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Detección del virus del Dengue, Chikungunya y Zika en pacientes con síndrome febril en el departamento de Piura, Perú

Autores:

Jorge Derek Tantaleán Yépez	-	0000-0002-6919-1015
José Fernando Sánchez Carbonel	-	0000-0001-7422-8073

Asesores:

Juana del Valle	-	0000-0002-6011-5040
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ABSTRACT

Background: Arboviral diseases has reemerged in the last years affecting primarily the coast of Peru. Piura is an endemic region of Dengue, where the recent appearance of Chikungunya (CHIKV) and Zika (ZIKV) has been renewed.

Aim: To assess the presence of DENV, CHIKV, and ZIKV in serum samples of patients with acute febrile illness in a locality of Piura, Peru and describe associated clinical features.

Methodology: Descriptive cross-sectional study, sampling was done for convenience where it was obtained a total of 496 serum samples from patients with acute febrile illness were collected from 18 primary care centers. All samples were analyzed via real-time RT-PCR to detect DENV, CHIKV, and ZIKV. In addition, positive samples for DENV were also processed for serotypes 1 to 4 classification via RT-PCR.

Results: DENV was the most common arbovirus detected in 170/496 (34.3%), followed by ZIKV in 39/496 (7.9%) and CHIKV in 23/496 (4.6%). Among the 170 samples positive for DENV, serotype 2 was the most predominant type present in 97/170 (57.1%) of samples, followed by the serotype 3 in 9/170 (5.3%). Headaches, muscle pain, and joint pain were the most common symptoms associated with fever in patients with DENV and ZIKV. No symptoms predominance was observed in patients with CHIKV.

Conclusions: DENV is considered the most frequent arbovirus in Peru and the number of cases has increased dramatically in the last 5 years. However, it is not the only arbovirus that circulates along the northern coast of Peru. It has also been determined the presence of ZIKV and CHIKV in our population, which may suggest the circulation of other arboviruses that have not been detected.

Keywords: Peru, arbovirus, Dengue, Chikungunya, Zika, PCR

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BACKGROUND

In the last years, the incidence of emerging and reemerging arboviral diseases has increased producing a serious social and economic impact in Latin America [1-4]. Dengue (DENV), Chikungunya (CHIKV) and Zika (ZIKV) are arboviruses of great concern due to their impact on public health especially in low-income countries [3,5,6]

Dengue which is considered the predominant arboviral disease in Peru has experienced a rapid prevalence increase in the last five years. In 2017, the number of cases has tripled compared to the previous year being the Peruvian coasts the most affected areas [7]. Piura which is located on the Northwestern coast of Peru registered 26.2% of cases in 2016 and is by far the region with the higher prevalence of DENV, followed by La Libertad (17.2%), Ayacucho (12.1%) and Loreto (10.8%) [8,9]. However, due to the limited laboratory confirmation rate and the number of asymptomatic infected patients, data available at the national level are still limited [10].

In 2013, the first native case of Chikungunya was reported on the Caribbean Island of San Martin [11]. Furthermore, CHIKV has also experienced a rapid spread and by the year 2016 more than 152 000 cases of Chikungunya fever have been reported in Latin America, with most cases reported in Bolivia, Ecuador, Colombia, Venezuela and Peru [12]. In Peru, the first case of CHIKV was reported in 2015 in Tumbes, and since then the number of cases has increased rapidly especially in neighbor regions such as Piura [13,14].

Zika was detected in Peru for the first time in 2016, and until now more than 865 confirmed cases have been registered [15,16]. ZIKV have been reported in 10 of Peru's 25 departments

including Cajamarca, Ica, La Libertad, Madre de Dios, and Piura. However, the impact of Zika in the Peruvian communities has acquired special attention in the last year due to the potential effects on pregnancy. Since ZIKV was first reported in Peru, more than 200 confirmed cases of pregnant women affected by the virus have been observed [16].

The clinical features of these three arboviruses represent a challenge to physicians due to their similarities upon presentation. Most of these patients present as a nonspecific acute febrile illness which can be associated with nausea, vomiting, muscle pain, joint pain, rash, abdominal pain among others [3,5,6]. Therefore, laboratory confirmation has become an essential tool for the etiological diagnosis of arboviruses. In addition, molecular techniques such as PCR have proven to be the most sensitive diagnostic method in the detection and confounding of infections by DENV, ZIKV and CHIKV[3,17,18].

In Peru, the National Center for Epidemiology, Prevention and Control of Diseases at the Ministry of Health have been performing DENV surveillance for the last 5 years; with CHIKV and ZIKV recently added in 2013 and 2016 respectively. However, due to the limited resources, the Laboratory of the National Institute is the only facility that provides laboratory confirmation for the country [16]. Consequently, despite an adequate strategy implemented at the national level, less than 50% of dengue cases are confirmed in the laboratory [8,9].

The present study was conducted in the locality of Piura, Peru to assess the presence of DENV, CHIKV, and ZIKV via real-time RT - PCR in patients with acute febrile illness and to describe epidemiological and clinical features in these patients.

Aim: To assess the presence of DENV, CHIKV, and ZIKV in serum samples of patients with acute febrile illness in a locality of Piura, Peru and describe associated clinical features.

MATERIALS AND METHODS

Patients and Sampling

Descriptive Cross-sectional study, where a convenience sampling was carried out, which was carried out by experienced personnel and trained in the health establishments who were responsible for filling out epidemiological files. Conducted in Piura in coordination with the *“Dirección Regional de Salud de Piura – Red de salud Morropón -Chulucanas”*. Piura is a coastal region in Northwestern Peru with an estimated population of 1 630 772 and has been extensively recognized as an endemic area for dengue. [8,9,18].

In this study, 496 patients with an acute febrile illness were evaluated, who met our inclusion criteria were studied from May to August 2016 (Figure 1). Patients from the following primary health care facilities were included in the study: Buenos Aires, Caserio Cereza, Charrasquillo, Chulucanas, La Alberca, La Bocona, La Encantada, La Matanza, La Pareja, La Quemazón, Laynas, Malacasi, Morropon, Paccha, Pueblo Nueva Esperanza, Salitral, San Juan de Bigote, Serran and Solsol

Inclusion criteria: Patients who presented to the to health establishment with acute febrile illness (greater than or equal to 38 C axillary temperature for less than 7 days) along with one or more of the following symptoms: chills, headache, dizziness, cough, sore throat, nausea and/or vomits, loss of appetite, back pain, dysuria, myalgias, arthralgias, retro-ocular pain, rash, melena, nasal bleeding, gums bleeding, petechiae, ecchymosis, blood tinged sputum, abdominal pain, chest pain, fatigue and altered mental status.

Exclusion criteria: Patients with samples inadequately conserved, or with an incomplete data sheet.

Ethics Statement

This study was approved by the Research Ethics Board of the *Hospital Regional de Cajamarca*, Peru. A written informed consent was signed before enrollment; for participants under 18 years old the informed consent was signed by parents or children caregivers before enrollment.

Samples

One serum sample per patient was collected by using Vacuette® TUBE Serum Separator Clot Activator (Vacuette, Greiner Bio-One, Kremsmünster, Austria); all the samples were stored at -80°C.

The Centers for Disease Control and Prevention (CDC, Fort Collins, CO, USA) provided positive controls for DENV, CHIKV, and ZIKV.

RNA extraction

From 200 µL of serum samples, RNA was extracted with the High Pure RNA Isolation Kit (Roche Applied Science, Mannheim, Germany), according to the manufacturer's instructions. After extraction viral RNA was eluted in 100 µl of nuclease free water to be processed or stored at -20°C until use.

Amplification by Real-time RT-PCR assay for Detection DENV, CHKV and ZIKV with taqman probe.

A one-step RT-PCR was performed using TaqMan with BHQ quencher probe at 125 nM and 250 nM of primers in a final volume of 20 µL. Five microliters of the extracted RNA was combined

with 15 µl of the master mix and the reverse transcription step was performed 95°C for 15 minutes, 60 cycles of 15 seconds at 95°C and 45 seconds at 60°C. All the procedure was performed in Light Cycler® 2.0 Instrument and data was analyzed with the LightCycler® Software 4.1 (Roche Diagnostic, Deutschland-Mannheim, Germany). The primers and the probe used for DENV, CHIKV, and ZIKV described by Leparc-Goffart et al., 2009 [19], Peyrefitte et al., 2005 [20] and Faye et al., 2013 [21] respectively.

Data analysis

Qualitative variables were reported as frequencies and percentages. All analyses were processed with the IBM Statistical Package for the Social Sciences (SPSS) software version 21.0 (SPSS, Chicago, IL, USA).

RESULTS

A total of 496 serum samples from patients with acute febrile illness were assessed for the presence of DENV, CHIKV, and ZIKV. Most of the patients were between 20 to 44 years old (31.3%) and between 5 to 19 years old (27.8%) with no gender predominance. DENV was the most common arbovirus detected in 170/496 (34.3%), followed by ZIKV in 39/496 (7.9%) and CHIKV in 23/496 (4.6%). **(Table 1)**

Furthermore, DENV positive samples were classified by serotypes 1 to 4 depending on their amplified primers. Among the 170 samples positive for DENV, serotype 2 was clearly the most predominant type as it was present in 97/170 (57.1%) of samples, followed by the serotype 3 in 9/170 (5.3%). No serotypes 1 or 4 were detected. **(Table 2)**

Clinical symptoms associated with the fever were registered by the attending physician in all 496 patients. Among the patients with DENV positive samples, headaches 89.4% (152/170), muscle pain 86.5% (147/170) and joint pain 84.1% (143/170) were the most common symptoms accompanying fever. Furthermore, sore throat and back pain were significantly more common in patients with DENV. In the group of patients with positive samples for ZIKV the most frequent symptoms upon presentation were muscle pain 89.7% (35/39), headaches 87.2% (34/39) and joint pain 82.1% (32/39). For the patients with CHIKV, no symptoms predominance was observed; the most common symptoms were headaches, joint pain, loss of appetite, back pain, retro-ocular pain and muscle pain in 74 to 87%. **(Table 3)**

DISCUSSION

A resurgence of arboviral diseases have been observed in Latin America and the most common etiologies are DENV, CHIKV, and ZIKV [1-3]. Peru is not an exception and despite the relatively new implementation of molecular techniques for the national epidemiological surveillance a high prevalence of these viruses have been reported in the last years with outbreaks primarily affecting the coasts of the country [7-9] However, the low laboratory confirmation rate for the national surveillance is especially worrisome as this may lead to an underestimation of these pathogens [9-10].

The national reports in the last 3 years have demonstrated that Piura region is an endemic area for dengue and other arboviruses [8,9,18]. Moreover, after El Niño phenomenon the region of Piura has been severely affected becoming an important scenario from arboviral diseases; thus highlighting the necessity for early detection and confirmation of febrile syndromes etiologies [23,24]. The Peruvian epidemiological reports from July 2017 reveals that

a total of 43719 cases of dengue has been observed in the first half of the year in Piura, which represents the 64.8% of national cases. However, due to the increased demand of laboratory confirmation after El Niño phenomenon, the laboratory of the Instituto Nacional de Salud has been able to confirm only 23.2% of samples, a rate even lower than the previous year [7,8] In addition, the most common serotypes circulating in this region are DENV-2 and DENV-3, but due to the low confirmation rates other serotypes may be neglected [7,10].

In our study population, DENV was the most prevalent arbovirus as we were able to detect it in 34.3% of patients with acute febrile illness. Furthermore, among these 170 positive samples, we observed a clear predominance of DENV-2 in 57.1% followed by DENV-3 in 5.3%, no DENV-1 and DENV-4 were detected. This serotype distribution is similar to the national reports of 2017; however, we were not able to determine the serotype in 64 samples (37.6%) even though we used molecular characterization via real-time RT-PCR. We believe this might be explained due to possible mutations in the DENV circulating in Peru. The presence of mutant variants of DENV can occur as intra-epidemic events sometimes enhancing the replication of specific serotypes [25,26]. This new variant of DENV may contain specific sequences which cannot be amplified by conventional primers for serotypification [20,26,27].

CHIKV cases have also been increasing in 2017 after the outbreaks in Tumbes, Piura, and Ancash [7]. So far in 2017, Piura is again the most affected territory with 379 confirmed cases and 410 probable cases of CHIKV. We were able to detect CHIKV in 23 samples of patients with acute febrile illness.

Suspected autochthonous Zika cases have been reported in 10 departments of Peru and in 2017 a total of 5760 cases of ZIKV have been registered with only an 8.5% of laboratory-confirmed cases. In Piura, only 27 cases have been reported and no autochthonous cases have

been confirmed [12]. However, we believe that the low confirmation rate of our national surveillance may be diminishing the presence of ZIKV in North coast of Peru. In our series, we detected ZIKV in 39 of 496 samples a high number of cases if compared with the national reports in the region. However, due to the recent implementation of CHIKV and ZIKV surveillance and the lack of recent studies describing the prevalence of these viruses in Peru it is difficult for us to compare our results.

We also reported preliminary information about clinical features in patients with acute febrile illness upon presentation in our population. Among the patients with DENV positive samples, headaches 89.4% (152/170), muscle pain 86.5% (147/170) and joint pain 84.1% (143/170) were the most common symptoms accompanying fever. Similarly, for patients with ZIKV the most frequent symptoms were muscle pain 89.7% (35/39), headaches 87.2% (34/39) and joint pain 82.1% (32/39). This information demonstrates once more the similar presentation these both pathogens can have and highlights the importance of laboratory confirmation.

Interestingly, for CHIKV no predominant symptom was observed being headaches, joint pain, loss of appetite, back pain, retro-ocular pain and muscle pain in 74 to 87% of patients. Even though we observed that a sore throats and back pain were significantly more common in patients with DENV compared to the other arboviruses, due to our limited sample we cannot conclude that these clinical features can categorically aid for a DENV diagnosis.

CONCLUSIONS

In conclusion, the underreporting and low confirmation rates of arboviruses remain a surveillance limitation for Peru, which lead to an underestimation of DENV, CHIKV, and ZIKV. Piura is one of the most affected regions for arboviral disease, especially after the El Niño phenomenon. Our study indicates that DENV is considered the most frequent arbovirus in Peru and the number of cases has increased dramatically in the last 3 years. However, it is not the only arbovirus that circulates along the northern coast of Peru. It has also been determined the presence of ZIKV and CHIKV in our population, which may suggest the circulation of other arboviruses that have not been detected, so it is necessary to expand more studies.

Limitations

This study has two main limitations. First, since we only included patients from primary care facilities in an outpatient setting, it is likely that we might have neglected more severe cases. Second, since the study was designed for DENV, CHIK and ZIKV detection via real-time PCR, it is possible that other arboviral etiologies might be present in the negative samples of patients with acute febrile illness. More importantly, as we only collected clinical features from the attending physician we cannot establish causality between the arboviruses detected on the samples and the clinical presentation.

This study, since it is not probabilistic, can not be extrapolated to the rest of the population.

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Attachments

Figure 1: Flow of the study for PCR confirmed DENV, ZIKV and CHIKV in patients with acute febrile illness

Demographics	Totality of cases	Real-time RT-PCR Confirmed
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Table 1. Demographics and PCR confirmed a DENV, ZIKV and CHIKV in patients with acute febrile illness

		DENGUE (n = 170)		ZIKA (n = 39)		CHIKUNGUNYA (n = 23)	
		n	(%)	n	(%)	n	(%)
Age (years)							
0 a 4	39 (7,9)	10	2.00	4	0.8	4	0.8
5 a 19	138 (27,8)	40	8.1	7	1.4	5	1.0
20 a 44	155 (31,3)	55	11.1	15	3.0	6	1.2
45 a 59	86 (17,3)	30	6.0	7	1.4	3	0.6
≥ 60	78 (15,7)	35	7.1	6	1.2	5	1.0
Gender							
Female	270 (54,4)	89	17.9	23	4.6	15	3.0
Male	226 (45,6)	81	16.3	16	3.2	8	1.6

Table 2. DENV Serotypes in positive samples via Real time RT-PCR

	TOTAL CASES OF DENGUE n = 170(%)	PCR Real Time Confirmed				
		DENV1 n (%)	DENV2 N (%)	DENV3 n (%)	DENV4 n (%)	Undetermined n (%)
Positives	170 (34.3)	0 (0.0)	97 (57.1)	9 (5.3)	0 (0.0)	64 (37.6)
Negatives	326 (65.7)	0 (0.0)	73 (42.9)	161 (94.7)	0 (0.0)	106 (62.4)

Table 3. Clinical symptoms in patients with positive samples for DENV, ZIKV, and CHIKV.

Clinical symptoms	Total n= 496 (%)	Real-time RT-PCR Confirmed cases					
		DENGUE (n = 170)		ZIKA (n = 39)		CHIKUNGUNYA (n = 23)	
		n	(%)	n	(%)	n	(%)
Chills	3(0,6)	1	0.2	0	0.0	0	0.0
Headache	444(89,5)	152	30.6	34	6.9	20	4.0
Dizziness	1(0,2)	0	0.0	1	0.2	0	0.0
Cough	1(0,2)	1	0.2	0	0.0	0	0.0
Sore throat	184(37,1)	50	10.1	11	2.2	11	2.2
Nausea and/or	251(50,6)	86	17.3	20	4.0	13	2.6

vomits							
Loss of appetite	312(62,9)	104	21.0	23	4.6	18	3.6
Back pain	270(54,4)	105	92.5	23	4.6	17	3.4
Dysuria	1(0,2)	0	0.0	0	0.0	0	0.0
Myalgia	419(84,5)	147	29.6	35	7.1	17	3.4
Arthralgia	396(79,8)	143	28.8	32	6.5	19	3.8
Retro-ocular pain	337(67,9)	118	23.8	27	5.4	17	3.4
Rash	89(17,9)	26	5.2	8	1.6	6	1.2
Melena	2(0,4)	0	0.0	0	0.0	0	0.0
Nasal bleeding	9(1,8)	3	0.6	1	0.2	1	0.2
Gums bleeding	3(0,6)	2	0.4	1	0.2	0	0.0
Petechiae	11(2,2)	3	0.6	0	0.0	1	0.2
Ecchymosis	2(0,4)	1	0.2	1	0.2	0	0.0
Blood tinged sputum	1(0,2)	0	0.0	0	0.0	0	0.0
Abdominal pain	22(4,4)	7	1.4	1	0.2	1	0.2
Dolor Torácico / Disnea Chest pain	5(1,0)	2	0.4	0	0.0	0	0.0
Fatigue	3(0,6)	0	0.0	1	0.2	0	0.0
Altered Mental Status	1(0,2)	1	0.2	0	0.0	1	0.2