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Original Article

Effect of serotypes on clinical manifestations of dengue fever in adults

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Background and purpose: Dengue fever (DF) is a major public health issue. However, it is unclear whether different dengue virus serotypes (DENV) are associated with different clinical manifestations and outcomes. This study investigated the association between viral serotype and clinical manifestations of DF.

Methods: Adult patients with DENV-2 and DENV-3 who were treated at Kaohsiung Medical University Hospital and Kaohsiung Municipal Hsiao-Kang Hospital, Kaohsiung, Taiwan, from January 1998 to September 2007 were enrolled. The patients' demographic data, underlying diseases, clinical manifestations, laboratory data, and disease outcomes were retrospectively analyzed.

Results: 294 patients had DENV-2 and 91 had DENV-3. The median age was 50 years, and 45.7% of patients were men. Patients with DENV-3 were more likely to have a malignancy ($p = 0.011$), myalgia ($p = 0.03$), skin rash ($p < 0.001$), ascites ($p = 0.04$), and fever ($p = 0.003$) than patients with DENV-2. Patients with DENV-3 had their lowest levels of white blood cells and platelets, and peak plasma activated partial thromboplastin time (aPTT) 1 day later than patients with DENV-2. DENV-2 infection was associated with a higher monocyte count and more prolonged aPTT early in the clinical course. Infection by DENV-2 more commonly occurred as a secondary infection, while infection by DENV-3 was more common as a primary infection ($p < 0.001$). There were no differences between the groups in organ involvement, disease severity, duration of hospital stay, and medical expenditure.

Conclusion: The symptoms, signs, and laboratory findings appear to be different for patients infected with DENV-2 and DENV-3, but there is no difference in outcomes.

Key words: Dengue; Dengue hemorrhagic fever; Dengue virus

Introduction

Dengue fever (DF) is one of the most common mosquito-borne viral diseases of the tropical and subtropical regions of Asia, the Pacific islands, South America, Central America, northern Australia, and Africa. There is a long history of cyclic outbreaks in the more than 100 countries where this disease is endemic [1]. DF is a major public health problem in Asian countries, where the case fatality rate ranges from 0.5% to 3.5%. The disease can manifest as subclinical infection, mild disease, or severe and fatal dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS). The more

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severe the disease, the fewer patients there will be, as the disease presents in an 'iceberg' formation [2].

Dengue virus (DV) is the pathogen responsible for DF. DV is a positive-sense single-strand RNA virus of the *Flaviviridae* family, and has 4 distinct serotypes (dengue virus type 1 [DENV-1], DENV-2, DENV-3, and DENV-4). DV is transmitted by *Aedes aegypti* and *Aedes albopictus* [1,2]. Most dengue infections are asymptomatic or cause mild undifferentiated fever and/or rash in infants and young children [2], and most patients in Southeast Asia with confirmed dengue infection are children [3]. However, in southern Taiwan, most patients are adult. These patients usually experience typical DF, which is generally self-limiting, and is characterized by high fever, pain in 5 characteristic areas (severe frontal headache, retro-orbital pain, bone pain, myalgia, and arthralgia), and maculopapular rash [2]. Typical laboratory findings include leukopenia with an increase of monocytes and atypical lymphocyte count, thrombocytopenia, elevated aminotransaminase, and prolonged activated partial thromboplastin time (aPTT). Some patients with DF experience bleeding complications [2].

Viral factors can affect the severity of the disease and the outcome [2], but there are few data about the clinical manifestations of the different DV serotypes in adults. It is possible that different DV serotypes manifest differently in adults. This study examined the effect of different DV serotypes on the manifestations of DF in adults of southern Taiwan.

Methods

The medical records of all patients diagnosed with dengue infection between January 1998 and December 2007 at the Kaohsiung Medical University Hospital and Kaohsiung Municipal Hsiao-Kang Hospital Kaohsiung, Taiwan, were retrospectively reviewed. All patients older than 15 years with serotype-confirmed DV infection were included. Diagnosis was confirmed by the Centers for Disease Control (CDC) of Taiwan based on 1 of the following criteria: positive reverse-transcriptase polymerase chain reaction (RT-PCR) for dengue virus RNA; positive DV isolation; detection of dengue-specific immunoglobulin (Ig) M antibody ≥1:40 in acute phase serum; or a 4-fold increase of IgG antibody titers in the convalescent phase by enzyme-linked immunosorbent assay (ELISA) [4]. Laboratory identification of the serotype and the presence of primary or secondary infection were supported by the CDC.

DENV serotypes were detected by a primer set targeting the region of the capsid (C) gene via real-time RT-PCR after the region of the non-structural protein 5 genes had been targeted by another primer set to detect all flaviviruses. Four sets of serotype-specific primers targeting the C gene were used to differentiate the DENV serotypes of the positive samples [5]. Primary and secondary DENV infections were differentiated by envelope/membrane-specific capture IgM and IgG ELISA in acute phase and convalescent phase serum samples. Infection was defined as primary DENV infection if the ratio of the IgM to IgG was ≥1.2 and secondary DENV infection if the ratio was <1.2 [6].

Data collected included the patients' basic demographic characteristics, past medical history, manifestations (symptoms and signs), imaging findings, laboratory data, treatments, duration of hospital stay, and medical expenditure. Patients who had not visited a foreign country within the dengue incubation period were classified as having indigenous infection.

The fever onset day was defined as day 1 of the disease course; fever was considered to have subsided when the body temperature was no higher than 37.5°C. Laboratory data of red blood cell and white blood cell (WBC) counts, coagulation profiles, and liver function tests were recorded in parallel with the fever days. Plasma leakage was characterized by pleural effusion diagnosed by chest radiograph or ascites diagnosed by ultrasound (US) or computed tomography (CT). Pseudocholecystitis was defined as a self-limiting illness with positive Murphy sign and thickened gallbladder wall diagnosed by US or CT. Associated diseases were determined by analysis of system-specific symptoms and signs. Diagnosis of DHF was made according to the World Health Organization criteria of: plasma leakage syndrome with hemoconcentration (hematocrit ≥20% above baseline), pleural effusion, or ascites; fever lasting for 2 to 7 days; thrombocytopenia (platelet count, <100,000 cells/mm^3); and hemorrhagic tendency demonstrated by a positive tourniquet test or spontaneous bleeding [7].

Statistical analysis

Data were analyzed by the Statistical Package for the Social Sciences for Windows (Version 13.0; SPSS, Inc., Chicago, IL, USA). Continuous data are presented as mean ± standard deviation (SD) for normally distributed data and as median and range for non-normally distributed data. Categorical data are presented as numbers and percentages. Non-parametric tests, including

the Mann-Whitney *U* test for 2 unpaired groups and the Kruskal-Wallis test for ≥3 unmatched groups, were used for analysis of non-normally distributed data. Categorical data were compared by chi-squared test, with or without Fisher's exact test. Survival analysis was performed by the Kaplan-Meier method with the log-rank test or Wilcoxon test. A p value ≤0.05 was considered to be statistically significant.

Results

Demographic characteristics

401 serotype-confirmed patients were enrolled, including 15 patients with DENV-1, 294 with DENV-2, 91 with DENV-3, and 1 with serotype-4. As the number of patients with DENV-1 or DENV-4 were too few to be representative, these patients were excluded, so only 385 patients with DENV-2 and DENV-3 were enrolled for analysis.

Men and women were equally represented, and the median age was 50 years (Table 1). The distribution of age and sex did not differ between patients with DENV-2 and DENV-3. Malignancy was more common in the DENV-3 group (Table 1).

Clinical manifestations

These patients had most of the classic symptoms of DF (Table 2), including fever (99.7%), chills (82.9%), bone pain (77%), myalgia (67.8%), upper respiratory tract symptoms (73.6%), gastrointestinal symptoms (96.9%), and skin rash (34.6%). Among the patients with hemorrhagic manifestations, petechiae were the most common sign (38.8%). When petechiae were excluded, 33.5% of patients presented with any sign of bleeding (Table 2).

There was no significant difference between the DENV-2 and DENV-3 groups for most symptoms. Myalgia, ascites, and skin rash were significantly more common in the DENV-3 group, and bone pain tended to be more common in the DENV-2 group. There was no significant difference between the groups in the incidence of pleural effusion, effects on organ systems, or incidence of hepatitis (Table 3). Patients with DENV-3 had a tendency to have a higher incidence of acute hepatitis with serum aminotransferase (alanine aminotransferase and/or aspartate aminotransferase) levels more than 5-fold the upper limit of normal ($p = 0.06$, data not shown).

Laboratory findings

Serial blood samples taken on days 3 to 8 of the febrile period showed that the lowest median WBC count was on day 4 ($2.8 \times 10^3/\mu L$) for DENV-2 patients and on day 5 ($2.6 \times 10^3/\mu L$) for DENV-3 patients (Fig. 1). There were no differences in WBC counts on comparable days between the groups. The lowest median platelet count was on day 6 ($39 \times 10^3/\mu L$) for DENV-2 patients and on day 7 ($43.5 \times 10^3/\mu L$) for DENV-3 patients. There were no differences in platelet counts on comparable days between the groups.

The median monocyte count was higher in the DENV-2 group than the DENV-3 group from days

Table 1. Demographic profile and underlying diseases of patients with dengue virus serotypes (DENV) 2 and 3.

Variable	DENV-2 (n = 294) No. (%)	DENV-3 (n = 91) No. (%)	Total (n = 385) No. (%)	p^a
Age (years; median [range])	49 (15-96)	52 (16-82)	50 (15-96)	0.606[b]
Sex				
Male	138 (46.9)	38 (41.8)	176 (45.7)	0.386
Female	156 (53.1)	53 (58.2)	209 (54.3)	
Indigenous disease	292 (99.3)	89 (97.8)	381 (99.0)	0.238[c]
Underlying disease				
Malignancy	7 (2.4)	8 (8.8)	15 (3.9)	0.011
Diabetes mellitus	22 (7.5)	8 (8.8)	30 (7.8)	0.684
Chronic hepatitis C virus	8 (2.7)	2 (2.2)	10 (2.6)	1.000
Chronic hepatitis B virus	15 (5.1)	8 (8.8)	23 (6.0)	0.194
Chronic kidney disease	3 (1.0)	1 (1.1)	4 (1.0)	1.000
Cardiovascular disease[d]	49 (16.7)	19 (20.9)	68 (17.7)	0.357

[a] χ^2 test.

[b] Mann-Whitney *U* test.

[c] Fisher's exact test.

[d] Hypertension, heart failure or ischemic heart disease.

Table 2. Clinical presentations of patients with dengue virus serotypes (DENV) 2 and 3.

Presentation	DENV-2 (n = 294) No. (%)	DENV-3 (n = 91) No. (%)	Total (n = 385) No. (%)	p^a
Fever	294/294 (100)	90/91 (98.9)	384/385 (99.7)	0.236[b]
Chills	160/190 (84.2)	48/61 (78.7)	208/251 (82.9)	0.319
5-ache symptoms[c]	244/247 (98.8)	78/80 (97.5)	322/327 (98.5)	0.599[b]
Bone pain	175/220 (79.5)	42/62 (67.7)	217/282 (77.0)	0.051
Myalgia	91/144 (63.2)	48/61 (78.7)	139/205 (67.8)	0.030
Upper respiratory tract symptoms[d]	129/180 (71.7)	44/55 (80.0)	173/235 (73.6)	0.220
Gastrointestinal symptoms[e]	191/195 (97.9)	59/63 (93.7)	250/258 (96.9)	0.103[b]
Skin rash	85/291 (29.2)	47/91 (51.6)	132/382 (34.6)	<0.001
Petechiae	114/294 (38.8)	35/90 (38.9)	149/384 (38.8)	0.985
Any bleeding sign	97/294 (33.0)	32/91 (35.2)	129/385 (33.5)	0.701[b]
Pleural effusion	35/294 (11.9)	10/91 (11.0)	45/385 (11.7)	0.812
Ascites	6/294 (2.0)	6/91 (6.6)	12/385 (3.1)	0.040[b]
Gallbladder wall thickening	10/294 (3.4)	5/91 (5.5)	15/385 (3.9)	0.361[b]

[a]χ^2 test.
[b]Fisher's exact test.
[c]Myalgia, arthralgia, bone pain, retro-orbital pain, or headache.
[d]Cough, rhinorrhea, nasal stuffiness, or sore throat.
[e]Abdominal pain, right upper quadrant pain, diarrhea, or nausea/vomiting.

Table 3. Organ involvement of patients with dengue virus serotypes (DENV) 2 and 3.

Organ system	DENV-2 (n = 294) No. (%)	DENV-3 (n = 91) No. (%)	Total (n = 385) No. (%)	p^a
Cardiovascular				
Chest pain	6/294 (2.0)	4/91 (4.4)	10/385 (2.6)	0.256[b]
Angina	2/294 (0.7)	1/91 (1.1)	3/385 (0.8)	0.556[b]
Transient bradycardia	4/294 (1.4)	0/91 (0)	4/385 (1.0)	0.577[b]
New-onset arrhythmia	0/294 (0)	1/91 (1.1)	1/385 (0.3)	0.236[b]
Hepatobiliary system				
Pseudocholecystitis	3/294 (1.0)	1/91 (1.1)	4/381 (1.0)	1.000[b]
Acute hepatitis[c]	13/294 (4.4)	6/91 (6.6)	19/385 (4.9)	0.410[b]
Polyneuropathy[d]	3/294 (1.0)	1/91 (1.1)	4/385 (1.0)	1.000[b]
Psychiatric[e]	2/294 (0.7)	1/91 (1.1)	3/385 (0.8)	0.556[b]

[a]χ^2 test.
[b]Fisher's exact test.
[c]Alanine aminotransferase >200 IU/L (normal range, 10-40 IU/L).
[d]New-onset numbness/paresthesia and positive neuron conducting velocity test.
[e]New-onset depression.

3 to 8, and was significantly higher on days 3 and 4. This was similar for aPTT. The median aPTT for the DENV-2 and DENV-3 groups reached a peak on day 6 and day 7, respectively. The median daily aPTT was more prolonged for the DENV-2 group from days 3 to 6, but this difference disappeared by day 7. The frequency of hemoconcentration was similar between the 2 groups ($p = 0.707$, data not shown).

Outcomes

DENV-2 infection was more commonly associated with secondary infection ($p < 0.001$). Disease outcomes of number of patients with DF and DHF, duration of hospital stay, and associated medical expenditure were similar for the 2 groups (Table 4). None of the patients experienced DSS and all patients survived the infection. Infection with DENV-3 was associated with more febrile days than infection with DENV-2 ($p = 0.003$) [Fig. 2 and Table 4]. The patients in the DENV-2 and DENV-3 groups received similar treatments, such as amounts of fluid administration ($p = 0.626$), platelet transfusion ($p = 0.77$), and fresh frozen plasma transfusion ($p = 0.117$).

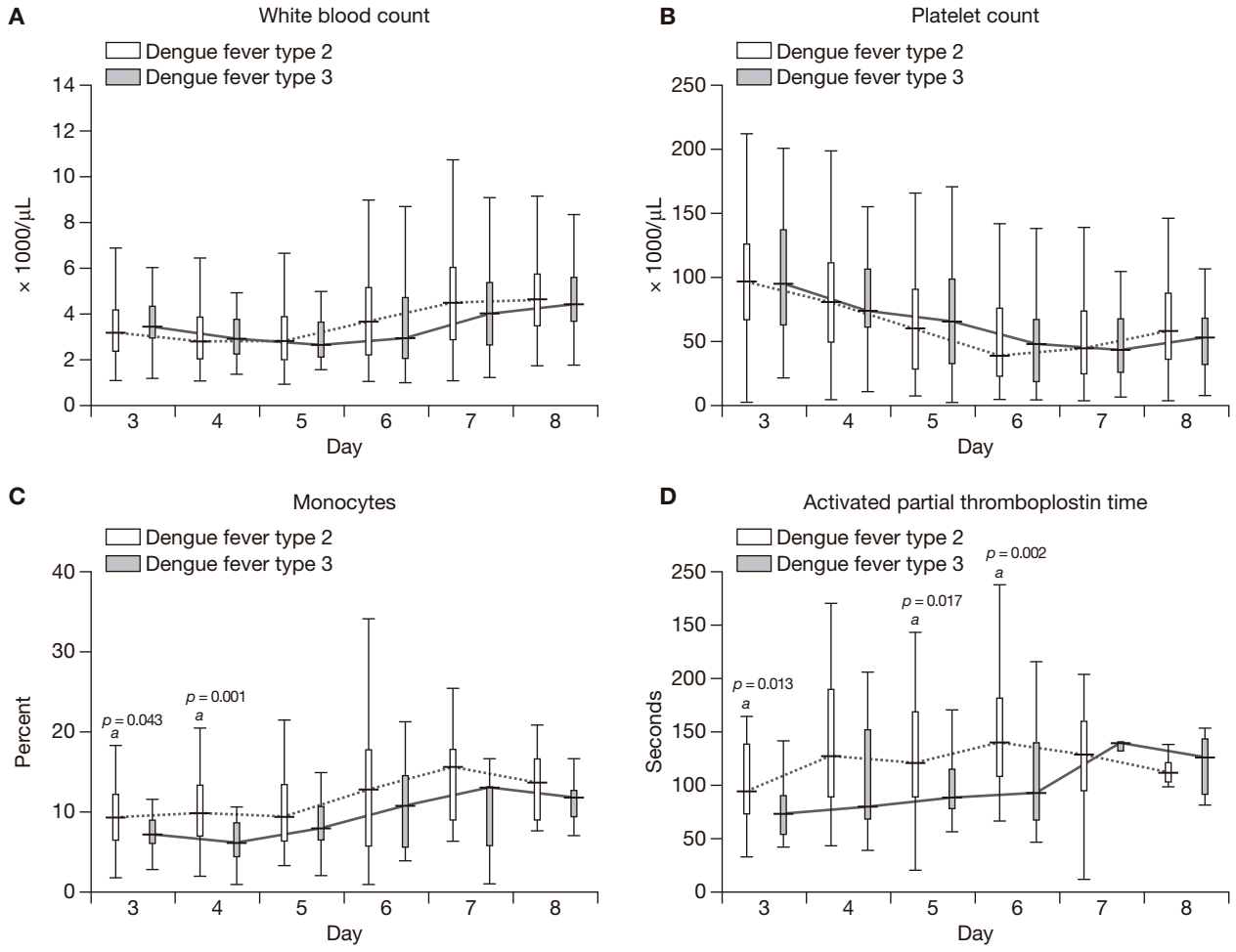

Fig. 1. Comparison of the kinetic change of (A) white blood cell count (normal range, 4-10 × 103/μL); (B) platelet count (normal range, 130-500 × 103/μL); (C) monocyte percentage (normal range, 0-10%); and (D) activated partial thromboplastin time (normal range, 24.0-36.8 sec) in peripheral blood between adult patients with dengue virus serotype 2 and 3 infection during fever days 3 to 8. Data represent the median ± range.
[a]$p \leq 0.05$.

Discussion

These data clearly demonstrate that infection by DENV-2 and DENV-3 has different clinical manifestations in adults. DENV-3 infection occurred more frequently in patients with malignancy. There are 2 possible explanations for this finding. One explanation could be that outbreaks of DENV-2 and DENV-3 occurred in different districts of Kaohsiung, with differing prevalences of malignancy. However, the outbreak areas were similar. The other reason is that DENV-3 may have a preference for replication in an environment that might also be suitable for the development of malignancy. Activation of mast cells and basophils has been implicated in the pathogenesis of DF [8]. Many allergic diseases are also associated with mast cell activation [9] and skin rash is a common manifestation of many allergic diseases. The results showed that DENV-3 infection was more associated with skin rash than DENV-2. Therefore, it appears that different DENVs may have different pathogeneses. Furthermore, monocytes are the principal targets of the dengue virus [10]. It has previously been reported that DENV-2 can infect human monocytes more efficiently than DENV-3 and might replicate more rapidly [10]. This study also showed that DENV-3 infection was associated with a more prolonged suppression of WBCs, monocytes, platelets, and aPTT. Therefore, there might be some differences between DENV-2 and DENV-3 that affects the disease manifestations. Given the limitations of this study, these findings must be confirmed in the future.

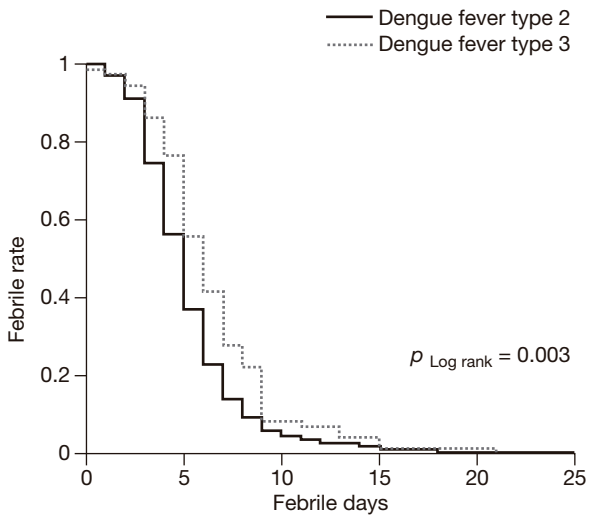

Fig. 2. Fever clearance time of dengue fever serotypes 2 (n = 224) and 3 (n = 72) by Kaplan Meier analysis.

DV infection can cause inflammation of the liver, with thickening of the gallbladder wall, ascites, and pleural effusion [11]. The detection of DV antigens in hepatocytes and the recovery of DV particles from liver biopsies support the view that DV is hepatotropic [12]. These results show that DENV-3 infection is more associated with acute hepatitis in adults, similar to that previously reported for children [13]. However, this study did not find that DENV-2 infection causes more shock, plasma leakage, and complications of fluid overload, in contrast to the results for children [13]. Ascites and pleural effusion are 2 primary signs of plasma leakage. In this study, DENV-3 was more associated with ascites, but not pleural effusion, than DENV-2. The reason for this is not clear. It is possible that DENV-3 might have a preference for the peritoneum, but this speculation needs further study.

Bleeding episodes are related to several factors, including vasculopathy, thrombocytopenia, platelet dysfunction, and prothrombin-complex deficiency [14]. Immune-mediated destruction of platelets [15,16] and platelet consumption caused by the release of high levels of platelet-activating factor from monocytes [17] have been implicated in causing thrombocytopenia during DV infection. Thrombocytopenia and coagulopathy appear to predispose patients to bleeding episodes. This study found that DENV-2 infection was associated with a higher monocyte count and more prolonged aPTT in association with thrombocytopenia in the early stage of disease. However, there were no differences in hemorrhagic manifestations between DENV-2 and DENV-3 infection.

This retrospective study demonstrates that DENV-2 and DENV-3 have different clinical manifestations in adults from southern Taiwan. However, this difference is valid only after exclusion of confounding factors. Factors such as age, presence of secondary viral infections, and chronic diseases can alter the manifestations of DF [18,19]. Chronic diseases such as bronchial asthma and diabetes, hypertension, and renal insufficiency have been suggested as risk factors for DHF [2,18]. This study showed no difference in the incidence of these chronic diseases between the 2 groups. This analysis of the association of clinical manifestations and secondary infections also indicated no statistically significant difference between the 2 groups. Therefore, the conclusion that DENV-2 and DENV-3 have different clinical manifestations appears to be valid.

There was no difference in outcomes caused by the different serotypes. The clinical outcomes and medical expenditure did not differ according to viral

Table 4. Outcomes of patients with dengue fever serotypes (DENV) 2 and 3.

Outcome	DENV-2 (n = 294) No. (%)	DENV-3 (n = 91) No. (%)	Total (n = 385) No. (%)	p^a
Final diagnosis				
Dengue fever	236/294 (80.3)	75/91 (82.4)	311/385 (80.8)	0.65
Dengue hemorrhagic fever	58/294 (19.7)	16/91 (17.6)	74/385 (19.2)	
Primary/secondary infection				
Primary	29/129 (22.5)	38/74 (51.4)	67/203 (33.0)	<0.001
Secondary	100/129 (77.5)	36/74 (48.6)	136/203 (67.0)	
Fever (days; median [range]/no.)	5 (1-25)/224	6 (0-21)/72	5 (0-25)/296	0.003[b]
Duration of hospital stay (days; median [range]/no.)	6 (2-29)/215	5.5 (3-21)/70	6 (2-29)/285	0.507[b]
Medical expenditure (NT$; median/no.)	17,204/215	14,986/70	16,876/285	0.747[b]

[a]χ^2 test.
[b]Mann-Whitney *U* test.

serotype. However, this study was hospital-based and excluded patients from the community who had subclinical infections. Therefore, the results only apply to patients with relatively severe DV infection. Severe DHF is associated with secondary infection. Infection with DENV-2 superimposed on previous DENV-1 infection carries the highest risk for development of DHF [20-23]. Although this study found that DENV-2 infection occurred more frequently as a secondary infection, there was not more DHF in the DENV-2 group. Therefore, other factors of host characteristics or the interaction of risk factors could also be important. The interactions of risk factors (host, viral, and epidemiologic) determine the occurrence of a DHF epidemic [2]. High vector density, high virus circulation, and a more susceptible host population (at risk for secondary infection) also contribute to the occurrence of DHF [2]. DENV-2 DHF can occur 16 to 20 years after primary infection by DENV-1 [24]. Previous reports have shown that when DENV-1 and DENV-2 outbreaks arise 20 years apart, the ratio of adults with DF to adults with DHF/DSS was 24:1 [25,26]. DENV-1 outbreaks occurred in Kaohsiung during 1987 and 1988 [27], 15 years before the 2002 DENV-2 outbreak. This study showed that the ratio of DF to DHF was 4:1. This different DF/DHF ratio in this study could be due to different time intervals between the first and second infections. If so, DHF could occur more frequently with a shorter time interval between infections, and the DF/DHF ratio could be even lower. An alternative explanation is that this hospital-based study included only 2 hospitals. If the study expanded into the community, more DF patients would be included and the ratio of the DF/DHF would be higher. Therefore, the relationship between the time intervals of different dengue outbreaks and the DF/DHF ratio needs further study.

This study employed a retrospective design. All the data were recorded in the patients' chart and should therefore not be subject to recall bias. Furthermore, both DF and DHF begin with a sudden rise in body temperature [2], so the first febrile day should be obvious. It is therefore likely that the finding that patients with DENV-3 had more febrile days is valid.

DENV-2 and DENV-3 infection can manifest differently with regard to symptoms, signs, and laboratory findings, but there were no differences in outcomes. DV serotype is important in disease manifestation.

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