Early clinical and biological features of severe clinical manifestations of dengue in Vietnamese adults

Pham Thai Binh [a,e,f,g,*], Severine Matheus [b], Vu Thi Que Huong [c], Xavier Deparis [d], Vincent Marechal [e,f,g]

[a] Infectious Disease Department, People's Hospital 115 of Ho Chi Minh City, Viet Nam
[b] Centre National de Référence des Arbovirus et Virus Influenza, Institut Pasteur Guyane, Cayenne, French Guiana
[c] Microbiology and Immunology Department, Pasteur Institute of Ho Chi Minh City, Viet Nam
[d] Département d'Epidémiologie et de Santé Publique, Institut de Médecine Tropicale du Service de Santé des Armées à Marseille, France
[e] Centre de Recherche des Cordeliers, Université Pierre et Marie Curie – Paris 6, UMRS 872, Paris F-75006, France
[f] Université Paris Descartes, UMRS 872, Paris F-75006, France
[g] INSERM, U872, Paris F-75006, France

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ABSTRACT

Background: Dengue is a major public health problem in both children and adults in Vietnam, but dengue severity in adults has been poorly investigated.
Objectives: To describe severe manifestations of dengue in Vietnamese adults and to identify early indicators of these manifestations.
Study design: A prospective longitudinal study was carried out from July to October, 2007, in People's Hospital 115, Ho Chi Minh City, Vietnam.
Results: One hundred ninety-five clinically suspected dengue patients were enrolled. 151 of these were laboratory-confirmed using serum samples on day 3 after onset of fever for RT-PCR and/or virus isolation. Dengue was associated with plasma leakage in 51 patients (33.8%), gallbladder thickening in 30 patients (20%), spontaneous bleeding in 127 patients (84.1%), and internal bleeding in 37 patients (24.5%). Several early indicators were associated with severe manifestations of dengue. Frequent vomiting ($\geq$3 times a day), marked lymphopenia, thrombocytopenia, and elevated liver enzymes on day 3 after onset of fever were significantly associated with plasma leakage and gallbladder thickening. Increased alanine aminotransferase level in plasma on day 3 was significantly associated with internal bleeding. Gallbladder thickening and internal bleeding were more common with specific serotypes.
Conclusion: Several severe manifestations of dengue were observed in Vietnamese adults. These manifestations were associated with early clinical and biological indicators. This information may be useful for clinicians to better monitor adult dengue patients, particularly in tropical areas where health resources may be limited.

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1. Background

Dengue has emerged as a major public health problem in both children and adults in Vietnam. Early diagnosis and regular monitoring in conjunction with appropriate supportive therapy is the best approach to decrease mortality from dengue. However, it is difficult to establish an early diagnosis and prognosis of dengue, especially in adults. World Health Organization (WHO) criteria for dengue hemorrhagic fever (DHF)[1,2] are adapted to the late phase of illness. This classification may be useful for a retrospective classification, but early indicators of the most severe forms of the disease are lacking. Furthermore, our knowledge of dengue is primarily based on clinical observations in children. Clinical descriptions and early indicators of the most severe forms of dengue still need to be established for adults.

2. Objectives

The objectives of this study were to describe severe manifestations of dengue in Vietnamese adults and to identify early indicators of these manifestations.

3. Study design

3.1. Patients

From July to October, 2007, a prospective longitudinal study was carried out in People's Hospital 115, Ho Chi Minh City, Vietnam. A total of 195 clinically suspected dengue cases (15 through 65 years of age) were consecutively enrolled after their consent was obtained. At enrollment, these patients presented with sudden onset of high continuous fever, lasting less than three days, associated with intense headache or arthralgia or myalgia, without rhinitis or signs of other clinically obvious alternative diagnosis. Laboratory-confirmation of dengue was established using serum samples on day 3 after onset of fever for reverse transcriptase polymerase chain reaction (RT-PCR) or virus isolation. The onset of fever was defined as day 1 of illness. The first day when fever disappeared (axillary temperature below 37°5) was assigned as the defervescent day.

3.2. Virus isolation and identification

For virus isolation, undiluted serum (0.05 ml per tube) on day 3 was inoculated into duplicate tubes of C6/36 (*Aedes albopictus*) cells and incubated at 28 °C for seven to fourteen days. Thereafter, cells were harvested and dengue viruses detected and typed using an indirect immunofluorescence assay with monoclonal antibodies specific for each dengue serotype (Centers for Diseases Control and Prevention, Colorado, USA).[3]

3.3. Reverse transcriptase polymerase chain reaction

Dengue viral RNA was detected using nested RT-PCR as described by Lanciotti and collaborators.[4] Dengue viral RNA was extracted from serum samples on day 3 using the QIAgen viral RNA mini kit (Qiagen) according to the manufacturer's instructions, and stored at −70 °C. Extracted dengue viral RNA was converted to cDNA using RNA amplification with a highly conserved primer pair, i.e. D1 (forward) and D2 (reverse).[4] Dengue virus sequences were amplified and typed using second-round amplification with primer D1 and four serotype-specific primers (TS1, TS2, TS3, and TS4).

3.4. Assessment of early biological features of dengue

On day 3 after onset of fever, blood samples were analyzed for complete blood count (CBC), plasma electrolytes, plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT), and prothrombin time (PT). As determined by normal hematological values for Vietnamese persons, cutoff values of white blood cell count (<4000/μL), neutrophil count (<1700/μL), lymphocyte count (<1000/μL), lymphocyte count (<500/μL) were used to define leucopenia, neutropenia, lymphopenia, and marked lymphopenia, respectively. A cutoff value for platelets (<100,000/μL) was used to define thrombocytopenia.[1,2] Plasma AST and ALT normal level was ≤40 Unit/L and the normal PT was >70%.

3.5. Assessment of severe clinical manifestations of dengue

Plasma leakage, gallbladder thickening and internal bleeding were used to assess dengue severity. On day 5, when plasma leakage (ascites and/or pleural effusion), and gallbladder thickening were usually observed,[5–7] abdominal ultrasound examination was used to detect these signs and other abnormalities, and was repeated later, if necessary. Internal bleeding involving gastrointestinal and genitourinary tracts were recorded with daily macroscopic examination of vomitus, stool, and urine.

3.6. Statistical analysis

Data were entered and analyzed using SPSS version 13.0 for Windows. In univariate analysis, chi-square or Fisher exact tests were used to compare binary variables and *t*-test was used to compare continuous variables. Variables with *p* value <0.25 in univariate analysis were subsequently entered in logistic regression models to identify independent associations. Backward approach was used to build final fitted models. The Hosmer and Lemeshow test was used to access goodness-of-fit of models.[8]

4. Results

Laboratory-confirmed dengue was observed in 151 of 195 clinically suspected cases in patients 15 through 55 years of age. Dengue-1, -2, -3 and -4 were detected in 49, 41, 38, and 4 patients, respectively. Concurrent infections with dengue-1 and dengue-4, with dengue-2 and dengue-3, and with dengue-3 and dengue-4 were detected in 17, 1, 1 patients, respectively (Table 1).

4.1. Clinical and biological features of dengue in adults

Table 2 describes clinical and biological features of dengue in Vietnamese adults compared to febrile adults without dengue. On day 3, dengue was significantly associated with leucopenia and lymphopenia in peripheral blood count (*p* < 0.05). Hyponatremia (<135 mEq/L) and hypokalemia (<3.5 mEq/L) were found in 19.4% and 69.8%, respectively. Plasma potassium levels below 3.0 and 2.5 (mEq/L) were observed in 28.6% and 3.2%, respectively. A significant increase in the occurrence of spontaneous bleeding (*p* = 0.008) and plasma leakage (*p* = 0.001) was observed in dengue patients compared to patients without confirmation of dengue. The mean duration of fever was significantly longer in dengue than in patients with presumed viral (non-dengue) illness (*p* < 0.001). Furthermore, as shown in Table 3, mucosal bleeding usually occurred before fever disappeared. No fatal cases and no dengue shock syndrome or encephalopathy were observed.

4.2. Early clinical and biological features of severe clinical manifestations of dengue

4.2.1. Univariate analysis

As shown in Table 2, dengue was manifested by several severe manifestations including plasma leakage (33.8%), gallbladder thickening (20%), or internal bleeding (24.5%). There was a significant association between gallbladder thickening and plasma leakage

Table 1
Dengue virus detection using serum samples on day 3 after onset of fever for RT-PCR and/or virus isolation.

Dengue virus typing results	Number of patients		
	Overall	RT-PCR	Virus isolation
Dengue-1	49	46	28
Dengue-2	41	41	10
Dengue-3	38	38	15
Dengue-4	4	4	1
Dengue-1 and dengue-4	17		
Dengue-1		17	12
Dengue-4		17	0
Dengue-2 and dengue-3	1		
Dengue-2		0	1
Dengue-3		1	1
Dengue-3 and dengue-4	1		
Dengue-3		1	1
Dengue-4		1	1
Total tested patients	195	194	106