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The correlation of clinical and subclinical presentations with dengue serotypes and plasma viral load: the case of children with dengue hemorrhagic fever in Vietnam

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ABSTRACT

Numerous studies have been carried out on patients afflicted with dengue hemorrhagic fever (DHF), but various issues related to the disease, including the characteristics of the dengue virus (DENV), remain unclear. To address this deficiency, the current research was conducted to determine the correlation of clinical and subclinical presentations with dengue serotypes and plasma viral load. This prospective cohort study, which was performed at Tien Giang General Hospital from 2009 to 2014, involved 481 children who were under 15 years of age and had DHF for less than 72 hours. Results showed that among the patients, the highest proportion were composed of those suffering from DENV-1 infection (44.7%). The progression of the disease to dengue shock syndrome (DSS) owing to infection with DENV-2 and DENV-1 was significantly higher than that caused by infection with DENV-3 and DENV-4. No statistically significant differences in DENV viremia were found between the non-shock DHF and DSS groups. Finally, no correlation was found between dengue plasma viral load and clinical and subclinical presentations. The findings led to the conclusion that dengue serotypes can be used as a basis in ascertaining the prognosis of DSS and DHF.

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INTRODUCTION

Dengue fever (DF) is an acute infection caused by the dengue virus (DENV) and transmitted to humans by *Aedes* mosquitoes, mainly *Aedes aegypti* (World Health Organization, 2009). These mosquitoes cause about 390 million dengue cases in the world, of which 96 million (67-136 million) are severe cases; out of these extreme instances, 90% occur in the age group under 15 years (Bhatt *et al.*, 2013). In Vietnam, DF incidence is equally critical and accompanied with huge treatment costs given that it is a tropical monsoon country characterized by ongoing urbanization and a population with limited knowledge of the disease (Nguyen *et al.*, 2019; Guzmán

and Kouri, 2002). As estimated in 2017, the cost of treating a child with dengue averages at about 151 USD - a situation that seriously affects the developing economy of Vietnam (Pham *et al.*, 2016).

Clinical and subclinical symptoms are very important aspects in the diagnosis of DF and its complications, especially severe dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Symptoms such as plasma loss and coagulopathy, for example, render DHF patients prone to hypovolemic shock, which eventually results in severe bleeding and death. Many patients afflicted with DHF have died despite the release of World Health Organization (2009) guidelines on the diagnosis and treatment of DF, thus motivating a number of studies on factors related to dengue and dengue-induced death. Some of these determinants are virulence, infection and reinfection, local factors (age, nutritional status, gender, etc.), pre-existing severe illnesses (gastrointestinal hemorrhage, liver failure, metabolic acidosis, etc.), and management issues (late detection and treatment, inappropriate management, uncontrolled monitoring) (Guzmán and Kouri, 2002; Ha, 2003; Houghton-Trivino *et al.*, 2010). Understanding these factors plays a critical role in managing and improving the treatment and prognosis of DHF.

Prospective studies in Latin America and Southeast Asia concluded that most cases of DHF shock are associated with secondary immune responses. Factors such as originating virus and patient location are likewise critical contributors to severe illness. The amount of virus in the blood may also be an essential aspect because viral concentrations in DSS cases are often high. In some countries, different levels of virulence are considered a culprit in various consequences (Tang *et al.*, 2010). These findings underscore the necessity of looking into the association between a patient's body and DENV as well as immunological factors because such explorations are expected to enhance the prognosis of dengue patients. Correspondingly, the current work determined the relationship of dengue serotypes and DENV infection levels with clinical and subclinical DHF symptoms in children. This association is an important new issue with respect to diagnosing and managing potentially fatal DF.

MATERIALS AND METHODS

Study design and setting

This prospective cohort research was carried out at the Pediatrics Department of Tien Giang General Hospital from 2009 to 2014. Children with DF were selected on the basis of the following criteria: (1) the presence of one or more clinical symptom markers, such as abdominal pain, persistent vomiting, clinical fluid accumulation, mucosal bleeding, lethargy, restlessness, and liver enlargement (>2 cm) corresponding with criteria in the World Health Organization (1997) guidelines for DHF; (2) ages below 15 years; (3) DF lasting less than 72 hours; and (4) consent from the children's families. The exclusion criteria were the existence of diseases such as liver failure, kidney failure, nephrotic syndrome, heart failure, and congenital heart disease and the refusal of families to participate in the research.

Sample size

To ensure the validity of results, the minimum sample size was calculated using the formula recommended by the WHO (Lachenbruch *et al.*, 1991)

$$N = \frac{Z_{\alpha/2} \times P(1-P)}{d^2}$$

This study assumed a 95% confidence interval ($Z_{1-\alpha/2}$ = 1.96) with a 5% margin of error (d). The prevalence of DHF and DSS in children was calculated on the basis of Ha's (2003) study on isolated DENV strains, for which the author determined the ratio of DHF and DSS incidence to population size as follows: 16/84 = 0.19.[6] Therefore, the minimum sample size appropriate for the present study was 236 children. For accurate results, we endeavored to recruit twice this number of patients, but a total of 481 patients were found eligible for inclusion.

Data collection and analysis

Data were collected through face-to-face interviews with the patients' guardians and a review of the patients' medical records. The interviewer first explained the meaning of the study as well as the projected benefits and risks encountered over the course of the research. Information was then collected on the following patient characteristics: age and gender; clinical symptoms, such as vomiting, abdominal pain, mucosal bleeding, bleeding under the skin, temperature, liver enlargement, and shock; and subclinical symptoms, namely, white blood cell count, aspartate aminotransferase (ASP) and alanine aminotransferase (ALT) concentrations, platelet count, hematocrit (HCT) level, and DENV serotype. These data were uploaded onto Microsoft Excel (v. 2007) for storage and statistical calculations. The relationship among the patients' clinical characteristics, DENV serotype, and DENV concentration in the blood was determined via a chi-square test to run on the Statistical Package for the Social Sciences (v. 16.0).

Ethical considerations

This study was approved by the ethics committee of the Tien Giang General Hospital. The patients were informed that participation in this study is completely voluntary and that their identities will be kept anonymous. The guardians were asked to sign a statement confirming that they willingly took part in the research.

RESULTS AND DISCUSSION

Clinical characteristics

Table 1 presents the observed clinical symptoms of the 481 patients, of which 40.1% exhibited vomiting; most of these patients (22.4%) started vomiting from day 3 of disease onset. Abdominal pain was experienced by 29.5% of the patients, out of whom the highest number (12.3%) suffered from this symptom on the third day from disease occurrence. A total of 38.5% of the patients presented with subcutaneous hemorrhaging, with most (19%) presenting this symptom on day three of the disease. The same timing was observed as to mucosal hemorrhaging, which manifested in 19.4% out of 38.7% of the patients. Hepatomegaly was a commonly occurring symptom, manifesting in 20.4% of the patients and occurring most often on the fourth day from disease onset.

Subclinical characteristics

Table 2 shows the lowest average white blood cell count was 4.72±1.96/mm (Nguyen *et al.*, 2019), which occurred among most of the patients on days 4 and 5 of the disease. The highest AST concentration was 68.58±9.00 U/L, which manifested in the majority of the patients (93.6%) also on the fourth and fifth days. The second and third highest proportions of patients developed this symptom on the third and sixth days, respectively. The highest average ALT concentration was 40.61±7.17 U/L, found in 93.6% of the sample and occurring on the fourth and fifth days from disease occurrence. The lowest platelet count was 80.19±33.91/mm (Nguyen *et al.*, 2019), manifesting in nearly all the children on days 4 to 6 of the disease. The highest average HCT concentration was 42.86±4.30%.

DENV concentrations

Table 3 shows the average daily viral dengue concentration in the patients was 3.5×10^8 (copies/ml). The comparison of virus types showed no significant differences in DENV concentration (p = 0.645), but the highest occurring dengue variant was DENV-3 infection, whereas the lowest was DENV-4 infection. The average DENV concentrations differed in terms of the time of DENV infection, with the highest concentration occurring on day 1 of the fever. Viral con-

Table 1: Distribution of Patients According to Clinical Manifestations

Clinical manifestations		N	%
Vomiting	No	288	59.9
	Yes	193	40.1
	Day 1^{st}	21	4.4
	Day 2^{nd}	64	13.3
	Day 3^{rd}	108	22.4
Abdominal pains	No	339	70.5
	Yes	142	29.5
	Day 2^{nd}	40	8.3
	Day 3^{rd}	59	12.3
	Day 4^{th}	26	5.4
	Day 5^{th}	17	3.5
Bleeding under the skin	No	296	61.5
	Yes	185	38.5
	Day 2^{nd}	31	6.4
	Day 3^{rd}	91	19.0
	Day 4^{th}	41	8.5
	Day 5^{th}	22	4.6
Mucosal bleeding	No	295	61.3
	Yes	186	38.7
	Day 2^{nd}	39	8.1
	Day 3^{rd}	93	19.4
	Day 4^{th}	31	6.4
	Day 5^{th}	23	4.8
Highest day temperature	Mean ± SD	38.62 ± 0.66	
	Day 1^{st}	22	4.6
	Day 2^{nd}	166	34.5
	Day 3^{rd}	217	45.1
	Day 4^{th}	41	8.5
	Day 5^{th}	14	2.9
	Day 6^{th}	21	4.4
Liver enlargement	No	383	79.6
	Yes	98	20.4
	Day 3^{rd}	16	3.3
	Day 4^{th}	38	7.9
	Day 5^{th}	31	6.5
	Day 6^{th}	13	2.7
DSS	No	457	95
	Yes	24	5
	Day 3^{rd}	2	0.4
	Day 4^{th}	11	2.3
	Day 5^{th}	10	2.1
	Day 6^{th}	1	0.2

Table 2: Distribution of Patients According to Subclinical Symptoms (N = 481)

Characteristic		N	%
DENV serotype	DENV-1	215	44.7
	DENV- 2	92	19.1
	DENV-3	61	12.7
	DENV-4	113	23.5
Characteristic	Time		
White cell counts (highest)	Mean ± SD	4.72 ± 1.96/mm^3	
	Day 2^{nd}	60	12.5
	Day 3^{rd}	76	15.8
	Day 4^{th}	105	21.8
	Day 5^{th}	102	21.2
	Day 6^{th}	84	17.5
	Day 7^{th}	54	11.2
AST (highest)	Mean ± SD	68.58 ± 9.0 U/L	
	Day 3^{rd}	31	6.4
	Day 4^{th}	198	41.2
	Day 5^{th}	252	52.4
ALT (highest)	Mean ± SD	40.61 ± 7.17 U/L	
	Day 3^{rd}	31	6.4
	Day 4^{th}	198	41.2
	Day 5^{th}	252	52.4
Platelet (lowest)	Mean ± SD	80.19 ± 33.91/mm^3	
	Day 2^{nd}	11	2.3
	Day 3^{rd}	12	2.5
	Day 4^{th}	84	17.5
	Day 5^{th}	143	29.7
	Day 6^{th}	181	37.6
	Day 7^{th}	50	10.4
Hct (highest)	Mean ± SD	42.86 ± 4.30 %	
	Day 2^{nd}	13	2.7
	Day 3^{rd}	83	17.3
	Day 4^{th}	131	27.2
	Day 5^{th}	160	33.3
	Day 6^{th}	83	17.3
	Day 7^{th}	11	2.3

centration in the blood decreased on days 2 and 3, with the difference between these days being statistically significant (p = 0.01). Average DENV levels in cases of DENV reinfection (3.7×10^8 copies/ml) were higher than cases of initial infection (3.2×10^8 copies/ml), but the difference was non-significant (p = 0.513).

Correlation of clinical and subclinical characteristics with DENV type and concentration

Table 4 presents the results on the relationship between clinical characteristics and DENV serotypes. No correlation was found between DHF and vomiting, abdominal pain, and hepatomegaly in the children (p > 0.05). Subcutaneous hemorrhaging and mucosal hemorrhaging most frequently occurred in patients with DENV-1 infection, followed by those infected with DENV-3 and DENV-2. The lowest manifestations were found in the children infected with DENV-4. The difference among these incidences was statistically significant (p = 0.01).

Table 5 shows that no correlation was found between DHF and the average DENV concentration in the patients exhibiting clinical symptoms (p > 0.05).

The relationship between subclinical characteristics and DENV type and concentration

The average number of leukocytes and platelets is lowest in the patients infected with DENV-2, followed by the group with DENV-1 infection. The difference between these groups was statistically significant (p = 0.01). The highest average HCT was observed in the DENV-2 group, followed by the DENV-1 group, also with the difference being statistically significant (p = 0.01). No correlation was found between the highest concentrations of AST and ALT and different types of DENV (Table 6).

The patients participating in this study were identified as potentially afflicted with DHF, with the proportion of patients who were likely to develop DSS being approximately 5%. Out of these patients, 3% and 2.1% suffered from shock on days 4 and 5 of the disease, respectively. The rate of DSS found in the current study is much lower than that observed by Hung (2004), whose investigation involved 62 children under 12 months of age with DHF, including no-shock DHF (grade II, 69.3%) and DHF accompanied by shock (grade III, 15 cases; grade IV, four cases). Among the patients, 30.7% showed improved management of DF diagnosis and treatment. The findings of the present study are also lower than those derived in research in Brazil (i.e., 6.5%) (Vicente *et al.*, 2016).

The distribution of patients, according to DENV serotype, was as follows. The most prevalent was DENV-1 (44.7%), followed by DENV-4 (23.5%), DENV-2 (19.1%), and DENV-3 (12.7%). Tuan *et al.* (2012) found that the occurrence rates of DENV-1, DENV-2, DENV-3, and DENV-4 in Vietnam are 62.8%, 27.4%, 8.8%, and 0.9%, respectively. As can be seen, DENV-1 is the predominant disease-causing serotype, as was similarly found by Rathakrishnan *et al.* (2012) and Vicente *et al.* (2016) (77.3%). In Thailand, Fried *et al.* (1994) found that DENV-4 still has a relatively low prevalence (36%) in the South-

Table 3: DENV Concentrations

DENV concentrations (copies/ml) Mean		Mean 3.5×10^8	P-value*
Day has fever	Day 1^{st}	9.4×10^8	0.010
	Day 2^{nd}	4.5×10^8	
	Day 3^{rd}	1.5×10^8	
History of DF or DHF	No	3.2×10^8	0.513
	Yes	3.7×10^8	
DENV serotype	DENV-1	4.6×10^8	0.645
	DENV-2	1.4×10^8	
	DENV-3	8.4×10^8	
	DENV-4	0.5×10^8	

(*) Chi-square test

Table 4: Relationship between Clinical Characteristics and DENV Serotypes (N = 481)

Clinical manifestations	DENV serotype	DENV-1	DENV-2	DENV-3	DENV-4	Total	P-value*
Vomiting	Yes	88 (45.6)	33 (17.1)	23 (11.9)	49 (25.4)	193	0.700
	No	127 (44.1)	59 (20.5)	38 (13.2)	64 (22.2)	288	
Abdominal pains	Yes	65 (45.8)	28 (19.7)	21 (14.8)	28 (19.7)	142	0.560
	No	150 (44.2)	64 (18.9)	40 (11.8)	85 (25.1)	339	
Mucosal bleeding	Yes	110 (59.1)	31 (16.7)	43 (23.1)	2 (1.1)	186	0.010
	No	105 (35.6)	61 (20.7)	18 (6.1)	111 (37.6)	295	
Bleeding under the skin	Yes	114 (61.6)	30 (16.2)	40 (21.6)	1 (0.5)	185	0.010
	No	101 (34.1)	62 (20.9)	21 (7.1)	112 (37.8)	296	
Liver enlargement	Yes	45 (45.9)	18 (18.4)	8 (8.2)	27 (27.6)	98	0.400
	No	170 (44.4)	74 (19.3)	53 (13.8)	86 (22.5)	383	
DSS	Yes	14 (58.3)	8 (33.3)	1 (4.2)	1 (4.2)	24	0.027
	No	201 (44.0)	84 (18.4)	60 (13.1)	112 (24.5)	457	

(*) Chi-square test

Table 5: Relationship between Clinical Characteristics and DENV Concentrations

Clinical manifestations		n	Mean DENV concentration (copies/ml)	P-value*
Vomiting	Yes	193	$2.80x10^8$	0.18
	No	288	$4.19x10^8$	
Abdominal pains	Yes	142	$2.65x10^8$	0.52
	No	339	$4.05x10^8$	
Mucosal bleeding	Yes	186	$5.27x10^8$	0.19
	No	295	$2.60x10^8$	
Bleeding under the skin	Yes	185	$4.72x10^8$	0.38
	No	296	$2.95x10^8$	
Liver enlargement	Yes	98	$6.77x10^8$	0.34
	No	383	$2.83x10^8$	
DSS	Yes	24	$3.44x10^8$	0.96
	No	457	$3.64x10^8$	

(*) Chi-Square test

Table 6: Relationship between Subclinical Characteristics and DENV Serotypes

Subclinical	DENV serotype-	n	Mean	SD	P-value*
White cell count ($/mm^3$)	1	215	4.53	2.49	0.01
	2	92	4.32	1.76	
	3	61	5.19	2.45	
	4	113	5.15	2.03	
AST concentrations (U/L)	1	215	65.85	64.98	0.38
	2	92	75.19	70.36	
	3	61	80.49	58.96	
	4	113	61.98	33.49	
ALT (U/L)	1	215	40.33	53.34	0.50
	2	92	41.79	65.44	
	3	61	50.29	11.97	
	4	113	34.95	37.87	
Platelet ($/mm^3$)	1	215	77.89	36.75	0.01
	2	92	73.61	30.95	
	3	61	83.26	31.31	
	4	113	88.27	30.43	
Hct (%)	1	215	43.27	4.41	0.01
	2	92	43.96	4.30	
	3	61	42.33	3.79	
	4	113	41.50	4.01	

(*) Chi-square test

ern region, which is a finding supported by research in other parts of the world (Ha, 2003; Vicente *et al.*, 2016; Que *et al.*, 2012).

A statistically significant difference in DENV serotype prevalence was found between DHF and DSS ($p = 0.027$, $p<0.05$), which differs from the survey results derived in Thailand (Fried et al. 2010), where no difference in serotypes between dengue groups was discovered. The current results indicated that patients with DENV-1 and, particularly, DENV-2 had higher shock rates than the rest of the patients. This finding is similar to that derived by Tuan *et al.* (2012), who recorded a higher and more severe case of shock in the DENV-2 infected group than in the DENV-1 infected group Tuan *et al.* (2012). The present work is also consistent with studies conducted in Taiwan and Thailand (Fried *et al.*, 1994; Chen *et al.*, 2007).

The limitations of this study are worth noting. First, the subjects were selected on the basis of DHF classification, but no clear distinction exists among DF, DHF, and DSS cases; thus, it is possible to confuse DF and DHF (Hadinegoro, 2012) given that patients afflicted with these conditions display the same symptoms as those observed in other infections. Second, the study was conducted in only one hospital, thus preventing generalizability to the entire Vietnam or other parts of the world. Finally, because this work found no correlation among plasma concentrations of DENV, this precludes approaches to evaluating severe cases of DHF for enhanced management. The strengths of the research include a five-year data collection period, complete with virus isolation and serotyping. In addition, the study monitored patients over the course of several days, thus acquiring complete data on the timing of clinical symptoms and a more general view of DHF and DSS cases.

CONCLUSIONS

The susceptibility of patients infected with DENV-2 and DENV-1 was significantly higher than those infected with the other virus types. Subcutaneous hemorrhaging and mucosal hemorrhaging occurred most frequently in patients with DENV-1 infection, followed by those infected with DENV-3 and DENV-2. These symptoms manifested themselves to the lowest extent in the DENV-4 cases. The difference among the subjects was statistically significant ($p = 0.01$). The average number of leukocytes and platelets was lowest in the DENV-2 patients, followed by the DENV-1 group, with the difference between them being statistically significant ($p = 0.01$). The highest average HCT was observed in the group infected with DENV-2, followed by the patients infected with DENV-1. The difference between these groups was also statistically significant ($p = 0.01$). No correlation was found between DENV serotype and vomiting, abdominal pain, hepatomegaly, and AST and ALT levels in the children with DHF ($p > 0.05$). DENV concentrations were unassociated with the clinical and subclinical symptoms examined.

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