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Association of combinations of interleukin-10 and pro-inflammatory cytokine gene polymorphisms with dengue hemorrhagic fever

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ABSTRACT

Pro-inflammatory and anti-inflammatory cytokines have been shown to play an important role in dengue disease pathogenesis. In the present study, to find out whether single nucleotide polymorphisms (SNPs) in the pro-inflammatory and anti-inflammatory cytokine genes are associated with dengue disease severity, SNPs in *TNF*, *IFNG*, *IL1B*, *IL8*, *IL0*, *IL17A* and *IL17F* genes were investigated using polymerase chain reaction based methods in 132 dengue (DEN) cases [87 dengue fever (DF), 45 dengue hemorrhagic fever (DHF) cases] and 108 apparently healthy controls (HC) from Pune, Maharashtra, western India. Under recessive genetic model (C/C vs. T/T + T/C), the *TNF* rs1799964 C/C genotype was significantly associated with DEN [$P = 0.014$, OR with 95% CI 3.07 (1.18–7.98)]. Frequency of T/C genotype of *IL17F* rs763780 was significantly lower in DEN group as compared to HC [$P = 0.033$, OR with 95% CI 0.43 (0.19–0.95)]. Under overdominant genetic model (A/T vs. A/A + T/T), *IL8* rs4973 A/T genotype was negatively associated with DHF compared to HCs [$p = 0.029$, OR with 95% CI 0.43 (0.20–0.93)]. Under overdominant genetic model, A/G genotype of *IL10* rs1800871 was significantly negatively associated with DHF compared to DF cases [$p = 0.014$, OR with 95% CI 0.35 (0.15–0.84)]. Significantly higher frequency of the combined genotype *IL10* A/A-*IFNG* A/T and lower frequency of the combined genotypes *IL10* A/G-*IL1B* A/A, *IL10* A/G-*IL8* A/T and *IL10* A/G-*IL17F* T/T were observed in DHF cases compared to DF. The results suggest that heterozygous genotypes of *IL8* rs4973 and *IL10* rs1800871 are associated with reduced risk of DHF. Combinations of *IL10* rs1800871 and pro-inflammatory cytokine genotypes influence the risk of DHF.

1. Introduction

Dengue, caused by dengue virus (DENV), is a leading cause of hospitalization and deaths in Asian and Latin American countries [1]. Infection with DENV results in diverse clinical manifestations ranging from mild form of the disease namely dengue fever to severe forms of the disease including dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). The diversity in the outcome of DENV infection is affected by the infecting serotype/genotype of the virus, host immune status, host genetics and environmental factors [2].

Immune response resulting from DENV infection has shown to be the major contributor in disease pathogenesis. Pro-inflammatory and anti-inflammatory cytokines have been identified as the main culprits in the process leading to dengue disease severity (Table 1). Higher concentrations of various cytokines such as interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin (IL)-6, IL-8 and IL-10 have been reported in DHF cases as compared to DF cases [3].

TNF-α and IL-1β, endogenous pyrogens and pro-inflammatory cytokines, are responsible for the induction of fever during DENV infections [4]. Infection of granulocyte macrophage colony stimulating factor macrophage (GM-CSF Mϕ) with DENV results in production of higher levels of TNF-α, IL-1β and other pro-inflammatory cytokines leading to increase in vascular permeability, a characteristic symptom of DHF [5]. DENV also activates nucleotide binding domain leucine rich repeat family PYD containing protein 3 (NLRP3) inflammasome in GM-CSF Mϕ resulting in massive production of IL-1β, which is responsible for high fever in DENV infected patients [6]. Infection of endothelial cells (EC) with DENV infection predisposes EC to TNF-α induced apoptosis enhancing vascular permeability [7].

IFN-γ is a T helper 1 (Th1) cytokine known to be important in dengue disease pathogenesis [3,8]. It has been shown that the relative proportion of IFN-γ and TNF-α produced by DENV serotype specific or cross reactive T cell clones correlate with disease severity [9,10]. IL-8, a pro-inflammatory cytokine, acts as a chemoattractant for neutrophils. Higher levels of IL-8 have been reported in serum samples of DHF cases [11,12]. IL-8 levels have been reported to be associated with neutrophil degranulation and haemodynamic variables in dengue cases [13]. IL-8 is known to affect cytoskeleton

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Table 1
Relevance of different cytokines to dengue disease severity and effect of single nucleotide polymorphisms in the cytokine coding genes on cytokine production and function.

Cytokine	Relevance to dengue disease severity	Single nucleotide polymorphisms	Effect on cytokine production/function
TNF-α	Induces fever, enhances vascular permeability, induces apoptosis of endothelial cells	rs1799964 C/T	Allele 'C' associated with higher TNF-α levels
		rs1800629 G/A	Allele 'A' associated with higher TNF-α levels
IL1-β	Induces fever, enhances vascular permeability	rs16944 A/G	Allele 'A' associated with higher transcriptional activity
IFN-γ	Restricts dengue virus replication	rs2430561 A/T	Allele 'T' associated with higher IFN-γ levels
IL-8	Affects cytoskeleton and tight junction reorganization and influence vascular permeability. Associated with neutrophil degranulation and haemodynamic variables in dengue cases	rs4973 T/A	Allele 'A' associated with higher IL-8 levels
IL-17A and IL-17F	Enhance tissue mediated pro-inflammatory response in combination with other pro-inflammatory cytokines	rs2275913 G/A	Allele 'A' associated with higher production of IL-17
		rs763780 T/C	Allele 'C' associated with reduced expression and activity of IL-17F
IL-10	Associated with size of pleural effusion and decay of platelets in dengue cases. Consistently higher levels in DHF cases	rs1800871 A/G	Allele 'A' associated with higher levels of IL-10

and tight junction reorganization and influence the permeability of microvascular endothelial monolayers [14]. IL-17 family of cytokines has been reported to be associated with autoimmune diseases. IL-17A and Il-17F enhance tissue mediated pro-inflammatory response in synergistic association with other cytokines such as TNF-α, IL-1β and IFN- γ [15]. A recent study has reported elevated levels of IL-17 in severe dengue cases [16]. Consistently, elevated plasma levels of IL-10, an anti inflammatory cytokine, has been reported in patients with severe dengue [3]. The plasma levels of IL-10 have been shown to be associated with size of pleural effusion and decay of platelets in dengue patients [17,18].

Single nucleotide polymorphisms (SNPs) in the cytokine genes are known to affect cytokine production (Table 1). The variant allele (A) of *TNF* rs1800629 polymorphism has been shown to be associated with DHF in Cuban and Venezuelean populations [19,20]. Our recent study has shown that the C/C genotype of *TNF* rs1799964 polymorphism is associated with symptomatic dengue [21]. An intron polymorphism (A/T) in the *IFNG* gene (rs2430561) has been shown to influence IFN-γ levels [22,23]. Polymorphisms in the promoter region of the *IL10* gene (rs1800871) have been shown to be associated with dengue [20]. Polymorphisms in the *IL17A* and *IL17F* genes have been shown to be associated with asthma and other inflammatory diseases [24,25].

Genetic association between dengue disease severity and various host gene polymorphisms has been extensively studied in Cuban, Thai, Venezuelean and Caucasian populations and genetic markers that are associated with severe forms of dengue have been identified [26]. Studies involving association of cytokine gene polymorphisms and dengue disease are lacking in Indian populations, which are genetically diverse and overlap with hapmap populations [27]. Genetic associations reported for one population may not hold true for the other population. In the present study, we investigated whether polymorphisms in the *TNF*, *IFNG*, *IL1B*, *IL8*, *IL17A*, *IL17F* and *IL10* genes are associated with development of DF or DHF in a group of DENV infected patients from western India.

2. Study subjects and methods

2.1. Study subjects

The Institutional Human Ethics Committee approved the study and a written informed consent was obtained from the study participants. One hundred and thirty-two subjects (mean age ± standard deviation (SD) 30.7 ± 13.1) (84 males and 48 females) who had a history of hospitalization for dengue during 2007–2010, and had been laboratory confirmed by dengue specific IgM ELISA were included in the study. Dengue specific ELISA was performed using an IgM capture ELISA kit developed and manufactured by the National Institute of Virology, Pune, India and being used by national vector borne disease control programme (NVBDCP), Government of India. Based on the presence of at least two of the DHF defining criteria of the World Health Organization, 1999 [28], 45 cases had DHF (mean age ± SD 29.6 ± 14.8) (34 males and 11 females) and 87 cases had DF (mean age ± SD 31.3 ± 12.4) (50 males and 37 females). Since most of the samples were collected retrospectively, it was not possible to investigate the infecting serotypes and immune status as a part of this study. However, acute sera from 51 dengue cases (28 DF and 23 DHF cases) were available and subjected to anti DENV IgG capture ELISA (E-DEN02G, Panbio diagnostics, Windsor, Australia) to distinguish primary/secondary cases. Apparently healthy controls (HCs) consisted of 108 subjects (mean age ± SD 31.3 ± 13.2) (67 males and 41 females) from the same geographical area, living in and around Pune, Maharashtra, Western India and were not related to each other. At the time of sample collection, HCs had no known history of hospitalization for dengue like illness in past. When the HCs were tested for their exposure to DENV by DENV specific IgG using commercial kit (E-DEN01G, Panbio diagnostics), 82.0% of the HCs had IgG antibodies against DENV.

2.2. Genotyping of cytokine gene polymorphisms

DNA was isolated from the white blood cells using salting out procedure. *TNF* (rs1799964 & rs1800629), *IFNG* (rs2430561) and *IL10* (rs1800871) were investigated using PCR based methods [23,29–31]. *IL1B* (rs16944), *IL8* (rs4973), *IL17A* (rs2275913) and *IL17F* (rs763780) were genotyped using Taqman genotyping assays (Applied Biosystems) using the protocol described by the manufacturer.

2.3. Statistical analysis

Allele, genotype and combined genotype frequencies were calculated by direct counting. Genotype frequency distributions were tested for their confirmation to Hardy–Weinberg equilibrium using Chi square test. Allele, genotype, and combined genotype frequencies were compared between different study groups using Chi square test or Fisher's exact test as appropriate. For allele and

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