

Original Paper

The Association of Cytokines with Severe Dengue in Children

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Received 20 May, 2014 Accepted 16 July, 2014 Published online 31 July, 2014

Abstract: Background: Dengue virus infection is a major public health problem. A hypothesis put forward for severe dengue is the cytokine storm, a sudden increase in cytokines that induces vascular permeability. Previous studies and our recent meta-analysis showed that IL-6, IL-8, IFN γ , TNF α , VEGF-A and VCAM-1 are associated with dengue shock syndrome. Therefore, in this study we aim to validate the association of these cytokines with severe dengue.

Methods & Findings: In a hospital based-case control study in Vietnam, children with dengue fever, other febrile illness and healthy controls were recruited. Dengue virus infection was confirmed by several diagnostic tests. Multiplex immunoassay using Luminex technology was used to measure cytokines simultaneously. A positive association with dengue shock syndrome was found for VCAM-1, whereas a negative association was found for IFN γ . Furthermore, multivariate logistic analysis also showed that VCAM-1 and IFN γ were independently correlated with dengue shock syndrome.

Conclusion: IFN γ and VCAM-1 were associated with dengue shock syndrome, although their role in the severe dengue pathogenesis remains unclear. Additional studies are required to shed further light on the function of these cytokines in severe dengue.

Key words: Association, cytokine, dengue, severity, shock

INTRODUCTION

Dengue virus infection is still a major public-health burden in tropical and subtropical areas of the world, particularly in South-East Asia and the Western Pacific regions. Annually, around 50 million dengue fever cases occur worldwide, including more than 500,000 cases of severe dengue [1, 2]. So far, there is no licensed antiviral drug or vaccine available against dengue virus infection, although some potential candidates are currently being evaluated [3]. Dengue virus is a single-stranded RNA virus, composed of an isosahedral nucleocapsid that is coated by a lipid envelope and is transmitted by the *Aedes* mosquito [2]. Four serotypes of the dengue virus exist, that is DENV-1 to DENV-4 of the Flavivirus genus. Since infection with one serotype does not provide protection

against the others, a secondary infection is more likely to be severe [3].

Dengue virus infection can be diagnosed using cell culture, serology, viral NS1 protein test or a PCR-based method [1]. Dengue virus infection ranges from asymptomatic or mild dengue fever (DF) to dengue hemorrhagic fever (DHF) to dengue shock syndrome (DSS) according to the 1997 World Health Organization (WHO) classification [4]. DHF is characterized by increased vascular permeability, hemorrhagic manifestations, thrombocytopenia, hepatomegaly and circulatory failure. DSS is defined by all symptoms of DHF plus clinical evidence of shock, such as narrow pulse pressure (< 20 mmHg), hypotension for age and cold clammy skin [4]. Mortality in dengue virus infection is mostly due to the severe forms of the disease, especially DSS, with a case-fatality rate exceeding 5% in some

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areas [5].

Knowledge regarding the development of severe forms of dengue virus infection is limited [6]. A popular hypothesis on the pathogenesis of severe dengue is the occurrence of a “cytokine storm,” that is, alterations of cytokine levels that can induce the malfunction of endothelial cells [7]. Vascular permeability of endothelial cells will consequently lead to the plasma leakage characteristic of DHF and DSS. In a previous study, we discovered that vascular endothelial growth factor A (VEGF-A) was significantly increased in patients with severe dengue fever [8]. Plasma levels of vascular cell adhesion molecule 1 (VCAM-1) are also reported to be significantly higher in DHF than DF [9, 10]. Our recent meta-analysis also showed that the cytokines interferon gamma (IFN γ), interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis factor alpha (TNF α) are associated with DSS, although the number of studies upon which the conclusion was based is small [11]. Because these six specific cytokines show a positive association with DSS in particular, we have chosen to further validate the relation of these cytokines in the present study.

METHODS

Study design

This is a hospital-based case control study set in Vietnam. Cases and controls were recruited in the Children’s Hospital No. 2 in Ho Chi Minh City and the Center for Preventive Medicine in Vinh Long Province.

Selection criteria and dengue classification

The inclusion criteria were: patients between 6 months and 15 years of age with suspected dengue virus infection and hospitalization within the first five days of illness. Exclusion criteria were: patients below 6 months or above 15 years of age, hospitalization after five days of illness, and patients without confirmed diagnosis of dengue infection. Patients with confirmed dengue fever were classified as cases. Patients with fever, but with negative serological, Real Time PCR and virological diagnosis for dengue virus infection, were defined as having other non-dengue febrile illness (OFI). A healthy control group was used to serve as a baseline. The healthy control group was recruited from school children living in Ho Chi Minh City with negative dengue diagnostic test results and no symptoms of dengue or other diseases, between 3 and 10 years of age. Sampling was performed upon study enrollment. The hospital staff recorded the clinical symptoms of each patient daily. Additionally, laboratory tests were performed by the hospital staff as a daily routine. Sampling for cases

and OFI was performed upon enrollment in the hospital. Sampling for controls was performed at enrollment in the study. Data on full blood count were also collected. The combined data were used to classify the dengue patients in the categories DF, DHF and DSS according to the 1997 WHO guidelines [5]. According to the criteria, DF is an acute fever with at least two of the following symptoms: retro-orbital pain, myalgia, headache, leucopenia, rash and hemorrhagic manifestations. DHF is characterized by acute fever lasting 2 to 7 days and other symptoms such as bleeding, thrombocytopenia (< 100,000 cells) or signs of plasma leakage (hemoconcentration, ascites and pleural effusion). DSS manifests itself with signs of shock, such as hypotension with cool and clammy skin, weak and rapid pulse or a narrowing of the pulse pressure (< 20 mmHg). In 2009, the WHO established new guidelines, separating dengue cases into non-severe dengue and severe dengue based on severe bleeding and/or severe organ impairment [1]. In our study, however, the 1997 guidelines were still in use at the time of sample collection in 2006 and 2007 and classification was based upon the 1997 guidelines.

Ethical considerations

This study was approved by the institutional ethical review committees of the Institute of Tropical Medicine, Nagasaki University, and the Pasteur Institute in Ho Chi Minh City. Written informed consent from parents and assent from subjects was obtained by a study research nurse. All biological materials collected were anonymized after completion of demographic and clinical data collection.

Dengue diagnosis

Plasma samples for cytokine measurement were obtained from dengue patients around the transition period of fever to defervescence. The plasma was divided into two tubes: one for dengue diagnostic testing and the other for cytokine measurement. The plasma samples for cytokine measurement were stored at -80°C . Several diagnostic tests were performed to confirm dengue virus infection. During the acute phase of the disease, blood was drawn from patients to confirm dengue virus infection by virus isolation or RNA detection with reverse transcription PCR. Dengue virus isolation was performed using the C6/36 cell line of *Aedes albopictus* and viral identification via immunofluorescence with monoclonal antibodies supplied by the Centers for Disease Control and Prevention (Fort Collins, Colorado, USA) [12]. For the reverse transcription PCR, a Ready-To-Go reverse transcriptase PCR kit (Amersham, Massachusetts, USA) was used to detect the dengue virus genome [13]. After the febrile period, when the virus was no longer detectable in the blood, anti-

dengue virus IgM and IgG antibodies were assessed by ELISA (in-house kit of the Pasteur Institute, Ho Chi Minh City, Vietnam) to confirm dengue virus infection. An IgM/IgG ratio of < 1:8 indicated a secondary dengue infection [14]. For dengue serotype determination, reverse transcription PCR and virus isolation were used [15].

Cytokine Measurement

Since a limited volume of blood was collected from the children, we performed a multiplex immunoassay using polystyrene beads based on Luminex technology. The Procarta Immunoassay Kit—Polystyrene Beads (Affymetrix, Santa Clara, California, USA) was used according to the guidelines provided by the manufacturers. The filter plate was read on the Luminex 100 instrument (Luminex, Austin, Texas, USA).

Statistical Analysis

All data were stored in a computerized database (MS Excel 2007, Microsoft, Redmond, Washington, USA). Descriptive and general statistics were calculated for all patients in the study. Skewness and kurtosis were tested to assess normal distribution of the variables. For continuous normally distributed variables, a one way ANOVA was performed to compare multiple groups, while the groups were assessed pair-wise using the *Student's t test*. For non-normal distributed variables, the Kruskal-Wallis test was performed on all groups, whereas the Mann Whitney test was used to compare groups head to head. The Chi-square test was applied to categorical variables. Additionally, a multiple logistic regression was performed. Finally, the distribution of the cytokines was assessed for non-severe versus severe dengue patients using the Mann Whitney test for non-continuous variables and the *Student's t test* for continuous variables. Statistical significance was set at $p < 0.05$. All statistical tests were performed on the SPSS 20.0 (SPSS Inc., Chicago, Illinois, USA) statistical program.

RESULTS

Study population characteristics

The characteristics of the study population are summarized in Table 1. A total of 67 patients were enrolled in this study, including 5 healthy controls, 5 OFIs and 57 dengue patients. According to the 1997 WHO classification, the 57 dengue patients were classified as 6 DF, 18 DHF and 33 DSS patients. No significant differences in age or sex were found between the groups. Dengue serotype was comparable for all dengue patients. Dengue virus 4 was found to be completely lacking, and serotypes were undetectable in 37 out of 57 cases. In total, 43 out of 57

dengue patients had a secondary DV infection, but no significant differences were found among the groups. Hematocrit was significantly higher ($p < 0.005$) in the DSS group compared to all the other groups and in the DHF group compared to the OFI group. A significantly lower platelet count was found ($p < 0.049$) in the DSS group compared to the control and OFI groups. White blood cell count did not differ among the groups.

The cytokines IL-6 and TNF α showed no significant difference among groups. IL-8 was significantly higher ($p < 0.040$) in the DSS group compared to the control group and OFI groups and in the DHF group compared to the control and DF groups. IFN γ levels were significantly decreased ($p < 0.045$) in the DSS group compared to the DF group. VEGF-A was significantly higher in the DSS group compared to the OFI group. Additionally, VCAM-1 levels were significantly elevated ($p < 0.045$) in the DSS group compared to the OFI and DF groups.

Cytokine levels of severe dengue versus non-severe dengue

Univariate analysis was first carried out to differentiate DSS from DHF and DF combined. The distribution of the cytokine values is shown in Figure 1. The patients were divided into a non-severe dengue group (DF and DHF combined, $n = 24$) and a severe dengue group (DSS, $n = 33$). The levels of IL-6, IL-8, TNF α and VEGF-A did not differ between the groups. However, we observed a significantly higher level of VCAM-1 ($p < 0.037$) and a lower level of IFN γ in the severe dengue group compared to the non-severe dengue group.

To take the effects of all variables into account for the association with DSS, a multivariate logistic regression was carried out. After correction for age, sex, white blood cell count (WBC) and the other cytokines, IFN γ ($p < 0.04$) and VCAM-1 ($p < 0.029$) were significantly associated with DSS (Table 2).

Primary infection versus secondary infection

The study participants were separated into groups based on primary infection and secondary infection. The results in Table 3 show that 14 patients had a primary dengue virus infection, whereas 50 patients had a secondary infection. No significant difference in cytokine level was found between the groups.

DISCUSSION

Six cytokines (IL-6, IL-8, VEGF-A, VCAM-1, TNF α and IFN γ) were measured with the aim of validating their association with dengue severity. Several cytokines were

Table 1. Clinical characteristics and laboratory parameters

| Characteristics | Controls N = 5 | OFI N = 5 | DF N = 6 | DHF N = 18 | DSS N = 33 | P value |
|-----------------------------|-------------------|-------------------|--------------------|------------------|--------------------|------------------------------|
| Age (years) | 5.6 ± 3.9 | 8.0 ± 4.6 | 9.8 ± 2.9 | 8.6 ± 3.2 | 8.6 ± 3.4 | 0.386 |
| Female | 3 (60%) | 2 (40%) | 3 (50%) | 9 (50%) | 17 (51%) | 0.981 |
| DV serotypes | na | na | | | | |
| DV-1 | | | 1 (16.7%) | 4 (22.2%) | 3 (9.1%) | 0.170 |
| DV-2 | | | 1 (16.7%) | 4 (22.2%) | 2 (6.1%) | — |
| DV-3 | | | 1 (16.7%) | 3 (16.7%) | 1 (3%) | — |
| DV-4 | | | 0 (0%) | 0 (0%) | 0 (0%) | — |
| Unknown | | | 3 (49.9%) | 7 (38.9%) | 27 (81.8%) | — |
| Secondary/Primary Infection | na | na | | | | |
| Primary | | | 1 (16.7%) | 4 (22.2%) | 9 (27.3%) | 0.623 |
| Secondary | | | 5 (83.3%) | 14 (77.8%) | 24 (72.7%) | — |
| HCT (%) | 38.7 (34–44) | 41.2 (37–43) | 40.6 (38–46) | 42.9 (37–45) | 43.6 (37–53) | < 0.005 ^{a,b,c,d,f} |
| PLT (× 10 ³) | 94 (65–164) | 126 (114–216) | 119 (81–138) | 70.7 (11–116) | 43.5 (10–102) | < 0.049 ^{a,b} |
| WBC (× 10 ³) | 4.3 (2.3–4.5) | 7.0 (2.3–7.6) | 3.0 (2.1–5.6) | 3.5 (1.2–10.5) | 4.1 (0.7–10.4) | 0.175 |
| IL-6 | 1.25 (0.79–2.61) | 0.70 (0.31–7.31) | 1.26 (0–2.19) | 1.20 (0–8.77) | 1.33 (0–4.65) | 0.864 |
| IL-8 | 1.76 (0.7–2.65) | 1.55 (0.73–4.69) | 2.51 (1.93–4.08) | 3.60 (0–12.80) | 3.40 (1.38–18.11) | < 0.040 ^{a,b,e,g} |
| IFN γ | 0.43 (0.23–3.26) | 0.21 (0.14–30.73) | 13.46 (0.34–30.73) | 1.38 (0–146.01) | 0.53 (0.14–6.61) | 0.045 ^c |
| TNF α | 3.03 (0–4.81) | 1.94 (0.93–2.49) | 1.65 (0.49–3.73) | 1.74 (0.22–3.81) | 1.35 (0–6.95) | 0.687 |
| VEGF-A | 8.45 (3.42–13.22) | 6.69 (2.92–13.42) | 7.14 (3.91–16.38) | 10.77 (0–29.01) | 13.42 (3.42–72.65) | 0.029 ^b |
| VCAM-1 | 3444.0 ± 1300.1 | 2564.2 ± 1784.2 | 2785.2 ± 1514.5 | 3605.9 ± 1666.5 | 4159.7 ± 1045.5 | < 0.045 ^{b,c} |

OFI, other febrile infections; DF, dengue fever; DHF, dengue hemorrhagic fever; DSS, dengue shock syndrome; DV, dengue virus; HCT, hematocrit; PLT, platelets; WBC, white blood cells; IL-6, interleukine 6; IL-8, interleukine 8; IFN γ , interferon gamma; TNF α , tumor necrosis factor alpha; VEGF-A, vascular endothelial growth factor; VCAM-1, vascular cell adhesion molecule 1. Cytokine concentrations in pg/mL. One way ANOVA and *student's t test* for continuous variables normally distributed, Kruskal-Wallis test and Mann-Whitney test for continuous variables not-normally distributed, χ^2 test for categorical variables.

Mean ± SD, number (percentage of total), median (minimum–maximum).

^a between control and DSS patients

^b between OFI and DSS patients

^c between DF and DSS patients

^d between DHF and DSS patients

^e between control and DHF patients

^f between OFI and DHF patients

^g between DF and DHF patients

positively associated with DSS compared to the OFI or control group, such as IL-8, VEGF-A and VCAM-1. For IL-6 and TNF α , no differences were found among groups. Interestingly, IFN γ was significantly decreased in DSS patients compared to DF patients. In the logistic regression analysis, IFN γ showed a negative association with severe dengue, both in the univariate and multivariate analysis. In contrast, VCAM-1 had a positive association with DSS in the univariate analysis and the adjusted analysis. We did not find any significant difference between the groups for cytokine levels of patients with primary dengue infection and those with secondary dengue infection.

Cytokine IL-6 is secreted by T cells, macrophages,

NK cells and activated endothelial cells to stimulate innate immune response. IL-6 is a potent mediator of fever and acute phase reactions. Additionally, IL-6 facilitates the coagulation cascade together with other pro-inflammatory cytokines. Some studies found increased levels of IL-6 in severe dengue versus non-severe dengue, while others failed to show this association [9, 16–19]. In our study, no difference was found in IL-6 levels in any of the analyses.

IL-8 is a pro-inflammatory chemokine, which enhances vascular permeability and activates the innate immune system. It is produced by monocytes, endothelial cells and hepatocytes. Elevated levels of IL-8 have been observed in DSS patients compared to non-shock dengue

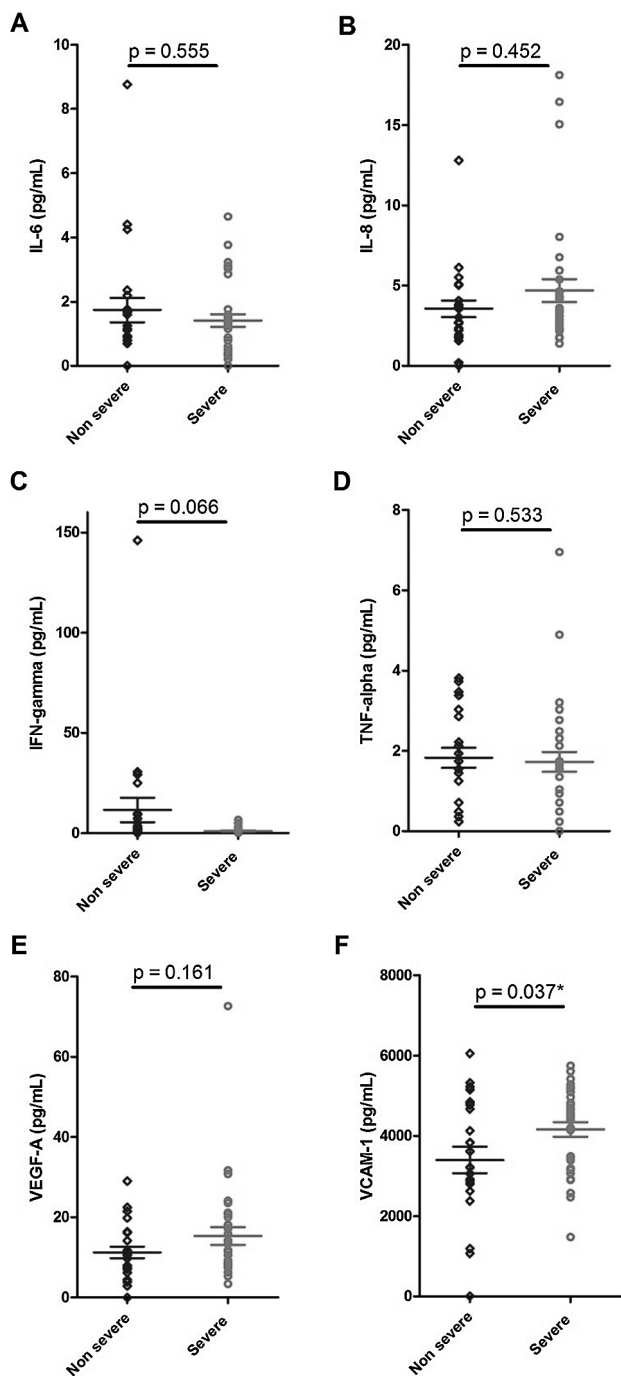


Fig. 1. Cytokine levels between the groups: non-severe dengue (DF/DHF) and severe dengue (DSS). The Mann Whitney test was carried out to assess differences of IL-6, IL-8, IFN γ , TNF α and VEGF-A between the groups. *Student's t test* was performed to compare the levels of VCAM-1. Scatter plot graphs with standard errors are shown.

patients and healthy controls [9, 20, 21]. Our findings confirm this association, as we found significantly higher lev-

Table 2. Multivariate logistic regression model, DSS versus non-shock cases (DF/DHF)

| Parameter (n) | Adjusted OR (95% CI) | P value |
|-----------------------|----------------------|---------|
| Age | 0.99 (0.76–1.30) | 0.97 |
| Sex | 1.01 (0.24–4.23) | 0.99 |
| WBC ($\times 10^3$) | 1.00 (0.68–1.48) | 0.99 |
| IL-6 | 1.07 (0.40–2.89) | 0.89 |
| IL-8 | 0.83 (0.70–1.33) | 0.83 |
| IFN γ | 0.63 (0.40–0.99) | 0.043* |
| TNF α | 1.12 (0.59–2.14) | 0.73 |
| VEGF-A | 1.01 (0.93–1.12) | 0.74 |
| VCAM-1 | 1.00 (1.00–1.01) | 0.029* |

DSS, dengue shock syndrome; DF, dengue fever; DHF, dengue hemorrhagic fever; OR, odds ratio; WBC, white blood cells; IL-6, interleukine-6; IL-8, interleukine-8; IFN γ , interferon gamma; TNF α , tumor necrosis factor alpha; VEGF-A, vascular endothelial growth factor A; VCAM-1, vascular cell adhesion molecule 1. Corrected for age, sex, WBC, IL-6, IL-8, IFN γ , TNF α , VEGF-A and VCAM-1 in the adjusted OR.

els of IL-8 in the DSS and DHF groups compared to the DF, OFI and control groups. IL-8 might be involved in severe dengue pathogenesis, since dengue-infected endothelial cells in vitro are observed to secrete IL-8 [22, 23]. Endothelial cells play a distinct role in the progression to severe dengue, because the activation and cell death of endothelial-cell lead to vascular permeability, plasma leakage and hemorrhage. Yet, it is difficult to establish the role of IL-8 in severe dengue pathogenesis, since the evidence of endothelial cells apoptosis in vivo is very limited [24]. Also, the fast recovery of DHF patients makes structural damage to endothelial cells implausible.

IFN γ is mainly produced by T cells and NK cells. Its main effects are monocyte and macrophage activation [25]. IFN γ has been associated with severe dengue [9, 26–28]. It appears that IFN γ mostly peaks between the time of declining viral load and the moment of defervescence and plasma leakage, which indicates that the adaptive immunity has been activated. Activation of the adaptive immunity can in turn enhance the cytokine production of NK cells, leading to high levels of IFN γ in DF. Therefore it has been proposed that high levels of IFN γ indicate recovery in DF. In our study, IFN γ was particularly high in DF compared to DSS, a finding consistent with previous studies [29–33]. Furthermore, IFN γ showed a negative association with severe dengue in the multivariate logistic analysis.

VCAM-1 is an endothelial surface molecule expressed when endothelial cells are activated by cytokines such as TNF α . VCAM-1 has been found at elevated levels in DHF patients compared to DF patients and controls [10,

Table 3 Primary infection vs. secondary infection

| Characteristics | Primary Infection N = 14 | Secondary Infection N = 50 | P value |
|-----------------|-----------------------------|-------------------------------|---------|
| IL-6 | 1.59 (0–3.11) | 1.13 (0–8.77) | 0.137 |
| IL-8 | 3.72 (0–8.03) | 2.98 (0.19–18.11) | 0.178 |
| IFN γ | 1.06 (0–30.73) | 0.48 (0.14–146.01) | 0.255 |
| TNF α | 1.03 (0–3.47) | 1.70 (0–6.952) | 0.066 |
| VEGF-A | 13.18 (0–24.12) | 10.14 (2.92–72.65) | 0.188 |
| VCAM-1 | 3817.77 \pm 1532.35 | 3728.73 \pm 1400.61 | 0.717 |

OFI, other febrile infections; DF, dengue fever; DHF, dengue hemorrhagic fever; DSS, dengue shock syndrome; DV, dengue virus; HCT, hematocrit; PLT, platelets; WBC, white blood cells; IL-6, interleukine 6; IL-8, interleukine 8; IFN γ , interferon gamma; TNF α , tumor necrosis factor alpha; VEGF-A, vascular endothelial growth factor; VCAM-1, vascular cell adhesion molecule 1. Cytokine concentrations in pg/mL. One way ANOVA and *student's t test* for continuous variables normally distributed, Kruskal-Wallis test and Mann-Whitney test for continuous variables not-normally distributed, χ^2 test for categorical variables. Mean \pm SD, number (percentage of total), median (minimum–maximum).

34, 35]. Our results are consistent, showing a significant difference in VCAM-1 for the DSS group compared to the OFI and DF groups. The distribution of VCAM-1 levels between the non-severe and severe dengue group also showed a significant increase. VCAM-1 mediates the adhesion of lymphocytes and cells of the innate immune system to the vascular endothelium to facilitate chemotaxis [34]. Yet, it is unclear if VCAM-1 is the cause or result of activated endothelium. Analysis of endothelial cell factors may help to determine the role of VCAM-1 in severe dengue pathogenesis.

TNF α has pro-inflammatory, vascular permeability-enhancing and coagulation-activating effects. Elevated levels of TNF α have been found in DHF compared to DF [9, 26, 27]. However, their role in the pathogenesis of DSS remains unclear. It has been proposed that TNF α is secreted by dengue virus-infected monocytes, which induce endothelial cell production of reactive nitrogen and oxygen species, leading to apoptotic cell death and therefore hemorrhage [36, 37]. In our analysis, TNF α was not significantly different in the DSS group. Other studies have shown that TNF α is particularly high in DSS patients during defervescence [33]. But that could be due to different sampling times, leading to lower levels of TNF α in DSS. The low level of TNF α could furthermore be explained by a possible difference in population or virus serotype.

VEGF-A is a cytokine that stimulates vasculogenesis and angiogenesis. It effectively enhances vascular permeability and also activates the coagulation system by upregulating the production of tissue factor [38, 39]. Recent studies on dengue virus infection have reported elevated levels of VEGF-A in DHF patients during the time of plasma leakage [8, 40–42]. However, other studies produced contradicting results, reporting lower levels of VEGF-A in severe dengue patients [9, 43]. In our study, VEGF-A lev-

els were significantly elevated in DSS patients compared to OFI patients, a finding consistent with most previous studies.

Previous studies have shown that secondary dengue infections carry a higher severity risk [6]. According to the cytokine storm hypothesis, cytokine levels are assumed to be elevated in a secondary dengue infection [7]. However, no difference in cytokine levels between the primary and secondary infection was found in our study, probably due to the small sample size. Further studies are required to clarify this hypothesis.

In this study, we collected plasma samples from children infected with dengue virus in South Vietnam. The children were carefully monitored and their symptoms and laboratory results were recorded. Our sample therefore consists of trustworthy data on DSS for children in Vietnam. Since the plasma samples from the children were only available in small amounts, we took advantage of multiplex immunoassay technology to measure the cytokines simultaneously. It appears that the cytokines IL-8, IFN γ , VEGF-A and VCAM-1 are dengue-specific cytokines, but it is difficult to hypothesize the exact role of these cytokines for DSS. In the multivariate analysis, only IFN γ and VCAM-1 were associated with severe dengue, IFN γ showing a negative association. It is possible that synergistic reactions between these cytokines occur, making it harder to identify the contribution of each cytokine to severe dengue pathogenesis [6, 44].

There were certain limitations in interpreting the results of cytokines, because several differences in cytokine levels were observed between our study and other studies. In this study, only a single plasma sample per patient was used. Since the sampling time differs in each patient and cytokine levels in dengue virus infection are reported to be dynamic, this can result in differences. Since severe mani-

festations of dengue usually occur around the time of defervescence, it is especially critical to take sampling time into account. The ideal sampling time is immediately after the onset of the disease, but most patients are hospitalized after a few days of illness. Another limitation in our study involved the low number of participants in the control, OFI and DF groups. Since our study was a case control study and we primarily focused on the DSS compared to the DHF group, we used the remaining DF samples. Thus, the non-significant associations between DF and DHF/DSS should be interpreted with caution. Finally, a large number of patients is necessary to find significant differences in cytokine level, since cytokines are only present in plasma in very small amounts. A good setting would be a large prospective study in which blood samples are drawn from the patients every day of illness. Additional research needs to be done to shed further light on the role of cytokines in severe dengue pathogenesis.

In conclusion, IFN γ and VCAM-1 were associated with dengue shock syndrome, while TNF α , IL-6, IL-8, and VEGF-A were not. Additional large prospective studies are required to further investigate the function of these cytokines in severe dengue.

COMPETING INTERESTS

The authors have no competing interests with commercial or other affiliations.

ACKNOWLEDGMENTS

This study was supported in part by the Japan Initiative for Global Research Network on Infectious Diseases (J-GRID) for KH. The funder had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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