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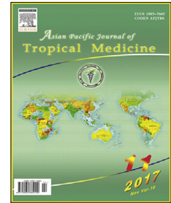

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# Association of *TNFA* (−308G/A), *IFNG* (+874 A/T) and *IL-10* (−819 C/T) polymorphisms with protection and susceptibility to dengue in Brazilian population

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ABSTRACT

**Objective:** To evaluate gene polymorphisms and their association with susceptibility to dengue.

**Methods:** A retrospective case-control study was performed with 262 subjects, comprising 78 dengue fever (DF) patients, 49 dengue hemorrhagic fever (DHF) patients and 135 healthy controls. Genotypic and allelic profiles were identified using polymerase chain reaction based in real time and amplification-refractory mutation system.

**Results:** We observed a protective association of *IL-10* (−819 C/T) C allele ($P = 0.028$, $OR = 0.56$, $CI = 0.34–0.91$) against DHF, while the C/T ($P = 0.047$, $OR = 2.10$, $CI = 1.01–4.38$) and T/T ($P = 0.008$, $OR = 3.82$, $CI = 1.38–10.59$) genotypes were associated with DHF and DF, respectively. The dominant model *TNFA* −308 GA + AA ($P = 0.043$, $OR = 0.45$, $CI = 0.20–1.00$) genotypes were found to have protective effect against dengue infection. A protective association among the *IFNG* (+874 A/T) A/T genotype against DF ($P = 0.02$, $OR = 0.46$, $CI = 0.24–0.89$) and DHF ($P = 0.034$, $OR = 0.43$, $CI = 0.19–0.95$) was observed. When the studied single-nucleotide polymorphism was analyzed in combination, the combination GTA ($P = 0.022$, $OR = 2.95$, $CI = 1.18–7.41$) was statistically significantly associated with susceptibility to DF and the combination GCT ($P = 0.035$, $OR = 0.28$, $CI = 0.08–0.90$) with protection against the development of DHF.

**Conclusions:** This research identifies the association of the *IFNG* (+874 A/T), *TNFA* (−308G/A), *IL-10* (−819 C/T) genotypes as a factor for protection, susceptibility and severity to dengue.

## 1. Introduction

Dengue is a public health problem and its incidence has a wide geographical spread [1,2]. It is endemic in more than 100 countries and the World Health Organization estimated a 50–100 million dengue infections reported worldwide each year [3]. However, a cartographic study estimated that there are approximately 390 million dengue cases per year around the world including symptomatic and asymptomatic [4]. According to the Pan American Health Organization in 2015, Brazil was the country that reported most cases of dengue in the Americas with 1 649 008 of suspected dengue records and an incidence rate of 820.27 cases [5].

Dengue infection presents diverse a wide spectrum of clinical presentation, from asymptomatic and mild dengue fever (DF), to the most serious forms: dengue hemorrhagic fever (DHF) and dengue shock syndrome. DHF is characterized by increased vascular permeability, followed by vascular leakage, which promotes the appearance of hemorrhagic manifestations and

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thrombocytopenia, while dengue shock syndrome includes hypotension and hypovolemic shock [6]. Clinical manifestations of dengue to severe clinical conditions may lead to death [7].

The mosquito *Aedes aegypti* is the main vector transmitting the viruses that causes dengue in tropical and subtropical regions, and there are four serotypes distinct: DENV 1–4 [8]. Environmental factors, the serotype/genotype of dengue virus, the immune response and genetic background of host have significant influence on the development of clinical manifestations of dengue, as well as in disease severity [9]. Additionally, single nucleotide polymorphisms (SNPs) in cytokine genes have significantly contributed to the comprehension of the physiopathology and the role of host genetic in dengue infection [10].

There are various factors associated with the development of dengue, and the host immune response has been highlighted as a genetic biomarker for the disease with the production of several cytokines [11]. Therefore, polymorphisms in genes coding can influence the production and function of these proteins, protection, susceptibility or disease progression [12].

Tumor necrosis factor alpha (TNF-$\alpha$) is a pro-inflammatory cytokine involved in several physiological processes, immune conditions and tumor growth. TNF-$\alpha$ has been associated with DHF and dengue shock syndrome influencing the activity in endothelial cells, induction of inflammatory mediators, recruitment of inflammatory cells, survival of inflammatory cells, induction of tissue-destructive enzymes, apoptosis, among others [13]. The SNP −380G/A (rs1800629) has been reported to directly affect TNF-$\alpha$ expression in autoimmune and infectious diseases [14].

Interleukin 10 (IL-10) presents a pleiotropic role with immune regulation and inflammatory in infectious diseases. In DENV pathogenesis, IL-10 has immunomodulatory activity with consequences in persistent infection viral enable an inflammatory that promotes aggravation of infection [15]. There are few studies investigating the role of polymorphisms of *IL-10* gene (SNP −819C/T-rs1800871) in the pathogenesis of dengue.

Interferons are a family of pleiotropic cytokines which are produced by T helper cells and natural killer cells during the initial phase of infection. Interferon-gamma (IFN-$\gamma$) is noteworthy due to its essential role in the regulation of the inflammatory response [16], in which it enhances the transcription of genes involved in antiviral response and antitumor activity [17]. The increase of *IFNG* expression was identified as a consequence of a functional polymorphism A/T (rs2430561), located at the +874 position in the first intron [18].

The investigation of SNPs in pro-inflammatory cytokines such as TNF-$\alpha$ and IFN-$\gamma$, as well as the anti-inflammatory cytokine of IL-10, has been associated with the variation of cytokine levels in the immune response. In this study, we investigated the possibility of SNPs from *IL-10*, *TNFA* and *IFNG* gene regions (−819C/T, −308G/A and +874A/T, respectively) influence the susceptibility to infection or dengue progression in a sample of Brazilian patients.

## 2. Materials and methods

### 2.1. Study design and samples

Dengue patients attended in the city of Arapiraca by Unified Health System, Northeast Brazil, during the years between 2010 and 2015 were recruited for this research. The patients were classified by medical records and clinical laboratory results which were obtained at hospital or health center. The classification of dengue cases were in accordance with the criteria of World Health Organization guidelines [6]. DF was characterized by the presence of high fever accompanied by the following symptoms: myalgia, severe headache, retro-orbital, abdominal pain, arthralgia or rash. The DHF has the same clinical condition with hemorrhagic manifestations. We recruited patients in hospitals, who presented medical records of hemorrhagic manifestations and thrombocytopenia were less than 80 000/mm$^3$. Case population was positive for ELISA anti-dengue IgM realized in the Municipal Laboratory of Arapiraca (Dengue IgM Capture Elisa, PanBio, Brazil).

Control population was a group of healthy volunteer's blood donor. They all reported no history, signs and symptoms of dengue infection, and consequently without hospitalization. The laboratories tests in this group were performed by using immunochromatographic rapid test (Bioeasy/Abon, Brazil) and enzyme linked immunosorbent assay (Dengue IgM Capture Elisa, PanBio, Brazil). This retrospective case-control study was reviewed and approved by the Research Ethics Committee of Federal University Alagoas, and consent from all study participants was obtained (Protocol: 1.073.204).

### 2.2. DNA extraction and genotyping

Genomic DNA was extracted from peripheral blood in anticoagulant solution Ethylenediamine tetraacetic acid, and it was performed in accordance to the manufacturer instructions (Qiagen FlexiGene® DNA Handbook, Qiagen, Germany). For patients with dengue laboratory confirmed before, samples were obtained from swab oral mucosa cells for NaCl solution extraction method [19]. DNA was quantified in a BioPhotometer plus (Eppendorf® AG, Hamburg, Germany), and visualized in a 1% agarose gel electrophoresis stained with ethidium bromide. The DNA samples were stored at −20 °C.

Polymorphisms in the *TNFA* gene (−308G/A – rs1800629) and *IL-10* gene (−819 C/T – rs1800871) were genotyped by real-time polymerase chain reaction (PCR), performed by allelic discrimination method using TaqMan assays (Applied Biosystem®, California, USA). Amplification of the target DNA was performed in Step One Plus equipment (Applied Biosystem®, California, USA) with the following conditions: 95 °C for 10 min, followed by 40 cycles of 92 °C for 15 s and 60 °C for 1 min. Data were analyzed by Step One Plus software.

The polymorphism of *IFNG* (+874A/T – rs2430561) gene was identified by amplification refractory mutation system – PCR. The primer sequences were as follows [20]: *IFNG* primer A allele, 5′-TTCTTACAACACAAAATCAAATCA-3′; *IFNG* primer T allele, 5′-TTCTTACAACACAAAATCAAATCT-3′; GH (growth hormone) internal control 1, 5′-GCCTTCCCAACCATTCCCTTA-3′; GH (growth hormone) internal control 2, 5′-TCACGGATTTCTGTTGTGTTTC-3′; and *IFNG* generic primer, 5′-TCAACAAAGCTGATACTCCA-3′. Amplification of the target DNA was performed in a thermocycler (Esco technologies®, USA) under the following conditions: heating at 95 °C for 3 min, 10 cycles of denaturation at 95 °C for 15 s, annealing at 65 °C for 50 s, elongation at 72 °C for 40 s, followed by 20 cycles of denaturation at 95 °C for 20 s, annealing at 55 °C for 50 s, elongation at 72 °C for 50 s, final elongation at 72 °C for 7 min, and final hold at 4 °C for 5 min. Amplification refractory mutation system – PCR

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