The role of different serotypes and dengue virus concentration in the prognosis of dengue shock syndrome in children

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ABSTRACT
Dengue haemorrhagic fever (DHF) is a burden of disease in tropical countries, caused by any one of four-dengue virus (DENV) serotypes (DENV-1 to DENV-4). Although there have been many studies on patients with DHF, many things remain unclear, including the role of DENV serotypes and DENV concentration. The objective of this study was to determine the role of different serotypes and DENV concentration in the prognosis of dengue shock syndrome. This was a prospective cohort study, conducted to show information relating to patients' conditions, such as hematocrit, platelet, leukocytes, and DENV concentration and the differences between DENV serotypes. The study also expressed the relationship between two groups, DHF without shock and DHF with shock, in terms of immune status, different DENV serotypes, and DENV concentration. Two-hundred and thirty-four patients were serologically confirmed as having a DENV infection. On hospital admission day (fever within 72 hours), results showed that almost all patients had a secondary dengue infection (76.5 %). DENV-1 accounted for the highest number of cases (61.11 %), and DENV-4 accounted for the lowest (0.43 %). No statistically significant difference was found when comparing the two groups (DHF with shock and DHF without shock) or when comparing the groups of different DENV serotypes. The study concluded that different DENV serotypes or DENV concentration in the first day of hospitalization (fever within 72 hours) cannot be used for prognostic of DSS.

INTRODUCTION
Dengue fever (DF) is a burden of disease and is a cause of mortality, especially in tropical regions such as Southeast Asia, Africa, and the Western Pacific (Guzman et al., 2010). Dengue virus (DENV)–of the family Flaviviridae–is transmitted predominantly by Aedes aegypti mosquitoes and is caused by any one of four DENV serotypes (DENV-1 to DENV-4) (Simmons et al., 2012).

About 50–80 million people each year are estimated to suffer from dengue infection, and at least 12,000–
24,000 deaths occur, mainly among children under 15 years of age (World Health Organization, 2011; Simmons et al., 2012; Bhatt et al., 2013; Stanaway et al., 2013). The years 2017–2018 witnessed a worldwide drop in the number of DF cases, but this was followed by a growth in cases in 2019, especially in Australia, Cambodia, China, Laos, Malaysia, the Philippines, Singapore, and Vietnam, countries that are endemic with DF (World Health Organization, 2019). Approximately 500,000 cases every year lead to severe dengue in the form of dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS) (Guzman et al., 2010). Although DSS and vascular leakage is often mentioned in adult conditions, research in 2012 showed that these syndromes can generally be more severe in children (The et al., 2012). However, if no appropriate activities are managed, severe DF may result in rapid death, particularly in children (Huy et al., 2010; Sam et al., 2013). The rate of mortality due to DSS ranges from <1% to >10, depending on the severity of DF and the experience of healthcare personnel (Wills et al., 2005; Bunnag and Kalayanarooj, 2011; Gupta et al., 2011).

In Vietnam, after a presence of more than five decades, DHF is becoming more and more complex and is developing faster. Epidemics are also developing, as well as there being an increase in the number of infected people and the proportion of mortality. DHF is an endemic disease and is an important challenge not only for Vietnam’s public health activities but also for many other countries. Despite the burden of DHF and the increasing studies on DF and the experience of healthcare personnel, there still remain some unclear things, such as the pathogenetic nature of the disease, the interaction between the immune system and DHF/DSS, the preventive vaccine, and the relationship of DENV concentration and DENV serotype with DHF/DSS, etc. Therefore, this study was conducted with the objective of investigating more closely some of the above problems: the role of different serotypes and dengue virus concentration in the prognosis of dengue shock syndrome in children.

MATERIALS AND METHODS

Sample collection and selection

The prospective cohort study was conducted using data collected from Tien Giang Hospital. The study took into account children who all had the following characteristics:

1. Being hospitalized in Tien Giang Hospital from December 2009 to November 2012
2. Being aged between 18 months old and 18 years
3. Having a fever within 72 hours and
   • Being diagnosed with general DHF on hospital admission.

The sample size was defined by the following formula from (Lwanga et al., 1991).

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

Where \( \alpha \) is the significant level of the test (the probability of type I error) in any hypothesis test. The coefficient \( Z_{(1 - \alpha/2)} \) in this study was chosen as 1.96. \( P \) is the anticipated population proportion; in this study, a \( P \) value of 0.5 was considered safe in the sampling, and the margin error (d) was 5%. According to the formula, there needed to be at least 385 cases for DHF with shock and DHF without shock. Patients who were not diagnosed with DHF did not complete the surveyor did not give consent were excluded from the study.

Laboratory methods

An amount of venous blood was collected from each child on hospital admission day and was put into EDTA-containing tubes for testing. DHF was confirmed by testing NS1 enzyme-linked immunosorbent assay (ELISA) and testing for dengue IgG/IgM by ELISA in each blood sample. The different dengue virus serotypes were determined using real-time reverse transcription-polymerase chain reaction (RT-PCR). Blood parameters continued to be monitored every day until the patients had clinical and hematological improvement.

The study also calculated the average hematocrit, platelet, leukocytes, and DENV concentration for each patient; this was also recorded.

Statistical analysis

For descriptive analyses, the study used number and percentage for categorical variables including gender, the severity of illness, immune status, day of fever, and distribution of DENV serotype. Continuous data were expressed as average and standard deviation (SD) and min-max. The study used a chi-squared test to compare univariate categorical data and an independent sample t-test or one-way analysis of variance (ANOVA) to analyze the relationship among groups for continuous variables. All analyses were performed with the statistical software STATA 10.0.

Ethical considerations
RESULTS AND DISCUSSION

In total, there were 513 admissions during the study period, collected from December 2007 to November 2009. Blood samples were tested for dengue IgG/IgM by ELISA, RT-PCR, and NS1 ELISA. Of the cases, 234 (45.6%) were IgG positive, and the remaining 279 cases (54.4%) were negative because of negative diagnosis tests or undefinable results.

Table 1 shows the baseline characteristics of the 234 study participants. There were no significant differences concerning gender characteristics with the ratio male: female = 1.2:1. Of the 234 cases that presented DHF, only 15 (6.41%) were discovered as having a shock. In terms of immune status, 23.05% of the cases were a primary infection while most (76.5%) were a secondary infection. Most children were febrile at day 2 and day 3 (97 cases (41.45%) and 119 cases (50.85%), respectively). All four serotypes were detected, but with a different distribution. DENV-1 was predominant in 143 cases, accounting for up to 61.11% of cases; this was followed by DENV-3 with 55 cases (23.5%), and DENV-2 with 35 cases (14.96%). Only one case was caused by DENV-4, belonging to the group DHF with shock.

Table 2 shows the subclinical characteristics of the children in the study on hospital admission day, including hematocrit, platelet, leukocytes, and DENV concentration. Of the 234 children, the mean hematocrit value was 39% (range [min–max]: 25.3%–53.0%). The mean platelet value on hospital admission day was 168,100/mm$^3$ (range [min–max]: 25,000/mm$^3$–372,000/mm$^3$), while the mean leukocytes value was 4,500/mm$^3$ (range [min–max]: 1,200/mm$^3$–21,000/mm$^3$). DENV concentration was measured by day of fever and by immune status. DENV concentration was the highest at day 2 of fever with 7.74E + 08 (range [min–max]: 0–4.01E + 10). The higher DENV concentration was found in the secondary infection group, with the mean DENV concentration being 5.24E + 08. There was no statistically significant difference in terms of DENV concentration between the three groups of the day of fever or between the two groups of immune status; $p$-values were 0.2981 and 0.1268, respectively.

Table 3 shows the differences of DENV serotype, immune status, and DENV concentration between the two groups, DHF with shock and DHF without shock.

With regard to the relation between subclinical characteristics and different DENV serotypes, Table 4 demonstrates the mean of each subclinical characteristic, including hematocrit, platelet, leukocytes, and DENV concentration for each serotype. There was no association between the three subclinical characteristics and DENV concentration with the different DENV serotypes; all $p$-values in the test were more than 0.05.

Characteristics of the study sample

Of the 234 children shown to be positive with dengue by the IgG/IgM (ELISA) test, the study results show that the proportion of DHF cases without shock (93.59%) was much higher than DHF cases with shock. This result is lower than Nguyen et al. (2004), who studied 62 infants under 12 months who were diagnosed with DHF, including two groups: 69.3% of children with DHF without shock (level II) and 30.7% of children with DHF with shock (15 cases with level III and four cases with level IV).

Regarding immune status, the results illustrate that most of the cases were DENV secondary infections.
Table 2: Subclinical characteristics of study participants on hospital admission day

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
<th>Min - Max</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haematocrit (%)</td>
<td>39.0 ± 4.5</td>
<td>25.3 - 53.0</td>
<td></td>
</tr>
<tr>
<td>Platelet (1000/mm³)</td>
<td>168.1 ± 67.7</td>
<td>25 - 372</td>
<td></td>
</tr>
<tr>
<td>Leukocytes (1000/mm³)</td>
<td>4.5 ± 2.3</td>
<td>1.2 - 21</td>
<td></td>
</tr>
</tbody>
</table>

**DENV concentration (copies/ml)**

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Min - Max</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>4.34E+08 ± 2.84E+09</td>
<td>0 - 4.01E+10</td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>3.50E+08 ± 6.04E+08</td>
<td>0 - 2.01E+09</td>
<td>0.298</td>
</tr>
<tr>
<td>Day 2</td>
<td>7.74E+08 ± 4.31E+09</td>
<td>0 - 4.01E+10</td>
<td></td>
</tr>
<tr>
<td>Day 3</td>
<td>1.70E+08 ± 7.87E+08</td>
<td>0 - 7.72E+09</td>
<td></td>
</tr>
<tr>
<td>Primary infection</td>
<td>1.43E+08 ± 5.49E+07</td>
<td></td>
<td>0.127</td>
</tr>
<tr>
<td>Secondary infection</td>
<td>5.24E+08 ± 2.42E+08</td>
<td></td>
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</tr>
</tbody>
</table>

Table 3: Factors related to the severity of DHF

<table>
<thead>
<tr>
<th>Factors</th>
<th>DHF with shock n (%)</th>
<th>DHF without shock n (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DENV serotype</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DENV-1</td>
<td>12 (80%)</td>
<td>131 (59.82%)</td>
<td>0.121</td>
</tr>
<tr>
<td>DENV-2</td>
<td>2 (13.33%)</td>
<td>33 (15.07%)</td>
<td>0.855</td>
</tr>
<tr>
<td>DENV-3</td>
<td>1 (6.67%)</td>
<td>54 (24.66%)</td>
<td>0.112</td>
</tr>
<tr>
<td>DENV-4</td>
<td>0 (0%)</td>
<td>1 (0.46%)</td>
<td>0.793</td>
</tr>
<tr>
<td><strong>Immune status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary infection</td>
<td>2 (12.33%)</td>
<td>53 (24.2%)</td>
<td>0.337</td>
</tr>
<tr>
<td>Secondary infection</td>
<td>13 (86.67%)</td>
<td>166 (75.8%)</td>
<td></td>
</tr>
<tr>
<td>DENV concentration (copies/ml)</td>
<td>9.08E+07 ± 4.58E+09</td>
<td>4.58E+08 ± 2.93E+09</td>
<td>0.088</td>
</tr>
</tbody>
</table>

Table 4: Relation between subclinical characteristics(SC) and DENV serotype

<table>
<thead>
<tr>
<th>SC/DENV serotype</th>
<th>DENV-1</th>
<th>DENV-2</th>
<th>DENV-3</th>
<th>DENV-4</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hct (%)</td>
<td>38.7 ± 4.4</td>
<td>40.5 ± 4.9</td>
<td>38.9 ± 4.3</td>
<td>36</td>
<td>0.175</td>
</tr>
<tr>
<td>Platelet (1000/mm³)</td>
<td>168.8 ± 67.7</td>
<td>152.6 ± 65.9</td>
<td>176.0 ± 68.7</td>
<td>175</td>
<td>0.457</td>
</tr>
<tr>
<td>Leukocytes (1000/mm³)</td>
<td>4.4 ± 2.4</td>
<td>4.1 ± 1.7</td>
<td>4.9 ± 2.3</td>
<td>4.8</td>
<td>0.397</td>
</tr>
<tr>
<td>DENV Concentration (copies/ml)</td>
<td>4.599E+08 ± 5.930E+08</td>
<td>9.268E+07 ± 5.83E+06</td>
<td>2.119E+08</td>
<td>0.872</td>
<td></td>
</tr>
</tbody>
</table>

In comparison, with Tang et al. (2010) study on 353 DENV-positive patients hospitalized in South China, this result is totally different. In Tang et al.’s study, the proportion of primary infection was more than that of secondary infection (60.1 % and 39.9%, respectively). Children were mainly hospitalized when having fever on day 2 and day 3 with rates of 41.45% and 50.85%, respectively; very few patients were hospitalized for day 1 fever (7.69%). Characteristics of different DENV serotypes during hospital admission day (fever within 72 hours)

The patient distribution by different DENV serotypes was as follows: the highest proportion was DENV-1 (61.11%), followed by DENV-3 (23.5%), DENV-2 (14.96%), and only one case was caused by DENV-4 (0.41%). Compared with the study of Tuan (2009), the distribution of different DENV serotypes among patients was that DENV-1, DENV-2, DENV-3, and DENV-4 accounted for 62.8%, 27.4%, 8.8%, and 0.9%, respectively. It can clearly be seen from these two studies that DENV-1 is...
the predominant serotype causing DHF, and these results are also similar to those of Rathakrishnan et al. (2012). This is also the conclusion in Huong et al. (2012); that is, that DENV-4 is still the serotype with the lowest proportion of DHF cases in southern Vietnam.

No statistically significant difference was found when comparing different DENV serotypes between the two groups, DHF without shock and DHF with shock ($p > 0.05$). In contrast, Tuan (2009) showed that the number of cases of DHF with shock caused by DENV-2 was seen much more, and patient condition was more severe than cases caused by DENV-1.

There is no statistically significant difference in terms of immune status between the two groups, DHF without shock and DHF with shock ($p = 0.337$). The same result is seen in Bozza et al. (2008) ($p = 0.3989$), although the ability of those secondarily infected with severe DHF was greater than the mild DHF group at rates of 42% and 28%, respectively.

Regarding hematocrit, platelet, leukocytes, and DENV serotype concentration on hospital admission day, no statistically significant difference was found among the four different serotypes, DENV-1, DENV-2, DENV-3, and DENV-4.

**Characteristics of DENV concentration on hospital admission day (fever within 72 hours)**

The mean DENV concentration was $4.34E + 08 \pm 2.84E + 09$ (copies/ml), and no statistically significant difference was found among the four different serotypes ($p=0.8717$). The mean DENV concentration on hospital admission day in the group DHF with shock was higher than in the group DHF without shock; however, this difference was not statistically significant ($p = 0.084$). Houghton-Trivino et al. (2010) on 38 patients with DHF and DF also showed that there was no association between DENV concentration and the severity of DHF or DF.

DENV concentration means were seen to be different by fever day (day 1, day 2, or day 3); among them, mean DENV concentration was highest on day 2, but the difference among these fever days was not statistically significantly different ($p = 0.2981$).

Regarding immune status, mean DENV concentration in secondary infection cases was higher than in primary infection cases ($5.24E + 08 \pm 2.42E + 08$ and $1.43E + 08 \pm 5.49E + 07$).

**CONCLUSIONS**

After analysis, the results show that there was no significant difference when comparing different DENV serotypes, immune status, and subclinical characteristics between the two groups, DHF with shock and DHF without shock. According to this research, different DENV serotypes and DENV concentration on hospital admission day (fever within 72 hours) still cannot be used for the prognosis of DSS.

**REFERENCES**


