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The correlation between clinical manifestations and cytokine concentrations in Vietnamese children with dengue hemorrhagic fever

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ABSTRACT

Background: All over the world, dengue hemorrhagic fever (DHF) causes mortality each year. To determine the development and severity of DHF, healthcare professionals usually record and monitor clinical manifestations and the functioning of the immune system. Nevertheless, many issues remain unclear as to the influence of cytokine concentrations on clinical symptoms.
Objective: This study was aimed at ascertaining the correlation between clinical manifestations and cytokine concentrations in children with DHF.
Materials and methods: A prospective cohort study was conducted involving 234 patients who were serologically diagnosed with dengue virus infection in Tien Giang Hospital. The patients' clinical symptoms, such as fever duration on hospital admission, vomiting, abdominal pain, mucosal bleeding, bleeding under the skin, hepatomegaly, and high bodily temperature, were documented every day from admission to discharge. Cytokine concentrations were detected and measured via multiplex microbead immunoassay. The correlation between cytokine concentration and each clinical manifestation was then identified.
Results: Interleukin-6 (IL-6) concentration affected the manifestation of abdominal pain in the patients, and high concentrations of IL-2, IL-4, and IL-13 increased the occurrence of hepatomegaly. Temperature was influenced by IL-5, IL-10, and IL-12 concentrations.
Conclusion: The findings confirmed the correlation between cytokine concentrations and certain clinical manifestations.

Keywords: Clinical manifestations, concentrations, concentrations, cytokine, dengue hemorrhagic fever, Vietnam.

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INTRODUCTION

Dengue hemorrhagic fever (DHF) causes the deaths of afflicted individuals each year, especially in tropical countries, such as those in Southeast Asia, Africa, and the Western Pacific, where conditions are conducive to the spread of the disease [1]. The dengue virus (DENV) is classified into four serotypes (DENV-1 to DENV-4), which are transmitted predominantly by *Aedes aegypti* mosquitoes [2]. Over 230 million dengue fever cases, more than 2 million instances of severe disease, and 21,000 deaths have been recorded, indicating that the illness places half of the world's population at risk [3]. Research probed into the clinical manifestations that help healthcare practitioners warn the public against the development of DHF, and some epidemiological studies confirmed and illustrated the important role of the immune system in predicting the severity of dengue and its inducement of clinical manifestations [4-7]. In other works, monocytes infected by DENV were activated to stimulate inflammatory mediators, such as plasma cascade systems, neutrophils, and especially cytokines [8,9].

Cytokine secretion by specific cells of the immune system is seen as an adaptively immunological response. These substances have been corroborated as biomarkers of hepatitis, sepsis, and several types of hemorrhagic fever [10-12]. Their essential functions in DHF infection have also been consolidated in clinical research. Some cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), IL-8, IL-10, and interferon gamma (IFN-γ), have been detected in patients suffering from severe dengue [13-16]. Certain forms of these substances, including IL-6, are produced by DENV-infected fibroblasts [17], while others, such as IL-8, can activate neutrophils [18]. These cytokines have been verified as potential predictors or prognostic biomarkers of dengue progression and severity [19,20].

With consideration for the above-mentioned issues, we conducted the present research to closely evaluate the correlation of cytokine concentration detected during DHF progression and the emergence of clinical manifestations. The objective is to help doctors obtain evidence for accurate prognoses and diagnoses as well as appropriate treatments.

MATERIALS AND METHODS

Sample selection

This prospective cohort study was conducted in Tien Giang Hospital, and child patients were enrolled for participation in the research on the basis of the following criteria: (1) hospitalization in the chosen hospital from 2011 to 2015, (2) consent from the family as regards participation, (3) fever contracted within 72 hours, and (4) provisional diagnosis of DHF on hospital admission.

The sample size was defined using the formula below, which was presented in the guidelines of the World Health Organization [21]:

$$N=\frac{Z^2_{(1-\frac{\alpha}{2})} x\, p(1-p)}{d^2}$$

where α denotes the significance level of a test (with a probability of type I error occurring). Coefficient $Z_{(1-\alpha/2)}$ was set at 1.96, and the margin of error (*d*) established was 5%. Similar to Juffrie et al.'s [22] analysis, our examination of the patients' plasma sample on hospital admission revealed that IL-8 concentration increased in 16.9% of the cases, with concentrations fluctuating between 20 and 482 pg/mL and the mean concentration being 30 mg/mL. Accordingly, there needed to be at least 216 patients involved in the current work.

Patients who were not diagnosed as having DHF, who failed to complete the survey, whose parents withheld consent, or who displayed other comorbidities (liver failure, renal failure, nephrotic syndrome, heart failure, congenital heart defect, etc.) were excluded from the research.

Laboratory tests

Upon hospital admission, venous blood was drawn from the patients, after which the samples were analyzed to confirm DHF via enzyme-linked immunosorbent assay (ELISA) for the detection of IgG/IgM antibodies and nonstructural protein 1 (NS1). Different DENV serotypes were identified using real-time reverse transcription-polymerase chain reaction (RT-PCR). Cytokine concentration in the blood was measured in each child for whom DHF was confirmed. The test was conducted at the Oxford University Clinical Research Unit of the National Hospital for Tropical Diseases at Ho Chi Minh City. Such concentration was detected through multiplex microbead immunoassay, which uncovered 10 types of cytokines in the patients' bloodstreams: IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IFN-γ, and TNF-α. The patients' clinical manifestations were followed up and documented every day until hospital discharge.

Statistical analyses

The study expressed the following categorical variables in number and percentage: fever duration on hospital admission, vomiting, abdominal pain, mucosal bleeding, bleeding under the skin, hepatomegaly, and high bodily temperature. Continuously recorded data, such as the concentration of each type of cytokine, were expressed as means and standard deviations (SDs). The Kruskal–Wallis test with Bonferroni–Dunn post hoc significance testing was carried out to compare univariate categorical data with continuous variables. Univariate/multivariate linear regression was conducted to analyze the correlation between cytokine concentrations and temperature levels. All the analyses were performed using the Statistical Package for the Social Sciences (v. 18.0).

Ethical considerations

The study's protocol on human research was approved before the initiation of the investigation.

RESULTS

In total, 234 patients participated in the research from 2011 to 2015. Aside from testing the patients' blood samples to detect dengue through IgG/IgM and NS1 ELISA and RT-PCR, the clinical symptoms of each patient were detected and tracked to ensure the provision of necessary treatment. Table 1 shows the characteristics and clinical symptoms of the patients as well as the number of cases examined and the percentages of individual groups exhibiting each clinical manifestation. As previously indicated, the multiplex microbead immunoassay identified IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IFN-γ, and TNF-α in patient plasma. Tables 2 to 8 illustrate the correlation between cytokine concentration and each of the clinical symptoms presented in Table 1.

Table 1. Baseline characteristics of the study participants

Variable	n	%	Variable	n	%
Fever day on hospital admission			**Abdominal Pain**		
Day 1	18	7.69	No	156	66.7
Day 2	97	41.45	Yes	78	33.3
Day 3	119	50.85	Day 2	16	6.8
Vomiting			Day 3	22	9.4
No	145	61.9	Day 4	26	11.1
Yes	89	38.1	Day 5	9	3.8
Day 1	13	5.6	Day 6	5	2.1
Day 2	27	11.5	**Hepatomegaly**		
Day 3	46	19.7	No	194	82.9
Day 4	2	0.9	Yes	40	17.1
Day 5	1	0.4	Day 3	4	1.7
			Day 4	19	8.1
			Day 5	13	5.6
			Day 6	4	1.7

Table 1. Baseline characteristics of the study participants (cont.)

Variable	n	%	Variable	n	%
Mucosal Bleeding			**Bleeding under the skin**		
No	52	22.2	No	54	23.1
Yes	182	77.8	Yes	180	76.9
Day 2	37	15.8	Day 2	31	13.2
Day 3	92	39.3	Day 3	87	37.2
Day 4	31	13.2	Day 4	41	17.5
Day 5	14	6.0	Day 5	14	6.0
Day 6	8	3.4	Day 6	7	3.0
Temperature					
Highest temperature	38.97 ± 0.38				
The day of highest temperature					
Day 1	8	3.4			
Day 2	62	26.5			
Day 3	104	44.4			
Day 4	41	17.5			
Day 5	14	6.0			
Day 6	5	2.1			

Table 2. Correlation between cytokine concentration and vomiting

Variable	Group Yes (Mean pg/ml ± SD)	Group No (Mean pg/ml ± SD)	p-value
IL-1	1.46 ± 1.25	1.52 ± 3.09	0.86
IL-2	12.52 ± 16.30	12.06 ± 15.12	0.83
IL-4	1.23 ± 1.28	1.39 ± 1.96	0.51
IL-5	7.41 ± 19.12	6.33 ± 17.64	0.67
IL-6	33.51 ± 39.10	32.53 ± 61.07	0.89
IL-10	20.08 ± 35.30	30.04 ± 84.65	0.21
IL-12	2.31 ± 2.18	2.71 ± 5.42	0.51
IL-13	10.61 ± 13.02	10.37 ± 12.08	0.88
TNF-α	28.72 ± 69.05	26.95 ± 64.91	0.84
INF-γ	1.23 ± 0.89	1.24 ± 1.22	0.99

Table 3. Correlation between cytokine concentration and abdominal pain

Variable	Group Yes (Mean pg/ml ± SD)	Group No (Mean pg/ml ± SD)	p-value
IL-1	1.93 ± 4.17	1.28 ± 0.98	0.18
IL-2	13.49 ± 17.65	11.60 ± 14.39	0.38
IL-4	1.57 ± 2.51	1.21 ± 1.15	0.23
IL-5	5.72 ± 12.90	7.25 ± 20.34	0.54
IL-6	48.03 ± 79.95	25.34 ± 31.39	**0.01**
IL-10	28.92 ± 56.36	24.92 ± 76.27	0.68
IL-12	2.40 ± 2.33	2.64 ± 5.23	0.69
IL-13	11.47 ± 14.21	9.96 ± 11.44	0.38
TNF-α	29.01 ± 55.99	26.93 ± 71.16	0.82
INF-γ	1.44 ± 1.60	1.13 ± 0.72	0.19

Table 4. Correlation between cytokine concentration and mucosal bleeding

Variable	Group Yes (Mean pg/ml ± SD)	Group No (Mean pg/ml ± SD)	p-value
IL-1	1.36 ± 1.09	1.96 ± 5.02	0.39
IL-2	12.26 ± 15.45	12.15 ± 16.03	0.96
IL-4	1.26 ± 1.13	1.56 ± 3.02	0.49
IL-5	6.92 ± 19.24	6.10 ± 14.03	0.77
IL-6	31.42 ± 54.11	38.10 ± 52.39	0.43
IL-10	28.24 ± 76.82	19.29 ± 38.47	0.42
IL-12	2.61 ± 4.88	2.39 ± 2.60	0.75
IL-13	10.53 ± 12.38	10.23 ± 12.69	0.88
TNF-α	29.96 ± 72.55	19.44 ± 36.88	0.32
INF-γ	1.21 ± 0.85	1.32 ± 1.73	0.53

Table 5. Correlation between cytokine concentration and bleeding under the skin

Variable	Group Yes (Mean pg/ml ± SD)	Group No (Mean pg/ml ± SD)	p-value
IL-1	1.32 ± 1.07	2.08 ± 4.92	0.27
IL-2	11.43 ± 14.71	14.92 ± 17.95	0.19
IL-4	1.20 ± 1.09	1.77 ± 2.98	0.17
IL-5	6.87 ± 19.41	6.32 ± 13.46	0.84
IL-6	31.95 ± 55.25	36.09 ± 48.49	0.62
IL-10	28.39 ± 77.18	19.11 ± 38.17	0.39
IL-12	2.56 ± 4.91	2.56 ± 2.53	0.99
IL-13	9.85 ± 11.71	12.49 ± 14.49	0.22
TNF-α	30.76 ± 73.58	17.16 ± 30.61	0.18
INF-γ	1.18 ± 0.84	1.41 ± 1.71	0.36

Table 6. Correlation between cytokine concentration and hepatomegaly

Variable	Group Yes (Mean pg/ml ± SD)	Group No (Mean pg/ml ± SD)	p-value
IL-1	1.76 ± 0.98	1.44 ± 2.76	0.47
IL-2	18.97 ± 18.81	10.84 ± 14.45	**0.01**
IL-4	1.84 ± 1.35	1.22 ± 1.79	**0.04**
IL-5	7.27 ± 12.58	6.63 ± 19.16	0.84
IL-6	48.43 ± 91.52	29.70 ± 41.57	0.21
IL-10	33.73 ± 62.73	24.71 ± 71.65	0.46
IL-12	2.92 ± 2.00	2.49 ± 4.83	0.57
IL-13	15.44 ± 14.01	9.44 ± 11.85	**0.01**
TNF-α	29.91 ± 53.82	27.15 ± 68.79	0.81
INF-γ	1.35 ± 0.64	1.21 ± 1.17	0.48

Table 7. Correlation between cytokine concentration and temperature (univariate linear regression)

Variable	β	β_s	R	R^2	p-value
IL-1	-0.006	-0.25	0.025	0.001	0.71
IL-2	0.003	0.93	0.93	0.009	0.16
IL-4	0.008	0.25	0.25	0.001	0.71
IL-5	-0.002	-0.076	0.076	0.006	0.25
IL-6	0.000	-0.015	0.015	0.000	0.82
IL-10	-0.001	-0.141	0.141	0.02	**0.03**
IL-12	0.009	0.066	0.066	0.004	0.32
IL-13	0.005	0.105	0.105	0.011	0.10
TNF-α	0.000	0.027	0.027	0.001	0.67
INF-γ	0.012	0.022	0.022	0.001	0.73

Table 8. Correlation between cytokine concentration and temperature (multivariate linear regression)

Variable	β	β_s	R	R^2	p-value
IL-1	-0.052	-0.226	0.28	0.078	0.35
IL-2	-0.012	-0.332	0.28	0.078	0.25
IL-4	-0.01	-0.031	0.28	0.078	0.91
IL-5	-0.008	-0.253	0.28	0.078	**0.01**
IL-6	0.001	0.054	0.28	0.078	0.46
IL-10	-0.001	-0.168	0.28	0.078	**0.03**
IL-12	0.028	0.212	0.28	0.078	**0.03**
IL-13	0.019	0.398	0.28	0.078	0.14
TNF-α	0.001	0.073	0.28	0.078	0.30
INF-γ	0.100	0.190	0.28	0.078	0.20

DISCUSSION

Characteristics of clinical manifestations

Of the 234 children, 50.85% were hospitalized after contending with a fever for three days, but only 7.69% were taken to the hospital during the first day of fever occurrence. Among the participants, 89 (40%) suffered from vomiting, which manifested most frequently on day 3 after disease onset. This symptom persisted up to day 5 for one child. These results are lower than those derived by Priyadarshini et al. [23] in India and by Neto et al. [24] in Brazil, with the authors reporting that 70.9% and 71.8% of their respondents exhibited vomiting, respectively.

Abdominal pain was a typical occurrence in Neto et al.'s [24] study, with 75% of the children and adolescents exhibiting this symptom. Contrastingly, in the current work, only 33.3% of the cases suffered from abdominal pain, which emerged on different days after disease onset. The highest number of patients (11%) contracted this symptom on day 4. Bleeding under the skin and mucosal bleeding are symptoms constantly observed in DHF patients [25], and this research is no exception. Among the cases, 76.9% presented with signs of bleeding under the skin; the highest proportion of the participants (37.2%) manifested this symptom on day 3. Of the patients, 77.8% presented with mucosal hemorrhaging from days 2 to 6 after disease contraction, and up to 39.2% had this clinical manifestation on day 3. Compared with Neto et al.'s study, the present research found a lower number of patients afflicted with hepatomegaly (37.5% vs. 17.1%, respectively). Similar to the case of abdominal pain, this clinical manifestation arose most frequently on day 4.

The patients' temperatures were monitored continuously from admission to discharge. The highest temperature measured was 38.97±0.38°C. Table 1 shows that 44.4% of the DHF patients had the highest temperature on day 4 and that 26.5% of the patients exhibited such temperature on day 3. On day 6, five cases of fever were recorded.

Correlation between cytokine concentration and vomiting

After cytokine concentrations were measured, a comparison was made with respect to the 10 substances identified between two groups of patients: vomiting and non-vomiting participants. Table 2 illustrates that no statistically significant difference was found ($p > 0.05$) between the groups, leading

to the conclusion that cytokine concentration and vomiting were uncorrelated.

Correlation between cytokine concentration and abdominal pain

As shown in Table 1, 78 of the patients suffered from abdominal pain. Similar to what was done for the correlation between cytokine concentration and vomiting, a comparison was made between the patients with and without abdominal pain. Table 3 indicates that the IL-6 concentration in the group with abdominal pain was statistically significantly higher than that in the group without this symptom ($p=0.01$, $p < 0.05$). For all other cytokines, no correlation between concentration and abdominal pain was found.

Correlation between cytokine concentration and mucosal bleeding

A total of 182 patients developed mucosal bleeding during hospitalization. Table 4 presents the same results as those on vomiting (Table 2); that is, no correlation existed between cytokine concentration and mucosal bleeding.

Correlation between cytokine concentration and bleeding under the skin

Among the patients, 180 manifested with bleeding under the skin, but as displayed in Table 5, no statistically significant difference between groups (bleeding, no bleeding) in terms of cytokine concentration was found ($p>0.05$).

Correlation between cytokine concentration and hepatomegaly

Out of the sample, 40 cases were diagnosed with hepatomegaly. The comparison of the hepatomegaly and non-hepatomegaly groups showed that IL-2, IL-4, and IL-13 concentrations in the former were higher than those in the latter. These differences were statistically significant, as reflected in the following p-values: 0.01, 0.04, and 0.01 for IL-2, IL-4, and IL-13 concentrations, respectively. No correlation between the remaining cytokines and hepatomegaly was detected.

Correlation between cytokine concentration and temperature

As uncovered in the univariate linear regression, IL-10 exerted the strongest influence on the temperature of the patients, as reflected by a correlation coefficient of 0.03 (Table 7). This result is consistent with previous studies on IL-10, which has been considered a prognostic biomarker of DHF/dengue shock syndrome [11,12,26,27,34]. The difference in IL-10 concentrations can explain the 2% variation in temperature among the DHF patients ($R^2=0.020$). The multivariate linear regression (Table 8) showed that the model can explain 7.8% ($R^2=0.078$) of the variations in patients' temperature on the basis of the concentrations of only three cytokines: IL-5, IL-10, and IL-12 ($p = 0.01$, 0.03, and 0.03, respectively).

CONCLUSION

The results indicated that IL-6 concentration affected the manifestation of abdominal pain and that the high concentrations of IL-2, IL-4, and IL-13 elevated the occurrence of hepatomegaly. The patients' temperatures were affected by IL-5, IL-10, and IL-12 concentrations. On the grounds of the findings, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, and IL-13 may be used as reference in determining the prognosis regarding the aforementioned clinical symptoms. No other correlation was noted between cytokine concentration and the other clinical manifestations, such as vomiting, mucosal bleeding, and bleeding under the skin.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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