exact test was used if the number of any cell was <5. *P* values were corrected according to the number of specificities tested and the number of comparisons performed, with a level of significance established as $pC < 0.05$. Relative risk with 95% confidence intervals (CI) was calculated as the odds ratio (OR), according to Woolf's method [19]. Linkage disequilibrium between HLA and TNF alleles was evaluated using the χ^2-test.

3. Results

Allele and genotype frequencies of TNF-α promoter's polymorphisms (positions −238 and −308) in the whole group of Chagas' disease patients and healthy controls are shown in Table 1. Observed and expected frequencies were in Hardy–Weinberg equilibrium in both studied groups. The whole group of patients showed increased frequency of −308 T2 (A) allele when compared to healthy controls ($pC = 0.008$, OR = 3.03, 95% CI = 1.29–7.12).