

Characteristics of a dengue hemorrhagic fever outbreak in 2001 in Kaohsiung

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A dengue outbreak occurred in Kaohsiung City starting in July in 2001. We studied the clinical profile of all patients admitted to Kaohsiung Veterans General Hospital during this outbreak from July 2001 to January 2002. A total of 25 cases of clinically suspected dengue fever were treated during this period, and 13 of them were confirmed by laboratory results (13/25; 52%). Eleven of the 25 patients (11/25; 44%) were admitted. The mean age of the patients with laboratory confirmation of infection was 53 years (range, 7 to 85 years). Headache (7/13; 53.8%), bone pain (8/13; 61.5%), myalgia (10/13; 76.9%), abdominal pain (7/13; 53.8%), and skin rash (9/13; 69.2%) were the most common presentations. A high proportion of patients were classified as having dengue hemorrhagic fever (DHF) [6/13; 46.2%] and 2 of these patients had dengue shock syndrome (DSS) based on the World Health Organization criteria. Pretibial petechia (6/13; 46.1%), gastrointestinal bleeding (6/13; 46.1%), and hemoptysis (4/13; 30.8%) were the most common hemorrhagic manifestations. The average hospital stay was 7.1 days. Thrombocytopenia was very common and 84.6% patients had a platelet count less than 100,000/mm³. Monocytosis was found in all patients. Few patients required blood or platelet concentrate transfusion. The 2 patients who developed DSS both survived. All patients recovered completely without any obvious sequela. In conclusion, there was a high percentage of DHF among patients in the dengue outbreak in 2001. Increasing rates of DHF compared to previous reports from Taiwan may be a sign of hyperendemicity (multiple serotypes present) of the dengue virus in Kaohsiung City and its greater likelihood elsewhere in Taiwan. Prevention and control of both dengue fever and DHF have thus become increasingly important.

Key words: Dengue, dengue hemorrhagic fever, epidemiology, signs and symptoms

Dengue fever is a mosquito-borne febrile disease caused by any 1 of 4 serotypes of dengue virus, which belongs to the family *Flaviviridae*, and is transmitted by *Aedes aegypti* and *Aedes albopictus* [1]. The earliest outbreak of dengue fever that can be traced in Taiwan occurred in 1902. Several outbreaks have been reported since 1981 in southern Taiwan (1987, 1988, 1991, 1994, 1998) [2]. Dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) have also been sporadically reported in the past [3]. A dengue outbreak occurred in Kaohsiung City starting in July in 2001 and ending in January in 2002. This study analyzed data on patients treated from July 2001 to January 2002 including clinical manifestations and laboratory results. The proportion of DHF/DSS was compared with that from previous

outbreaks, and the serotypes of dengue virus were determined.

Materials and Methods

All patients hospitalized at Kaohsiung Veterans General Hospital due to dengue fever during the outbreak in 2001 in Kaohsiung were included in this retrospective study. Clinically suspected dengue fever was defined as the presence of fever and any 2 of the following symptoms: headache, retro-orbital pain, myalgia, polyarthralgia, skin rash, nausea and vomiting, and hemorrhagic manifestation. Blood samples were drawn in the acute stage (within 5 days after symptoms onset) and the convalescent stage (14th to 40th day after symptom onset) and were sent to the Center for Disease Control of the Department of Health in Taiwan. The diagnosis of dengue fever was confirmed based on meeting 1 of the following criteria: (1) virus isolation; (2) positive

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result of real-time polymerase chain reaction; and (3) positive result of enzyme-linked immunosorbent assay (ELISA) serologic test.

Real-time polymerase chain reaction was done with a Merck stratagene MS4000 system using dengue pre-M area as a primer. The following 2 methods were used for virus isolation: (1) mosquito cell lines — the C6/36 clone of *Aedes albopictus* cells was chosen for virus isolation; and (2) mosquito inoculation. A serology result was considered positive if the immunoglobulin M (IgM) titer was $\geq 1:40$. In the case where IgM was negative, a dengue infection was diagnosed if there was a 4-fold rise in an acute phase compared with convalescent phase anti-dengue IgG titer.

The diagnosis of DHF was made based on the finding of hemoconcentration (increase hematocrit $\geq 20\%$), evidence of transudation, thrombocytopenia (platelet $\leq 100,000/\text{mm}^3$) and hemorrhagic manifestation. Rapid and weak pulse, narrowing of the pulse pressure to less than 20 mm Hg or hypotension were the parameters used to establish the diagnosis of DSS [1]. Data collected on patients included age, gender, residential area, the duration from symptom onset to admission, and average admission days. Clinical manifestations and their frequency were analyzed, and the time sequence since symptom onset was estimated. The ratio of DHF to dengue fever was determined, and serotype was confirmed by virus isolation. The laboratory data were also compared and analyzed.

Results

In total, there were 25 cases of clinically suspected dengue fever treated at the hospital during the study period. Among these, 13 (52%) were confirmed by laboratory findings and 11 patients were admitted. The age of these patients at the time of laboratory diagnosis ranged from 7 to 85 years, and a mean of 53 years. There was no significant difference in the mean age of patients with dengue fever (50 ± 16.5 years) and DHF/DSS (55 ± 26.4 years) [$p=0.368$ by t test]. The male-to-female ratio was 1.6:1. The peak incidence was in the 30 to 50 years in males and 50 to 70 years in females. An analysis of monthly dengue incidence showed the peak incidence occurred between October and December 2001 (Fig. 1). The duration of disease onset to admission ranged from 1 to 7 days with an average of 4.3 days, and the average admission time was 7 days.

Clinical manifestations (Table 1) included myalgia (10/13; 76.9%), skin rash (9/13; 69.2%), headache

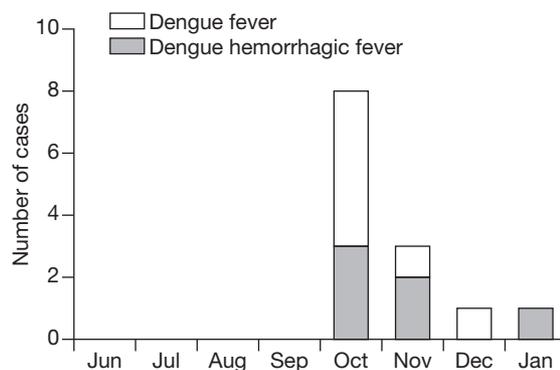


Fig. 1. Monthly distribution of dengue incidence during the 2001 outbreak in Kaohsiung (n = 13).

(7/13; 53.8%), general weakness (7/13; 53.8%), bone pain (8/13; 61.5%), abdominal pain (7/13; 53.8%), anorexia (8/13; 61.5%), cough (3/13; 21.4%), vomiting (2/13; 15.4%), diarrhea (2/13; 15.4%), and retro-orbital pain (2/13; 15.4%). All patients had fever and 4 of them (4/13; 30.8%) had fever with a peak higher than 39°C . The fever reached its peak at an average of 2.8 days after symptom onset. Seven patients developed a second fever peak at an average of 5.6 days after resolution of the initial fever. This fever displayed the so-called “saddle back” fever pattern. The duration of fever ranged from 3 to 12 days, with an average of 5.6 days. The initial presentations included headache, bone pain and myalgia, cough, and retro-orbital pain, in combination with fever attacks. General weakness, abdominal pain and anorexia usually developed on the second or third day. Skin rash, vomiting and diarrhea developed just after the first fever peak subsided. The intensity of the

Table 1. Clinical manifestations of 13 patients with dengue fever in the 2001 dengue outbreak in Kaohsiung

Signs and symptoms	No. of cases (%)
Skin rash	9 (69.2)
Headache	7 (53.8)
General weakness	7 (53.8)
Bone pain and arthralgia	8 (61.5)
Muscle pain	10 (76.9)
Abdominal pain	7 (53.8)
Chilliness/cold sweating	6 (46.2)
Anorexia	8 (61.5)
Nausea/vomiting	2 (15.4)
Cough	3 (21.4)
Retro-orbital pain	2 (15.4)
Diarrhea	2 (15.4)
Hepatosplenomegaly	3 (21.4)
Sore throat	1 (7.7)

Table 2. Hemorrhagic manifestations of 13 patients with dengue fever in the 2001 dengue outbreak in Kaohsiung

Hemorrhagic manifestation	No. of cases (%)
Petechia	6 (46.1)
Gastrointestinal bleeding	6 (46.1)
Hematuria	3 (23.1)
Epistaxis	3 (23.1)
Gum bleeding	3 (23.1)
Hemoptysis	4 (30.8)
Subconjunctiva hemorrhage	1 (7.7)
Ecchymosis	2 (15.4)

bone pain was usually described by the patients as being at an intensity that was unbearable and that they had never experienced before. This manifestation may be a very helpful characteristic in differentiating dengue from other types of viral infections. A maculopapular or scarlatiniform skin rash usually developed on the trunk on the third to fifth day just after the first fever peak subsided, and then spread to the face and extremities.

Hemorrhagic manifestation (Table 2) occurred in 8 patients (8/13; 61.5%), and included petechia on the extremities (6/13; 46.1%), gastrointestinal bleeding (6/13; 46.1%), hemoptysis (4/13; 30.8%), hematuria (3/13; 23.1%), epistaxis (3/13; 23.1%), gum bleeding (3/13; 23.1%), and trunk ecchymosis (2/13; 15.4%). According to the World Health Organization diagnostic criteria, DHF was diagnosed in 6 patients (6/13; 46.2%) and 2 of them had DSS (2/13; 15.4%). The hemorrhagic manifestation developed on the third to fifth day after symptom onset, usually within 1 day of defervescence. Evidence of capillary leakage was seen in 5 cases (5/13; 38.5%), with pleural effusion in 2 and ascites in 3.

Dengue virus serotype 2 was isolated from 4 patients with DHF. Dengue virus serotype 3 was isolated from 2 patients. One of these patients had dengue fever and the other, who had previously developed dengue fever in 1988, developed severe DSS.

Abnormal laboratory data are important indicators of dengue fever. Leukopenia (white blood cells $<4000/\text{mm}^3$) developed in 8 patients (8/13; 61.5%), and 11 patients (11/13; 84.6%) developed thrombocytopenia (platelet $<100,000/\text{mm}^3$). Severe thrombocytopenia (platelet $<20,000/\text{mm}^3$) was found in 5 patients (5/13; 38.5%) all of whom had hemorrhagic manifestations requiring platelet transfusion. All patients presented with monocytosis ($>7\%$), and 9 (9/13; 69.2%) had atypical lymphocytes in their peripheral blood. Platelet counts in the 11 hospitalized patients were followed daily. The lowest platelet counts were found between the third to seventh day. Hemoconcentration (increased hematocrit

$\geq 20\%$) developed in 5 patients (5/13; 38.5%). A significant prolongation of partial thromboplastin time (PTT) [increased by more than 2-fold vs control] was seen in 7 patients (7/13; 53.8%), but the prothrombin time (PT) was normal in all patients. Increased aminotransferase was seen in 9 patients (9/13; 69.2%). New-onset prerenal azotemia developed in 4 patients (4/13; 30.8%).

Discussion

Dengue viruses belong to the genus *Flavivirus* of the family *Flaviviridae*. They are subgrouped into 4 serotypes, dengue virus 1, 2, 3, and 4. Dengue virus infections cause a broad spectrum of illness, ranging from asymptomatic, mild undifferentiated fever, to classical dengue fever, as well as dengue fever with hemorrhagic manifestations [1]. Infection by a dengue virus can also result in severe life-threatening conditions such as DHF and DSS, which are still major causes of mortality among children in southeast Asia [4]. Seroepidemiologic studies several decades ago in Thailand first showed that DHF occurred predominantly in young children experiencing a secondary dengue infection. This observation led to the proposal that the development of DHF is determined by host immune factors related to previous exposure to dengue viruses. The development of non-neutralizing cross-reactive antibodies could cause the antibody-dependent enhancement of heterotypic-secondary dengue infection in Fc-receptor bearing cells, known as the sequential infection hypothesis of DHF/DSS [5]. Immune activation including complement activation, monocyte/macrophage and lymphocyte activation, and cytokine production may also be involved [6]. An alternative hypothesis that may account for severe dengue disease involves virus variation and virulence [7]. Available evidence suggests that both viral and host immune factors are involved in the pathogenesis of severe dengue disease. Unfortunately, the role of each is not fully understood and the lack of an animal model makes this a difficult area to study [8].

Hemostatic defects in DHF/DSS are multifactorial mechanisms that include thrombopathy, coagulopathy, and vasculopathy [9]. Thrombocytopenia is common in dengue fever, and is a constant finding in DHF/DSS [10]. In this outbreak, the majority of patients (11/13; 84.6%) had platelet count less than $100,000/\text{mm}^3$. The depression in the bone marrow observed in dengue fever in the acute stage may account for thrombocytopenia

[11]. In addition, direct infection of megakaryocytes by dengue virus could lead to an increased destruction of platelet cells [12]. The presence of antibodies directed against platelets was demonstrated in the sera of patients in this study, and the antibody isotype that showed cross-reactivity with platelets appeared to be IgM. A previous study found that the antiplatelet IgM levels were higher in sera of patients with DHF/DSS than in other dengue fever patients, particularly during the acute phase [13].

Coagulopathy is also found in most DHF cases and PTT and thrombin time are more frequently abnormal than PT [14]. In our study, PT was normal in all patients with or without hemorrhage, while PTT prolongation was observed in 53.8% of patients. Disseminated intravascular coagulation, which occurs in DHF/DSS patients and consumes both platelet and clotting factors, may contribute to the prolongation of PTT [15]. In 1 study, a reverse of the tissue plasminogen activator/plasminogen activator inhibitor-1 ratio was found in the acute stage of DHF/DSS [9]. Tissue plasminogen activator increase in dengue virus infection may result from virus infection of human monocytes and endothelial cells [16]. Besides, a high serum level of production of cytokines such as tumor necrosis factor (TNF) is found in the acute stage of dengue patients [17]. TNF has been shown to induce the promotion and subsequent inhibition of plasminogen activation in humans [18]. Therefore, both virus infection and immune response induced by dengue virus may disturb the fibrinolysis system and cause hyperfibrinolysis in the acute stage of dengue virus infection.

Outbreaks of dengue fever are generally cyclic in nature [19]. The earliest record found was in a Chinese encyclopedia of disease symptoms and remedies, first published during the Chin Dynasty (265 to 420 A.D.) and formally edited in 610 A.D. (Tang Dynasty) and again in 992 A.D. (Northern Sung Dynasty) [20]. The disease was called water poison by the Chinese and was thought to be somehow connected with flying insects associated with water. An epidemic trend of dengue virus infection in which large outbreaks have occurred about every 3 years since 1981 was recognized when an outbreak occurred on Liuochyou Shiang, a small island near Taiwan in Pintung County. Different serotypes have been recognized in the previous outbreaks [2]. This may be a risk factor for the development of hyperendemicity. The large ratio of unapparent to apparent infections, reported to be as high as 160:1 in Thailand, may result in significant

under-reporting of outbreak-related infections from hospital-based surveillance [21].

In our study, DHF developed in a very high proportion of patients (6/13; 46.2%). This ratio is higher than previously reported in other hospitals in Taiwan (about 2 to 6%) [22], although inter-hospital variation or bias may exist. DSS developed in 2 patients (2/13; 15.4%) in this study and both survived. This case-fatality rate was lower compared with previous reports [23]. Analysis of monthly dengue incidence in this outbreak showed a peak incidence between October and December, similar to previously reported outbreaks (Fig. 1). The dramatic emergence of epidemic DHF has become a global public health problem. The resurgence appears to be closely associated with demographic and societal changes over the past 50 years [24]. The factors responsible for this situation include unprecedented global population growth, uncontrolled urbanization, especially in tropical developing countries, lack of effective mosquito control methods [25], increased air travel [26], and changes in public health policy that placed emphasis on high-technology mosquito control methods rather than on larval source reduction, the only effective method [25].

Unfortunately, tools available to prevent dengue infection are very limited. Effective disease prevention programs must have several integrated components, including active laboratory-based surveillance, emergency response, education of the medical community to ensure effective case management, community-based integrated mosquito control, and effective use of vaccines when they become available [27]. Active surveillance is needed to monitor secular trends and to provide an early-warning or predictive capability for epidemic transmission. Space sprays with insecticides to kill adult mosquitoes are not usually effective unless they are used indoors [28]. The most effective way to control the mosquitoes is larval source reduction, including elimination or cleaning of water-holding containers that serve as the larval habitats [25].

Changes in the pattern of epidemiology from non-endemicity (no endemic disease) or hypoendemicity (1 serotype present) to hyperendemicity (multiple serotypes present) have been observed in many countries in Southeast Asia. Increased incidence of severe forms of disease such as DHF and DSS is also a characteristic feature of hyperendemicity.

In this regard, our finding of a high percentage of DHF in the dengue outbreak in 2001 compared to

previous outbreaks may be a sign that hyperendemicity (multiple serotypes present) of dengue has developed in Kaohsiung City and is thus increasingly likely elsewhere in Taiwan. Therefore, the need for prevention and control of dengue and DHF has become more urgent.

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