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The role of cytokines in the prognosis of children with dengue shock syndrome in Vietnam

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ABSTRACT

Dengue shock syndrome (DSS) is a severe complication of dengue hemorrhagic fever (DHF) and can lead to death, but DSS incidence and effects are difficult to predict via clinical examination. With consideration of this issue, this research determined the role of cytokines in the severity and prognosis of DHF in children. This prospective cohort research involved 234 patients who were aged 18 months to 18 years old, admitted to Tien Giang General Hospital, and diagnosed as having DHF. The cytokine levels of the patients were recorded from admission to discharge. Almost all the patients (>67%) exhibited increased interleukin 10 (IL-10), IL-6, and IL-2, whereas only 0.43% (the lowest) presented with elevated IL-12. Concentrations of IL-2, IL-4, IL-13, and tumor necrosis factor-α were highest i the patients infected with DENV-1, followed by those infected with DENV-3 and DENV-2. IL-12 concentration decreased with increasing days of fever occurrence. No correlation was found between cytokine concentrations in the first day of hospitalization and shock in the DHF patients. The concentration of cytokines during the first day of hospitalization (i.e., patients with a fever lasting less than 72 hours) cannot be used as a guide in determining DSS prognosis.

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INTRODUCTION

Although dengue hemorrhagic fever (DHF) has a low rate of mortality, it is one of the diseases regarded as transmitted by arthropod vectors, which account for the highest percentage. DHF is endemic to more than 120 tropical and subtropical countries, thus rendering 55% of the global population susceptible to the risk of infection (World Health Organization, 2009). The disease has existed for nearly a century, yet its pathogenesis has yet to be fully understood, and the main determinant of its progression into severe cases has not been clearly defined. One such complication is dengue shock syndrome (DSS), which can lead to death. The incidence and effects of this form of the disease are difficult to predict

through clinical examination.

Researchers have recently suggested that different cytokines play a role in the formation of severe DHF complications and the diagnosis of DHF shock. The fact that the cytokine system is very complex and that these molecules seem to be interesting indicators are relevant to disease severity and prognosis (Kleiner *et al.*, 2013).

MATERIALS AND METHODS

This prospective cohort study was conducted at Tien Giang General Hospital in Vietnam from December 2009 to November 2012. The participants selected were patients who were aged 18 months to 18 years, had a history of fever lasting less than 72 hours and were clinically suspected of having DHF. To determine a suitable sample size, this research used a formula that compares two ratios with a desired reliability of 95% [α = 0.05 Z (1-α/2) = 1.96, β = 10% Z (1-β) = 1.28]. The equation was adopted on the basis of Mustafa's (2001). In the group with elevated concentrations of cytokines, the incidence rates of DHF with shock (P1) and non-shock DHF (P2) were 0.63 and 0.09, respectively [P = (P1 + P2)/2]. In line with this, the study needed at least 15 patients each under the shock and non-shock groups. According to Hung *et al.* (2004), however, the incidence rate of shock accompanied DHF is 20.5%. Thus, the acceptable minimum sample size is N $\geq$ 75.

$$N = \frac{\left[Z_{1-\alpha/2}\sqrt{2P(1-P)} + Z_{1-\beta}\sqrt{P_1(1-P_1)+P_2(1-P_2)}\right]^2}{(P_1-P_2)^2}$$

Venous blood samples were taken from each patient daily from the date of admission to date of discharge. Serologic tests for dengue virus (DENV) infection, namely, enzyme-linked immunosorbent assay (ELISA) for immunoglobulin M (IgM)/immunoglobulin G (IgG) (anti-DENV IgM/IgG ELISA) and the comparison of nonstructural protein-1 detection via reverse transcription-polymerase chain reaction and ELISA were conducted to confirm all cases of DHF. Cytokine concentration was detected using multiplex microbead immunoassay, which enabled the simultaneous detection of 10 cytokines: interleukin-1β (IL-1β), IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-13, interferon gamma-γ (IFN-γ), and tumor necrosis factor-α (TNF-α). Table 1 below shows the threshold of increase in cytokines obtained from the research of Hung *et al.* (2004) and Kleiner *et al.* (2013) on children.

Statistical analysis

Data were analyzed using Stata version 10.0. Descriptive statistics were used to express frequencies, and percentages were adopted to represent the characteristic variables of the sample. Quantitative variables with a normal distribution were compared by subjecting two patient groups (shock-accompanied DHF and non-shock DHF) to Student's t-test and three patient groups to the analysis of variance. A p-value of less than 0.05 (<0.05) was considered significant.

Ethical considerations

This study was approved by the Scientific Research Committee of Tien Giang General Hospital. The collected data were used only for the purposes of this study. Participation was voluntary and anonymous, and the participants were asked to sign an informed consent form prior to enrollment in the research.

RESULTS AND DISCUSSION

From December 2009 to November 2012, 234 patients were confirmed to be infected with DENV and were included in the study. Table 2 shows the characteristics of the participants.

Table 3 presents the results on differences in serum cytokine concentrations between the non-shock and shock-accompanied DHF groups, and Table 4 lists the average cytokine concentrations measured on the first day of hospitalization.

The findings regarding the relationship between cytokines and disease severity, DENV serotype and fever duration are displayed in Table 5, Table 6 and Table 7.

Sample characteristics

The rate of disease incidence among the patients in the non-shock DHF group (93.59%) was higher than that in the shock-accompanied DHF group (6.41%), which is also considerably lower than the percentage reported by Mustafa *et al.* (2001) for 58 cases. The author found an incidence rate of 69.1% in the non-shock DHF patients (levels I and II) and a rate of 30.9% among the patients suffering from shock-accompanied DHF (13 cases of level III, 13 cases of level IV). Most of the patients in the present study (76.5%) were re-infected at a rate of 23.5%. This result contrasts with that of Tang *et al.* (2011), whose survey on 353 hospitalized DENV patients in Southern China uncovered re-infection and infection rates of 39.9% and 60.1%, respectively. Among the patients in the current work, 41.45% and 50.85% were hospitalized upon contracting a fever on days 2 and 3, respectively. Only a few (7.69%) were hospitalized upon fever occurrence on day 1.

Table 1: Threshold of increase in cytokines obtained

Cytokine	Threshold of increase (pg, ml)
IL-1	4.04 ± 3.53
IL-2	1.8 ± 2.4
IL-4	0.2 ± 0.5
IL-5	3.1
IL-6	1.4 ± 2.2
IL-10	0.3 ± 0.9
IL-12p70	34.5
IL-13	12.0 ± 5.0
TNF-α	0.8 ± 1.2
IFN-γ	4.1 ± 5.8

Table 2: Characteristics of the sample (n = 234)

Variable	n	%
Disease severity		
Non-shock	219	93.59
Shock	15	6.41
Infection status		
Infection	55	23.50
Re-infection	179	76.50
Fever day upon admission		
Day 1	18	7.69
Day 2	97	41.45
Day 3	119	50.85
Type DENV		
DENV-1	143	61.11
DENV-2	35	14.96
DENV-3	55	23.50
DENV-4	01	0.43

Table 3: Proportions of patients with increased cytokine concentrations on the first day of hospitalization

Cytokines	The proportion of patients with increased cytokines (n, %)			P
	Non-shock (n=219)	Shock (n=15)	Total n= 234	
IL-1	9 (4.11)	0 (0)	9 (3.85)	0.423
IL-2	151 (68.95)	08 (53.30)	159 (67.95)	0.210
IL-4	137 (62.56)	7 (46.67)	144 (61.54)	0.221
IL-5	88 (40.18)	6 (40.00)	94 (40.17)	0.989
IL-6	160 (73.06)	8 (53.30)	168 (71.79)	0.100
IL-10	163 (74.43)	8 (53.30)	171 (73.08)	0.075
IL-12	1 (0.46)	0 (0)	1 (0.43)	0.793
IL-13	61 (27.85)	4 (26,67)	65 (27.78)	0.921
INF-γ	122 (55.71)	8 (53.30)	130 (55.56)	0.858
TNF-α	100 (45.66)	5 (33.30)	105 (44.87)	0.353

Table 4: Average cytokine concentrations measured on the first day of hospitalization

Cytokine	Mean ± SD (pg/ml)	Maximum (pg/ml)
IL-1	1.237265 ± 2.640344	36.53
IL-2	11.96838 ± 15.75000	72.60
IL-4	1.064188 ± 1.842224	20.43
IL-5	6.477863 ± 18.2812	154.74
IL-6	32.63923 ± 53.85879	528.45
IL10	25.98675 ± 70.26549	826.80
IL12	2.296966 ± 4.591996	57.23
IL13	10.19949 ± 12.63749	59.78
INF-γ	27.35927 ± 66.48916	534.06
TNF-α	0.9754701 ± 1.244713	12.29

Table 5: Average cytokine concentrations in relation to disease severity

Cytokines	Mean ± SD (pg/ml)		P
	Non-shock	Shock	
IL-1	1.25 ± 0.18	0.99 ± 0.31	0.48
IL-2	11.83 ± 1.02	13.90 ± 5.94	0.73
IL-4	1.06 ± 0.12	1.05 ± 0.43	0.98
IL-5	6.32 ± 1.23	8.67 ± 5.08	0.65
IL-6	30.18 ± 2.71	68.40 ± 37.93	0.33
IL-10	24.66 ± 4.62	45.28 ± 24.26	0.41
IL-12	2.34 ± 0.31	1.63 ± 0.53	0.66
IL-13	10.06 ± 0.82	12.14 ± 4.61	0.66
INF-γ	26.60 ± 4.40	38.43 ± 22.09	0.66
TNF-α	0.99 ± 0.08	0.69 ± 0.21	0.22

Table 6: Average cytokine concentrations in relation to DENV serotype

Cytokines	Mean ± SD (pg/ml)				P
	DENV-1	DENV-2	DENV-3	DENV-4	
IL-1	1.50 ± 3.23	0.38 ± 0.65	1.09 ± 1.25	0	0.135
IL-2	14.39 ± 17.06	4.53 ± 10.68	10.61 ± 13.29	0	0.006
IL-4	1.32 ± 2.16	0.31 ± 0.81	0.88 ± 1.14	0	0.023
IL-5	6.89 ± 18.39	9.24 ± 28.37	3.76 ± 5.65	0	0.532
IL-6	34.63 ± 59.83	15.10 ± 33.51	39.21 ± 46.05	0	0.165
IL-10	25.16 ± 51.40	11.54 ± 29.70	37.79 ± 116.22	0	0.365
IL-12	2.71 ± 5.20	1.51 ± 4.89	1.74 ± 1.81	0	0.352
IL-13	12.11 ± 13.85	4.68 ± 7.83	8.90 ± 10.62	0	0.010
INF-γ	29.76 ± 66.61	3.06 ± 5.30	37.04 ± 82.94	0	0.101
TNF-α	1.11 ± 1.34	0.43 ± 0.74	0.97 ± 1.15	0	0.026

Table 7: Average cytokine concentrations in relation to fever duration

Cytokines	Mean ± SD (pg/ml)			P
	Day 1	Day 2	Day 3	
IL-1	1.43 ± 1.33	1.42 ± 3.78	1.05 ± 1.33	0.56
IL-2	16.43 ± 16.11	13.05 ± 16.68	10.40 ± 14.81	0.21
IL-4	1.47 ± 1.62	1.27 ± 2.40	0.83 ± 1.21	0.13
IL-5	13.72 ± 35.77	5.76 ± 14.89	5.96 ± 16.91	0.21
IL-6	52.45 ± 46.14	32.79 ± 66.92	29.51 ± 41.32	0.24
IL-10	15.32 ± 14.87	20.75 ± 43.93	31.86 ± 89.84	0.41
IL-12	5.60 ± 13.04	2.33 ± 3.62	1.76 ± 2.12	0.003
IL-13	13.61 ± 12.64	11.11 ± 13.23	8.93 ± 12.07	0.22
INF-γ	17.71 ± 22.29	27.27 ± 67.02	28.88 ± 70.61	0.80
TNF-α	1.20 ± 1.07	1.02 ± 1.46	0.90 ± 1.06	0.56

Rate of increase in cytokine concentrations in DHF patients

Of the subjects, 73.08% had increased IL-10, accounting for the highest percentage in the sample. The proportions of patients with increased IL-6 and IL-2 were 71.79% and 71.79%, respectively. The lowest proportion of patients with elevated IL-2 concentrations was 0.43%. This result is consistent with that derived by Rathakrishnan *et al.* (2012), who found lower concentrations of IL-12 in DHF patients than in healthy individuals.

The present study also found that the non-shock and shock-accompanied DHF patients all had comparable cytokine concentrations ($p > 0.05$) (Table 2). Pacsa *et al.* (2000) research on 246 patients revealed that all cases of levels III and IV DHF exhibited increased cytokine concentrations; such elevation was found among 91% to 95% of patients with levels I and II DHF.

The relationship between average cytokine concentration and disease severity

The average concentrations of IL-2, IL-5, and IL-13, and especially IL-6, IL-10, and INF-γ, in the group with shock-accompanied DHF, were higher than those in the non-shock group, but the difference was statistically nonsignificant ($p > 0.05$, t-test). Conversely, the average concentrations of IL-1, IL-4, IL-12, and TNF-α in the shock-accompanied DHF group were lower than those in the non-shock group. Again, however, the difference between the groups was statistically nonsignificant ($p > 0.05$, t-test). Bozza *et al.* (2008) discovered that concentrations of IL-1β, IFN-γ, IL-4, IL-6, IL-7, IL-13, and granulocyte-macrophage colony-stimulating factor are significantly higher among subjects with severe DHF than in those with mild DHF. The author also found that the concentration of macrophage inflammatory protein-1β was slightly higher in patients with mild DHF.

The relationship between average cytokines concentration and DENV serotype

The highest average concentrations of IL-2, IL-4, IL-13, and TNF-α were observed in the DENV-1–infected patients, followed by those infected with DENV-3 and DENV-2. The differences among these concentrations were statistically significant at 0.006, 0.023, 0.010, and 0.026, respectively. The average concentrations of IL-1, IL-5, IL-6, IL-10, IL-12, and INF-γ showed no statistically significant differences under various types of DENV infection ($p > 0.05$) (Table 5).

The relationship between average cytokines concentration and duration of fever

The highest average concentration of IL-12 was found in patients who developed a fever on day 1 of (5.60±13.04) disease. Then this concentration gradually decreased on day 2 (2.33±3.62) and day 3 (1.76±2.12). The difference among the concentrations was statistically significant ($p = 0.003$). The highest average concentrations of IL-1, IL-2, IL-4, IL-6, IL-13, and TNF-α also occurred in the patients who had a fever on day 1. Similarly, the concentrations slowly declined on days 2 and 3, but the difference was statistically nonsignificant ($p>0.05$). In contrast, the IL-10 and INF-γ concentrations tended to increase with the rising number of days with fever, with the lowest increase observed in the patients who developed this symptom on day 1. The concentrations increased on day 2 and were even higher in patients with have had a fever for three days, but the difference was statistically nonsignificant ($p > 0.05$). Chaturvedi *et al.* (2000) reported that TNF-α, IL-2, and IL-6 had the highest concentrations on day 2 and that IFN-γ con-

centrations occurred on day 2 and peaked on day 3. The author also stated that IL-10 and IL-5 levels increased on day 4, and IL-4 was elevated on day 6 after viral infection.

CONCLUSION

No difference in cytokine concentrations was found between the first day of admission and the day of shock complication occurrence in DHF patients. Increases in IL-10, IL-6, and IL-2 concentrations occurred among the majority of the subjects (>67%), and elevated IL-12 was exhibited by only 0.43% (the lowest) of the patients. The levels of IL-2, IL-4, IL-13, and TNF-α were highest in the DENV-1–infected group, followed by the DENV-3 and DENV-2 patients. The IL-12 concentrations decreased with the rising number of fever days.

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Disclosure

The authors have no conflicts of interests to declare.

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REFERENCES

Bozza, F. A., Cruz, O. G., Zagne, S. M. O., Azeredo, E. L., Nogueira, R. M. R., Assis, E. F., Kubelka, C. F. 2008. Multiplex cytokine profile from dengue patients: MIP-1beta and IFN-gamma as predictive factors for severity. *BMC Infectious Diseases*, 8(1):86–86.

Chaturvedi, U. C., Agarwal, R., Elbishbishi, E. A., Mustafa, A. S. 2000. Cytokine cascade in dengue hemorrhagic fever. *implications for pathogenesis*, 28:183–188. Pathogens and Disease.

Hung, N. T., Lei, H., Lan, N. T., Lin, Y., Huang, K., Lien, L. B., Halstead, S. B. 2004. Dengue Hemorrhagic Fever in Infants: A Study of Clinical and Cytokine Profiles. *The Journal of Infectious Diseases*, 189(2):221–232.

Kleiner, G., Marcuzzi, A., Zanin, V., Monasta, L., Zauli, G. 2013. Cytokine Levels in the Serum of Healthy Subjects. *Mediators of Inflammation*, pages 1–6.

Mustafa, A. S., Elbishbishi, E. A., Agarwal, R., Chaturvedi, U. C. 2001. Elevated levels of interleukin-13 and IL-18 in patients with dengue hemorrhagic fever. *Pathogens and Disease*, 30:229–233.

Pacsa, A. S., Agarwal, R., Elbishbishi, E. A., Chaturvedi, U. C., Nagar, R., Mustafa, A. S. 2000. Role of interleukin-12 in patients with dengue hemorrhagic fever. *Pathogens and Disease*, 28:151–155.

Rathakrishnan, A., Wang, S. M., Hu, Y., Khan, A. M., Ponnampalavanar, S., Lum, L. C. S., Sekaran, S. D. 2012. Cytokine Expression Profile of Dengue Patients at Different Phases of Illness. *PLOS ONE*, 7(12):e52215.

Tang, Y., Kou, Z., Zhang, F., Yao, X., Liu, S., Ma, J., Jin, X. 2011. Both Viremia and Cytokine Levels Associate with the Lack of Severe Disease in Secondary Dengue 1 Infection among Adult Chinese Patients. *PLOS ONE*, 5(12):e15631.

World Health Organization 2009. Dengue guidelines for diagnosis, treatment, prevention and control, Geneva.

