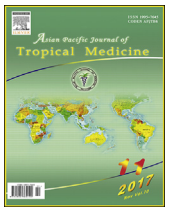
HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: http://ees.elsevier.com/apjtm



Original research https://doi.org/10.1016/j.apjtm.2017.10.009

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

Ana Caroline Melo dos Santos[1], Edilson Leite de Moura[1], Jean Moises Ferreira[1], Alexandre Wendell Araujo de Moura[1], Ailson Darlan Sales Ferreira[1], Rubens Pereira Bezerra[1], Diego de Siqueira Figueiredo[1], Karol Fireman de Farias[1], Tiago Gomes de Andrade[1,2], Elaine Virgínia Martins de Souza Figueiredo[1]✉

[1]*Molecular Biology and Gene Expression Laboratory, Federal University of Alagoas, Campus Arapiraca, Av Manoel Severino Barbosa, Bom Sucesso, Arapiraca, AL, 57309-005, Brazil*

[2]*Faculty of Medicine, Federal University of Alagoas, Campus AC Simões, Tabuleiro dos Martins, Maceió, Alagoas, Brazil*

ARTICLE INFO

Article history:
Received 15 Jul 2017
Received in revised form 23 Aug 2017
Accepted 24 Sep 2017
Available online 28 Oct 2017

Keywords:
Immune system
IFNG
TNFA
IL-10
Dengue
Polymorphism

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

First author: Ana Caroline Melo dos Santos, Institute of Biological Sciences and Health, Federal University of Alagoas, Maceió, Alagoas, CEP 57072-970, Brazil.
Tel: +55 82 99603 3197.
E-mail: anacaroline12305@gmail.com
✉Corresponding author: Elaine Virgínia Martins de Souza Figueiredo, Laboratory of Molecular Biology and Gene Expression, Federal University of Alagoas, CEP: 57309-005, Arapiraca, Brazil.
Tel: +55 82 99931 0827.
E-mail: elainevms@yahoo.com.br

Peer review under responsibility of Hainan Medical University.
Foundation project: This work was supported by the Fundação de Amparo à Pesquisa de Alagoas (FAPEAL) – Programa Primeiros Projetos (PPP/2011).

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-α) is a pro-inflammatory cytokine involved in several physiological processes, immune conditions and tumor growth. TNF-α 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-α 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-γ) 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-α and IFN-γ, 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

amplicons were then submitted to a 2% agarose gel electrophoresis stained with ethidium bromide (Amresco Inc., USA), and visualized under ultraviolet light.

2.3. Statistical analysis

The data were presented as mean and standard deviation. Hardy–Weinberg equilibrium was tested using goodness-of-fit *chi*-square test to compare the observed and expected genotype frequencies among cases and controls. Statistical analysis was performed using BioEstat version 5.0 for allelic frequencies and associations. For association analysis, a logistic regression model was carried out by using SNPstats software, the intrinsic factors that could influence the profile of the population and thus adjusted by the data in relation to age and gender. Codominant model, dominant model, recessive model, over dominant model and log-additive model were considered to evaluate the risk of dengue associated with each SNP. Akaike information criterion and Bayesian information criterion were used to determine the best model of inheritance. Odds ratios (*OR*) and 95% confidence intervals (*CI*) were calculated considering $OR < 1$ associated with protection and $OR > 1$ associated with susceptibility/risk. Values $P < 0.05$ were considered statistically significant. The power of sample size was calculated by G*power software version 3.0 using as test family: *chi*-squared test; statistical test: goodness-of-fit tests-contingency tables and type of power analysis (*post hoc*) [21].

3. Results

The genotypic distribution was based on the Hardy–Weinberg equilibrium. Seventy-eight patients with DF [age (32.77 ± 15.40) years], 49 [(35.40 ± 18.10) years] patients with DHF and 135 [age (22.40 ± 4.90) years] control subjects were recruited for this study. Tables 1 and 2 showed the genotypic and allelic frequencies of *TNFA* (−308G/A), *IL-10* (−819 C/T) and *IFNG* (+874 A/T) SNPs in dengue clinical forms and healthy controls.

Genotypic frequencies between group dengue (DEN) and controls were compared, and an association of protection with *TNFA* (−308G/A) polymorphism gene G/A + A/A was found in model dominant ($P = 0.043$, $OR = 0.51$, $CI = 0.26–0.99$). Comparing DF group and healthy controls for *TNFA* (−308G/A) polymorphism in a dominant model (GG *vs* G/A + A/A), G/A + A/A genotypes were positively associated with protection for DF ($P = 0.043$, $OR = 0.45$, $CI = 0.20–1.00$) (Table 3). Genotypic (Table 3) and allelic (Table 4) frequencies were not significantly different between DF and DHF.

To analyze the association of *IL-10* (−819 C/T) polymorphism with disease susceptibility, the genotype frequency was compared between DEN and controls. In a codominant model ($P = 0.014$, $OR = 4.07$, $CI = 1.52–10.85$), dominant model ($P = 0.040$, $OR = 1.78$, $CI = 1.02–3.11$) and recessive model ($P = 0.008$, $OR = 3.41$, $CI = 1.33–8.72$) the T/T genotype was significantly associated with DEN. The genotypic distribution of *IL-10* (−819 C/T) were compared between DF group and healthy controls, and a high frequency of genotype C/C was observed in group control. The T/T genotype of *IL-10* (−819 C/T) was significantly associated with DF but not with the severe form DHF (Table 1) in a co-dominant model ($P = 0.027$, $OR = 4.21$, $CI = 1.45–12.25$) and recessive model ($P = 0.008$, $OR = 3.82$, $CI = 1.38–10.59$). When compared DF group with DHF, the C/T genotype was significantly associated with the progression for DHF in an over dominant model ($P = 0.047$, $OR = 2.10$, $CI = 1.01–4.38$) (Table 3). Interestingly, the C allele was significantly associated with protection for DEN ($P = 0.018$, $OR = 0.62$, $CI = 0.43–0.91$) and DHF ($P = 0.028$, $OR = 0.56$, $CI = 0.34–0.91$) when compared to healthy controls (Table 4).

We also evaluated the association of *IFNG* (+874 A/T) polymorphisms with DEN group and healthy controls, in a codominant model ($P = 0.026$, $OR = 0.45$, $CI = 0.24–0.83$), dominant model ($P = 0.033$, $OR = 0.54$, $CI = 0.30–0.96$) and over dominant model ($P = 0.007$, $OR = 0.46$, $CI = 0.26–0.82$), the T/A genotype was significantly associated with protection infection for DENV. The T/A genotype of *IFNG* (+874 A/T) polymorphism was statistically associated with protection when

Table 1
Genotypic frequency of *TNFA*, *IL-10* and *IFNG* in healthy controls and dengue patient groups [*n*(%)].

Groups	*TNFA* −308 (G/A)			*IL-10*-819 (C/T)			*IFNG* +874 (A/T)		
	G/G	G/A	A/A	C/C	C/T	T/T	A/A	A/T	T/T
Control	99 (73.3)	33 (24.4)	3 (2.2)	72 (53.3)	55 (40.7)	8 (5.9)	43 (35.2)	64 (52.5)	15 (12.3)
DEN	102 (80.3)	24 (18.9)	1 (0.8)	54 (42.5)	54 (42.5)	19 (15.0)	54 (42.5)	46 (36.2)	27 (21.3)
DF	63 (80.8)	14 (17.9)	1 (1.3)	37 (47.4)	28 (35.9)	13 (16.7)	35 (44.9)	28 (35.9)	15 (19.2)
DHF	39 (79.6)	10 (20.4)	0 (0.0)	17 (34.7)	26 (53.1)	6 (12.2)	19 (38.8)	18 (36.7)	12 (24.5)

For *IFNG* it was used 122 controls due to used of PCR-ARMS did not getting amplification of some samples.

Table 2
Allelic frequency of *TNFA*, *IL-10* and *IFNG* in healthy controls and dengue patient groups [*n*(%)].

Groups	*TNFA* −308 (G/A)		*IL-10* −819 (C/T)		*IFNG* +874 (A/T)	
	G	A	C	T	T	A
Control	231 (85.6)	39 (14.4)	199 (73.7)	71 (26.3)	58 (37.2)	98 (62.8)
DEN	228 (86.3)	36 (13.7)	162 (63.7)	92 (36.3)	136 (39.7)	206 (60.3)
DF	140 (89.7)	16 (10.3)	102 (65.4)	54 (34.6)	94 (38.5)	150 (61.5)
DHF	88 (89.7)	10 (10.3)	60 (61.2)	38 (38.8)	42 (42.8)	56 (57.2)

For allelic frequencies of IFNG it was used 122 controls due to used of PCR-ARMS did not getting amplification of some samples.

Table 3
Association of genotype distributions with protection and susceptibility.

SNP	Model	Control *vs* DEN		Control *vs* DF		Control *vs* DHF		DF *vs* DHF	
		OR (95% *CI*)	*P*	*OR* (95% *CI*)	*P*	*OR* (95% *CI*)	*P*	*OR* (95% *CI*)	*P*
TNFA	Codominant	0.52 (0.26–1.05)	0.120	0.45 (0.20–1.03)	0.130	0.49 (0.19–1.27)	0.170	1.21 (0.48–3.02)	0.540
−308 (G/A)	Dominant	0.51 (0.26–0.99)	0.043	0.45 (0.20–1.00)	0.043	0.46 (0.18–1.18)	0.092	1.12 (0.45–2.77)	0.800
	Recessive	0.36 (0.03–3.92)	0.380	0.58 (0.05–6.32)	0.640	–	–	–	–
	Over dominant	0.54 (0.27–1.07)	0.072	0.46 (0.20–1.04)	0.054	0.50 (0.19–1.30)	0.140	1.23 (0.49–3.07)	0.660
	Log additive	0.53 (0.29–0.98)	0.039	0.50 (0.24–1.04)	0.052	0.45 (0.18–1.13)	0.073	1.03 (0.44–2.41)	0.950
IL-10	Codominant	4.07 (1.52–10.85)	0.014	4.21 (1.45–12.25)	0.027	3.37 (0.89–12.82)	0.170	1.05 (0.34–3.20)	0.140
−819 (C/T)	Dominant	1.78 (1.02–3.11)	0.040	1.62 (0.86–3.08)	0.130	1.83 (0.84–3.97)	0.120	1.78 (0.84–3.77)	0.130
	Recessive	3.41 (1.33–8.72)	0.008	3.82 (1.38–10.59)	0.008	2.66 (0.75–9.43)	0.140	0.71 (0.25–2.01)	0.510
	Over dominant	1.13 (0.65–1.97)	0.670	0.95 (0.50–1.83)	0.890	1.32 (0.61–2.83)	0.480	2.10 (1.01–4.38)	0.047
	Log additive	1.79 (1.18–2.73)	0.005	1.75 (1.09–2.82)	0.020	1.75 (0.97–3.18)	0.064	1.21 (0.30–2.02)	0.460
IFNG	Codominant	0.45 (0.24–0.83)	0.026	0.45 (0.22–0.92)	0.066	0.45 (0.19–1.07)	0.100	1.15 (0.50–2.61)	0.790
+874 (A/T)	Dominant	0.54 (0.30–0.96)	0.033	0.53 (0.27–1.03)	0.061	0.58 (0.26–1.29)	0.180	1.23 (0.58–2.60)	0.590
	Recessive	1.39 (0.64–3.05)	0.400	1.39 (0.56–3.44)	0.480	1.81 (0.66–5.00)	0.260	1.31 (0.54–3.23)	0.550
	Over dominant	0.46 (0.26–0.82)	0.007	0.46 (0.24–0.89)	0.020	0.43 (0.19–0.95)	0.034	1.02 (0.48–2.15)	0.960
	Log additive	0.80 (0.54–1.20)	0.290	0.79 (0.49–1.27)	0.330	0.91 (0.51–1.60)	0.730	1.18 (0.30–1.92)	0.500

Table 4
Association of allelic distributions with protection and susceptibility.

SNP	Allele	Control	Control *vs* DEN		Control *vs* DF		Control *vs* DHF		DF *vs* DHF	
			OR (95% *CI*)	*P*	*OR* (95% *CI*)	*P*	*OR* (95% *CI*)	*P*	*OR* (95% *CI*)	*P*
TNFA	G	231 (85.6)	1.06 (0.65–1.74)	0.880	1.47 (0.79–2.74)	0.270	1.48 (0.71–3.10)	0.370	1.00 (0.43–2.31)	0.840
−308 (G/A)	A	39 (14.4)								
IL-10	C	199 (73.7)	0.62 (0.43–0.91)	0.018	0.67 (0.43–1.03)	0.088	0.56 (0.34–0.91)	0.028	0.83 (0.49–1.41)	0.590
−819 (C/T)	T	71 (26.3)								
IFNG	T	58 (37.2)	1.11 (0.75–1.64)	0.650	0.94 (0.62–1.42)	0.860	1.19 (0.74–1.92)	0.530	1.26 (0.75–2.12)	0.440
+874 (A/T)	A	98 (62.8)								

For allelic frequencies of *IFNG* it was used 122 controls due to used of PCR-ARMS did not getting amplification of some samples.

Table 5
Combination frequency of TNFA, IL-10 and IFNG in healthy controls and dengue patient groups [n(%)].

No	Cytokines			Control	Frequencies (%)		
	TNFA	IL-10	IFNG		DEN	DF	DHF
1	G	C	A	41.48	38.85	35.43	40.61
2	G	C	T	21.09	19.75	21.65	16.53
3	G	T	A	11.10	14.55	20.24	12.45
4	G	T	T	12.40	14.79	12.43	20.19
5	A	C	A	4.94	5.05	5.20	4.08
6	A	C	T	5.04	4.42	3.10	0.00
7	A	T	A	3.95	2.59	1.95	–

For combinations it was used 122 controls due to used of PCR-ARMS did not getting amplification of some samples.

compared DF group with healthy controls for dengue infection ($P = 0.02$, $OR = 0.46$, $CI = 0.24–0.89$) and when compared DHF group with healthy controls ($P = 0.034$, $OR = 0.43$, $CI = 0.19–0.95$) in an over dominant model (Table 3). There were no observed differences in allelic and genotypic frequencies between DF and DHF (Tables 3 and 4).

Tables 5 and 6 showed the combinations distribution of the analyzed polymorphisms. *TNFA/IL-10/IFNG* GTA was associated with the susceptibility for dengue infection ($P = 0.022$, $OR = 2.95$, $CI = 1.18–7.41$) and *TNFA/IL-10/IFNG* GCT ($P = 0.035$, $OR = 0.28$, $CI = 0.08–0.90$) was significantly associated with protection for DHF. The *post hoc* power analyses in this paper were 68% (*TNFA* −308G/A), 99% (*IL-10* −819 C/T) and 99% (*IFNG* +874 A/T).

Table 6
Association of combinations distributions with protection and susceptibility.

No	Cytokines			Controls *vs* DEN		Controls *vs* DF		Controls *vs* DHF		DF *vs* DHF	
	TNFA	IL-10	IFNG	*OR* (95% *CI*)	*P*	*OR* (95% *CI*)	*P*	*OR* (95% *CI*)	*P*	*OR* (95% *CI*)	*P*
1	G	C	A	1.00	–	1.00	–	1.00	–	1.00	–
2	G	C	T	0.60 (0.28–1.26)	0.180	0.89 (0.38–2.10)	0.790	**0.28 (0.08–0.90)**	**0.035**	0.42 (0.17–1.05)	0.066
3	G	T	A	1.28 (0.68–2.42)	0.450	**2.95 (1.18–7.41)**	**0.022**	0.79 (0.23–2.71)	0.710	0.59 (0.24–1.46)	0.250
4	G	T	T	2.22 (0.97–5.08)	0.060	1.04 (0.48–2.23)	0.920	1.14 (0.48–2.67)	0.770	1.94 (0.92–4.10)	0.086
5	A	C	A	0.82 (0.22–2.98)	0.760	0.72 (0.15–3.38)	0.680	0.48 (0.09–2.72)	0.410	1.06 (0.31–3.57)	0.930
6	A	C	T	1.04 (0.30–3.66)	0.950	0.86 (0.17–4.40)	0.860	0.08 (0.00–4.91)	0.230	0.83 (0.13–5.23)	0.840
7	A	T	A	0.22 (0.03–1.41)	0.110	0.29 (0.04–2.00)	0.210	–	–	–	–

For combinations it was used 122 controls due to used of PCR-ARMS did not getting amplification of some samples.
The values in bold show comparisons with a significant association.

4. Discussion

We investigated the potential association of the *TNFA* (−308G/A), *IFNG* (+874 A/T) and *IL-10* (−819 C/T) polymorphism with the development of dengue in a Brazilian sample. Individual genetic variations may affect the host response to infection, in this context, several studies have investigated the role of cytokine gene polymorphisms in susceptibility to diseases including dengue [22]. In infection dengue, the polymorphisms genetics could mediate the production of cytokines, and susceptibility or progression to infection. Here, our findings suggested a role of genetic polymorphisms of the *TNFA* (−308G/A), *IL-10* (−819) and *IFNG* (+874 A/T) on dengue infection in a sample from the Brazilian population.

The significant role of TNF-α in DENV immunopathogenesis has been reported [23]. In this study, we identified that the dominant model GA + AA was linked with protection to dengue, additionality to our results. In a case control study on Malaysia, the G/A + A/A *TNFA* (−308G/A) genotype was associated with reduced risk for DHF [24]. The presence of A allele polymorphism has been related to a higher *TNFA* gene expression level [25]. Therefore, our findings suggest a possible correlation between the presence of high-expressing *TNFA* −308A allele and protection against DF. The TNF-α cytokine in high and medium concentrations may inhibit the DENV replication in human dendritic cells and this inhibition may decrease the dengue infection [23].

Interestingly, the Brazilian studies that investigated the role of several cytokines polymorphisms concluded that *TNFA* (−308G/A) were not related with predisposition to dengue [26,27]. Studies conducted in population Thai [28] and Mexican [29] did not identify association of the polymorphism of this cytokine with dengue. Some studies have shown the association of the A allele of *TNFA* (−308G/A) polymorphism with severity of dengue in Cuban population with secondary infection with DENV-2 [30]. In a study conducted in Thai children, the same allele was associated with the risk of bleeding [31]. Another study performed in Brazil identified a significant association of *TNFA* allele A with no persistence of the symptoms of dengue in convalescence [32]. In Sri Lanka, the G/G genotype was identified as a risk factor for the development of DHF [10]. In our study, the G/G genotype had a high frequency in the case group compared with the control group, however, there was no significantly statistical difference.

IL-10 is anti-inflammatory cytokine which has been considered as key in the control of host immune response by regulate the production of several pro inflammatory cytokines [33]. Likely the IL-10 is regulated at transcriptional level by several polymorphisms in the promotor region in this gene, among them the polymorphism −819 [15,33]. In our study, the T/T genotype of the SNP *IL-10* (−819 C/T) was significantly associated with susceptibility to DF infection, whereas the C/T genotype was linked with the progression for DHF differently from other studies performed which did not identify association in the populations Brazilian [26], Singhalese [10] and Cuban [30]. Nevertheless, the heterozygous genotype was previously associated with protection for DF in India [34]. In this study, we found an association between C allele and protection against the development for the DHF. Other studies, including one performed in a sample from the Brazilian population, did not find any allelic association [24,26,34].

Interferons are cytokines that play a complex role in host resistance because of the action of pathogens, the IFN-γ is able to upregulate the expression of Fcγ receptors on monocytes and macrophages, thus, may facilitate viral replication [35]. The presence of +874 A/T polymorphism in *INF-γ* gene has been shown to influence gene transcriptional level. Genotypes TT, T/A and AA were associated with high, intermediate and low production of *IFNG*, respectively, wherein the T allele was associated high levels [36]. Our findings suggest a possible association between heterozygosity for *IFNG* (+874 A/T) polymorphism and a protector effect against DF and DHF. The A allele previously showed a possible association with the non-persistence of symptoms during thirty days, in primary dengue and persistence of symptoms in secondary dengue [32], in a Brazilian patients cohort. Yet in population Brazilian, the genotype heterozygosity in comparison of group control and dengue was linked with protection [27]. However, earlier studies did not find associations between this SNP and dengue severity [30,34]. The T allele has been associated with increased indoleamine-pyrrole 2,3-dioxygenase activity [37], the growth of this enzyme activity promotes a high conversion of tryptophan to kynurenine, a molecule involved in DHF, for present increased significantly in patients with DHF [38]. The intermediate expression A/T genotype may not increase indoleamine-pyrrole 2,3-dioxygenase activity, accordingly causing a low level of kynurenine that may act in effect protector against DHF.

We also performed a combinations analysis to investigate the combinatory effect among the cytokines polymorphism. Our findings showed an association between *TNFA/IL-10/IFNG* GTA combinations and the susceptibility for dengue infection. While the *TNFA/IL-10/IFNG* GCT combinations were associated with a protector effect against DHF. The diversity genetic between populations and miscegenation of population Brazilian may explain the different results presented in this study.

We identified associations between polymorphisms in *TNFA* (−308G/A), *IFNG* (+874 A/T) and *IL-10* (−819) genes with dengue infection and clinical category for the disease. This outcome provides evidence that genetic polymorphisms in immune system affect susceptibility or protect to clinical phenotypes of dengue and may be considered as good prognostic markers. Yet it is important that further studies investigated asymptomatic individuals and linked with factors clinical and laboratorial, genetics, immunological are needed. In addition, gene expression of cytokines in patients with dengue associated with polymorphisms may be an opportunity to observe the synchronized biological relevance and interaction of genes in susceptibility, progression or protection a disease.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

This work was supported by the Fundação de Amparo à Pesquisa de Alagoas (FAPEAL) – Programa Primeiros Projetos (PPP/2011).

We thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (Grant number: 1532477) by supported scholarship the ACMS. This work was financed by

Fundação de Amparo à Pesquisa de Alagoas (FAPEAL), nº protocol: 20110906-011-0025-0080. The authors acknowledge the help rendered by Laboratory Municipal de Arapiraca – AL in sample collection and performing IgM ELISA. The authors thank Laboratory de Analysis Clinics Dr. Edler Lins (LACEL) by performing IgM ELISA in control group.

In memory of the researcher Alysson Felix da Silva, student of the course of Biological Sciences of UFAL and member of our research group.

References

[1] Brady OJ, Gething PW, Bhatt S, Messina JP, Brownstein JS, Hoen AG, et al. Refining the global spatial limits of dengue virus transmission by evidence-based consensus. *PLoS Negl Trop Dis* 2012; **6**(8): e1760.

[2] Grange L, Simon-Loriere E, Sakuntabhai A, Gresh L, Paul R, Harris E. Epidemiological risk factors associated with high global frequency of inapparent dengue virus infections. *Front Immunol* 2014; **5**: 280.

[3] WHO. *Dengue and severe dengue*. Geneva: World Health Organization; 2013. [Online] Available from: http://www.who.int/mediacentre/factsheets/fs117/en/ [Accessed on 15th February, 2016]

[4] Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. *Nature* 2013; **496**: 504-507.

[5] PAHO WHO. Dengue: PAHO/WHO data, maps and statistics. Washington: Pan American Health Organization. Available from: http://www.paho.org/hq/index.php?option=com_topics&view=rdmore&cid=6290&Itemid=40734. [Accessed on 15th February, 2016].

[6] WHO. *Dengue hemorrhagic fever: diagnosis, treatment, prevention and control*. 2nd ed. Geneva: World Health Organization; 1997. Available from: http://apps.who.int/iris/bitstream/10665/41988/1/9241545003_eng.pdf.

[7] WHO. *Dengue: guidelines for diagnosis, treatment, prevention, and control*. Geneva: WHO; 2009. doi: WHO/HTM/NTD/DEN/2009.1.

[8] Normile D. Surprising new dengue virus throws a spanner in disease control efforts. *Science* 2013; **342**(6157): 415.

[9] Herrero LJ, Zakhary A, Gahan ME, Nelson MA, Herring BL, Hapel AJ, et al. Dengue virus therapeutic intervention strategies based on viral, vector and host factors involved in disease pathogenesis. *Pharmacol Ther* 2013; **137**(2): 266-282.

[10] Fernando AN, Malavige GN, Perera KL, Premawansa S, Ogg GS, De Silva AD, et al. Polymorphisms of transporter associated with antigen presentation, tumor necrosis factor-α and interleukin-10 and their implications for protection and susceptibility to severe forms of dengue fever in patients in Sri Lanka. *J Glob Infect Dis* 2015; **7**(4): 157.

[11] Mohsin SN, Mahmood S, Amar A, Ghafoor F, Raza SM, Saleem M. Association of FcγRIIa polymorphism with clinical outcome of dengue infection: first insight from Pakistan. *Am J Trop Med Hyg* 2015; **93**(4): 691-696.

[12] Harapan H, Fajar JK, Wahyuniati N, Anand JR, Nambaru L, Jamil KF. Non-HLA gene polymorphisms and their implications on dengue virus infection. *Egypt J Med Microbiol* 2013; **14**(1): 1-11.

[13] Kalliolias GD, Ivashkiv LB. TNF biology, pathogenic mechanisms and emerging therapeutic strategies. *Nat Rev Rheumatol* 2016; **12**(1): 49.

[14] Elahi MM, Asotra K, Matata BM, Mastana SS. Tumor necrosis factor alpha – 308 gene locus promoter polymorphism: an analysis of association with health and disease. *BBA – Mol Basis Dis* 2009; **1792**(3): 163-172.

[15] Tsai TT, Chuang YJ, Lin YS, Wan SW, Chen CL, Lin CF. An emerging role for the anti-inflammatory cytokine interleukin-10 in dengue virus infection. *J Biomed Sci* 2013; **20**(1): 40.

[16] Bream JH, Ping A, Zhang X, Winkler C, Young HA. A single nucleotide polymorphism in the proximal IFN-gamma promoter alters control of gene transcription. *Genes Immun* 2002; **3**(3): 165.

[17] Conti-Freitas LC, Foss-Freitas MC, Mamede RCM, Foss NT. Interferon-gamma and interleukin-10 production by mononuclear cells from patients with advanced head and neck cancer. *Clinics (Sao Paulo)* 2012; **67**(6): 587-590.

[18] Teixeira LK, Fonseca BP, Barboza BA, Viola JP. The role of interferon-gamma on immune and allergic responses. *Mem Inst Oswaldo Cruz* 2005; **100**: 137-144.

[19] Abrão MG, Billerbeck AEC, Nishi MY, Marui S, Mendonça BBD. Standardization of DNA extraction with NaCl from oral mucosa cells: application in PROP1 gene study. *Arq Bras Endocrinol Metab* 2005; **49**(6): 978-982.

[20] Perrey C, Pravica V, Sinnott PJ, Hutchinson IV. Genotyping for polymorphisms in interferon-γ, interleukin-10, transforming growth factor-β1 and tumour necrosis factor-α genes: a technical report. *Transpl Immunol* 1998; **6**(3): 193-197.

[21] Santos ACMD, de Moura EL, Ferreira JM, Santos BRCD, Alves VDM, de Farias KF, et al. Meta-analysis of the relationship between TNF-α (– 308G/a) and *IL-10* (– 819C/t) gene polymorphisms and susceptibility to dengue. *Immunol Investig* 2017; **46**(2): 201-220.

[22] Chapman SJ, Hill AV. Human genetic susceptibility to infectious disease. *Nat Rev Genet* 2012; **13**(3): 175-188.

[23] Shi YJ, Jiang ZY, Zeng K. Effect of IL-6 and TNF-alpha on dengue virus infection of human dendritic cells. *Chin J Cell Mol Immunol* 2006; **22**(4): 469-471.

[24] Sam SS, Teoh BT, Chinna K, AbuBakar S. High producing tumor necrosis factor alpha gene alleles in protection against severe manifestations of dengue. *Int J Med Sci* 2015; **12**(2): 177-186.

[25] Abraham LJ, Kroeger KM. Impact of the-308 TNF promoter polymorphism on the transcriptional regulation of the TNF gene: relevance to disease. *J Leukoc Biol* 1999; **66**(4): 562-566.

[26] Xavier-Carvalho C, Gibson G, Brasil P, Ferreira RX, de Souza Santos R, Cruz OG, et al. Single nucleotide polymorphisms in candidate genes and dengue severity in children: a case–control, functional and meta-analysis study. *Infect Genet Evol* 2013; **20**: 197-205.

[27] Feitosa RNM, Vallinoto ACR, Vasconcelos PFDC, Azevedo RDSDS, Azevedo VN, Machado LFA, et al. Gene polymorphisms and serum levels of pro-and anti-inflammatory markers in dengue viral infections. *Viral Immunol* 2016; **29**(7): 379-388.

[28] Vejbaesya S, Luangtrakool P, Luangtrakool K, Kalayanarooj S, Vaughn DW, Endy TP, et al. TNF and LTA gene, allele, and extended HLA haplotype associations with severe dengue virus infection in ethnic Thais. *J Infect Dis* 2009; **199**(10): 1442-1448.

[29] García-Trejo AR, Falcón-Lezama JA, Juárez-Palma L, Granados J, Zúñiga-Ramos J, Rangel H, et al. Tumor necrosis factor alpha promoter polymorphisms in Mexican patients with dengue fever. *Acta Trop* 2011; **120**(1): 67-71.

[30] Perez AB, Sierra B, Garcia G, Aguirre E, Babel N, Alvarez M, et al. Tumor necrosis factor–alpha, transforming growth factor–β1, and interleukin-10 gene polymorphisms: implication in protection or susceptibility to dengue hemorrhagic fever. *Hum Immunol* 2010; **71**(11): 1135-1140.

[31] Chuansumrit A, Anantasit N, Sasanakul W, Chaiyaratana W, Tangnararatchakit K, Butthep P, et al. Tumour necrosis factor gene polymorphism in dengue infection: association with risk of bleeding. *Paediatr Int Child Health* 2013; **33**(2): 97-101.

[32] Dettogni RS, Tristão-Sá R, dos Santos M, da Silva FF, Louro ID. Single nucleotide polymorphisms in immune system genes and their association with clinical symptoms persistence in dengue-infected persons. *Hum Immunol* 2015; **76**(10): 717-723.

[33] Perovic D, Perovic V, Pravica V, Bonaci-Nikolic B, Mijanovic R, Bunjevacki V. Evaluation of cytokine genetic polymorphisms in adult patients with common variable immunodeficiency: a single-center study. *Immunol Lett* 2016; **176**: 97-104.

[34] Alagarasu K, Bachal RV, Tillu H, Mulay AP, Kakade MB, Shah PS, et al. Association of combinations of interleukin-10 and

pro-inflammatory cytokine gene polymorphisms with dengue hemorrhagic fever. *Cytokine* 2015; **74**(1): 130-136.

[35] Boehm U, Klamp T, Groot M, Howard J. Cellular responses to interferon-g. *Annu Rev Immunol* 1997; **715**: 749-795.

[36] Pravica V, Perrey C, Stevens A, Lee JH, Hutchinson IV. A single nucleotide polymorphism in the first intron of the human IFN-γ gene: absolute correlation with a polymorphic CA microsatellite marker of high IFN-γ production. *Hum Immunol* 2000; **61**: 863-866.

[37] Raitala A, Pertovaara M, Karjalainen J, Oja SS, Hurme M. Association of interferon-gamma+ 874 (T/A) single nucleotide polymorphism with the rate of tryptophan catabolism in healthy individuals. *Scand J Immunol* 2005; **61**(4): 387-390.

[38] Cui L, Lee YH, Thein TL, Fang J, Pang J, Ooi EE, et al. Serum metabolomics reveals serotonin as a predictor of severe dengue in the early phase of dengue fever. *PLoS Negl Trop Dis* 2016; **10**(4): e0004607.