JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Clinical manifestations of primary and secondary dengue in Paraguay and its relation to virus serotype

Dolores Lovera[1,2], Celia Martínez-Cuellar[1,2], Fernando Galeano[1,2], Sara Amarilla[1], Cynthia Vazquez[3], Antonio Arbo[1,2]

[1] Department of Pediatrics, Institute of Tropical Medicine, Asuncion, Paraguay
[2] National University of Asuncion, Asuncion, Paraguay
[3] Central Laboratory of Public Health, Asuncion, Paraguay

Abstract

Introduction: Dengue virus (DENV) infection is currently a major cause of morbidity in the world, and its incidence has mainly increased during the last years in Latin America, including Paraguay. The objective of this study was to compare the clinical and laboratory findings of dengue and assess whether the serotype is a risk factor for severity.

Methodology: We included patients ≤ 15 years old hospitalized with dengue at the Institute of Tropical Medicine, from Paraguay, from 2007 to 2018. Patients were classified according to the 2009 WHO dengue classification guidelines and were stratified by serotype into three groups according to the hospitalization period: the 2007 epidemic (DENV-3), the 2012-2013 epidemic (DENV-2) and the 2016-2018 epidemic (DENV-1).

Results: Of 784 children hospitalized for dengue, 50 cases (6.3%) were caused by DENV-3, 471 (60%) by DENV-2, and 263 (33.5%) by DENV-1. Myalgias and headache were associated with DENV-3 cases and abdominal pain was associated with DENV-2 cases. Bleeding was observed mainly in DENV-1 and 2 cases. Patients with DENV-2 infections experienced more severe disease, required fluid expansion more frequently, and exhibited more often shock and admission in the ICU. Secondary cases of dengue were more severe that primary cases. Patients with infection by DENV-2 had longer hospital stays (5.1 ± 2.8 days) when compared to DENV-3 o DENV-1 infection cases (2.9 ± 1.6 days and 4.36 ± 2.7 days, respectively) ($p < 0.05$).

Conclusions: The DENV serotype has a profound impact on the clinical manifestations and severity of dengue. DENV-2 infections were associated more frequently to requirement of fluid expansion, shock, and longer hospital stay.

Key words: Dengue; children; serotype; Paraguay.

J Infect Dev Ctries 2019; 13(12):1127-1134. doi:10.3855/jidc.11584

(Received 19 April 2019 – Accepted 08 August 2019)

Introduction

Dengue is one of the most prevalent arboviral infections [1]. It is transmitted by mosquitoes of the genus *Aedes*, mainly *A. aegypti*. Each year, approximately 50 to 80 million cases of dengue occur throughout the world with 500000 people requiring hospitalization and dengue being responsible for 12,000 to 24,000 death per year [1-3].

Dengue virus (DENV) has four serotypes, named from 1 to 4 [2]. Infections with different serotypes cause identical clinical syndromes [4]. Multiple factors have been suggested to contribute to severe dengue, such as age of patients [5], viral load [6], secondary infections [7], and serotypes [8]. Previous reports of dengue in children have suggested that infections with DENV-2 and DENV-3 are related to more severe forms of the disease than the other serotypes [9-12], while others authors have reported that DENV- 1 causes more severe forms of the illness [13,14]. Additionally, there would be some differences in the severity of the disease depending on the geographic region where dengue is found. In fact, it has been reported that DENV-2 and DENV-3 infections from non-Southeast Asia regions, as well as DENV-2, DENV-3 and DENV-4 from the Southeast Asia region, resulted in a greater percentage of severe infections [15].

Paraguay is a country with a high-endemicity epidemiological profile of dengue, with periodic significant epidemic outbreaks [16]. The country has been affected by different DENV serotypes in the last decade, with a well-differentiated pattern showing a particular serotype circulating virtually exclusively during the different epidemics. Therefore, the objective of this study was to analyze in Paraguay the clinical and laboratory characteristics of dengue cases based on

serotype and to determine if there is an association between the serotypes and dengue severity.

Methodology

Study design, population and setting

This was an observational, descriptive and retrospective study and was conducted at the Institute of Tropical Medicine of Paraguay. This institution is the main reference center for infectious and tropical diseases. All patients ≤ 15 years old hospitalized in the pediatric service with a diagnosis of dengue between 2007 and 2018 were included.

Definition of the circulating serotype

The circulating serotype in each epidemic was determined through sentinel surveillance at reference centers. At each sentinel center, samples were obtained from 10% of patients who meet the criteria for suspected cases of dengue as well as from all critical patients who required admission to the hospital. The samples were sent to the Central Public Health Laboratory where the determination of the circulating DENV serotype was carried out through the RT-PCR method [17].

Serological and dengue antigen detection studies

In most patients, when the evolution time of the fever was less than 5 days, demonstration of DENV NS1 protein by immunochromatography (Bioeasy, Standard Diagnostics INC, Republic of Korea) was done. Independently of evolution time, qualitative detection of IgG and IgM antibodies specific to dengue (anti-DENV IgG and IgM; SD Dengue IgM and IgG Capture enzyme-linked immunosorbent assay, StandardDiagnostics INC, South Korea) was performed in the majority of hospitalized cases. All the tests were carried out according to the manufacturer's' instructions.

Stratification of patients according to serotypes

Given that the circulation of DENV serotypes in the country has followed a very characteristic pattern, with virtually a single serotype circulating in different yearly epidemics, we were able to study the dengue patient population based on the year of hospitalization and the DENV serotype. Thus, hospitalized patients diagnosed with dengue during 2007 corresponded to DENV-1, hospitalized patients diagnosed with dengue during 2012-2013 corresponded to DENV-2, while hospitalized patients with diagnosis of dengue during 2016-2018 corresponded to DENV-1.

Definitions

We used the PAHO/WHO 2009 dengue definitions [4]. A suspicious case was confirmed by laboratory tests or by epidemiological link (in the epidemic period or in the context of the outbreak). Laboratory confirmation comprises at least one of the following laboratory results: presence of the NS1 antigen, de novo seroconversion of anti-DENV IgM antibodies, four-fold increase of IgG titers by neutralization test or virus genome identification by RT-PCR.

Severe dengue was defined by the occurrence of plasma leakage and/or fluid accumulation that led to shock (Dengue Schock Syndrome) or respiratory distress; and/or severe bleeding; and/or severe organ impairment. The nonsevere dengue group was divided into patients with and without warning signs. Cases of dengue with warning signs included the following symptoms and/or signs: abdominal pain, persistent vomiting, oral intolerance, clinical accumulation of fluid, bleeding of mucous membranes, lethargy/restlessness, precordial pain, hepatomegaly or laboratory data, such as thrombocytopenia (platelets less than 100,000/mm^3), and hemoconcentration, documented by the hematocrit increased up to 20% or more [18]. Only cases of dengue with warning signs or severe criteria were hospitalized.

Cases of dengue were defined as primary when patients at admission exhibited positive RT-PCR or NS1 antigen capture test with positive anti-DENV IgM and negative anti-DENV IgG. In the absence of positive RT-PCR or NS1 antigen test, the new detection of anti-DENV IgM with negative anti-DENV IgG was also considered a primary infection. Patients with positive RT-PCR or NS1 antigen test with positive anti-DEN IgG (with or without anti-DEN IgM positive) were considered to have a secondary DENV infection.

Statistical analysis

We compared the characteristics of patients infected with different DENV serotypes. Gender, age, presence of warning signs, laboratory findings and fluid therapy were particularly addressed. We used the chi-square test to compare proportions, and Student t test for categorical variables. Significant findings in univariate analysis were further assessed by multivariate logistic regression with correction for age and gender, with results given as Relative Risk (RR) and 95% confidence intervals (CIs).

Ethical clearance

This study was approved by the Ethics Committee of the Institute of Tropical Medicine. Informed consent

was not obtained from parents or legal guardians because this is a retrospective study based on secondary data that did not use patient identification data.

Results

General characteristics

During the study period, 784 patients aged ≤ 15 years were admitted to the Institute of Tropical Medicine with a diagnosis of DENV infection. Demographic, clinical and laboratory data of the population are shown in Table 1. The average age of the DENV patients admitted was 9.84 ± 4.46 years. The most frequently affected age group were children aged > 5 (84.3% of the total). Both genders were equally affected (male / female ratio 1:1).

When analyzing the clinical findings, fever was present in virtually all cases (98.5%). Other common symptoms of DENV disease such as headache, myalgia, and exanthema were observed in 59.4%, 46% and 38% of the patients, respectively. Gastrointestinal manifestations were prevalent in our series. Vomiting was observed in 506 patients (64.5%), and abdominal pain was found in 511 cases (65.2%). Furthermore, 25% of patients (n = 196) presented some type of bleeding (epistaxis, gingivorrhagia, hematuria, or metrorrhagia).

Regarding the laboratory data, the mean number of white blood cells at admission was 5,160 ± 3,427/mm^3, the average level of hemoglobin was 14 ± 3.2 g/dL and the platelet counts were 87,691 ± 77,676/mm^3. Liver involvement was frequent. The levels of alanine aminotransferase and aspartate aminotransferase at admission were 94 ± 329 IU/mL (normal up to 35 IU/mL) and 150 ± 465 IU/mL (normal up to 40 IU/mL), respectively.

Three hundred and sixty-one patients (46%) were admitted with signs of shock or developed shock during their hospitalization. The rest of the patients presented warning or severe signs without shock (n = 387, 49%). Seventy children (8.9%) required admission in the intensive care unit (ICU). The mortality in this series was 0.5% (5 patients).

Clinical manifestations and serotypes

The mean age of cases to DENV-1, -2 and -3 was 9.3 ± 5 years, 10.1 ± 4.1 years and 10.5 ± 2.9 years, respectively ($p > 0.5$) (Table 2). Similarly, no differences were found in the gender. When symptoms were comparatively analyzed (Table 2), rash was more frequent in those affected by DENV-2 ($p < 0.05$, RR 2.5, 95% CI 1.5-4.2). In contrast, patients with DENV-3 infections presented myalgia ($p < 0.05$, RR 1.58, 95% CI 1.3-1.9) and headache ($p < 0.05$, RR 1.25, 95% CI 1.1-1.5) more frequently. Regarding gastrointestinal manifestations, patients infected with DENV-2 presented abdominal pain more frequently when compared with those infected with DENV-3 ($p < 0.01$, RR 1.58, 95% CI 1.27-1.97) or DENV-1 ($p < 0.01$, RR 1.58, 95% CI 1.41-1.79). The presence of bleeding was similar among those infected by DENV-2 and DENV-1 ($p = 0.5$, RR 1.25, 95% CI 0.95-1.65) but higher when compared with DENV-3 ($p < 0.01$; RR 2.8, 95% CI 1.28-6.12). Finally, the presence of shock was significantly associated with infection by DENV-2 ($p < 0.05$, RR 12.5, 95% CI 4.2-37.5). Visceral (unusual) complications such as myocarditis, severe hepatitis and encephalitis were also analyzed in these DENV patients with no differences found between the different serotypes (Table 3).

Regarding the laboratory characteristics of DENV cases, the presence of thrombocytopenia < 100,000/mm^3 was significantly higher in DENV-1 infections ($p = 0.014$, RR 1.2, 95% CI 1-1.4). However, the incidence of hypoalbuminemia < 3.5 g/dL ($p < 0.05$, RR 1.9, 95% CI 1.3-2.6) and prolonged activated partial thromboplastin time (APTT)($p = 0.049$; RR 1.66, 95% CI 1-2.8) were higher in DENV-2 infections. The frequency of liver involvement (measured in terms of

Table 1. Demographic, clinical and laboratory findings in patients with dengue.

Characteristic	N° of patients N = 784 (%)
Age (mean ± SD; years)	9.84 ± 4.46
Age groups	
< 5 years	122/775 (15,7)
>5 years	653/775 (84.3)
Male	398 (50.8)
Female	386 (49.2)
Signs/symptoms	
Headache	466 (59.4)
Myalgias	361 (46)
Nausea/vomiting	506 (64.5)
Bleeding	196 (25)
Rash	298 (38)
Abdominal pain	511 (65.2)
Laboratory	
WBC/mm^3 (mean ± SD)	5,160 ± 3,427
Hemoglobin (g/dL) (mean ± SD)	14 ± 3.2
Platelets/mm^3 (mean±SD)	87,691 ± 77,676
Urea (mg/dL)	19.9 ± 22.5
Sodium (mEq/L)	130.7 ± 32.2
Potassium (mEq/L)	5.55 ± 13.69
ALT (IU/mL, mean ± SD)	94 ± 329
AST (IU/mL, mean ± SD)	149.9 ± 464.8

ALT: alanine aminotransferase; AST: aspartate aminotransferase; WBC: white blood cells.

increased transaminases) was similar in all three serotypes (Table 2).

Patients with infection by DENV-1 and DENV-2 had hemoconcentration more frequently than those suffering from DENV-3 infection ($p < 0.01$, RR 6.45, 95% CI 3.41-12.22). Incidentally, patients with DENV-2 infections required fluid expansion more frequently ($p < 0.01$, RR 1.17, 95% CI 1.1-1.2) and had more frequent admission in ICU ($p < 0.01$; RR 2.65, 95% CI 1.46-4.8) (Table 4). Finally, patients with DENV-2 infection had longer hospital stays (5.1 ± 2.8 days) when compared to patients with DENV-3 (2.9 ± 1.6 days) or DENV-1 (4.36 ± 2.7 days) infection ($p < 0.05$) (Table 2).

Characteristics of primary and secondary DENV disease according to serotype

We analyzed the pattern of secondary DENV infections (Tables 4 and 5). When the epidemic by DENV-3 occurred in Paraguay in 2007, the majority of the cases were primary as cases of DENV in Paraguayan children by 2007 were sporadic. After 2007, there were no cases of infection with DENV-3. Thus, the majority of secondary infections were caused by DENV-1 or DENV-2. When comparing the clinical presentation of cases with primary infection (n = 204) versus secondary infection (n = 27) caused by DENV-1 (Table 4), there were no differences in the frequency of general symptoms. However, abdominal pain was identified more frequently in cases of secondary infection ($p = 0.006$, RR 1.65, 95% CI 1.2-2.2) (Table 5). Regarding laboratory tests findings, an increase in liver enzymes ($p = 0.027$, RR 2.9, 95% CI 1.1-7.5) and hypoalbuminemia ($p = 0.004$, RR 3.1, 95% CI 1.4-6.8) were significantly associated with secondary infection.

Table 2. General characteristics of patients with dengue according to serotype.

Variables	DENV-1 N = 263 (%)	DENV-2 N = 471 (%)	DENV-3 N = 50 (%)	P value[a]	RR (95% CI)
Clinical variables					
Age (years ± SD)	9.3 ± 5	10.1 ± 4.1	10.5 ± 2.95	>0.5	
Male sex	131(49.8)	240 (51)	24 (48)	>0.5	-
Female sex	132 (50.2)	231 (49)	26 (52)	>0.5	
Fever	256 (97.3)	465 (99)	50 (100)	NS	-
Rash	26 (9.9)	259 (55)	11 (22)	**< 0.05**	2.5 (1.5-4.2)
Myalgia	99 (37.6)	226 (48)	38 (76)	**< 0.05**	1.58 (1.3-1.9)
Headache	125 (47.5)	301 (64)	40 (80)	**< 0.05**	1.25 (1.1-1.5)
Vomiting	155 (58.9)	320 (68)	27 (54)	0.51	1.09 (0,8-1.4)
Abdominal pain	126 (47.9)	358 (76)	24 (48)	**< 0.01**	1.58 (1.2-1.9)
Bleeding	59 (22.4)	132 (28)	5 (10)	< 0.1	2.24 (0.9-5.3)
Fluid expansion requirement	221(84)	462 (98)	0 (0)	**< 0.01**	1.17 (1.1-1.2)
Shock	5 (1,9)	353 (75)	3 (6)	**< 0.05**	12,5 (4.2-37.5)
PICU	12 (4,6)	57 (12)	1 (2)	**< 0.01**	2,65 (1.46-4.8)
Deaths	1 (0,4)	3 (0,6)	1 (2)	0.29	3,14 (0.3-29.6)
Length of stay (days ± SD)	4.36 ± 2.7	5.1 ± 2.8	2.9 ± 1.6	**< 0.05**	
Laboratory variables					
Platelets < 100.000	150 (57)	224 (48)	13 (26%)	**0.01**	1.2 (1-1.4)
AST ≥ 5 times normal	34/231 (14,7)	41/442 (9)	2/26 (7.7)	0.33	1.9 (0.5-7.5)
ALT≥ 5 times normal	13/231 (5.6)	25/442 (6)	2/26 (7.7)	0.67	1.4 (0.3-5.7)
AST ≥ 1000	3/231 (1.3)	8/442 (1.8)	0 (0)	0.62	1.4 (0.4-5.2)
ALT ≥ 1000	2/231 (0.9)	4/442 (1)	0 (0)	1.05	1.1 (0.2-5.7)
Albumin < 3.5 g	28/88 (31.8)	159/269 (57.9)	0/0 (0)	**< 0.05**	1.9 (1.3-2.6)
Prolonged aPTT	14/86 (16.3)	58/215 (27)	1/10 (10)	**< 0.05**	1.66 (1-2.8)

[a]*P* value of chi-square test for categorical variables and T of Student for continuous variables; those in bold are significant.

Table 3. Visceral complications in patients with Dengue according to serotype.

Complication	DENV-1 N = 263(%)	DENV-2 N = 471(%)	DENV-3 N = 50 (%)	P value	RR (95% CI)
Severe hepatitis	3 (1.1)	8 (1.7)	1 (2)	0.62	1.7 (0.2-16.5)
Myocarditis	2 (0.8)	7 (1.5)	1 (2)	0.41	2.6 (0.2-28.5)
Encephalitis	2 (0.8)	4 (0.5)	2 (4)	0.06	5.3 (0.8-36.5)

Table 4. Clinical and laboratory characteristics of patients with secondary DENV-1.

Variables	Primary cases N = 204 (%)	Secondary cases N = 27 (%)	P value	RR (IC95%)
Clinical variables				
Fever	202 (99)	27 (100)	1	-
Rash	17 (8)	4 (15)	0.27	1.78 (0.6-4.9)
Myalgia	76 (37)	10 (37)	0.98	0.99(0.6-1.7)
Headache	93 (46)	16 (59)	0.18	1.3 (0.9-1.8)
Vomit	113 (55)	19 (70)	0.14	1.27(1-1.7)
Abdominal pain abdominal	87 (42)	19 (70)*	0.006	1.65(1.2-2.2)
Bleeding	42 (21)	6 (22)	0.84	1.08(0.5-2.3)
Shock	5 (3)	1 (3.7)	0.7	1.5(0.2-12.5)
Expansions	167 (82)	27 (100)*	< 0.05	0.82 (0.68-0.9)
PICU admission	7 (3)	3 (11)	0.07	3.2 (0.9-11.8)
Decease	1 (0.5)	0 (0)	0.88	-
Days in hospital (± SD)	4,4 ± 2,7	4,3 ± 2,7	0.5	
Laboratory variables				
Leukocytes (mm^3) (X ± DE, mm^3)	4,757 ± 3,108	4,751 ± 3,098	0.5	
Hematocrit (%) ((%)(X ± DS,%)	40.8 ± 4.6	41.3 ± 4.2	0.5	
Hemoconcentration	89 (44)	14 (52)	0.42	1.2 (0.8-1.8)
Platelets (mm^3) (< 100.000/mm^3)	118 (58)	19 (70)	0.21	1.22(0.9-1.6)
AST ≥ 5 times	13 (6)	5 (18.5)*	0.027	2,9(1.1-7.5)
ALT ≥ 5 times	3 (2)	3 (11)*	0.003	0.56 (0.3-1.2)
AST ≥ 1000	1 (1)	1 (3.7)	0.09	7.6 (0.5-117.3)
ALT ≥ 1000	0 (0)	1 (3.7)	0.12	-
Albumin < 3.5 g/dL	17 (8)	7 (26)*	0.0049	3.1 (1.4-6.8)
Prolonged aTTP	4 (4)	1 (3.7)	0.56	1.9 (0.2-16.3)

AST: aspartate aminotransferase test; ALT: alanine aminotransferase test; aTTP: activated partial thromboplastin time; *Significant differences.

Table 5. Clinical and laboratory characteristics of patients with secondary DENV-2.

Variables	Primary cases N = 136 (%)	Secondary cases N = 253 (%)	P	RR
Clinical variables				
Fever	135 (99)	248 (98)	0.477	1.01 (1-1)
Rash	78 (57)	136 (54)	0.52	1.06(0.9-1.3)
Myalgias	63 (46)	124 (49)	0.59	0.94 (0.8-1.2)
Headache	81 (59.5)	169 (67)	0.14	1.1 (1-1.3)
Vomit	88 (65)	187 (74)*	0.049	1.15 (1-1.3)
Abdominal pain	95 (70)	209 (82.6)*	0.003	1.2 (1-1.3)
Bleeding	25 (18)	71 (28)*	0.033	1.5 (1-2.3)
Shock	96 (70.5)	191 (75)	0.26	1.07 (0.9-1.2)
Expansions	129 (95)	248 (98)*	0.044	1.04 (1-1.1)
PICU	17 (12.5)	36 (14)	0.63	1.14 (0.7-2)
Deceases	0 (0)	3 (1)	-	-
Days in hospital (± SD)	5.1 ± 2.8	5.1 ± 2.8		
Laboratory variables				
Leukocytes/mm^3 X ± DE)	5,522 ± 3,617	5,522 ± 3,617	0.5	
Hematocrit (%)	41.3 ± 6.9	41.4 ± 6.7	0.5	
Hemoconcentration	77 (56.6)	183 (72)*	0.001	1.3 (1.1-1.5)
Platelets< 100.000/mm^3	96 (70.6)	212 (84)*	< 0.05	0.62 (0.5-0.8)
AST ≥ 5 times	10 (7)	25 (10)	0.4	1.4 (0.7-2.7)
ALT ≥ 5 times	3 (2)	18 (6)*	0.04	3.24 (1-10.8)
AST ≥ 1000	3 (2)	4 (1.6)	0.66	1.4 (0.3-6.1)
ALT ≥ 1000 ≥ 1000	1 (0.7)	2 (0.8)	0.95	1.1 (0.1-11.9)
Albumin< 3.5 gr/dL	34 (25)	97 (38)*	0.007	1.5 (1.1-2.1)
Prolonged aTTP	14 (10)	38 (15)	0.19	1.46 (0.8-2.6)

AST: aspartate aminotransferase test; ALT: alanine aminotransferase test; aTTP: activated partial thromboplastin time; *Significant differences.

These findings showing a greater illness severity are consistent with the higher fluid therapy rates in secondary cases of DENV-1 ($p < 0.05$, RR 0.82, 95% CI 0.68-0.98) and a higher trend to shock ($p = 0.07$) (Table 4). However, overall the frequency of shock was low in patients with primary or secondary infection caused by DENV-1.

Similar results were observed when secondary cases of DENV-2 infection were analyzed (Table 5). Markers of severe disease such as vomiting ($p = 0.049$; RR 1.15, 95% CI 1-1.3), abdominal pain ($p = 0.003$; RR 1.2, 95% CI 1-1.3) and presence of bleeding ($p = 0.033$; RR 1.5, 95% CI 1-2.3) were found more frequently in secondary cases. In addition, secondary cases showed more frequently thrombocytopenia < 100,000/mm^3 ($p < 0.05$; RR 0.62, 95% CI 0.5-0.8), increase in liver enzymes ($p = 0.04$, RR 3.24, 95% CI 1-10.8) and hypoalbuminemia ($p = 0.007$; RR 7.5, 95% CI 1.1-2.1). This increase in severity was translated into higher rates of fluid treatment in secondary cases of DENV-2 ($p = 0.044$; RR 1.04, 95% CI 1-1, 1). However, the incidence of established shock was similar in both primary and secondary cases caused by DENV-2 (Table 5).

Discussion

The recent introduction and establishment of multiple circulating serotypes of DENV in several regions of the world, including South America, has increased the importance of understanding and characterizing the role of each serotype in the clinical presentation and evolution of DENV disease [8-15].

This cross-sectional study showed that the distribution of age groups and gender was similar in infections by different DENV serotypes. Among the general symptoms, children hospitalized with DENV-3 infection had a higher prevalence of musculoskeletal manifestations (myalgias) ($p< 0.05$; OR 1.58, 95% CI 1.3-1.9) and headache ($p < 0.05$; RR 1.25, 95 % CI 1.1-1.5) compared to infections with DENV-1 and DENV-2. Halsey *et al*. [8] had similar findings in a study performed in several Latin American countries, which included 466 patients under the age of 20. However, Yung *et al*. [13] in a study of adult population in Singapore did not find differences in the frequency of musculoskeletal manifestations and headache among infections with the DENV-1, -2 or -3.

Cutaneous manifestations were reported in 38% of the patients. Individuals with DENV-2 infection presented cutaneous manifestations more frequently than those infected with other serotypes. This is in correspondence with the findings of Halsey *et al*. [8]. Although the frequency of vomiting was similar in DENV cases caused by different serotypes, the presence of abdominal pain was more frequent in infections with DENV-2. This phenomenon was previously reported by Thomas *et al*. [19] in a Martinique population and Kumaria R [20] in India. However, in the study by Halsey *et al*. [8] this finding was most frequently associated with DENV-3. However, as in this previous study only 16 pediatric patients with DENV-2 infection were included, results might be biased and may not be comparable to our study. Although only 21.7% of the patients in the present series had hemorrhagic manifestations, they were more frequent in infections with DENV-1 and DENV-2 compared to infections with DENV-3.

The analysis of the laboratory profile of DENV cases showed some interesting differences. Although there was no difference in the white blood cell count in cases related to different serotypes, a finding previously reported by other authors [21], the frequency of thrombocytopenia (platelets less than 100,000/mm^3)was higher in infections with DENV-1 and DENV-2 compared to DENV-3 (0.014, RR 1.2, 95% CI 1-1.4). Rodrigues da Costa Faria *et al*. [22] in Brazil, also found that cases of DENV-2 exhibited higher decrease in platelets number. However, Balmaseda *et al*. [11] found that the incidence of thrombocytopenia was higher in infections with DENV-1 compared to cases of DENV-2. Additionally, Fried *et al*. in Thailand [23] did not find differences in the incidence of thrombocytopenia when they compared cases of DENV caused by the four serotypes.

Two additional findings were highly related to the viral serotype. Hypoalbuminemia and hemoconcentration were observed more frequently in DENV-1 and DENV-2 infections. Since both factors are an expression of the capillary leakage phenomenon, this could explain the severity increase of DENV-1 and DENV-2 infections compared to DENV-3. In relation to these observations, 98% of patients with DENV-2 infection and 84% with DENV-1 infection required one or more fluid treatments with physiologic saline, which was more than the required by patients with DENV-3 infection. Furthermore, the presence of shock, which represents the most severe expression of the disease, was significantly associated with DENV-2 infection (< 0.05, RR 12.5, 95% CI 4.2-37.5).

The relationship between DENV serotypes and the severity of disease has previously been addressed in a number of works. Several chracteristics influence the severity of the disease such as age of patients, viral load, secondary infection, and the force of infection observed in a particular epidemic period [5-7, 15, 22-24].

However, it is probably that the serotype may be the main factor that influences the disease severity. In this sense, the associationof severity increase with DENV-2 was previously reported by Vaughn *et al*. [10] in Thailand, Balmaseda *et al*. [11] in Nicaragua and Thomas *et al*. [12, 19] in Martinique. However, Yung *et al*. [13] in Singapore and Anantapreecha *et al*. [14] in Thailand reported that DENV-1 was most frequently associated with severe forms.

Our study showed also that secondary cases of DENV infection, both caused by serotype 1 and 2, were more severe than primary cases and confirm partly the pioneering observations of Halstead [25] that cases of secondary dengue may be more severe than the primary cases. One of the important hypotheses that explain the severity of secondary cases of DENV is that after an infection by a certain serotype a long-lasting homotypic immunity is generated for subsequent episodes of infection with the same serotype and a short-lived heterotypic immunity against different serotypes causing the primary infection. However, long-lasting antibodies do not neutralize DENV of different serotypes but improve the entry of DENV into cells that express the Fc receptor, a phenomenon known as antibody-dependent enhancement or ADE [26]. It is therefore possible that in Paraguay, where successive epidemics and different serotypes have occurred in the last decade, a phenomenon of immune enhancement could be taking place.

Several reasons can explain why DENV severity varies in different parts of the world and in the same location. Studies of our group have shown that severe cases of DENV exhibit a particular profile of cytokines (higher levels of IFN I) [27]. It is likely that differences in the cytokine profile in relation to the viral serotype and within a serotype occur depending on whether it is a primary or secondary infection. Moreover, the DENV polyprotein shows a 30% divergence among the four serotypes. Within each serotype there are several genotypes that have a geographical distribution. Some data indicate that genetic changes in DENV strains could directly affect the expression of disease in infected humans [28]. In this sense, it has been shown that DENV-2 of Asian origin replicates to higher titers in human dendritic cells, infects *A. aegypti* mosquitoes more efficiently and is transmitted at a higher rate than American DENV-2 strains [29-30]. The change of genotype within the same serotype can occur in the same country over the years. This phenomenon was observed with DENV-4 in Puerto Rico where three major epidemics occurred in 1982, 1986 and 1998; however, the last two epidemics were caused by different subtypes [31]. Similar changes of subtypes of DENV-2 were observed in Brazil and Peru where the circulation of a different lineage of American/Asian DENV-2 genotype was the cause of more severe forms [32,33].

This study has several limitations. The retrospective design may have influenced the data collection of different characteristics of the patients. However, because the same medical team dealt with all of the patients, this aspect may have been minimized. Another limitation is that the study site is a reference center. This may have caused a population bias resulting in the most seriously ill patients being referred to the institution. However, as the same situation was present during the different epidemics, the study findings cannot be explained by this single factor.

Finally, this study which covers several DENV outbreaks occurring in a period of more than a decade has allowed a comparation of DENV cases infected by three different serotypes and is providing useful data for a more complete analysis of the topic. Our study showed a severity increase of infections caused by DENV-2 as well as in secondary DENV cases. These data may contribute to the implementation of preventive measures during DENV epidemics.

References

1. Stanawa S, Shepard DS, Undurraga EA, Halasa YA, Luc E Coffeng LE, Brady OJ, Hay SI, Bedi N, Bensenor IM, Castañeda-Orjuela CA, Chuang TW, Gibney KB, Memish ZA, Rafay A, Ukwaja KN, Yonemoto N, Murray CJL (2016) The global burden of dengue: an analysis from the Global Burden of Disease Study 2013. Lancet Infect Dis 16: 712-723.
2. Wilder-Smith A, Ooi E-E, Horstick O, Wills B (2019) Dengue. Lancet 393: 350-363.
3. Suaya JA, Shepard DS, Siqueira JB, Martelli CT, Lum LCS, Tan LH, Kongsin S, Jiamton S, Garrido F, Montoya R, Armien B , Huy R, Castillo L, Caram M, Sah BK, Sughayyar R, Tyo KR, Halstead SB (2009) Cost of dengue cases in eight countries in the Americas and Asia: a prospective study. Am J Trop Med Hyg 80: 846-855.
4. World Health Organization (WHO) (2009) Dengue: Guidelines for diagnosis, treatment, prevention and control. New ed. Geneva. Switzerland: World Health Organization.147 p.
5. Dinh The T, Le Thi Thu T, Nguyen Minh D, Tran Van N, Tran Tinh H, Nguyen Van Vinh C, Wolbers M, Dong Thi Hoai T, Farrar J, Simmons C, Wills B (2012) Clinical features of Dengue in a large Vietnamese cohort: intrinsically lower platelet counts and greater risk for bleeding in adults than children. PLoS Negl Trop Dis 6: e1679.
6. Nunes PCG, Nogueira RMR, Heringer M, Chouin-Carneiro T, Damasceno Dos Santos Rodrigues C, de Filippis AMB, Lima MDRQ, Dos Santos FB (2018) NS1 antigenemia and viraemia load: potential markers of progression to dengue fatal outcome? Viruses 10: E326
7. Guzmán MG, Kouri G, Bravo J, Soler M, Martínez E (1991) Sequential infection as risk factor for dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) during the 1981

dengue hemorrhagic cuban epidemic. Mem Inst Oswaldo Cruz.86: 367.
8. Halsey ES, Marks MA, Gotuzzo E, Fiestas V, Suarez L, Vargas J, Aguayo N, Madrid C, Vimos C, Kochel TJ, Laguna-Torres A (2012) Correlation of serotype-specific dengue virus infection with clinical manifestations. PLoS Negl Trop Dis 5: e1638
9. Anders KL, Nguyet NM, Chau NV, Hung NT, Thuy T, Lien LB, Farrar J, Wills B, Hien TT, Simmons CP (2011) Epidemiological factors associated with dengue shock syndrome and mortality in hospitalized dengue patients in Ho Chi Minh city, Vietnam. Am J Trop Med Hyg 84 :127-134
10. Vaughn DW, Green S, Kalayanarooj S, Innis B, Nimmannitya S, Suntayakorn S, Endy TP, Raengsakulrach B, Rothman AL, Ennis FA, Nisalak A (2000) Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. J Infect Dis 181: 2-9.
11. Balmaseda A, Hammond SN, Perez L, Tellez Y, Saborio ST, Mercado JC, Cuadra R, Rocha J, Perez MA, Silva S, Rocha C, Harris E (2006) Serotype-specific differences in clinical manifestations of dengue. Am J Trop Med Hyg 74: 449-456
12. Thomas L, Najioullah F, Besnier F, Valentino R, Rosine J, Cesaire R, Cabié A, and the Working Group on Dengue (2014). Clinical presentation of dengue by serotype and year of epidemic in Martinique. Am J Trop Med Hyg 91: 138-145.
13. Yung CF, Lee KS, Thein TL, Tan LK, Gan VC, Wong JGX, Lye DC, Ng LC, Leo YS (2015). Dengue serotype-specific differences in clinical manifestation, laboratory parameters and risk of severe diseases in adults, Singapore. Am J Trop Med Hyg 92: 999-1005
14. Anantapreecha S, Chanama S, Auegoonp AA, Naemkhunthot S, Sa-Ngasang A, Sawanpanyalert P, Kurane I (2005) Serological and virological features of dengue fever and dengue haemorrhagic fever in Thailand from 1999 to 2002. Epidemiol Infect 133: 503-507.
15. Soo KM, Khalid B, Ching SM, Chee HY (2016) Meta-analysis of dengue severity during infection by different dengue virus serotypes in primary and secondary infections. PLoS ONE 11: e0154760.
16. Lovera D, Araya S, Mesquita MJ, Avalos C, Ledesma S, Arbo A (2014). Prospective applicability study of the new dengue classification system for clinical management in children. Pediatr Infect Dis J. 33: 933-935.
17. Santiago GA, Vergne E, Quiles Y, Cosme J, Vazquez J, Medina JF, Medina F, Colón C, Margolis H, Muñoz-Jordan JL (2013). Analytical and clinical performance of the CDC Real Time RT-PCR assay for detection and typing of dengue virus. PLoS Negl Trop Dis 7: e2311.
18. Lovera D, Martinez de Cuellar C, Araya S, Amarilla S, Gonzalez N, Aguiar C, Acuña J, Arbo A (2016) Clinical characteristics and risk factors of dengue shock syndrome in children. Pediatr Infect Dis J 35: 1294–1299.
19. Thomas L, Verlaeten O, Cabie A, Kaidomar S, Moravie V, Martial J, Najioullah F, Plumelle Y, Fonteau C, Dussart P, Césarie R (2008) Influence of the Dengue serotype, previous dengue infection, and plasma viral load on clinical presentation and outcome during a Dengue-2 and Dengue-4 Co-Epidemic. Am J. Trop. Med. Hyg 78: 990-998.
20. Kumaria R (2010) Correlation of disease spectrum among four Dengue serotypes: a five years hospital based study from India. Braz J Infect Dis 14: 141-146.
21. Carmo AMS, Suzuki RB, Cabral AD, Costa RT, Massari GP, Riquena MM, Fracasso HA, Eterovic A, Marcilli A, Speranca MA (2017) Co-circulating serotypes in a dengue fever outbreak: differential hematological profiles and phylogenetic relationships among viruses. J Clin Virol 90: 7-13.
22. Rodrigues da Costa Faria N, Fiestas Solorzano E, de Souza LJ, Nogueira RMR, de Bruycker-Nogueira F, Chouin-Carneiro T, Santos Simões JB, da Rocha Queiroz Lima M, de Oliveira Pinto LM, Kubelka CF, Heringer M, de Azeredo EL, Barreto Dos Santos F (2017) Analysis of clinical and laboratory alterations related to dengue case severity: comparison between serotypes 2 and 4 in Brazil. Am J Trop Med Hyg 97: 137-145.
23. Fried JR, Gibbons RV, Kalayanarooj S, Thomas SJ, Srikiatkhachorn A, Yoon I-K, Jarman RG, Green S, Rothman AL, Cummings DAT (2010) Serotype-specific differences in the risk of dengue hemorrhagic fever: an analysis of data collected in Bangkok, Thailand from 1994 to 2006. PLoS Negl Trop Dis 4: e6.
24. Nishiura H, Hoang PL, Tran NTT, Phan GT, Quoc Le H, Tran BQ, Van Nguyen N, de Vries PJ (2011) Age-specificity of clinical dengue during primary and secondary infections. PLoS Negl Trop Dis 5: e1180.
25. Halstead SB (1979) *In vivo* enhancement of Dengue virus infection in rhesus monkeys by passively transferred antibody. J Infect Dis 140: 527-533.
26. Guzman MG, Gubler DJ, Izquierdo A, Martinez E, Halstead SB (2016) Dengue infection. Nat Review 2: 1-25.
27. Talarico LB, Byrne AB, Amarilla S, Lovera D, Vázquez C, Chamorro G, Acosta PL, Ferretti A, Caballero MT, Arbo A, Polack FP (2017) Characterization of type I Interferon responses in dengue and severe dengue in children in Paraguay. J Clin Virol 97: 10-17.
28. Ko HY, Li YT, Chao DY, Chang YC, Li ZT, Wang M, Kao CL, Wen TH, Shu PY, Chang GJ, King CC (2018). Inter- and intra-host sequence diversity reveal the emergence of viral variants during an overwintering epidemic caused by dengue virus serotype 2 in southern Taiwan. PLoS Negl Trop Dis 12 : e0006827.
29. Anderson, JR, Rico-Hesse R (2006) *Aedes aegypti* vectorial capacity is determined by the infecting genotype of dengue virus. Am J Trop Med Hyg 75: 886-892.
30. Hanley KA, Nelson JT, Schirtzinger EE, Whitehead SS, Hanson CT (2008) Superior infectivity for mosquito vectors contributes to competitive displacement among strains of dengue virus. BMC Ecol 8: 1.
31. Bennett SN, Holmes EC, Chirivella M, Rodriguez DA, Beltran M, Vorndam V, Gubler DJ, McMilan WO (2003) Selection-driven evolution of emergent dengue virus. Mol Biol Evol 20: 1650-1658.
32. Michelli Faria Oliveira MF, Galvão Araújo JM, Ferreira OC Jr., Ferreira DF, Lima DB, Santos FB (2010) Two lineages of dengue virus type 2, Brazil. Emerg Infect Dis 16: 576-578.
33. Heringer M, Nogueira RM, de Filippis AM, Lima MR, Faria NR, Nunes PC, Nogueira FB, dos Santos FB (2015). Impact of the emergence and re-emergence of different dengue viruses' serotypes in Rio de Janeiro, Brazil, 2010 to 2012. Trans R Soc Trop Med Hyg 109: 268-274.

Corresponding author
Antonio Arbo, MD, MSc
Department of Pediatrics, Institute of Tropical Medicine
Av, Venezuela and Florida, Asuncion, Paraguay
Phone: 00595981404843
Fax: 0059521292164
E-mail: antonioarbo@hotmail.com

Conflict of interests: No conflict of interests is declared.