Journal of Clinical Virology 90 (2017) 7–13

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Co-circulating serotypes in a dengue fever outbreak: Differential hematological profiles and phylogenetic relationships among viruses

Andreia Moreira dos Santos Carmo[a,b], Rodrigo Buzinaro Suzuki[a,c], Aline Diniz Cabral[a], Renata Torres da Costa[a], Gabriela Pena Massari[a], Michele Marcondes Riquena[a], Helio Augusto Alves Fracasso[a], Andre Eterovic[d], Arlei Marcili[e,f], Márcia Aparecida Sperança[a,*]

[a] Centro de Ciências Naturais e Humanas, Universidade Federal do ABC, São Bernardo do Campo, 09606-070 São Paulo, Brazil
[b] Secretaria do Estado da Saúde do Estado de São Paulo, Instituto Adolfo Lutz, Centro de Laboratório Regional VIII, Santo André, 09040-160 São Paulo, Brazil
[c] Discipline of Parasitology, Marília Medical School, Marília, 17519-030, São Paulo, Brazil
[d] Universidade Federal do ABC, Centro de Ciências Naturais e Humanas, Santo André, 09210-170 São Paulo, Brazil
[e] Departamento de Medicina Veterinária Preventiva e Saúde Animal, Faculdade de Medicina Veterinária, Universidade de São Paulo, 05508-270 São Paulo, Brazil
[f] Universidade de Santo Amaro, 04829-300 São Paulo, Brazil

ARTICLE INFO

Article history:
Received 14 June 2016
Received in revised form 12 October 2016
Accepted 1 March 2017

Keywords:
pattern of dengue outbreak
hematological profile
dengue
molecular epidemiology
genotype sequence analysis
phylogeny

ABSTRACT

Background: Dengue virus, represented by four distinct, genetically diverse serotypes, is the etiologic agent of asymptomatic to severe hemorrhagic diseases. The spatiotemporal dynamics of dengue serotypes and its association to specific diseases vary among the different regions worldwide. By 2007, and in São Paulo State, Brazil, dengue-case concentration in urban centers had changed to increased incidence in small- and medium-sized towns, the case of Marília.
Objectives: The aim of this article was to distinguish dengue serotypes circulating during the 2007 Marília outbreak and define their association to demographic and hematological patient profiles, as well as the phylogenetic relationships among the different viruses.
Study design: PCR amplicons corresponding to the junction of capsid and dengue pre-membrane encoding genes, obtained from dengue serologically positive patients, were sequenced. Hematological and demographic data of patients with different Dengue serotypes were evaluated by univariate and bivariate statistics. Dengue PCR sequences were used in phylogenetic relationships analyzed for maximum parsimony.
Results: Molecular typing confirmed co-circulation of the dengue serotypes 1 (DENV1) and 3 (DENV3), which presented divergent correlation patterns with regard to hematological descriptors. The increase in atypical lymphocytes, a likely indication of virus load, could be significantly associated to a decrease in leukocyte counts in the DENV3 group and platelet in the DENV1. Phylogenetic reconstitution revealed the introduction of DENV1 from northern Brazil and local divergence of DENV3 by either microevolution or viral introduction from other geographical regions or both.
Conclusions: Dengue dynamics showed regional molecular-epidemiologic specificity, which has important implications for introduction of vaccines, disease management, and transmission control.

1. Background

Dengue, a highly morbid viral disease transmitted to humans by mosquitoes of the genus *Aedes* sp., is widely distributed throughout tropical and subtropical regions. The causative agent of dengue (DENV), an arbovirus from the family Flaviviridae and genus *Flavivirus*, presents four antigenically distinct serotypes, DENV1–4 [1,2]. Each serotype presents genetic variants associated to specific geographic and temporal distribution patterns [3–6].

Abbreviations: AGE, age; AL%, percentage of atypical lymphocytes; C, Capsid; DENV, Dengue virus; IBGE, Instituto Brasileiro de Geografia e Estatística; LEU, leukocyte; PLA, platelets; prM, Pre-membrane; SEX, gender.

* Corresponding author at: Universidade Federal do ABC, Center for Natural and Human Sciences, Rua Arcturus, 03-Jardim Antares, Bloco Delta, Sala 226, Laboratórios 105/107, CEP 09606-070 São Bernardo do Campo, São Paulo, Brazil. Tel.: +55 11 2320 6253/6187.
E-mail address: marcia.speranca@ufabc.edu.br (M.A. Sperança).

http://dx.doi.org/10.1016/j.jcv.2017.03.001
1386-6532/

Patients infected by DENV serotypes develop a wide spectrum of clinical signs, ranging from asymptomatic to severe, which, according to the latest revision of the World Health Organization, can be classified as probably dengue, dengue with warning signs, and severe dengue [7]. Severity can be associated to the occurrence of a previous heterotypic infection [8,9], viral strain and titer [10], number and temporal sequence of circulating serotypes [11], and host genetic background [12]. These features are dependent on spatial, temporal, and local population characteristics. Moreover, in medium-sized towns with populations ranging from 100,000 to 500,000 inhabitants, dengue outbreaks are long-lasting (more than 4 months) [13,14]. Thus, in order to improve disease control and the application of vaccines, dengue epidemiology assaying should be regional.

Since 2000, there has been a substantial increase in the spread of *Aedes aegypti* in the Americas, with Brazil, a country of continental dimensions, accounting for more than 70% [15]. Until mid-2007, the adult population of urban and peri-urban locations was the most affected by dengue outbreaks. However, from then on, there was a regional change in the pattern, with a significant increase in severe dengue cases among children under 15 years. This was the case of the northeast and Rio de Janeiro State in 2008/2009 [16].

In São Paulo State, and since the appearance of the first cases in 1987, occurrence was confined to cycles of outbreaks in various urban centers, but with a constant increase in disease severity. Introduction of the DENV1 serotype occurred in 1987, DENV2 in 1997, DENV3 in 2002, and DENV4 in 2011. By 2007, concentration in large urban centers had changed to increased incidence in small- and medium-sized towns. Marília, with 216,745 inhabitants (IBGE statistics, 2010) and a large population flow due to economic relevance, is a medium-sized town in western São Paulo State, around 450 km from the capital. The first severe cases of dengue hemorrhagic fever occurred in 2007, during an outbreak that reached 550/100,000 inhabitants. There has been an increase in numbers in subsequent epidemics.

Units of the Adolfo Lutz Institute monitor DENV circulation in São Paulo State, through laboratory tests and viral isolation. According to current regulations of the dengue control program by the surveillance center of São Paulo (http://www.cve.saude.sp.gov.br/htm/zoo/deng07_n2012.htm), dengue is confirmed by laboratory testing (serological status, viral isolation, PCR sequencing, and immunohistochemistry), up to the point that incidence reaches 150 cases/100,000 inhabitants in centers with populations varying between 150,000 and 249,999 residents. Hereon, diagnosis is only according to clinical criteria. Infected patients are simply notified without laboratory confirmation. Only severe cases warrant laboratory investigation. Official epidemiological information about the first severe Marília dengue outbreak in 2007 revealed the co-circulation of DENV1 and DENV3 serotypes, without any information as to (i) serotype status, (ii) association of demographic and hematological profiles with both serotypes, and (iii) virus genetic characteristics and phyletic relationships.

2. Objectives

The aim of this study was to determine the genetic characteristics of the DENV serotypes circulating during the 2007 Marília outbreak, their association to demographic and hematological profiles, and the subsequent phylogenetic reconstruction of virus relationships.

3. Study design

3.1. Patient and laboratory data

The subjects included in this study comprised 327 febrile patients under attendance at the Hemocenter of Marília during a dengue outbreak (March 7 to June 4, 2007). Infection was confirmed by enzyme-linked immunosorbent assaying of non-structural 1 (NS1) antigen ("Focus Diagnostics Dengue NS1 Antigen DxSelect™ Assay") [17]. All human samples used in the study were discarded after routine laboratory exams. Research protocol was approved by the Ethics Committee on Human Research of the Marília School of Medicine (number 069/03). Demographic data (gender and age), plus hematological conditions at first attendance (leukocyte and platelet counts and percentage of atypical lymphocytes), were obtained for each patient.

3.2. Dengue molecular diagnosis

Molecular diagnosis and plasma typing of 327 dengue-positive serological samples were by reverse transcription (RT) followed by the polymerase chain reaction (PCR) for amplification of the junction of the dengue capsid and pre-membrane encoding genes (CprM) [18]. PCR positive amplicons were sequenced by the Sanger

Table 1
Demographic and hematological status of DENV1- and DENV3-positive patients in the 2007 Marília outbreak.

Variable		DENV1	DENV3	Test	*P*-value
SEX	% males	58.1	46.8	1.4	0.2328
AGE	Mean	32.5	36.9	19,050	0.0485
	Standard deviation	16.70	19		
	Median	31	33		
	Range	(6–85)	(8–78)		
AL%	Mean	3.98	3.58	1618.0	0.6602
	Standard deviation	5.09	3.70		
	Median	2	3		
	Range	(0–21)	(0–18)		
LEU	Mean	3.53	3.52	1577.0	0.5164
	Standard deviation	0.16	0.18		
	Median	3.57	3.50		
	Range	(3.08–3.83)	(3.15–3.93)		
PLA	Mean	5.14	5.10	1464.0	0.2098
	Standard deviation	0.24	0.23		
	Median	5.17	5.13		
	Range	(4.12–5.55)	(4.00–5.42)		

Demographic and hematological status of patients from the two groups based on dengue virus-serotype testing (DENV1 $N = 43$, DENV3 $N = 79$), by gender (SEX), age in years (AGE), percentage of atypical lymphocytes (AL%), leukocyte and platelet counts (LEU and PLA, both in log10 cells/ml), for 122 patients. Except for SEX (χ^2 applied to a two-way contingency table), all test values are from Mann–Whitney U comparison among groups. Five contrasts in Bonferroni correction were considered when calculating critical *P*-values $P = 0.05/5 = 0.01$. No significant differences among groups were detected.