International Journal of Infectious Diseases 102 (2021) 97–102

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

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

Dengue-chikungunya coinfection outbreak in children from Cali, Colombia, in 2018–2019

Jaime E. Castellanos[a,*], Nayeli Jaimes[a], Carolina Coronel-Ruiz[a], Juan Pablo Rojas[b], Luis F. Mejía[b], Víctor H. Villarreal[b], Luz E. Maya[b], Luz M. Claros[b], Cielo Orjuela[b], Eliana Calvo[a], María Victoria Muñoz[b], Myriam L. Velandia-Romero[a]

[a] Grupo de Virología, Universidad El Bosque, Bogotá, Colombia
[b] Fundación Clínica Infantil Club Noel, Cali, Colombia

ARTICLE INFO

Article history:
Received 17 July 2020
Received in revised form 7 October 2020
Accepted 8 October 2020

Keywords:
Dengue fever
Chikungunya fever
RT-PCR
Serology
Arbovirus

ABSTRACT

Objective: To identify the arbovirus involved in febrile cases identified in a pediatric clinic in Cali, Valle del Cauca province, Colombia, and study the clinical characteristics.
Methods: A descriptive, prospective study enrolled 345 febrile children for 12 months in a pediatric clinic. Serum samples and medical record registers documenting signs and symptoms were analyzed to detect DENV, CHIKV, and ZIKV by reverse transcription-polymerase chain reaction and serology methods. Diagnosis at the time of admission and discharge were compared based on laboratory test results.
Results: All patients were diagnosed as severe dengue at admission. Molecular detection and serology tests identified 143 CHIKV-positive (41.4%), 20 DENV-positive (5.8%), and 123 DENV-CHIKV coinfection patients (35.7%). DENV or CHIKV serology test results of these double-infected patients yielded poor performance to confirm patient cases. ZIKV infection was detected in 5 patients (1.4%) as double or triple infections.
Conclusion: A sustained CHIKV circulation and transmission was confirmed causing febrile illness in children and indicating that this virus spreads even during the regular DENV season, leading to double infections and altering clinical symptoms. Specific clinical tests are necessary to closely identify the arbovirus involved in causing infectious diseases that can help in better treatment and mosquito-transmitted virus surveillance.

Introduction

Viral infections transmitted by *Aedes* mosquitoes are still a considerable health concern in tropical countries. Dengue is an endemo-epidemic disease in Colombia, which has reported the second highest number of cases in South America (Villar et al., 2015). The chikungunya virus (CHIKV) has been present in Africa and Asia for many years, and it was detected in America in 2013; since then, more than 2 million cases have been reported in the continent (Yactayo et al., 2016; PAHO, 2020). Although CHIKV belongs to the alphavirus genus from the Togaviridae family and dengue virus (DENV) belongs to the *Flavivirus* genus, both cause similar febrile syndromes and share many signs and symptoms, especially in children, which leads to misdiagnosis (Furuya-Kanamori et al., 2016). CHIKV infection causes higher fever than that caused by DENV infection and begins abruptly; patients have reported arthralgia/arthritis, rash, back pain, and severe myalgias that could develop into severe joint pain in adults and may last for weeks or months after acute infection (Burt et al., 2012). However, the disease is rarely fatal (Chahar et al., 2009). On the other hand, DENV infection could develop into a severe illness and sometimes could be fatal depending on the immune history of the patient (Bonifay et al., 2018).

Little is known about the clinical features of CHIKV infection in children with acute fever. However, some studies have reported differences in CHIKV infection in adults and children (Ritz et al., 2015) wherein the infection is more severe in children under two years of age (Sharma et al., 2018; Robin et al., 2010) and the worst cases could be seen in neonates (Pinzón-Redondo et al., 2016). High fever is common in both adults and children, and febrile convulsion may appear in children (Robin et al., 2008), although

* Corresponding author at: Grupo de Virología, Universidad El Bosque, Carrera 9 No. 131 A 02, Bloque O, Laboratorio 205, Bogota, Colombia.
E-mail address: castellanosjaime@unbosque.edu.co (J.E. Castellanos).

https://doi.org/10.1016/j.ijid.2020.10.022

there is not enough information about pediatric CHIKV infection. However, the well-documented 2006 epidemic that occurred on La Reunion Island indicated that asymptomatic CHIKV infection was significantly higher in children under 15 years of age (37%) than in adults (28%) (Sissoko et al., 2010). On the contrary, several studies have shown that severe CHIKV infection occurs mainly in children under two years of age (Sharma et al., 2018; Robin et al., 2010).

Because of the wide CHIKV circulation in areas where DENV has been steadily circulating and transmitting, cases of DENV-CHIKV coinfections have been frequently reported (Caron et al., 2012; Calvo et al., 2016; Vargas et al., 2018; Rückert et al., 2017; Mercado-Reyes et al., 2019), although there is no evidence of a higher severity in these DENV-CHIKV coinfection cases. The national surveillance study performed in Colombia during the 2016 Zika virus (ZIKV) epidemic found 34 cases of coinfection (0.14%), most of them with CHIKV-ZIKV coinfections, and only three DENV-CHIKV coinfections with two deaths (Mercado et al., 2018; Rueda et al., 2019); however, in 2018 and 2019, the Colombian Ministry of Health reported a decrease in CHIKV cases (800) in each year (24% confirmed by laboratory), and 1% of these cases originated from the province of Valle del Cauca (BES, 2018).

However, in a dengue endemic country like Colombia, the frequency of DENV and CHIKV infections and coinfections and their clinical manifestation in children are unknown. Thus, this study describes two outbreaks in a pediatric clinic; the first was caused by CHIKV, and the second was caused by DENV-CHIKV coinfection that occurred during the final quarter of 2018 and the first quarter of 2019 in Cali, Colombia, respectively; this period is usually endemic for DENV infections. We described in this work the laboratory strategy for case confirmation and the clinical features of infection in these acute febrile patients.

Methods

Study design and patients

This descriptive prospective study was performed in the pediatric setting in Fundación Clínica Club Noel ("the Clinic") located in Cali, capital of the western Valle del Cauca Colombian province, from July 2018 to August 2019. Individuals under 18 years of age presenting with fever or two symptoms such as rash, edema, headache, myalgias, or arthralgias were asked to participate. Children, adolescents, and parents were informed about the study, and those that accepted signed both the assent and consent forms. This research was reviewed by the Institutional Ethics Committee of Universidad El Bosque (Minutes 031-2017) and by the Ethics Committee of the Clinic (Resolution 12-2016). Clinical and personal data of children who met the aforementioned inclusion criteria were recorded, and blood samples were taken to evaluate hematimetric and biochemical variables. Immunochromatography-based rapid diagnostic tests were performed for dengue (Dengue Duo kit, SD Bioline, containing NS1 antigen detection and IgM/IgG antibodies). A serum aliquot was frozen and sent to the virology laboratory in Universidad El Bosque.

Sample processing and serology

Blood samples from febrile children were analyzed based on an algorithm previously reported to identify dengue cases (Castellanos et al., 2016; Castro-Bonilla et al., 2018; Velandia-Romero et al., 2019) using a combination of serology and molecular tests. Serological confirmation for dengue was performed by determining IgM levels using the UMELISA Dengue IgM Plus (Tecnosuma, Cuba UM2016), and anti-DENV high-affinity IgG antibodies (acute secondary antibodies) were detected by using the Capture IgG Elisa kit (Panbio Alere, 01PE10). Positive samples for any of the dengue antibodies were subject to DENV NS1 antigen detection (NS1 Dengue Early Elisa, Panbio Alere 01PE40) by following the manufacturer's instructions. IgM specific for CHIKV was evaluated using the Anti-CHIKV IgM Human Elisa kit (Abcam, ab177848), which reports sensitivity and specificity values of 90%.

Molecular detection of the virus

Viral RNA from DENV, CHIKV, and ZIKV were detected based on a previously reported, nested, multiplex, reverse transcription-polymerase chain reaction (RT-PCR) method (Calvo et al., 2016). Briefly, serum RNA was isolated using the RTP DNA/RNA Virus mini kit (Stratec, Molecular GMBH, Germany) by following the manufacturer's instructions. Five microliters of purified RNA were retrotranscribed and amplified in the first PCR round for DENV, CHIKV, and ZIKV using three outer primer pairs (Table 1) and the Luna Universal One-Step RT-PCR kit (New England Biolabs, USA E3000E5). This first amplification product was used as the template for the second PCR round in independent tubes and with a new set of primers for each virus and the four dengue serotypes. PCR products were separated in agarose gels and stained with ethidium bromide for imaging.

Data analysis

The clinical information obtained from medical records was registered in a database together with the virology laboratory test results. Descriptive statistics was used to analyze patient characteristics. Statistical analyses were performed using Stata software 13.0. Shapiro–Wilk test was performed to verify the normal distribution, and uni- and multivariate analyses were performed on nominal and ordinal variables. Chi-square and Fisher's exact tests established the differences between the patients grouped by virus involvement.

Results

Around 568 febrile children with a history of fever that met the inclusion criteria were invited to participate during the period from June 2018 to July 2019 in the clinic. Three hundred and forty-five children were enrolled after they or their parents signed the consent form (48.1% girls), and the median age was one year (range 0.9–5 years) (Table 2).

After admission and clinical examination, the presumptive diagnosis in every case was dengue disease. Confirmed discharge diagnosis was made after considering the laboratory test results. Of 345 patients, CHIKV infection was confirmed in 143 patients (41.4%) (127 by RT-PCR and 22 IgM positive), while dengue was confirmed in only 20 patients (5.8%) (15 by RT-PCR and six by serology). In a remarkable 123 patients (35.7%), a double DENV-CHIKV coinfection was detected (112 by RT-PCR and 11 by DENV or CHIKV serology) (Table 3). Moreover, two CHIKV-ZIKV coinfections were detected, one DENV-ZIKV coinfection was detected, and all three virus infections were detected in two patients. Results for dengue serology were unexpectedly low because only 20% and 50% of the samples from the DENV-CHIKV and DENV groups, respectively, had positive results. These 58 samples that were negative for any virus were grouped as other febrile illness.

Figure 1 shows the distribution of patients enrolled weekly (gray bars) and the laboratory-confirmed diagnosis from epidemiological week 20 of 2018 to week 26 of 2019, where 84.4% of the samples were confirmed viral etiology. Between June and October 2018 (considered as the dry season), a high number of CHIKV cases (64.3% of the total cases) was noted, while in mid-October, DENV infections began spreading without any variations in the number of CHIKV infections; therefore, the majority of confirmed cases of

Table 1
Nested RT-PCR for arbovirus detection (Calvo et al., 2016).

Virus	Reaction	Sequence 5→3	Amplicon (bp)
CHIKV	RT-PCR (outer)	$E1F_{10240}$ – ACGCAATTGAGCGAAGCAC $E1R_{10541}$ – CCAAATTGTCCYGGTCTTCCT	301
	PCR (inner)	$E1F_{10240}$ – ACGCAATTGAGCGAAGCAC $E1R_{10444}$ – CTGAAGACATTGGCCCCAC	204
DENV	RT-PCR (outer)	D_1 – AGTTGTTAGTCTRYGTGGACCGAC D_2 – TTGCACCAACAGTCAATGTCTTCAGGTTC	511
	Serotyping PCR (inner)	D_1 -TS1-CCCGTAACACTTTGATCGC	211
		D_1 -TS2-CGCCACAAGGGCCATGAACAGTTT	119
		D_1 -TS3-TAACATCATCATGAGACAGAGC	288
		D_1 -TS4-TTCTCCCGTTCAGGATGTTC	266
ZIKV	RT-PCR (outer)	MF_{944} – GGTCATGATACTGCTGATTGC ER_{1269} – CCACTAACGTTCTTTTGCAGAC	325
	PCR (inner)	MF_{944} – GGTCATGATACTGCTGATTGC ER_{1241} – AGTGTCTGACTGCTTGTCAAGG ER_{1109} – CTCTATGTCGACAGTCGGTTTG	297

arbovirus infections from November 2018 to March 2019 (75.2%) were DENV-CHIKV coinfections. The high number of CHIKV cases were reported during the driest months of June–October 2018, which is usually with low mosquito circulation, thus indicating a different virus-spreading pattern. Figure 1 shows the distribution of patients enrolled weekly (gray bars) and the laboratory-confirmed diagnosis from the epidemiological week 20 of 2018 to week 26 of 2019, where arbovirus infection in 84.4% of the samples was etiologically confirmed.

Rhinorrhea, cough, diarrhea, and respiratory symptoms were significantly more frequent in the CHIKV-confirmed patients ($p < 0.05$), which was probably related to the respiratory illness season at the end of the first quarter of 2018. Respiratory symptoms for both CHIKV and coinfected patients included nasal

Table 2
Characteristics of enrolled children, *n* (%).

Characteristics	All ($N = 345$)	OFI ($n = 58$)	CHIKV ($n = 143$)	DENV-CHIKV ($n = 123$)	DENV ($n = 20$)
Female	166 (48.1)	31 (53.4%)	65 (47.1%)	53 (43.1%)	12 (57.1%)
Age					
Nursing (<2 years)	164 (47.5%)	29 (50.0%)	72 (52.2%)	54 (43.9%)	6 (28.6%)
Preschool age (2–5 years)	69 (20.0%)	18 (31.0%)	27 (19.6%)	20 (16.3%)	4 (19.1%)
School age (5 to 11 years)	86 (24.9%)	10 (17.2%)	31 (22.5%)	37 (30.1%)	7 (33.3%)
Teenagers (12 to 18 years)	25 (7.5%)	1 (1.7%)	8 (5.8%)	12 (9.7%)	4 (19.1%)
Onset of illness (days)					
Less than 3 days	98 (28.5%)	15 (25.9%)	38 (27.5%)	40 (32.5%)	3 (14.3%)
3 to 6 days	210 (61.1%)	36 (62.1%)	87 (63.0%)	71 (57.7%)	14 (66.7%)
7 or more days	26 (7.6%)	3 (5.2%)	11 (8.0%)	9 (7.3%)	3 (14.3%)
Not reported	10 (2.9%)	4 (6.9%)	2 (1.5%)	3 (2.4%)	1 (4.8%)
Thrombocytopenia (<150.000 Plt/mm^3)					
Yes	81 (24.5%)	4 (7.3%)	28 (21.7%)	37 (30.3%)	10 (50.0%)
No	249 (75.2%)	50 (90.9%)	101 (78.3%)	85 (69.7%)	10 (50.0%)
Leukopenia (<500 cells/mm^3)					
Yes	70 (20.9%)	3 (5.4%)	29 (22.0%)	31 (25.4%)	7 (33.3%)
No	265 (79.1%)	52 (94.5%)	103 (78.0%)	91 (74.6%)	14 (66.7%)
Domicile					
Urban	312 (90.4%)	53 (91.4%)	123 (89.1%)	115 (93.5%)	17 (80.9%)
Rural	25 (7.5%)	2 (3.4%)	12 (8.7%)	8 (6.5%)	3 (14.3%)
Clinic's department					
Emergency room	292 (85.1%)	53 (91.4%)	115 (83.9%)	98 (80.3%)	21 (100%)
Observation ward	40 (11.7%)	5 (8.6%)	18 (13.1%)	17 (13.9%)	0 (0)
Hospitalization	4 (1.2%)	0 (0)	2 (1.5%)	2 (1.6%)	0 (0)
ICU	7 (2.0%)	0 (0)	2 (1.5%)	5 (4.1%)	0 (0)
Health insurance					
Subsidized	173 (51.0%)	30 (52.6%)	80 (59.7%)	53 (43.4%)	8 (38.1%)
Employees Contributory	158 (46.6%)	27 (47.4%)	53 (39.6%)	62 (50.8%)	13 (61.9%)
Patient handling					
Outpatient	152 (44.6%)	36 (62.1%)	57 (41.9%)	52 (42.6%)	6 (30.0%)
Inpatient	189 (55.4%)	22 (37.9%)	79 (58.1%)	70 (57.4%)	14 (70.0%)

OFI: other febrile illness: DENV: Dengue Virus; CHIKV: Chikungunya Virus.

Table 3
Description of discharge diagnostic of enrolled patients after serology and molecular tests.

Diagnosis[a]	*N* (%)	RT-PCR (*n* = 345)		CHIKV IgM[b] (*n* = 221)		DENV IgM[b] (*n* = 345)		DENV IgG[b] (*n* = 345)		DENV NS1 (*n* = 137)	
		Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
OFI	59 (17.1)	0 (0)	58 (100)	0 (0)	43 (0)	0 (0)	58 (100)	0 (0)	58 (100)	0 (0)	5 (8.6)
CHIKV	143 (41.4)	127 (92.0)	11 (7.9)	22 (15.9)	54 (39.1)	1 (0.7)	137 (99.3)	1 (0.7)	137 (99.3)	0 (0)	5 (3.6)
DENV-CHIKV	123 (35.7)	112 (91.1)	11 (8.9)	7 (5.7)	90 (73.2)	21 (17.1)	103 (83.7)	19 (15.4)	105 (85.4)	13 (10.6)	94 (76.4)
DENV	20 (5.8)	15 (71.4)	5 (2.4)	0 (0)	5 (2.4)	9 (42.9)	11 (52.4)	7 (30.0)	13 (61.9)	6 (28.6)	11 (52.4)

[a] Five samples are not shown in this table. One sample was DENV-ZIKV positive, two samples were CHIKV-ZIKV positive, and two samples were DENV-CHIKV-ZIKV positive.
RT-PCR: reverse transcriptase/polymerase chain reaction; **IgM:** Immunoglobulin M; **IgG:** Immunoglobulin G; **NS1:** Non-Structural Protein 1; **OFI:** Other Febrile Illness: **DENV:** Dengue Virus; **CHIKV:** Chikungunya Virus.
[b] Antibody capture ELISA test.

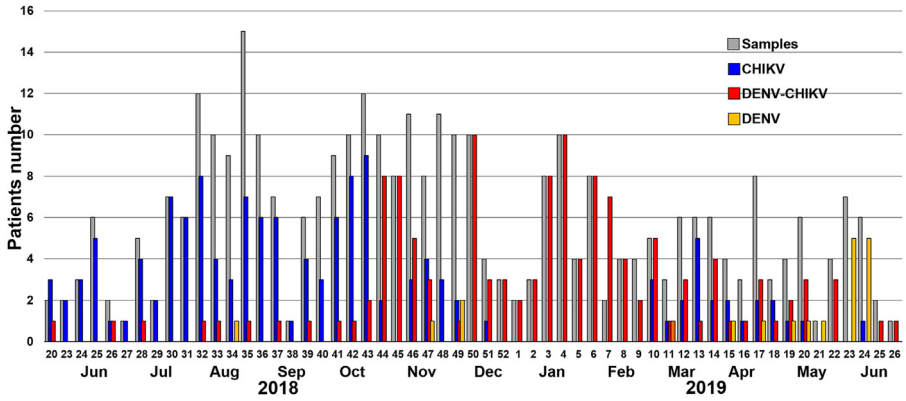

Figure 1. Distribution of arbovirus confirmed cases week-by-week from June 2018 to June 2019 in the Cali-Colombia pediatric Clinic.

flaring, intercostal retractions, wheezing, and rhonchi. Meanwhile, the coinfected patients more frequently presented with abdominal tenderness, rash, fever, and vomiting (Figure 2). These data suggest that an arboviral infection should not be ignored in patients with respiratory symptoms. A few neurological symptoms such as irritability, somnolence, hypotonia, and seizures were more frequent in CHIKV-confirmed patients.

Discussion

This prospective study evidenced that CHIKV is now endemic in Cali, Colombia, causing febrile disease in children and increasing the regular number of already high dengue fever cases in the second quarter each year. This city contributes 60% of the dengue cases in the Valle del Cauca province, which mainly affects children under 14 years of age (Hernández et al., 2016). In addition, CHIKV also spreads during the driest months of July and August when dengue transmission is low due to the low precipitation rates, indicating a phenomenon of persistence and transmission very different to that described for DENV; alternatively, even CHIKV could be modulating its circulation or transmission pattern. Clinically, although the set of symptoms is similar between the diagnostic groups, the intensity is different. However, the frequency of respiratory illnesses was higher in the CHIKV-confirmed patients than in those with a double infection, which is possibly related to the season of respiratory disease. Adults with CHIKV and DENV-CHIKV coinfection presented more severe joint and muscle symptoms with substantial movement restriction than adult dengue patients, although the frequency of symptoms was higher in the CHIKV-infected patients (Londhey et al., 2016). Although abdominal pain, rash, and vomiting were the most frequent symptoms reported in the double infection patients, they also presented with respiratory symptoms.

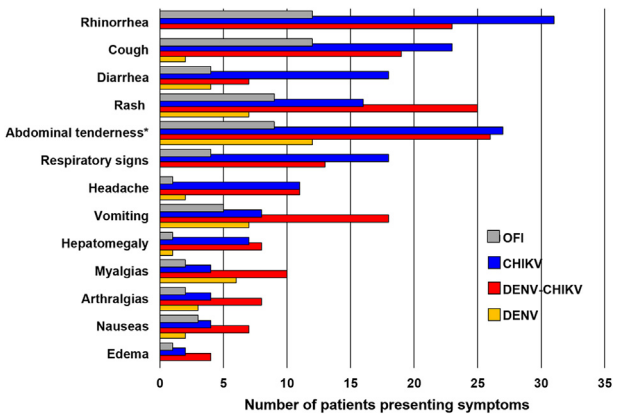

Figure 2. Description of symptoms reported during the admission consultation of enrolled children and classified after final virology and serology diagnostic. Other febrile illness (OFI), because tested negative in serology and virology procedures. *Abdominal tenderness in previous days or presence dunring the first consultation or presence during the first consultation.

Dengue has been endemo-epidemic since the last 40 years in Colombia due to high *Aedes aegypti* infestation rates in two-thirds of the country and poor sanitary development in many areas. These conditions led to a two-year CHIKV epidemic in 2014 after the virus was identified in the country, and the western province Valle del Cauca and its capital city Cali have been affected by arbovirosis. The same mosquito transmits both DENV and CHIKV, and therefore, double infections have been reported in these areas.

Previously, human double arbovirus infection was reported in natural mosquito populations, but the biological and epidemiological implications are not known. Multiple human infections could probably be acquired through a double-infected mosquito

bite or by two simultaneous bites from different mosquitoes causing a "real" coinfection. However, a "superinfection" could occur when a second infected mosquito bites a viremic individual. Experimentally, feeding mosquitoes with both viruses caused mixed midgut infection, but the DENV presence significantly increased CHIKV late replication a phenomenon also observed in salivary glands. However, the opposite was observed in early post-infection point times in these mosquitoes, indicating a facilitation process during the two simultaneous replication processes (Le Coupanec et al., 2017).

DENV-CHIKV coinfection prevalence in endemic countries is estimated between 1 to 19%, although most studies are based on serology data (Omarjee et al., 2014; Afreen et al., 2014). Using both RT-PCR and serology in our study, two-fifths of the febrile patients were confirmed for double DENV-CHIKV coinfection, the highest rate reported until today, especially in a children cohort, confirming the permanent and simultaneous CHIKV transmission in DENV endemic areas such as the Western Colombian province and its capital Cali.

Using a laboratory diagnostic algorithm, we confirmed the viral agent in 84.4% of the febrile cases, although only 11.1% of CHIKV and DENV-CHIKV patients tested positive in the CHIKV IgM test, endorsing the higher sensitivity of the molecular test in this pediatric population and the difficulties of using serology to confirm the CHIKV cases. Regularly, parents seek medical care in advance when their children have fever (median for this study, four days), which is a time point with high viremia and extremely low antibody response. The CHIKV IgM ELISA kit used was relatively less sensitive (13.1%) and reasonable in terms of specificity (81.4%). It is striking that CHIKV IgM-positive sample proportion was significantly lower in the DENV-CHIKV coinfection group (5.7%). The two-year study performed in Salvador (Brazil) evaluated two IgM ELISA kits that showed low sensitivities rates (4.0% and 10.3%) for acute samples, and this value rises until 95.7% in those samples taken four weeks after (Kikuti et al., 2019). Previous studies, along with this one, do not recommend the CHIKV IgM test as a part of the diagnostic arsenal to study febrile children in arbovirus endemic areas (Jain et al., 2017; Kikuti et al., 2019).

Surprisingly, only 20.8% of the DENV-confirmed samples tested positive in the different DENV serology tests. Moreover, the percentage of DENV-positive serology samples in the DENV-CHIKV group (17.1%) was significantly lower than that in the DENV-confirmed patients (42.9%). The first finding suggests that the immune response to DENV could be altered in those CHIKV-infected patients. Similar findings were described by Zaidi et al. (2018) as only half of nine out of 24 patients with double DENV-CHIKV infection patients had detectable DENV antibodies and most of them were children under 2 years of age. These authors proposed that a higher CHIKV replication rate induces an antiviral status hampering the DENV infection and immune response, according to their in vitro experiments. Conversely, they also speculated that memory DENV antibodies contribute more efficiently to clear this virus, leading to a lesser response. In conclusion, during DENV-CHIKV coinfection, DENV serology tests have the worst performance, suggesting that they could be useless.

As a result of the high dengue burden in Colombia, national guidelines establish that febrile cases in endemic areas should be addressed as dengue, and additional analyses for other viruses are not available. Hence, CHIKV cases or coinfections are not identified or reported, leading to inadequate patient care. This approach also negatively affects the virology surveillance and timely control of outbreaks. Hence, significant strengthening of clinical and laboratory activities is required to diagnose CHIKV and coinfections, which will help describe the spectrum of clinical manifestations in children coming from endemic areas and improve their medical care. For example, the use of nonsteroidal anti-inflammatory drugs, which are used in CHIKV fever but are banned during dengue cases because of their anticoagulant effect, could be of potential interest.

Conclusion

This study reports the steady CHIKV circulation in this endemic area causing mild and severe febrile disease in children. A strong warning should be sounded for medical personnel and institutions because most of the cases described here were presumptively diagnosed as dengue. A redefinition of the case criteria should also be discussed to allow clinicians to improve the distinguishing factors between CHIKV and DENV fevers and those caused during double infection, which will continue to be presented in this province and the country.

Limitations

This study has some limitations within which our findings need to be interpreted. First, the study enrolled only children, most of them under 5 years of age, from a private foundation that brings medical care to the poorest neighborhoods of Cali and near municipalities. This characteristic may distort the real data of CHIKV circulation. The nested RT-PCR method used to confirm diagnosis is a laboratory-developed test that does not have external evaluation, although both positive and negative controls were processed during each lot of tested sera and worked well. A greater effort is necessary to analyze other parameters such as viral load or immune mediators in the clinical characterization of patients.

Funding

This project was funded by Ministerio de Ciencia y Tecnologia de Colombia (Project 55696-2017) and Universidad El Bosque (PCI 2016-8343).

Conflict of interest

The authors have no financial or other interests regarding the submitted manuscript that might be construed as a conflict of interest.

Acknowledgements

The authors thank Liceth Castro Gómez from Clínica Club Noel for her valuable help with the patients' recruitment.

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