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Virus serotype and the severity of dengue infection in Vietnamese children at Children's Hospital 1, Ho Chi Minh City

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Abstract— Dengue hemorrhagic fever (DHF) is an endemic disease affecting tropical countries. When there is a change in predominating serotype, large outbreaks will occur. In Vietnam, there has been a significant increase in the number of DHF cases in 2022. Therefore, this study was conducted to investigate whether there have been any changes in the distribution of dengue serotypes and the severity of DHF among children in Vietnam.This case series report was conducted at Children's Hospital1 at Ho Chi Minh City from June 2022 to September 2022. Patients enrolled in the study were diagnosed with DHF based on WHO 2009 criteria and had positive RT-rPCR dengue results. Among 180 children enrolled in the study, 120 cases (66.7%) were caused by DENV-2, 58 (32.2%) by DENV-1, and 2 (1.1%) by DENV-4. The severity of disease varied, with the highest magnitude being DHF with warning signs (42.8%), followed by severe DHF (30.0%), and DHF (27.2%). DENV-2 infection was associated with more severe manifestations in terms of hemoconcentration (p=0.001), thrombocytopenia (p<0.001), abdominal pain (p=0,009), hepatomegaly (p=0,001). DENV-2 infection was more likely to result in shock compared to DENV-1 (p=0.036).DENV-2 was the predominating serotype, children infected with DENV-2 typically exhibited more severe manifestations compared to DENV-1. The relationship between shock, abdominal pain, hepatomegaly, hemoconcentration, thrombocytopenia, and dengue serotypes are statistically significant.

Keywords— Dengue hemorrhagic fever, dengue shock syndrome, dengue serotype, Vietnam, children.

1. Introduction

Dengue hemorrhagic fever (DHF) is caused by any of the four dengue serotypes (DENV-1, DENV-2, DENV-3, DENV-4). DHF is characterized by fever, hemorrhage and plasma leakage, shock, organ failure, which can be fatal if early detection and appropriate management is delayed. Currently, DHF has affected more than 120 countries and is one of the leading causes of hospitalization and death in Asian tropical and subtropical countries. Vietnam is located in the tropical monsoon and equatorial zone with all four dengue serotypes circulating. The humid and rainy climate during wet season creates favorable conditions for the activity of dengue virus, leading to annual epidemics.[1] It was estimated by WHO that 50 million dengue infections occur annually with a mortality rate of 1% and the incidence of DHF has continuously increased.[2] In the first six months of 2022, 1 million cases and 849 dengue-related deaths have been reported. In Vietnam, in the first five months of 2022, more than 25 thousand DHF cases were confirmed with 13 deaths. In April 2022 alone, the epidemic situation deteriorated rapidly with a sharp surge of

more than 10 thousand new cases reported, including severe cases and deaths.[3] Following the COVID-19 pandemic, the alarming rise in the number of DHF cases became a major public health concern. For this reason, we conducted this study at Children's Hospital 1 to investigate whether the changing distribution of circulating dengue serotypes be contributing to the complicated and unpredictable DHF situation? Children's Hospital 1, the largest pediatric hospital in the south of Vietnam, has a capacity of 1500 beds. It admits a high number of severe DHF cases from Ho Chi Minh City and provinces in the southern region.

2. Techniques

This was a case series report and was conducted on paediatric patients hospitalized at Children's Hospital 1 at Ho Chi Minh City from June 2022 to September 2022.Patients< 16 years oldwere included in the study if they met all three of the following criteria: satisfying the case definition criteria of dengue infection by WHO, having positive RT-rPCR dengue results andtheir families' consent.Within 24 hours of admission, 2 mL of blood would be taken from the children, stored in EDTA anticoagulant tubes, and transferred to the Nam Khoa Biotek Laboratory for RT-rPCR to identify dengue virus serotypes.

The blood samples were centrifuged at 3000 RPM for 10 min to isolate blood plasma and immediately analyzed by one-step reverse transcriptase real-time PCR (RT-rPCR) for DENV detection and for viral serotype identification that was referred from Gilberto A. Santiago (CDC) et al. Briefly, nucleic acids were extracted completely from 200µL plasma on the KingFisher FLEX system (Thermo, Waltham, MA, USA) using NKDNARNAprep-MAGBEAD extraction kits manufactured by Nam Khoa company. Extracted fluid (5 µL) was added into PCR 0.1 tubes of Rotor Gen Q MDx 5plex Platform (Qiagen, Kuala Lumpur, Malaysia), containing 20 µL one step RT rPCR mix made from Thermo AgPath-ID™ One-Step RT-PCR (Thermo, USA) with four specific primer pairs and four specific probes for four different DENV serotypes, that were: D1-F (CAA AAG GAA GTC GTG CAA TA), D1-R (CTG AGT GAA TTC TCT CTA CTG AAC), D1-PR (FAM-CAT GTG GTT GGG AGC ACG C-BHQ1) for DEN-1; D2-F (CAG GTT ATG GCA CTG TCA CGA T), D2-R (CCA TCT GCA GCA ACA CCA TCT C), D2-PR (HEX-CTC TCC GAG AAC AGG CCT CGA CTT CAA-BHQ1) for DENV-2; D3-F (GGA CTGG ACA CAC GCA CTC A), D3-R (CAT GTC TCT ACC TTC TCG ACT TGT CT), D3-PR (TexasRED-ACC TGG ATG TCG GCT GAA GGA GCT TG-BHQ2) for DENV-3 and; D4-F (TTG TCC TAA TGA TGC TGG TCG), D4-R (TCC ACC TGA GAC TCC TTC CA), D4-PR (CY5-TTC CTA CTC CTA CGC ATC GCA TTC CG-BHQ3) for DENV-4. All the primers and probes were ordered from Proligo (Sigma, Singapore). After this step, the PCR tubes were incubated in the Rotor Gen Q instruments (Qiagen, Malaysia) and ran with thermal cycles at 45 ◦C for 10 min to reverse transcriptase (RT), at 95 ◦C for 10 min to destroy the RT enzyme, 40 cycles with two thermal steps at 95 ◦C for 15 s followed by 60 ◦C for one minute in which we recorded fluorescent signals. The results were read and characterized together with DENV serotypes based on amplifying signals dyed with four different color channels: FAM for type 1, HEX for type 2, TexasRED for type 3, and CY5 for type 4. If signals were absent in all channels, we ran another assay using RT-rPCR mix manufactured by Nam Khoa company for house-keeping gene, RNAseP, to confirm the negativity of the sample.

During the research period, we collected 330 blood samples for RT-rPCR. Among these patients, 180 cases(54.5%) had positive results from RT-rPCR and were included in the studyfor description and comparison clinical and laboratory findingswith different DENV serotypes.

Epidemiological information (gender, age), weight, clinical symptoms (nausea, abdominal pain, bleeding, hepatomegaly, respiratory distress) and diagnosis at the time of admission, at the most severe manifestation, and at discharge were addressed. A patient was considered to have severe vomiting when vomiting ≥ 3 times/1 hour or ≥ 4 times/6 hours. Bleeding manifestations included subcutaneous hemorrhage, epistaxis, gingivorrhagia, gastrointestinal bleeding... Respiratory distresswas recorded when patients needed respiratory support. Biochemical tests included complete blood count, coagulation tests, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate, and albumin. Biochemical tests were performed by BECKMAN COULTER AU-680 machine at the Department of Biochemistry, CH 1. The complete blood count and coagulation test were performed by the Sysmex XN-2000 and STA R-Max machine, respectively, at the Department of Hematology Laboratory, CH 1.Hypoalbuminemiawas determined when albumin < 3.5 g/dL. The aPTT ratio was considered to increase when ≥ 1.3. The patients were described and analyzed according to two classifications: by shock status (including with and without shock) and by dengue serotypes (including DENV-1, DENV-2, DENV-3, DENV-4).

Differences between groups were analyzed using Fisher's exact test/Chi-square test for categorical variables and Student's t-test/ Wilcoxon Rank Sum test for continuous variables. Statistical analysis was conducted through STATA, and a two-tailed $p < 0.05$ was considered statistically significant.

3. Results

From June 2022 to September 2022, a total of 180 patients diagnosed withDHF based on positive results from RT-rPCR were enrolled in the study. Epidemiological, clinical and laboratory data of the population are shown in Table 1.

Table 1. Characteristics of shock and non-shock DHF patients.

Characteristics	Total subject n = 180	Shock n =40	Non-shock n = 140
Male	113 (62,8)	23 (57,5)	90 (64,3)
Female	67 (37,2)	17 (42,5)	50 (35,7)
Age	9 (5 – 12)	9,5 (9 – 12)	9 (5 – 12)
Overweight - Obese	54 (30,0)	15 (37,5)	39 (27,9)
Severe vomiting	39 (21,7)	10 (25,0)	29 (20,7)
Abdominal pain	52 (28,9)	24 (60,0)	28 (20,0)
Petechia	45 (25,0)	12 (30,0)	33 (23,6)
Mucosal bleeding	25 (13,9)	7 (17,5)	18 (12,9)
Hepatomegaly	96 (53,3)	40 (100,0)	56 (40,0)

Respiratory distress	16 (8.9)	10 (25,0)	6 (4,3)
Hct (%)	44,2 ± 5,2	48,7 ± 3,8	42,9 ± 4,9
White blood cell($\times 10^3/\mu L$)	3,5(2,7 – 4,6)	3,5(2,7 – 4,1)	3,5 (2,7 – 4,8)
Platelet ($\times 10^3/\mu L$)	76(32 – 143,5)	28(15,5 – 49)	98 (49,5 – 160,5)
Coagulation tests	***(n = 74)***	***(n = 35)***	***(n = 39)***
PT (%)	73(61 – 89,5)	68(60 – 90)	77 (64 – 89)
aPTT Ratio	1,4(1,2 – 1,8)	1,6(1,3 – 1,9)	1,4 (1,2 – 1,6)
Fibrinogen (g/L)	1,9(1,2 – 2,6)	1,6(1,0 – 2,3)	2,0 (1,5 – 2,7)
Liver enzymes	***(n = 131)***	***(n = 40)***	***(n = 91)***
Hepatic impairment	81 (61,9)	26 (65,0)	55 (60,4)
AST (U/L)	145(93 – 293)	148(98 – 316)	144 (86 – 290,5)
ALT (U/L)	61(33 – 118)	61(38 – 153)	60,5 (31 – 112)
Lactate (mmol/L)	***(n = 87)***	***(n = 40)***	***(n = 47)***
	2,3(1,9 – 3,1)	2,7(2,0 – 3,4)	2,1 (1,8 – 2,7)
Albumin (g/dL)	***(n = 47)***	***(n = 24)***	***(n = 23)***
	2,3(1,8 – 3,1)	1,9(1,7 – 2,3)	2,8 (2,3 – 3,4)
DENV-1	58 (32,2)	7 (17,0)	51 (36,4)
DENV-2	120 (66,7)	33 (83,0)	87 (62,1)
DENV-4	2 (1,1)	0 (0)	2 (1,4)

Data was presented as numbers (%) or means (±SD) or medians (interquartile range).

The highest number of patients belonged to the 5 – 10 years age group (47.8%) and the lowest number was in the under 5 years age group (5%). The youngest patient admitted was 6 months old. The severity of DHF was distributed as follows: 49 children (27.2%) had DHF, 77 children (42.8%) had DHF with warning signs, 54 children had severe DHF (30.0%).The highest admission rate (52.8%) was observed on day 3 and 4 of the disease.

There were 40 cases (22.2%) diagnosed with dengue shock syndrome (DSS). The occurrence of shock was highest on day 5 of the illness(52.5%) and lowest on day 3 (5%). Hepatomegaly was the most common clinical symptom in children with DHF at 53.3% and present in virtually all cases of DSS.

Hemorrhagic manifestations included spontaneous petechiae (25.0%) and mucosal bleeding (13.9%) in the form of epistaxisor gingivorrhagia (9.4%), upper gastrointestinal bleeding (hematemesis) (2.2%) and heavy menstrual bleeding (2.2%). The rate of respiratory distress was 8.9%, with the majority observed in the case of DSS (62.5%). Thrombocytopenia was found in 77.2% of DHF patients. Among the 74 cases performed coagulation tests, 62.2% showed coagulation abnormalities, with 29.7% in DSS group. The magnitude of increased aPTT ratio, decreased PT%anddecreased fibrinogen in DHF patients were 59.5%, 45.9%and 33.8%, respectively. Amongthe 131 cases evaluatedfor liver enzymes, 61.8% of patients had acute liver damage with 43.5% classified as mild, 11.5% as moderate, and 6.9% as severe. Among the 47

patients (26.1%) evaluatedfor serum albumin, 40 cases (85.1%) had hypoalbuminemia, with 55% in the shock group and 45% in non-shock group.

The majority of cases were caused by DENV-2 (66.7%), followed by DENV-1 (32.2%), DENV-4 (1.1%). Among the 40 cases ofDSS, the main dengue serotypes identified were DENV-2 (82.5%) and DENV-1 (17.5%). Patients infected with DENV-2 exhibited a wide spectrum of clinical presentations, including DHF, DHF with warning signs and DSS with severe shock. None of the patients infected with DENV-1 experienced severe shock. Two cases with infection by DENV-4 were classified as DHF with warning signs. We conducted an analysis on the epidemiological, clinical, and biochemical characteristics of the two common types, DENV-1 and DENV-2. The presence of shock was significantly associated with DENV-2 infection ($p = 0.036$). Patients infected with DENV-2 presented abdominal pain ($p = 0.009$) and hepatomegaly ($p = 0.01$) more frequently when compared with those suffering from DENV-1. The presence of an increase in hematocrit ($p < 0.001$) and thrombocytopenia ($p = 0.001$) was significantly higher in DENV-2 infection (table 2).

Table 2. Dengue serotypes and clinical manifestations of dengue patients.

Characteristics	**DENV-1 n = 58, (%)**	**DENV-2 n = 120, (%)**	**p**
Male	33 (56,9)	79 (65,8)	0,247[a]
Female	25 (43,1)	41 (34,2)	
Age	8,5 (5 – 11)	9 (6 – 12)	0,443 [a]
Overweight - Obese	17 (29,3)	36 (30,0)	0,925 [a]
Severe vomiting	9 (15,5)	28 (23,3)	0,228 [a]
Abdominal pain	9 (15,5)	41 (34,2)	**0,009** [a]
Petechia	16 (27,6)	28 (23,3)	0,538 [a]
Mucosal bleeding	10 (17,2)	17 (14,2)	0,592 [a]
Hepatomegaly	20 (34,5)	74 (61,7)	**0,001** [a]
Respiratory distress	3 (5,2)	12 (10,0)	0,277 [a]
Hct (%)	41,5(40 – 44)	45(42 – 48)	**<0,001** [a]
White blood cell ($\times 10^3/\mu L$)	3,4(2,4 – 3,9)	3,6(2,7 – 4,8)	0,074 [a]
Platelet ($\times 10^3/\mu L$)	106(67 – 164)	65(27,5 – 134,5)	**0,001** [a]
Coagulation tests	***(n = 17)***	***(n = 56)***	
PT (%)	77(67 – 91)	68,5(60 – 89)	0,284[b]
aPTT Ratio	1,4(1,2 – 1,8)	1,4(1,3 – 1,8)	0,774[b]
Fibrinogen (g/L)	2,2(1,4 – 2,7)	1,8(1,2 – 2,5)	0,502[b]
Liver enzymes	***(n = 33)***	***(n = 96)***	
Hepatic impairment	20 (60,6)	59 (61,5)	0,179[a]
AST (U/L)	198(96 – 537)	140,5(93 – 282,5)	0,256[b]
ALT (U/L)	60(41 – 189)	61(32,5 – 99,5)	0,466 [b]
Lactate (mmol/L)	***(n = 16)***	***(n = 69)***	
	2,8(1,9 – 3,1)	2,2(1,9 – 3,1)	0,387[b]
Albumin (g/dL)	***(n = 5)***	***(n = 40)***	

	2,3(2,2 – 2,4)	2,3(1,8 – 3,1)	0,759 [b]
DHF	25 (43,1)	24 (20,0)	**0,001** [a]
DHF with warning signs	20 (34,5)	55 (45,8)	0,237 [a]
DSS	7 (12,1)	31 (25,8)	**0,036** [a]

Data was presented as numbers (%) or means (±SD) or medians (interquartile range).
a : *Chi-square test* b *: Mann Whitney test*

4. Discussion

Dengue viruses consist of four serotypes: DENV-1, DENV-2, DENV-3, and DENV-4. All four serotypes can cause a wide range of illnesses, including DHF, DHF with warning signs and DSS. The severity of dengue epidemics is affected by the circulating serotypes.[2] In our study, we observed three circulating serotypes: DENV-1, DENV-2, and DENV-4. Among these, DENV-2 was the most prevalent, while DENV-4 was rare. The distribution of serotypes differed from previous studies. Tran Thanh Hai in a study in Tien Giang noted that DENV-1 was the most common serotype (44.7%) from 2008 to 2011, followed by DENV-4 (23,5%) and DENV-2 (19.1%), DENV-3 was the least (12.3%)[4]. In Ha Noi, Nguyen Manh Hung reported that DENV-1 (60.3%) was the predominant serotype during 2015 – 2017[5]. In 2018, Duong Q. Phan identified DENV-4 (68.5%) as the main circulating serotype in Quang Nam[6].

Among the warning signs of severe disease, hepatomegaly and abdominal pain are the two most common, in descending order of frequency. Notably, patients with infection by DENV-2 exhibited these symptoms more frequently compared to those infected with DENV-1, as previously observed by some authors. Additionally, markers of severe disease such as abdominal pain, excessive vomiting, hepatomegaly, and severe plasma leakage were observed more frequently in DENV-2 infection group compared toother serotypes.However, there was no significant difference in bleeding manifestation among different serotypes.[8–10] In contrast, Tran Thanh Hai noted that children infected with DENV-1 had a higher risk of bleeding compared to those infected with DENV-2 and DENV-4[4]. The frequency of shock was alsohigher in patients with DENV-2 infectioncompared to those with DENV-1 infection. This is in correspondence with the findings of Nguyen Minh Tuan in Vietnam, Lovera *et al* and Kalayanarooj *et al* in Thailand, Suppiah*et al* in Malaysia.[8–11].

Regarding the laboratory characteristics of DHF cases, patients infected with DENV-2 exhibitedhigher hematocrit and lower platelet countscompared to DENV-1, which is consistent with the findings of Tran Thanh Hai[4]. In a study on DHF children in Paraguay from 2007 to 2018, Lovera et al noted that patients with infection by DENV-1 and DENV-2 had hemoconcentrationand thrombocytopenia more frequently than those suffering from DENV-3 infection[9]. Hematocritis an indicator of plasma leakage, where a higher hematocrit indicates more plasma leakage from the capillaries. This suggestedthat cases of DENV-2 infection are more likely to develop capillary leakage which results in DSS, the most severe manifestation of the disease. Thrombocytopenia, acharacteristic of DHF and a warning sign of severe illness, occur due to various mechanisms, including platelets adhering to damaged vessel walls, consumption with clotting factors during disseminated intravascular coagulation, reduced platelet lifespan, or direct destruction by viruses or

specific antibodies attached to virus-infected platelets[12]. Conversely, Nguyen Minh Tuan did not find differences in hematocrit and platelet count when comparing among different DENV serotypes[8]. The frequency of liver involvement was similar in DENV-1 and DENV-2 serotypes, a finding previously noted by other authors[4,8,9].However, in a study conducted in Bangkok, Thailand from 1995 to 1999 Kalayanarooj et alreportedthat DENV-3 and DENV-4 had more degree of liver involvement, as demonstrated by higher mean values of AST and ALT.When considering the level of AST and ALT elevation > 200 U/L, the highest magnitude was observed in the DENV-3 and DENV-4 groups.[11]The exact mechanisms behind hepatic involvement in dengue infection are still under investigation and require further evidence.It could be a result of direct viral attack, particularly by viruses with a higher affinity for liver tissue, or dysregulated immunologic injury in response to the virus.[13]Some studies have reported that hypoalbuminemia is more common in the DENV-2 group compared to other virus types. Nguyen Minh Tuan found that hypoalbuminemia was associated with shock and were seen more frequently in DENV-2 compared to DENV-1 group.[8] In addition, Lovera *et al* noticed that hypoalbuminemia, an expression of capillary leakage phenomenon, were more frequently in DENV-1 and DENV-2 infection when compared to DENV-3[9]. However, in our study, we observed no difference in serum albumin among the virus serotypes. This may be due to the fact that our study only evaluated albumin in severe cases of DHF or DSS, sothis parameter only reflects albumin levels in severe dengue cases and cannot reflect the differences between different serotypes with all range of severity.

5. Conclusion

DENV-2 is the predominant circulating serotype, followed by DENV-1, and DENV-4 is rare. Infection with DENV-2 is associated with a higher incidence of dengue shock syndrome compared to DENV-1. Clinical signs indicative of severe DHF, such as abdominal pain, hepatomegaly, hemoconcentration, and thrombocytopenia, were more commonly observed in the DENV-2-infected group compared to DENV-1.

6. References

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8. Conflict of interest statement

There are no competing interest declared by the authors.