Research Article

Age-associated differences in clinical manifestations and laboratory parameters during a dengue virus type 4 outbreak in Argentina†

Short Title: Dengue virus type 4 in children vs. adults

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ABSTRACT

Infection by any of the four dengue virus (DENV) serotypes produces a wide spectrum of clinical illness in humans. Differences in clinical manifestation and severity have been associated with secondary heterologous infection, patient age and virus serotype. In this context, this retrospective study sought to analyze the presentation of dengue in patients during the 2014 DENV-4 outbreak affecting the City of Orán, Salta Province, Argentina. Demographic data, clinical manifestations and laboratory abnormalities of laboratory-confirmed dengue patients were compared between age groups and between patients with and without warning signs. Of 301 patients with laboratory-confirmed dengue, 37.9% presented dengue with warning signs. Although nearly half of all patients had secondary DENV infections, no severe dengue cases or deaths were reported. Furthermore, no association was found between incidence of warning signs and pre-existing immunity to DENV. Pediatric patients were least likely to present warning signs and showed significantly decreased risk of fever, retro-orbital pain, arthalgia, diarrhea and thrombocytopenia and higher risk of rash compared to older patients. Female patients of all ages were also at higher risk of developing several symptoms. The characterization of DENV-4 infection in humans, a DENV serotype recently reported in Argentina, revealed differences in clinical manifestations, laboratory parameters and the presence/absence of warning signs based on age group. Further investigation of these age-related differences should contribute to better assessment of dengue disease in at risk populations.

Keywords: dengue virus, epidemiology, pediatric population, disease outcomes, age-related differences

INTRODUCTION

Dengue virus (DENV) is a flavivirus that produces the most prevalent arthropod-borne viral disease in humans. After a lapse in the region-wide vector eradication put in place during the mid 20th century, DENV serotypes 1-4 have re-emerged in the Americas, making dengue a public health priority [1,2]. DENV infection produces a wide range of clinical manifestations including both asymptomatic and clinical infection [3]. The latter are grouped by the World Health Organization (WHO) into three levels of disease severity: dengue without warning signs (DWoWS), a febrile illness frequently associated with rash and joint pain; dengue with warning signs (DWWS), in which the patient may also experience hemorrhage, swelling, hepatomegaly and/or an increase in hematocrit and thrombocytopenia; and the potentially fatal severe dengue (SD), which may include severe bleeding, shock, organ failure and respiratory distress [4]. Differences in clinical manifestations and severity have been associated with secondary heterologous DENV infection [5], patient age [6-8] and virus serotype [9,10], though the underlying mechanisms of disease enhancement are still not fully characterized.

Since the re-emergence of DENV in Argentina in 1998, important outbreaks have occurred in 3-5 year cycles. The majority of cases occur in Northern provinces, where the sub-tropical climate and occasional winter frosts allow for seasonal proliferation of the most common DENV vector, *Aedes aegypti*. Though *A. aegypti* flight range is extremely limited [11], frequent domestic and commercial contact with bordering countries in which DENV is endemic can lead to DENV importation and the autochthonous spread of the virus. The city San Ramón de la Nueva Orán (also referred to as Orán) is the head of the homonymous department; it has a population of approximately 140,000 inhabitants and it is located in the province of Salta on the Northwestern border of Argentina, 270 km Northeast of the province

capital Salta and 30 km South of Bolivia (Figure 1). Since 1998, the province of Salta has experienced outbreaks of all four DENV serotypes; DENV-2 in 1998 [12], DENV-1 in 2002, DENV-3 in 2003 (specifically in Orán), both DENV-1 and DENV-2 between 2008-2014 [13,14], and DENV-4 in 2013 and 2014, the latter mainly affected the Northern departments, including the Department of Orán [13,15]. DENV-4 has been endemic in Central America since its introduction to the eastern Caribbean islands in 1981 [2]. In recent years, DENV-4 has been reported in increasingly southern latitudes in South America, and was reported for the first time in Argentina in 2010 in a single infection in the province of Santa Fe [16,17].

Dengue is considered a pediatric disease in endemic settings [18], where many adults have acquired immunity to multiple serotypes through repeated heterotypic infection. Because epidemiological studies are primarily conducted in endemic regions [19], studying dengue outbreaks in re-emergent, epidemic settings offers an interesting opportunity to evaluate clinical manifestations in adults and children who have had similar lifetime exposure to DENV. Furthermore, each DENV serotype represents a population of viral genotypes whose evolution may influence disease manifestation and patient prognosis. The current study describes the clinical manifestations and laboratory abnormalities of children and adults in the first autochthonous DENV-4 outbreak in Argentina.

MATERIALS AND METHODS

Study Population

This retrospective study was conducted at the regional public San Vicente de Paul Hospital between February and June 2014. San Vicente de Paul Hospital is the only high-complexity hospital in the Department of Orán, which provides medical assistance to the City of Orán and surrounding areas, with a catchment population of 170,000 persons. Four

private clinics also offer medical care in the municipality. During DENV outbreaks, an outpatient care center is created at San Vicente de Paul Hospital to address the unique needs of patients with probable dengue infections. The study´s inclusion criteria were: (1) presentation for treatment at San Vicente de Paul Hospital´s care center with symptoms suggestive of DENV infection; (2) laboratory confirmation of DENV infection by ELISA for IgM and NS1; and (3) residence in the municipality of Orán. All of the patients included in the study were treated as outpatients. Dengue cases were defined by both the presence of IgM (PanBio Dengue IgM Capture ELISA, Australia) and NS1 antigen (Platelia Dengue NS1 Ag, Bio-Rad, France) determined by ELISA in acute serum samples. Serotyping was performed at the provincial reference laboratory.

Data collection and study variables

Data were collected through epidemiological and clinical records and laboratory analysis performed during treatment at San Vicente de Paul Hospital´s care center.

Illness was defined as DWoWS when patients had laboratory-confirmed DENV infection and symptoms that did not fulfill the WHO definition of DWWS or SD. DWWS and SD were defined using the WHO guidelines for diagnosis. Demographic variables included sex and age: study participants aged 2-14 years were defined as children while patients 15 years and older were considered adults.

The clinical symptoms recorded were: fever, defined as an axial temperature greater than or equal to 38°C; headache; retro-orbital pain: localized pain behind the eye; bipalpebral edema: abnormal fluid accumulation in the internal tissue of the eyelid; conjunctival injection: congestion and dilation of blood vessels in the sclera causing ocular redness; myalgia: pain in response to gentle pressure during physical examination; arthralgia: localized joint pain; abdominal pain: tenderness or continuous pain in physical examination; rash: red skin

discoloration that disappears with pressure; itching; nausea; diarrhea: three or more loose stools per day; vomiting: three or more episodes per day; hepatomegaly: liver enlargement >2cm; jaundice; oligoanuria; cough; dyspnea; tachypnea: respiratory frequency greater than 20 breaths/minute; hemorrhagic syndrome: including mucosal bleeding, abundant menstruation and/or blood in urine. All clinical symptoms were considered absent or present.

Laboratory parameters were considered present or absent based of the following definitions: leukopenia -leukocyte count lower than 4,000/mm^3 and thrombocytopenia-platelet count lower than 150,000/ mm^3.

The pre-existing immunity to DENV was determined by PanBio Dengue IgG Capture ELISA (Australia), in order to distinguish between primary and secondary DENV infections.

Statistical Analysis

Data were analyzed using the chi-square test for categorical variables. Univariate logistic regression analyses were performed to investigate the association of relevant independent variables with the outcomes (e.g. DWoWS *vs.* DWWS) and odds ratios were calculated where appropriate. Multivariate analyses used a logistic regression model, adjusting odds ratios for age, sex and previous exposure to DENV. The Mann-Whitney test was used for continuous variables. P-values were calculated during regression analysis using a 2-tailed test. A p value <0.05 was considered significant for all the tests performed. Statistical analyses were performed using Stata/SE 13.0 for Windows (StataCorp LP, College Station, TX, USA).

Ethics Statement

This study was approved by San Vicente de Paul Hospital, Buenos Aires, Argentina, and Bioethics Committee of Facultad de Medicina, Universidad Nacional de Tucumán, Tucumán, Argentina.

RESULTS

Population Characteristics

Among the 301 patients with laboratory-confirmed dengue who received care at San Vicente de Paul Hospital between February and June 2014, 37.9% presented DWWS and 62.1% DWoWS. No SD cases or deaths were reported. Patient ages ranged from 2-86 years with a mean (standard deviation) age of 35.5 (16.9) years (Table I). The mean age of DWWS patients was 37.3 (17.2), while the mean age of DWoWS patients was 34.4 (16.6).The most frequent clinical manifestations were headache (94.7%), myalgia (93.0%), arthralgia (88.0%), retro-orbital pain (78.1%) and rash (54.5%). DWWS patients also exhibited significantly higher frequencies of conjunctival injection (56.1% *vs.* 29.4%), diarrhea (32.5% *vs.* 11.2%), cough (15.8% *vs.* 5.9%) and dyspnea (11.4% *vs.* 3.2%) compared to DWoWS patients. Though these symptoms are not warning signs, multivariate analyses revealed that conjunctival injection (OR=3.02; 95% CI=1.67–5.47; p<0.001) and diarrhea (OR=3.10; 95% CI=1.48–6.49; p=0.003) are associated with a significantly increased risk of DWWS. Among the patients who presented warning signs, 28.9% presented multiple warning signs. The most frequent warning signs were abdominal pain (27.9%) and thrombocytopenia (22.0%).

When pre-existing immunity to DENV was analyzed in the study population, we found that nearly half of patients had secondary dengue infections (47.1%; Table I). In addition, secondary infections were not correlated to DWWS (59.3% with DWoWS *vs.* 40.7% with DWWS; Table I).

Interestingly, 11% of the patients with laboratory-confirmed dengue did not report fever. These patients also reported significantly lower frequencies of headache, conjunctival injection and myalgia (Figure 2). All other symptoms were reported at frequencies similar to those reported by febrile patients (Table SI).

Association of clinical manifestations and age

Several reports have described an association of dengue severity with age [6,7]. In this study, no significant differences were found in the incidence of DWWS between age groups (26.5% for <15 years *vs.* 39.3% for >15 years; Table II), nor was there any significant correlation between the incidence of secondary infection and age groups (50.0% for <15 years *vs.* 46.8% for >15 years; Table II). Univariate and multivariate analyses revealed that patients over 15 years of age had a significantly increased risk of fever, retro-orbital pain, arthalgia, diarrhea and thrombocytopenia compared to younger patients. Conversely, pediatric patients (2-14) were two times more likely to present rash than older patients (Table II).

Clinical manifestations by sex

Previous studies have described differences in dengue presentation based on sex [18,20-22]. Nearly 56% of the participants in our study were female and these patients had a significantly higher rate of DWWS compared to men (43.1% *vs.* 31.3%; Table III). Although univariate analysis showed that females had an increased risk of developing DWWS, this risk was no longer significant when adjusted for age and pre-existing immunity to DENV (Table III). When previous exposure to DENV was studied, male and female patients were found to have similar rates of secondary infection. However, multivariate logistic regression model showed that female patients had a significantly increased risk of rash, nausea, diarrhea,

abdominal pain and leukopenia (Table III). All other symptoms were reported at rates similar to those reported by men.

DISCUSSION

This study found variations in disease severity, clinical manifestations and laboratory parameters between pediatric and adult patients with laboratory-confirmed DENV infections. While there is a body of published work describing a relationship between age and dengue severity, the nature of this relationship is unclear and may be complicated by confounding factors. A three-year study of hospitalized DENV patients in Nicaragua found that infants and children aged 4-6 years were more likely to develop severe disease than adults [6]. Similarly, a Cuban study reported that secondary DENV infections in children aged 3-15 years were 14.5 times more likely to be fatal than secondary infections in adults aged 16 to 39 years [23]. However, prospective school-cohort studies of DENV infections in children have reported approximately twice as many inapparent cases as symptomatic cases [24,25], and a recent review suggests that the majority of DENV infections are subclinical [19]. The current study found that children were significantly less likely to present fever, a symptom which is frequently considered the minimum symptom in defining symptomatic infections [19] and is critical in the WHO diagnostic guidelines [4].

The children in this study did not only present milder illness than adults, but they presented a distinct profile of clinical manifestations, characterized by significantly reduced risk of fever, retro-orbital pain, arthalgia, diarrhea and thrombocytopenia and increased risk of developing rash compared to adult patients. The absence of fever in almost 25% of children, especially accompanied by the dramatically reduced rates of thrombocytopenia, may cause these dengue cases to be overlooked. Mild and inapparent primary DENV

infection can prime a population for more severe disease in subsequent heterotypic outbreaks. In fact, longer intervals between heterotypic outbreaks have been associated with increased mortality [26,27]. Furthermore, viremic patients with inapparent illness have been shown to effectively transmit DENV to mosquitoes [28]. Given this background, asymptomatic, mild and non-febrile patients are vital to understanding and controlling DENV transmission and epidemic cycles in a public health context and highlight the need to monitor pediatric populations during outbreaks.

While half of children under 15 years old had secondary dengue infections, incidence of secondary infection was lower in younger children; in fact, 80 % of children under 10 years old had primary infections. Given that the 1998 outbreak of DENV-2 in Salta represented the first dengue cases reported in the country in over 80 years [12], the lack of association between incidence of secondary infection and age is unsurprising. Between 1998 and 2014, DENV circulated regionally in epidemic cycles, giving older children and adults in Oran a similar lifetime risk of exposure to DENV [13,14].

Virus serotype has been associated with disease severity and differential characteristic clinical manifestations [10,29,30]. Consistent with our findings, previous studies have described relatively mild disease during DENV-4 outbreaks [10,31-33]. A prospective six-year study of over 2,000 dengue infections found that, unlike serotypes 1-3, over half of DENV-4 infections in children were inapparent [27]. One 12-year retrospective study in Thailand found that DENV-4 was half as likely as DENV-2 or DENV-3 to result in severe disease during a secondary infection [9].These findings are consistent with the results of the current study, in which none of the patients developed severe disease despite a moderate incidence of secondary infections. Interestingly, 63% of the patients reported cutaneous symptoms, which is consistent with previous DENV-4 outbreaks, but represents a higher incidence than has been reported for other serotypes [10].

This study reports sex-based differences in the manifestations of DENV infection. Similar results have been reported in Mexico City [18],in French Caribbean islands [20] and in select Chinese localities [21]. One study in Korea, a country in which DENV is almost exclusively imported by travelers, found significantly higher DENV incidence in men [22]. Further investigation will be necessary to define whether these differences are due to physiological or to regional socio-behavioral factors.

In summary, clinical manifestations and laboratory abnormalities between pediatric and adult patients infected with DENV-4 exhibited significant differences. Variations were also noted in presentations in females compared to males. A better understanding of these differences should contribute to better assessment of emerging and re-emerging DENV infections in at risk regions.

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COMPETING INTERESTS

The authors have declared that no competing interests exist.

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FIGURE LEGENDS

Figure 1. Geographical location of the study. The city San Ramon de la Nueva Orán (Orán) is located in the province of Salta on the Northwestern border of Argentina, 270 km Northeast of the province capital Salta and 30 km South of Bolivia.

Figure 2. Differences in clinical manifestations between febrile and non-febrile patients. Patients with laboratory confirmed dengue who presented without fever also exhibited significantly lower frequencies of headache, myalgia and conjunctival injection (abbreviated as Conjun. Inj.). Data were analyzed using the chi-square test for categorical variables. A p value <0.05 was considered significant.

Table I. Demographics, constitutional, musculoskeletal, cutaneous, gastrointestinal, respiratory and hemorrhagic manifestations and laboratory parameters of patients, categorized by the WHO diagnostic guidelines.

Characteristics	Total n (%)	DWoWS n (%)	DWWS n (%)	p (Chi^2)
Demographic				
Number of patients	301	187 (62.1)	114 (37.9)	----
Mean age in years (StD)	35.5 (16.9)	34.4 (16.6)	37.3 (17.2)	0.133[a]
Age range in years	2-86	2-78	6-86	----
Female (%)	167 (55.5)	95 (50.8)	72 (63.2)	0.036
Constitutional	**296 (98.3)**	**182 (97.3)**	**114 (100)**	**0.078**
Fever	268 (89.0)	154 (82.4)	114 (100)	<0.001
Headache	285 (94.7)	177 (94.7)	108 (94.7)	0.975
Retro-orbital pain	235 (78.1)	140 (74.9)	95 (83.3)	0.085
Bipalpebral edema	6 (2.0)	3 (1.6)	3 (2.6)	0.536
Conjunctival injection	119 (39.5)	55 (29.4)	64 (56.1)	<0.001
Musculoskeletal	**283 (94.0)**	**171 (91.4)**	**112 (98.3)**	**0.016**
Myalgia	280 (93.0)	169 (90.4)	111 (97.4)	0.021
Arthralgia	265 (88.0)	162 (86.6)	103 (90.4)	0.335
Cutaneous	**190 (63.1)**	**114 (61.0)**	**76 (66.7)**	**0.320**
Rash	164 (54.5)	99 (52.9)	65 (57.0)	0.491
Itching	128 (42.5)	73 (39.0)	55 (48.3)	0.117
Gastrointestinal	**157 (52.2)**	**65 (34.8)**	**92 (80.7)**	**<0.001**
Nausea	113 (37.5)	43 (23.0)	70 (61.4)	<0.001
Diarrhea	58 (19.3)	21 (11.2)	37 (32.5)	<0.001
Vomiting	43 (14.3)	4 (2.1)	39 (34.2)	<0.001
Abdominal pain	84 (27.9)	18 (9.6)	66 (57.9)	<0.001
Hepatomegaly	4 (1.3)	1 (0.5)	3 (2.6)	0.123
Jaundice	3 (1.0)	1 (0.5)	2 (1.8)	0.301
Oligoanuria	2 (0.7)	0 (0)	2 (1.8)	0.069
Respiratory	**37 (12.3)**	**15 (8.0)**	**22 (19.3)**	**0.004**
Cough	29 (9.6)	11 (5.9)	18 (15.8)	0.005
Dyspnea	19 (6.3)	6 (3.2)	13 (11.4)	0.005
Tachypnea	7 (2.3)	4 (2.1)	3 (2.6)	0.783
Hemorrhagic syndrome	**5 (1.7)**	**0 (0)**	**5 (4.4)**	**0.004**
Laboratory abnormalities				
Leukopenia	111 (44.0)	54 (35.5)	57 (59.4)	<0.001
Thrombocytopenia	54 (22.0)	12 (8.0)	42 (44.2)	<0.001
Incidence of SI	**140 (47.1)**	**83 (59.3)**	**57 (40.7)**	**0.435**

Definition of abbreviations: DWWS= dengue with warning signs; DWoWS= dengue without warning signs; SI= secondary infection; StD= standard deviation.

[a] p-value was calculated using the Mann-Whitney Rank Sum test for non-categorical variables.

Table II. Characteristics of pediatric and adult patients and odds ratios for age-associated symptoms.

Characteristics	<15 years	≥15 years	p (Chi^2)	Crude OR			Adjusted OR[a]		
	n (%)	n (%)		OR	95% CI	p[b]	OR	95% CI	p[b]
Number of patients	34 (11.3)	267 (88.7)	---	---	---	---	---	---	---
Mean age in years (StD)	10.4 (3.3)	38.7 (15.1)	---	---	---	---	---	---	---
Age range in years	2-14	15-86	---	---	---	---	---	---	---
Female (%)	17 (50.0)	150 (56.2)	0.495	---	---	---	---	---	---
Incidence of DWWS	9 (26.5)	105 (39.3)	0.146	---	---	---	---	---	---
Incidence of SI	17 (50.0)	123 (46.8)	0.722	---	---	---	---	---	---
Symptoms									
Fever	26 (76.5)	242 (90.6)	0.013	3.0	1.22-7.27	0.017	3.0	1.21-7.26	0.017
Retro-orbital pain	20 (58.8)	215 (80.5)	0.004	2.9	1.37-6.11	0.005	2.9	1.35-6.05	0.006
Arthralgia	26 (76.5)	239 (89.5)	0.027	2.6	1.02-6.36	0.032	2.6	1.06-6.28	0.037
Rash	24 (70.6)	140 (52.4)	0.045	0.5	0.211-0.99	0.049	0.4	0.20-0.98	0.045
Diarrhea	1 (2.9)	57 (21.4)	0.010	9.0	1.15-65.34	0.036	8.9	1.19-67.98	0.034
Thrombocytopenia	1 (3.6)	53 (24.3)	0.013	8.7	1.15-65.36	0.036	8.9	1.17-67.06	0.034

Definition of abbreviations: StD= standard deviation; DWWS= dengue with warning signs; SI= secondary infection; OR= Odds ratio; CI= confidence interval.

[a] Logistic regression model, adjusted for sex and previous exposure to DENV.

[b] p-values were calculated during univariate or multivariate analyses using a 2-tailed test.

Table III. Characteristics of male and female patients and odds ratios for sex-associated symptoms.

Characteristics	Male	Female	p (Chi^2)	Crude OR			Adjusted OR[b]		
	n (%)	n (%)		OR	95% CI	p[c]	OR	95% CI	p[c]
Number of patients	134 (44.5)	167 (55.5)	----	---	---	---	---	---	---
Mean age in years (StD)	33.7 (16.9)	36.9 (16.7)	0.059[a]	---	---	---	---	---	---
Age range in years	2-78	4-86	----	---	---	---	---	---	---
Incidence of DWWS	42 (31.3)	72 (43.1)	0.036	1.7	1.03-2.67	0.037	1.8	0.81-4.07	0.147
Incidence of SI	59 (45.0)	81 (48.8)	0.520	---	---	---	---	---	---
Symptoms									
Rash	63 (47.0)	101 (60.5)	0.020	1.7	1.08-2.73	0.020	1.8	1.10-2.82	0.018
Nausea	38 (28.4)	75 (44.9)	0.003	2.1	1.27-3.34	0.003	2.1	1.26-3.37	0.004
Diarrhea	18 (13.4)	40 (24.0)	0.021	2.0	1.10-3.74	0.023	2.0	1.05-3.63	0.034
Abdominal pain	28 (20.9)	56 (33.5)	0.015	2.2	1.13-3.23	0.016	1.9	1.11-3.19	0.019
Leukopenia	40 (34.8)	71 (53.4)	0.003	2.2	1.28-3.59	0.004	2.2	1.29-3.65	0.004

Definition of abbreviations: StD= standard deviation; DWWS= dengue with warning signs; SI= secondary infection; OR= Odds ratio; CI= confidence interval.

[a] p-value was calculated using the Mann-Whitney Rank Sum test for non-categorical variables.

[b] Logistic regression model, adjusted for age and previous exposure to DENV.

[c] p-values were calculated during univariate or multivariate analysis using a 2-tailed test.

Figure 1

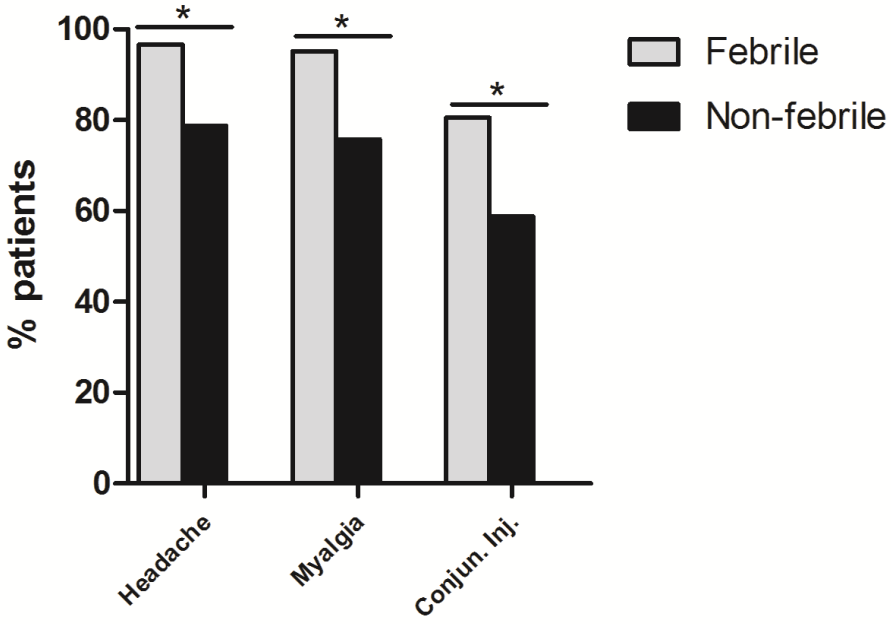

Figure 2