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Co-infection between Zika and different Dengue serotypes during DENV outbreak in Brazil

Cássia F. Estofolete[a,1], Ana C.B. Terzian[a,1], Tatiana E. Colombo[b,1], Georgia de Freitas Guimarães[a], Helio C. Ferraz Junior[a], Rafael A. da Silva[a], Gilmar V. Greque[c], Maurício L. Nogueira[a,*]

[a] São José do Rio Preto School of Medicine (FAMERP), Avenida Brigadeiro Faria Lima, 5416, Vila São Pedro, São José do Rio Preto, SP, CEP: 15090-000, Brazil
[b] Universidade Paulista, Campus de Sao Jose do Rio Preto, SP, Brazil
[c] São José do Rio Preto Regional School of Medicine Foundation (FUNFARME), Avenida Brigadeiro Faria Lima, 5416, Vila São Pedro, São José do Rio Preto, SP, CEP: 15090-000, Brazil

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ABSTRACT

Background: The recent introduction of new arboviruses in the Americas, as Zika virus (ZIKV) and Chikungunya virus (CHIKV), increased the risk of outbreaks and arboviral co-infections. Herein, we report twelve cases of co-infection of ZIKV and different DENV serotypes in a city located in the northwest region of São Paulo State, Brazil, which is hyper-endemic to Dengue.
Methods: Between January and November 2016, 1254 suspected cases of arboviral infection were available by our surveillance program in São José do Rio Preto. All suspected patients were examined and, when they were arboviral disease-suspectd, had sera separated and viral RNA analyzed by PCR/qPCR assays to determine the diagnosis of DENV 1-4, ZIKV, or CHIKV in the same samples. After the molecular results, twelve patients with ZIKV-DENV coinfection were identified and their clinical and laboratory characteristics were described.
Results: The mean between symptoms onset and collected sample of 3 days. DENV-1 was identified in seven co-infected patients and DEN2 in other five. Two patients presented alarm signs of Dengue and no one was hospitalized.
Conclusions: The constant presence of co-circulating arboviruses increases the chance of co-infection and demonstrates the importance of the differential diagnosis, especially during periods of arboviral outbreaks. The impact of this co-infection is known individual and collectively.

Introduction

Prior to the arrival and subsequent spread of Zika virus (ZIKV) in Latin America and the Caribbean, Dengue virus (DENV) was the predominant arbovirus [1]. With the recent increase of ZIKV and Chikungunya (CHIKV) circulation worldwide, several instances of human co-infection have been reported and characterized [2,3], being co-infection with more than one DENV serotype associated to more severe forms [4]. The São José do Rio Preto city is part of this context, it is located within the yellow fever transmission region, is hyper-endemic for dengue [5], and has confirmed cases of Saint Louis Encephalitis virus (SLEV) [6,7] and ZIKV [8]. In the past, the city reported cases of DENV co-infection (DENV-2/3) and co-infection among flaviviruses (DENV-3/SLEV) [7,9], suggesting that concurrent arboviruses circulation increases the chances of co-infection in the human population. This study provides a descriptive cases series of ZIKV-DENV coinfection detected during outbreak of Zika in an endemic area of Dengue.

Material and methods

Between January and November 2016, 1254 suspected cases of arboviral infection were available by our surveillance program in São José do Rio Preto, São Paulo, Brazil, among 25,896 reported

* Corresponding author.
E-mail addresses: cassiafestofolete@gmail.com (C.F. Estofolete), anacarolinaterzian@gmail.com (A.C.B. Terzian), taty_ec@hotmail.com (T.E. Colombo), gfggeorgia@gmail.com (G. de Freitas Guimarães), hcfjunior@gmail.com (H.C. Ferraz Junior), rafa_m112@hotmail.com (R.A. da Silva), greque@terra.com.br (G.V. Greque), mnogueira@famerp.br (M.L. Nogueira).
[1] These authors contributed equally to this work.

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cases. All suspected patients were examined by a physician and the illness was diagnosed as Dengue or Zika fever, based on Brazilian Ministry of Health criteria (Dengue as clinical signs compatible with dengue concurrent with an ongoing local DENV outbreak [10], and Zika fever based on the presence of macular or papular rash with two or more of following signs and symptoms: fever or conjunctival hyperemia without secretion and pruritus, polyarthralgia or joint edema [11]).

After, blood samples were collected, sera were separated and viral RNA was extracted using the QIAamp Viral RNA Mini kit (Qiagen) following the manufacturer's instructions. PCR/qPCR assays were applied to determine the diagnosis of DENV 1–4 [12], ZIKV [13], or CHIKV in the same samples. PCR was performed using Multiplex-Nested-PCR (M-N-PCR), to detect DENV 1–4 [12]. The first RT-PCR was performed using flavivirus generic primers, based on the sequence of non-structural protein type 5 (NS5). In the second PCR, nested assays, based on multiplex or conventional systems, were used with species-specific primers to detect and identify Dengue viroses (DENV 1–4). The RNA was initially tested for ZIKV by a TaqMan® RT-qPCR, as previously described [13] using the GoTaq® Probe1-Step RT-qPCR System (Promega). The qPCRs were performed using the Thermocycler StepOne Real-Time PCR System (Applied Biosystems). Description and performance characteristics of Zika virus real-time RT-PCR primer/probe sets (ZIKV 1086: 5′-CCGCTGCCCAACACAAG-3′; ZIKV 1162c: 5′-CCACTAACGTTCTTTTGCAGACAT-3′; ZIKV 1107-FAM: 5′-AGCCTACCTTGACAAGCAGTCAGACACTCAA-3′).

Twelve patients with ZIKV-DENV co-infection were identified and their clinical-laboratory characteristics were obtained. This study is part of an ongoing arbovirus surveillance program approved by the Ethical Review Board (CEP n° 02078812.8.0000.5415).

Table 1
Clinical and laboratory characteristics of ZIKV-DENV coinfection cases in São José do Rio Preto, São Paulo, Brazil, in 2016.

	N (SD)	% or media (SD)
Age (years)	46 (±15.08)	
Male	7/12	58
Female	5/12	42
Signs and symptoms		
Myalgia	10/12	83
Headache	9/12	75
Fever	7/12	58
Exanthema	7/12	58
Arthralgia	6/12	50
Nausea	3/12	25
Conjunctival hyperemia	3/12	25
Vomits	2/12	17
Abdominal pain	1/12	8
Neurological manifestations	0/12	0
Outcomes		
Alarm signs of dengue	2/12[a]	17
Severe dengue	0/12	0
Laboratory tests		
Hematocrit (%)	7/12	36 (±13.63)
Leukocytes (cells/mm^3)	7/12	3684 (±1,819)
Platelets (×10^3/mm^3)	7/12	169 (±66.1)
Virological tests		
DENV serotypes		
DENV-1	7/12	58
DENV-2	5/12	42
NS1 reagent	8/12	4
Time between symptoms onset and collected sample	3 (±1.64)	

[a] Alarm sign: abrupt platelets decrease.

Results

Of all 1254 avaliable patients, twelve were identified as co-infected by DENV and ZIKV and other 274 monoinfected by DENV (n = 135/274; 49.3%); being DENV-1: n = 41/135 (30.4%); DENV-2: n = 89 (65.9%); DENV-4: 5 (3.7%) or ZIKV (n = 139/274; 50.7%). The mean age was 46 years (±15.08 years, ranging 25–68 years), and 58% (7/12) were male. The most commonly reported signs and symptoms were myalgia (83%), headache (75%), fever (58%), exanthema (58%), and arthralgia (50%) (Table 1). Hemogram was performed in seven patients. The mean of hematocrit, leukocytes and platelets were 36% (±13.63), 3684/mm^3 (±1819), and 169×10^3/mm^3 (±66.1), respectively. Two patients presented alarm signs of Dengue (2/12, 17%), with abrupt platelets decrease. No patients were hospitalized, and the both patients with alarm signs were conducted closing in outward. The time mean between symptoms onset and collected sample was 3 days (±1.64, ranging 1–6 days). DENV-1 was detected in seven (7/12, 58%) patients, and DENV-2 in the other five (5/12, 42%). NS1 test was performed in 8 patients, and it was reagent in four (4/8, 50%). The descriptive panel of twelve cases is presented in Table 2.

Discussion

Since 2015, Brazil has been facing the challenge of the co-circulation of different arboviruses of public health importance, especially DENV and ZIKV [14]. DENV is responsible for major urban outbreaks, which are usually associated with the introduction of new serotypes [14,15]. In 2015–2016, in the city of São José do Rio Preto occurred a Dengue epidemic, characterized by DENV-1 and DENV-2 co-circulation (unpublished data). Concurrent ZIKV infections were detected in Dengue-suspected patients in the same period [8], creating epidemiologic conditions to co-infections (Table 3). We identified twelve cases of co-infection by DENV and ZIKV. The low identification of DENV-4 and, consequently, its low circulation associated with ZIKV introduction in a susceptible population and the fact of DENV-1 and DENV-2 had been introduced in our city in 1990 and 1998 [16,17] respectively, and have been circulating since then in lower or higher rates may explain the absence of co-infection by different DENV serotypes and DENV-4 and ZIKV.

Infections caused by arboviruses are commonly associated with any of three different and/or concurrent clinical syndromes: acute febrile illness, hemorrhagic fever, and encephalitis and neurological complications [18]. In our study, only mild and auto-limited cases were reported. There is different considerations about severity of DENV serotypes co-infection in literature, showing mild [19] and severe cases [20], while limited reports of ZIKV-DENV co-infection have been published [8]. Like this, the understanging of co-infection dynamics remains unclear, especially in terms immunopathological. In this context, clinicians have been facing diagnostic dilemmas with acute febrile syndrome and exanthema. The clinical profile presented by our co-infected patients shown that none exclusive characteristic might be identified as co-infection. It was the same than Dengue or Zika-patients, and the co-infection diagnosis was only possible due to systematic surveillance of arboviruses in the city, based on molecular tests.

Actually, the diagnosis of arboviral infection, especially ZIKV, is faced as a challenge, due to the probable low and short viremia levels [21] and difficulties to detection of the viral genome in clinical specimens. Besides, national guidelines [10,11] have been formulated to manage of diagnosis and treatment of Dengue or Zika, but none of them contemplate co-infections. Another aspect to be considered about co-infection is its recently association of the sexual intercourse as via of ZIKV transmission [22]. This route may be considered as a co-adjuvant in the co-infection cases. The mode of protection, in these cases, should be the same as the one adopted for

Table 2
Descriptive of clinical and laboratory characteristics of 12 ZIKV-DENV coinfection in São José do Rio Preto, São Paulo, Brazil, in 2016.

Patient	Age (years), gender	Time between symptoms onset and PCR	Sign and symptoms of arboviral illness	Laboratory tests[a]	Virological tests
1	39, female	3	Fever, arthralgia, exanthema	–	ZIKV PCR+ DENV-2+
2	25, male	1	Fever, nausea, vomits, conjunctival hyperemia, headache, myalgia, exanthema	–	ZIKV PCR+ DENV-2+
3	48, male	2	Fever, arthralgia, conjunctival hyperemia, headache, myalgia, exanthema	–	ZIKV PCR+ DENV-2+
4	47, female	1	Arthralgia, abdominal pain, conjunctival hyperemia, headache, myalgia, exanthema	Ht 42.3, leuko 5200, plat 255,000	ZIKV PCR+ DENV-2+
5	63, female	4	Fever, arthralgia, headache, myalgia, exanthema	Ht 34.8, leuko 1900, plat 109,000	ZIKV PCR+ DENV-1+ NS1+
6	27, male	2	Fever, myalgia, exanthema	–	ZIKV PCR+ DENV-1+
7	64, female	2	Vomits	Ht 33.1, leuko 5810, plat 246,000	ZIKV PCR+ DENV-1+
8	68, male	3	Fever, arthralgia, headache, myalgia. Abrupt platelets decrease	Ht 32.6, leuko 1860, plat 99,000	ZIKV PCR+ DENV-1+ NS1+
9	33, male	6	Fever, nausea, headache, myalgia. Abrupt platelets decrease	Ht 38.9, leuko 5520, plat 72,000	ZIKV PCR+ DENV-1+ NS1+
10	62, male	5	Fever, headache, myalgia,	Ht 37.1, leuko 1910, plat 142,000	ZIKV PCR+ DENV-1+ NS1+
11	41, male	1	Arthralgia, nausea, headache, myalgia,	–	ZIKV PCR+ DENV-2+
12	36, female	4	Fever, headache, myalgia, exanthema	Ht 37, leuko 3590, plat 195,000	ZIKV PCR+ DENV-1+

[a] Ht: hematocrit (%); leuko: leukocytes (cells/mm^3); plat: platelets (per mm^3).

Table 3
Reported or observed clinical and laboratory signs and symptoms in patients with ZIKV-DENV co-infection, 2015–2017.

Author, Year	Country	Number of Co-infection	Diagnosis Methods for ZIKV	Diagnosis Methods for DENV	Others
Estofolete et al, 2017	São José do Rio Preto, São Paulo, Brazil	12 cases	ZIKV PCR + in serum	DENV PCR + in serum	–
Chia et al., 2017 [25]	Singapore	5 cases	ZIKV PCR + in urine (4) or serum (1)	DENV PCR (4) + (serotypes 3 and 1) or NS1 reagent (1) in serum	–
Villamil-Goméz et al., 2016 [26]	Colombia	1 case	ZIKV PCR + in serum	DENV-2 PCR + in serum	CHIKV PCR + in blood
Villamil-Gómez et al., 2016 [26]	Colombia	1 case	ZIKV PCR + in serum	Dengue IgM reagent and DENV PCR + in serum	Chikungunya IgM reagent in blood
Dupont-Rouzeyrol et al., 2015 [2]	New Caledonia	2 cases	ZIKV PCR + in serum	DENV PCR + (Serotypes 1 and 3) in serum	
Iovine et al., 2017 [27]	Haiti	1 case	ZIKV PCR + in urine and saliva	DENV-2 PCR + in serum, urine, and saliva	Cell culture positive for ZIKV and DENV vRNA
Waggoner et al., 2016 [28]	Nicaraguan	6 cases	ZIKV PCR + in serum	DENV PCR + in serum	
		7 cases	ZIKV PCR + in serum	DENV PCR + in serum	CHIKV PCR + in serum

other sexually transmitted diseases [23]. Co-infected, the patients may be source of multiple arboviruses at the same time. The ability of *Aedes aegypti* mosquitoes to be co-infected and co-transmit arboviruses have been shown [24]. These factors together demonstrate the importance of the differential diagnosis, especially during periods of arboviral outbreaks.

In this way, the co-circulation of theses arboviruses has become a serious public health issue and challenge, in terms of transmission dynamics, vector competence, clinical spectrum, and health outcomes and complications. The pathogens detection and the appropriated infected patient treatment depend on a mutual effort. These efforts should include the patients who should seek the health service at the beginning of the symptoms; the health professionals that should be well trained to diagnose the diseases, the researches to develop accurate diagnostic tools and the governmental agencies to monitor and control the pathogens entrance in order to avoid/control outbreaks. We also believe that the use of clinical and epidemiological criteria associated with laboratory confirmation should be done even in the presence of a large DENV outbreak, as strategy to know better the implications of co-exposure and co-transmission to arbovirus, and their impact of this co-infection individual and collectively.

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Competing interests

None declared.

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