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Research note

Echovirus 30 detection in an outbreak of acute myalgia and rhabdomyolysis, Brazil 2016–2017

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ABSTRACT

Objectives: To describe an outbreak of acute myalgia accompanied by elevated levels of muscle enzymes that occurred in the northeast region of Brazil from December 2016 through to May 2017.
Methods: Clinical data were analysed and laboratory tests were performed in 86 specimens obtained from 52 individuals with suspected acute myalgia. A broader reactive enterovirus real-time RT-PCR followed by a semi-nested PCR amplification of partial VP1 gene were performed to identify the causative agent.
Results: Eighty-six clinical samples were received in our laboratory during the myalgia outbreak. Median age of individuals was 39 years. Sudden acute myalgia and dark urine were the most common symptoms. Creatine phosphokinase levels were elevated with mean value ~16 893 U/L. Human enterovirus was detected in 67% (58/86) of the patient's specimens (urine, serum, faeces and rectal swab). The enterovirus positivity per patient was 82.7% (43/52). Echovirus 30 (E-30) (82% of the typed specimens, 18/22; 76.4% (13/17) of the typed specimens per patient) was the main enterovirus identified. In addition to E-30, CV-A16 (1/22) and E-6 (3/22) were detected in 4% and 14% of the typed specimens, respectively. No deaths occurred.
Conclusion: The 2016–2017 outbreak of acute myalgia that occurred in the northeast region of Brazil can be associated with E-30. Despite the clinical manifestations, a favourable outcome was observed for all patients. **I.P. Sousa, Clin Microbiol Infect 2019;25:252.e5–252.e8**

Introduction

Although most human enterovirus (EV) infections are asymptomatic, these viral agents can cause several illnesses, such as meningitis, encephalitis, pleurodynia, acute flaccid paralysis, myositis and myocarditis [1]. Some enteroviruses, such as coxsackieviruses A9, B2, B3, B5 and B6, and echovirus 9, have also been associated with rhabdomyolysis development ([2] and references therein). Rhabdomyolysis results from the rapid breakdown of skeletal muscle fibres, which leads to leakage of potentially toxic cellular contents into the systemic circulation. The syndrome is characterized by muscle weakness, myalgia, dark urine and elevation of serum creatine phosphokinase (CPK) levels [3]. The aetiologies of rhabdomyolysis are extremely variable, with exercise, seizures, alcohol and infections presenting as common causes [3]. Recently, an outbreak of epidemic myalgia with high levels of CPK was observed in Japan and Brazil [4,5].

Methods

From December 2016 through May 2017, we received a set of 86 clinical specimens (35 faeces; 10 urine; 40 sera; 1 rectal swab) obtained from 52 patients from five states of the northeast region of Brazil, predominantly from the states of Bahia, Ceará, Paraíba, Pernambuco and Rio Grande do Norte (Table 1). Viral RNA was

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Table 1
Myalgia cases reported in Brazil, 2016–2017, and laboratory results

States	Patient. no.	Gender/ Age	Main symptoms/ CPK (U/L)	Source	Results by: rRT-PCR/RTsn-PCR (positive/specimens)	EV-positive rate (%) per specimen	Enterovirus typed
Bahia (48 specimens)	1	F/24	myalgia	stool/serum	+/–; +/+	stools (52.9; 9/17)	E-30 (serum)
	2	F/-	myalgia	stool/serum	–/–; +/+	urine (50; 1/2)	—
	3	M/-	myalgia	stool/serum	+/+; +/–	serum (86.2; 25/29)	—
	4	M/72	myalgia	stool/serum	–/–; +/+		CVA-16 (serum)
	5	F/28	dark urine; myalgia/4758	stool/serum	–/–; +/–		—
	6	F/32	dark urine; myalgia; fever	stool/serum	+/–; +/–		—
	7	M/42	dark urine; myalgia; fever	stool	+/–		—
	8	F/10	myalgia	stool/serum	+/+; +/+		E-6 (stool)
	9	M/76	myalgia	stool/serum	+/+; +/–		—
	10	M/63	myalgia/12 063	serum	+/+		—
	11	F/46	myalgia	serum	+/–		—
	12	F/8	myalgia; fever; headache/6174	serum	+/–		—
	13	F/68	myalgia	serum	+/–		—
	14	M/50	myalgia/3075	serum	+/+		E-6 (serum)
	15	M/10	myalgia/110	serum	+/–		—
	16	F/58	myalgia/4131	serum	+/–		—
	17	F/-	myalgia/22402	serum	+/–		—
	18	M/34	dark urine; myalgia	serum	+/+		—
	19	M/13	myalgia	serum	+/–		—
	20	M/17	myalgia	stool/serum	–/–; +/+		E-30 (serum)
	21	M/21	dark urine; myalgia/984	stool/serum	+/+; –/–		E-30 (stool)
	22	F/30	dark urine; myalgia/2700	serum	+/–		—
	23	F/48	dark urine; myalgia; fever	serum	+/+		E-30 (serum)
	24	M/20	myalgia/2541	serum	+/–		—
	25	M/75	myalgia/2000	serum	–/–		—
	26	M/39	myalgia/3592	stool/serum/urine	–/–; +/+; –/–		—
	27	M/43	myalgia	stool	+/–		—
	28	F/15	myalgia	stool/serum/urine	+/+; +/+; +/–		E-30 (serum)
	29	F/52	myalgia	stool/serum	–/–; –/–		—
	30	M/50	myalgia	stool/serum	–/–; +/+		E-30 (serum)
	31	M/54	myalgia	serum	–/–		—
	32	M/31	myalgia/30 740	stool	–/–		—
Ceará (28 specimens)	33	F/43	dark urine; myalgia/22 755	stool/serum	+/–; –/–	stool (54.5; 6/11)	E-6 (stool)
	34	F/58	myalgia	stool/serum/urine	+/–; +/–; +/–	urine (71.4; 5/7)	—
	35	M/19	dark urine; myalgia; fever/50 591	stool/serum/urine	+/+; +/+; +/+	serum (60; 6/10)	E-30 (stool/serum/urine)
	36	M/23	myalgia/74 137	stool/serum/urine	+/+; –/–; +/+		E-30 (stool/urine)
	37	M/70	myalgia; fever; headache	stool	+/+		E-30 (stool)
	38	F/44	myalgia; headache	stool	+/–		—
	39	F/15	myalgia	stool/serum/urine	+/–; +/+; +/–		—
	40	M/45	dark urine; myalgia	serum	+/–		—
	41	F/25	myalgia; fever	serum	–/–		—
	42	M/53	dark urine; myalgia/4508	stool/serum/urine	–/–; +/+; –/–		E-30 (serum)
	43	F/54	dark urine; myalgia/4500	stool/serum/urine	–/–; +/+; +/+		E-30 (serum/urine)
	44	M/37	dark urine; myalgia; fever	stool	–/–		—
	45	M/26	dark urine; myalgia; fever/80 000	stool/serum/urine	–/–; –/–; –/–		—
Pernambuco (4 specimens)	46	M/47	myalgia	stool	–/–		—
	47	F/-	myalgia	stool	+/+	stool (75; 3/4)	—
	48	M/29	myalgia	stool	+/–		—
	49	F/64	myalgia/209	stool	+/+		E-30 (stool)
Paraíba (2 specimens)	50	M/28	myalgia	stool	–/–	stool (50; 1/2)	—
	51	F/22	myalgia	stool	+/+		—
Rio Grande do Norte (4 specimens)	52	F/64	dark urine; myalgia	stool/serum/ urine/rectal swab	+/–; +/–; –/+; +/+	stool (100; 1/1) urine (0) serum (100; 1/1) rectal swab (100; 1/1)	E-30 (urine/rectal swab)

CPK, creatine phosphokinase; E-30, echovirus 30; EV, enterovirus; rRT-PCR, real-time RT-PCR; sn-PCR, semi-nested PCR.

extracted (Viral Nucleic Acid Extraction Kit, QIAmp; Qiagen, Hilden, Germany) directly from the clinical specimens and initially subjected to a broad-reactive real-time RT-PCR for human enteroviruses by an 'in-house' method that targets the 5′-conserved untranslated region of the EV genomes. EV-positive samples were then subjected to a semi-nested RT-PCR amplification of partial VP1 gene as described previously [6]. EV-positive amplicons (~350 nucleotides) were cycle-sequenced by the Sanger method using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Nucleotide sequences obtained were compared with those available at GenBank. This activity was considered as a public health response to the poliomyelitis/enterovirus surveillance and so did not require review by the review board.

Results

Initially, the specimens were obtained from suspected cases of epidemic myalgia occurring in individuals from three different families that were hospitalized in Salvador, State of Bahia, presenting sudden onset of severe pain in the cervical region, trapezius, followed by intense muscular pain in the arms, back, thighs and calves. In addition, patients exhibited dark-coloured urine and significant elevations of muscle enzymes, such as CPK (110 to 80 000 U/L; mean value ~16 893 U/L; ref. ~200 U/L). Other enzymes were measured in four of the patients, such as lactate dehydrogenase (mean value ~2173 U/L; ref. ~200 U/L) and transaminases. Both enzymes' levels were elevated. Many patients also presented fever and headache, apart from two patients that had renal disorder. The disease rapidly spread among families, suggesting that transmission occurred through contact or droplets. The number of cases increased quickly and other northeastern states were affected. In all, 86 clinical samples were received in our laboratory during the myalgia outbreak. Patient ages ranged from 8 to 76 years old (mean value 39 years) (Table 1).

Real-time RT-PCR for EV was performed and EV RNA was detected in 58 (67%) specimens with an EV-positive rate per state from 50%–75% (Bahia 63%; Ceará 62%; Pernambuco 75%; Paraíba 50% and Rio Grande do Norte 75%) (Table 1). EV-positive samples were then subjected to a semi-nested RT-PCR amplification of partial VP1 gene and EV-positive amplicons (32/58; 55.1%) were cycle-sequenced by the Sanger method. Nucleotide sequences obtained were compared with those available at GenBank and the analysis showed that Echovirus 30 (E-30) was the main EV detected in the outbreak (Table 1). In addition to E-30 (18/22; 82% of the typed specimens), the study identified CV-A16 (1/22; 4% of the typed specimens) and E-6 (3/22; 14% of the typed specimens) as well (Table 1). The EV-positive rates were more than 50% independent of the specimens analysed (Table 2). Interestingly, 66% of all E-30 identified in this study was from urine (4/18; 22.2%) or serum (8/18; 44.4%) specimens (Table 2). All the remaining E-30 were identified from faecal specimens: faeces (5/22; 27.8%) and rectal swab (1/22; 5.6%) (Table 2). Some samples that were detected in real-time RT-PCR, failed to produce amplicons and could not be typed (26/58; 44.9%).

Table 2
The source of enterovirus-positive specimens identified from patients presenting with acute myalgia

Type of specimens	EV-positive	EV-positive rates (%)	E-30-positive rate (%)
Stool	20	57.1 (20/35)	27.8 (5/18)
Serum	31	77.5 (31/40)	44.4 (8/18)
Urine	6	60 (6/10)	22.2 (4/18)
Rectal swab	1	100 (1/1)	5.6 (1/18)

EV, enterovirus; E-30, echovirus 30.

Discussion

In this study, we identified EV as the possible causative agent of an outbreak of acute myalgia occurring in the northeast region of Brazil. Enterovirus was detected in 82.7% (43/52) of the patients and in 67% (58/86) of the received specimens in our laboratory. Echovirus 30 was the main EV identified, corresponding to 82% (18/22) of the typed specimens and 76.4% (13/17) of the typed specimens per patient. Considering the total number of patients, this value corresponds to 25% (13/52). However, it is worth pointing out that the number of E-30 is probably higher than this because some EV-positive specimens did not produce EV-positive amplicons.

Although the classic symptoms of the rhabdomyolysis consist of muscle pain, weakness and dark urine, it is not common that all of these symptoms are present in the same patient [7]. However, in this study, the dark urine, myalgia and high levels of CPK were markedly present in most of the patients (Table 1).

Echovirus 30 has been one of the causes of paralysis, encephalitis, pancreatitis, and upper and lower respiratory diseases, gastroenteritis, hepatitis, diabetes 1 type and mainly viral meningitis in several countries [1,8–10]. These different outcomes associated with E-30 infections can reflect the adaptive capacity of this virus. Moreover, recombination events demonstrated in different studies are believed to play a substantial role in the infection and evolution of E-30 [10–12].

Enteroviruses have also been implicated in epidemic pleurodynia, an acute febrile illness characterized by chest pain, upper abdominal pain, headache and myalgia caused mainly by coxsackieviruses and echoviruses [1,8]. However, during the present outbreak, none of the patients showed the chest and abdominal pain typical of pleurodynia.

During the course of the clinical cases, patients voluntarily attended the local public health system walk-in clinics where the clinical samples (faeces/serum/urine) were collected. This activity was considered as a public health response to the myalgia outbreak verified in the northeast region of Brazil. As a result, it was not possible to obtain negative controls in the area, during the event, to perform statistical analysis. Furthermore, it has been shown that prevalence of enteroviruses, such as echovirus, in healthy populations may undergo fluctuations among different age groups [13,14].

In a recent study, Bandeira et al. suggested that the myalgia outbreak, which occurred in the State of Bahia, Brazil in 2016, was due to a toxic substance (Haff disease) present in fish consumed by patients [4]. However, samples of suspected fish or seafood showed negative results in tests performed for different aquatic toxins, biotoxins, heavy metals and cyanobacteria [4,15]. Furthermore, patients' specimens were examined in different laboratories for the presence of different aetiological agents (i.e bacteria, Zika virus, Chikungunya virus and parechovirus) [4,15]. It is worthwhile mentioning that, in contrast to previous reports [4], some patients presented fever as one of the symptoms (Table 1).

According to the available patient records, many of them did not ingest fish or seafood before the early symptoms. In conclusion, we document here that EV was the causative agent and that E-30 was the main EV detected in the outbreak of epidemic myalgia with rhabdomyolysis that occurred in Brazil from December 2016 to May 2017.

Limitations

The difficulty in accessing the patient records and the incomplete information were a study limitation. This problem prevented a more precise analysis of the outbreak.

Transparency declaration

The authors declare that they have no competing interests.

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