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Short Communication

Cytokine profiles in dengue fever and dengue hemorrhagic fever: A study from Indonesia

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Abstract

Recent studies have demonstrated that cytokine dysregulation has a critical role in the pathogenesis of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). The aim of this study was to investigate the association between tumor necrosis factor (TNF-α), interleukin 6 (IL-6), interleukin 10 (IL-10), and interleukin 17 (IL-17) with infection status, and severity of dengue. A prospective cross-sectional study was conducted at three hospitals in Gianyar regency and Denpasar municipality, Bali, Indonesia, from June to December 2022. Sixty-four dengue infected patients were involved. Patients' serum was tested for dengue infection using NS1 antigen rapid test, dengue virus immunoglobulin M (IgM) and immunoglobulin G (IgG) test, and reverse transcription polymerase chain reaction (RT-PCR). Cytokine levels (TNF-α, IL-6, IL-10, and IL-17) were measured using enzyme-linked immunosorbent assay (ELISA). Infection status was determined by combining serological and RT-PCR results, categorizing patients into primary and secondary infections. The present study found that DF patients had lower TNF-α, IL-6, and IL-17 but higher IL-10 levels compared to DHF patients (p<0.001). Elevated TNF-α, IL-6, and IL-17 levels were higher in secondary infection, while IL-10 level was higher in primary infection (p<0.001). In conclusion, cytokines play a crucial role in the interplay between cytokine dysregulation and dengue infection dynamics.

Keywords: Cytokine, dengue fever, dengue hemorrhagic fever, severity, infection status

Introduction

Dengue, despite its detrimental impact, yet remains neglected in tropical countries [1]. Originally endemic in Southeast Asia, dengue virus (DENV) infection has become a global concern, putting 3.9 billion people at risk, with the highest incidence in Asia [2]. In addition, global economic impact of dengue infection is estimated at around USD 8.9 billion annually [3].

Dengue infection caused by four DENV serotypes [4] and there are no specific clinical manifestations for each serotype, as severity can vary between dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) [5]. Furthermore, primary dengue infection typically leads to a mild or asymptomatic illness; in contrast, a secondary infection may result in a serious condition [6]. This phenomenon is described by antibody-dependent enhancement (ADE) theory and changes in pro- and anti-inflammatory mediators, commonly referred to as a cytokine storm [7].

Previous studies have proposed that cytokine dysregulation plays a crucial role in the pathogenesis of DHF and DSS [8]. Cytokines such as tumor necrosis factor (TNF-α), interleukin 6 (IL-6), interleukin 10 (IL-10), and interleukin 17 (IL-17) significantly impact severity of dengue

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Received: September 7, 2023 - Accepted: February 27, 2024 - Published online: March 8, 2024

infection [9,10]. The aim of this study was to investigate association and correlation of cytokine levels (TNF-α, IL-6, IL-10, and IL-17) to dengue infection status and severity.

Methods

Study design

A cross-sectional study was conducted at the Department of Internal Medicine, Faculty of Medicine and Health Sciences, Universitas Warmadewa, Bali, Indonesia, from June to December 2022. Samples were taken from the general hospitals at Kasih Ibu Denpasar, Puri Raharja and Sanjiwani Hospitals, which are situated in the Gianyar regency and Denpasar municipality. After giving written informed consent, hospitalized patients aged 18 years old who arrived with fever >38°C and at least one dengue symptom such as malaise, arthralgia, rash, retro-orbital discomfort were included in the study. Patients with a history of long-term health conditions, including diabetes mellitus, chronic renal disease, chronic lung disease, HIV/AIDS, and heart disease, were not included in our sample. Sera were collected during the acute phase (within the first five days of illness) and before discharge from the hospital. Each patient's demographic, clinical and hematological data as well as disease severity according to the WHO-SEARO 2009 guideline [1] were recorded.

Patients and criteria

Inclusion criteria of patients were as follows: (1) age ≥18 years old; (2) fever >38°C; and (3) accompanied by at least one symptom such as headache, myalgia, retroorbital pain, or vomiting. Patients with malignancy, autoimmune disease, coinfections or other inflammatory disorders were excluded from this study. Patients were categorized based on disease severity (DF and DHF), determined by hematocrit levels (hematocrit level ≥50% or a decrease of hematocrit level ≥20% post-fluid administration) and infection status (primary and secondary infection) using serology and reverse transcription polymerase chain reaction (RT-PCR) results.

Laboratory analysis, RNA extraction and DENV serotyping

Patients' serum was tested for dengue infection using NS1 antigen rapid test (Alere, Australia), Dengue Duo IgM/IgG Capture enzyme-linked immunosorbent assay (ELISA) (Standard Diagnostics, Bio line, Korea), and RT-PCR. Dengue viral ribonucleic acid (RNA) was extracted using QIAamp Viral RNA kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. The RT-PCR detection and serotyping were performed according to the method described earlier [11]. Dengue serotypes were identified using RT-PCR [11], which used an Applied Biosystems ProFlex PCR System (Thermo Fisher Scientific, MA, USA) and the One-Step RT-PCR Kit (Qiagen, Hilden, Germany) to target an area including the C-prM gene of DENV. Using a 1.5% agarose gel electrophoresis, the amplicons were visualized. For DENV-1, DENV-2, DENV-3, and DENV-4, the expected amplicon sizes were 482 bp, 119 bp, 290 bp, and 392 bp, respectively.

Cytokine levels (TNF-α, IL-6, IL-10, and IL-17) were measured using ELISA with Elabscience reagents (Bioassay Technology Laboratory, Shanghai, China). Infection status was determined by combining serological and RT-PCR results, categorizing patients into primary (negative serology, positive RT-PCR) and secondary infection (positive serology, positive RT-PCR). In addition, complete blood counts, including hemoglobin, hematocrit, erythrocyte count, platelet count, and leucocyte count, were also measured.

Statistical analysis

Continuous data were presented as mean and standard deviation, and categorical data were presented as percentages. To identify association of each outcome variable, Mann-Whitney's test and Chi-squared test for continuous and categorical data, were used respectively. SPSS version 25.0 software (IBM SPSS, Chicago, IL, USA) was employed for data analysis, with $p<0.05$ considered statistically significant.

Results

The present study enrolled 64 patients, predominantly female (n=37) with a median age of 26 years (**Table 1**). Demographic data, clinical manifestations, and laboratory parameters of included patients are detailed in **Table 1**. Fever onset occurred around day four, with DENV-3 being the most prevalent serotype (n=44). Common symptoms included headaches, retroorbital pain, and nausea.

Based on severity, the patients were classified by infection severity (32 patients with DF, 32 patients with DHF) (**Table 1**). No statistical differences were observed in sex, age, fever onset, and serotype between DF and DHF. As expected, retroorbital pain, nausea, vomiting and bleeding were more prevalent among DHF patients compared to DF patients. DHF patients also had longer hospital stays (p=0.001) (**Table 1**). Cytokine levels were significantly associated with DF and DHF patients. DF patients had lower TNF-α, IL-6, and IL-17 levels but higher IL-10 level compared to DHF patients (all had p<0.05) (**Table 1**).

Table 1. Demographic data, clinical manifestations, and laboratory parameters of the dengue patients and their comparations based on severity (DF vs DHF) (n=64)

Parameters	Total n (%)	DF (n=32) n (%)	DHF (n=32) n (%)	*p*-value
Sex				0.802
Male	27 (42.2)	13 (48.1)	14 (51.9)	
Female	37 (57.8)	19 (51.4)	18 (48.6)	
Age (year), mean±SD	29.1 (11.4)	30.2 (12.8)	27.3 (8.7)	0.124
Fever onset (day), mean±SD	3.9 (0.8)	3.9 (0.7)	3.7 (0.9)	0.222
Length of stay (day), mean±SD	5.4 (2)	4.8 (1.9)	6.4 (1.7)	0.001
Serotypes				0.664
DENV-1	6 (9.4)	4 (66.7)	2 (33.3)	
DENV-2	10 (15.6)	4 (40.0)	6 (60.0)	
DENV-3	44 (68.8)	22 (50.0)	22 (50.0)	
DENV-4	1 (1.6)	1 (100)	0 (0)	
Unknown	3 (4.7)	1 (33.3)	2 (66.7)	
Symptoms				
Headache	56 (87.5)	26 (46.4)	30 (53.6)	0.132
Retroorbital pain	30 (46.9)	9 (30.0)	21 (70.0)	0.004
Nausea	43 (67.2)	14 (32.6)	29 (67.4)	<0.001
Vomiting	23 (35.9)	3 (13.0)	20 (87.0)	<0.001
Bleeding	16 (25.0)	0 (0)	16 (100)	<0.001
Hemoglobin, mean±SD (g/dL)	14.27±1.57	13.58±1.29	14.89±1.55	0.001
Hematocrit, mean±SD (%)	43.11±5.26	40.58±4.32	45.41±5.03	<0.001
Erythrocytes, mean±SD (10^3/mL)	4.96±0.65	4.72±0.59	5.17±0.64	0.008
Leucocytes, mean±SD (10^3/mL)	3.05±1.14	2.90±1.04	3.18±1.23	0.162
Thrombocytes, mean±SD (10^3/mL)	93.66±44.61	99.07±42.42	88.75±46.62	0.613
TNF-α, mean±SD (pg/mL)	208.36±186.73	126.41±52.69	282.62±230.22	0.001
IL-6, mean±SD (pg/mL)	184±101.51	129.79±50.06	233.69±111.27	<0.001
IL-10, mean±SD (pg/mL)	130.12±74.12	173.14±83.37	91.15±32.49	<0.001
IL-17, mean±SD (pg/mL)	100.45±72.58	58.62±36.48	138.37±76.64	<0.001

DF: dengue fever; DHF: dengue hemorrhagic fever; DENV: dengue virus; TNF-α: tumor necrosis factor; IL-6: interleukin 6; IL-10: interleukin 10; IL-17: interleukin 17

Based on the infection status (22 patients had primary infection and 42 patients with secondary infection) (**Table 2**). The secondary infection had longer hospital stays and more severe symptoms compared to primary infection with more prevalent headache, nausea, vomiting and bleeding (**Table 2**). Our data indicated that patients with secondary infection had higher TNF-α (245.58 vs 126.08 pg/mL), IL-6 (213.57 vs 119.60 pg/mL), and IL-17 level (122.94 vs 50.74 pg/mL), yet lower IL-10 level (110.99 vs 172.43 pg/mL), compared to primary infection (all had p<0.05) (**Table 1**).

Discussion

The present study identified significant difference in cytokine levels between DF and DHF patients. DF patients had lower TNF-α, IL-6, and IL-17 levels but higher IL-10 levels compared to DHF patients. This pattern suggested a shift towards a pro-inflammatory state in DHF. Cytokines, especially TNF-α, IL-6, and IL-10, were associated with endothelial activation

mediated by CD4+ T helper 2 cells, while IL-17 originated from Th17 cells [12,13]. Prior studies have associated TNF-α and IL-6 to plasma leakage in dengue; yet most often the studies inadequately explained disease severity [14,15]. Contrary to a prior study that correlated DHF to increased IL-10 levels [16], the present study exhibited lower levels of IL-10. The source of this decrease remained elusive in the present study, but potential contributor could include various immune effector cell types, such as T helper cells, monocytes, B cells, cytotoxic T cells, and neutrophil cells [17,18].

Table 2. Association between demographic data, clinical manifestations, and laboratory parameters of the dengue patients with infection status (primary and secondary infection) (n=64)

Parameters	Total n (%)	Primary infection (n=22) n (%)	Secondary infection (n=42) n (%)	*p*-value
Sex				0.048
Male	27 (42.2)	13 (48.1)	14 (51.9)	
Female	37 (57.8)	9 (24.3)	28 (75.7)	
Age (year), mean±SD	29.1 (11.4)	30.1 (9.3)	28.5 (12.5)	0.631
Fever onset (day), mean±SD	3.9 (0.8)	4 (0.7)	3.7 (0.9)	0.113
Length of stay (day), mean±SD	5.4 (2)	4.1 (1.5)	6.2 (1.8)	<0.001
Serotypes				0.212
DENV-1	6 (9.4)	4 (66.7)	2 (33.3)	
DENV-2	10 (15.6)	4 (40.0)	6 (60.0)	
DENV-3	44 (68.8)	12 (27.3)	32 (72.7)	
DENV-4	1 (1.6)	1 (100)	0 (0)	
Unknown	3 (4.7)	1 (33.3)	2 (66.7)	
Symptoms				
Headache	56 (87.5)	16 (28.6)	40 (71.4)	0.011
Retroorbital pain	30 (46.9)	6 (20.0)	24 (80.0)	0.045
Nausea	43 (67.2)	6 (14.0)	37 (86.0)	<0.001
Vomiting	23 (35.9)	0 (0)	23 (100)	<0.001
Bleeding	16 (25.0)	0 (0)	16 (100)	0.001
Hemoglobin, mean±SD (g/dL)	14.27±1.57	13.58±1.17	14.58±1.64	0.011
Hematocrit, mean±SD (%)	43.11±5.26	40.25±4.12	44.40±5.25	0.002
Erythrocytes, mean±SD (10^3/mL)	4.96±0.65	4.83±0.61	5.02±0.67	0.452
Leucocytes, mean±SD (10^3/mL)	3.05±1.14	3.30±1.10	82.14±41.26	0.092
Thrombocytes, mean±SD (10^3/mL)	93.66±44.61	119.11±41.98	2.93±1.15	<0.001
TNF-α, mean±SD (pg/mL)	208.36±186.73	126.08±62.43	245.58±211.56	0.003
IL-6, mean±SD (pg/mL)	184±101.51	119.60±42.09	213.57±213.57	<0.001
IL-10, mean±SD (pg/mL)	130.12±74.12	172.43±68.17	110.99±69.24	<0.001
IL-17, mean±SD (pg/mL)	100.45±72.58	50.74±19.27	122.94±76.70	<0.001

DF: dengue fever; DHF: dengue hemorrhagic fever; DENV: dengue virus; TNF-α: tumor necrosis factor; IL-6: interleukin 6; IL-10: interleukin 10; IL-17: interleukin 17

In the present study, cytokines also showed a notable association with infection status — elevated TNF-α, IL-6, and IL-17 levels were higher in secondary infection, while IL-10 levels were higher in primary infection. These findings might be correlated to ADE phenomenon and cytokine storm [6,7]. ADE phenomenon increases DENV protein synthesis, generation, and release, along with elevated TNF-α and IL-6 production [19]. Cytokine storm is a shift of cytokine levels, causing endothelial breakdown, consequently leading to plasma leakage [20]. Cytokine storm in DENV infection is associated with a weak TNF-α correlation and moderate correlations for IL-6, IL-10, and IL-17, supporting the interplay between cytokines and dynamics of dengue infection.

Higher levels of TNF-α, IL-6, and IL-17, yet lower of IL-10 levels, were significantly correlated with symptoms such as nausea, vomiting, and bleeding, with varying strengths of correlation coefficients; yet, a notable finding in the present study was a strong correlation between IL-17 and bleeding in DF patients. IL-17 acts as a protective mediator in barrier immunity, supporting epithelial integrity by modulating tight junction proteins, subsequently linking and stabilizing epithelial cell connections to maintain barrier and exclude gut luminal contents and commensal microbes [21]. However, the present study introduced a new finding alongside common ones, as IL-8 levels were significantly higher in bleeding DF compared to non-bleeding DF, contributing to thrombocytopenia and increased bleeding [22].

The present study faced constraints due to restricted resources and funding, limiting inclusion of certain cytokines such as IL-8 and interferon. Inability to determine the serotype in three dengue-infected individuals raised concerns, possibly attributed to a human error. Furthermore, the present study focused solely on symptomatic individuals seeking hospital treatment, potentially overlooking asymptomatic cases. Moreover, COVID-19 pandemic exposed challenges, leading to patient withdrawals for reasons such as coinfections and personal concerns. Despite these limitations, the present study managed to yield substantial results.

Conclusions

Cytokines play a crucial role in interplay between cytokine dysregulation and dengue infection dynamics. DF patients had lower TNF-α, IL-6, and IL-17 levels but higher IL-10 levels compared to DHF patients. Patients with secondary infection of DENV had higher TNF-α, IL-6, and IL-17, yet with lower IL-10 levels compared to those with primary infection.

Ethics approval

The study protocol was approved by the Committee for Medical Research Ethics, Faculty of Medicine and Health Sciences, Universitas Warmadewa, Bali, Indonesia (Approval number: 46/UNWAR/FKIK/EC-KEPK/VI/2022).

Acknowledgments

None.

Competing interests

All the authors declare that there are no conflicts of interest.

Funding

The study was supported by Indonesian Ministry of Higher Education (Grant numbers: 160/E5/PG.02.00.PT/2022 and 0967/LL8/Ak.04/2022)

Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

How to cite

Masyeni S, Wardhana IMW, Nainu F. Cytokine profiles in dengue fever and dengue hemorrhagic fever: A study from Indonesia. Narra J 2024; 4 (1): e309 - http://doi.org/10.52225/narra.v4i1.309.

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