Dengue Virus Coinfection in Human Immunodeficiency Virus-1-Infected Patients on the West Coast of Mexico

Francisco Espinoza-Gómez,^{1,2} Iván Delgado-Enciso,^{2,3} Salvador Valle-Reyes,² Rodolfo Ochoa-Jiménez,¹
Conrado Arechiga-Ramírez,¹ José L. Gámez-Arroyo,¹ Roberto Vázquez-Campuzano,⁴
Carmen Guzmán-Bracho,⁴ Clemente Vázquez,² and Uriel A. López-Lemus^{2,5*}

¹Department of Internal Medicine, University Regional Hospital, Ministry of Health, Colima, Mexico; ²School of Medicine, University of Colima, Colima, Mexico; ³Cancer State Institute, Ministry of Health, Colima, Mexico; ⁴Department of Emerging Diseases and Emergencies, Institute for Epidemiological Diagnosis and Reference, Ministry of Health, Mexico City, Mexico; ⁵Center for Gene Therapy, Hematologic Malignancies and Stem Cell Transplantation Institute, City of Hope, Duarte, California

Abstract. Dengue virus infection in human immunodeficiency virus (HIV)-positive patients is not well studied. Previous reports suggest a transitory inhibition of the HIV-1 viral load, as well as a benign clinical progression of dengue. The follow-up of six HIV-1-infected patients, diagnosed and hospitalized with dengue virus infection in the State of Colima, Mexico, was carried out to analyze the progression of this viral coinfection. The presence of dengue virus serotype 1 was confirmed through molecular tests. No severe complications were observed in any of the patients during dengue virus infection. Significant alteration of the HIV-1 viral loads was not observed during dengue virus infection and 6 months after coinfection. Further studies are required to understand the pathology, as well as the clinical course, of these viral coinfections.

INTRODUCTION

Human immunodeficiency virus (HIV)-infected patients that live in tropical regions are exposed to infections, such as malaria or Chagas disease, that usually have a severe clinical progression and are difficult to diagnose.^{1,2} In theory, coinfection of HIV-1 with other tropical agents can worsen the clinical progression of HIV-1 infection.^{3,4} Nevertheless, in the case of coinfections of certain flaviviruses, such as the GB virus C (GBV-C), hepatitis C virus, or dengue virus (DENV), a reduction of the HIV-1 viral load has been observed.^{5–7} Despite the fact that HIV infection has a worldwide distribution and that extensive regions are endemic for DENV transmission, there are only seven reports on HIV/DENV coinfection,^{6,8–13} which describe 41 patients with this simultaneous infection.

We report herein on six cases of HIV-1-infected patients coinfecting with DENV in the State of Colima, Mexico, with a 6-month follow-up of the HIV-1 viral loads after coinfection.

METHODS

A total of six HIV-1-infected patients that were admitted to the University Regional Hospital of Colima with the clinical suspicion of DENV infection during the 2009 dengue epidemic in the State of Colima, Mexico, were followed up. Dengue diagnosis was confirmed through the detection of DENV NS1 antigen using the SD BIOLINE Dengue Duo rapid test kit (Yongin-si, Gyeonggi-do, Republic of Korea), following the manufacturer's protocol. This serological test detects both DENV NS1 antigen and IgM/IgG antibodies, corroborating a recent infection and exposure to the DENV sometime in the past. Table 1 shows the clinical manifestations of dengue fever, emphasizing that at the time of the medical evaluation, symptoms had begun 2–4 days earlier, with fever in all the cases (37.5–39.5°C) and abdominal pain, vomiting, and

headache in some of the patients. Even though some of the patients had a positive tourniquet test and/or capillary leak at the time of the medical checkup, none of the patients presented with severe clinical symptoms or required hospitalization. Their clinical condition only required the prescription of acetaminophen 500 mg every 6 hours for 3 days, with symptom cessation in all patients between the fifth and sixth day from disease onset, with no chronic malaise. Likewise, the patients did not show symptoms of viral hepatitis or any other chronically infectious disease and were seen 6 months after the acute episode to be evaluated for HIV viral burden.

Whole blood samples were taken from these patients and placed in tubes with ethylenediaminetetraacetic acid in our laboratory. The serum samples were stored at –80°C until their processing. For DENV identification, the serum samples underwent RNA extraction using the QIAamp Viral RNA kit (Qiagen, Hilden, Germany), following the manufacturer's specifications. RNA quality and integrity were evaluated through standard electrophoretic and spectrophotometric methods, respectively. 1 µg of previously extracted RNA underwent real-time polymerase chain reaction (RT-PCR) with the Qiagen OneStep RT-PCR kit. Consensus primers previously described for a conserved region of the NS5 gene of various species of flaviviruses, including the dengue viruses (DENV-1 to 4), were used.¹⁴ The RT-PCR conditions were an initial preincubation at 50°C for 30 minutes (RT condition), followed by an incubation at 94°C for 15 minutes (initial PCR activation); 37 cycles at 97°C for 1 minute (denaturation), at 60°C for 1 minute (annealing), and at 72°C for 1 minute (extension); and a final extension at 72°C for 10 minutes. Each reaction product (10 µL) underwent electrophoresis in agarose gels at 1% that contained SYBR Green I Nucleic Acid Gel Stain (Invitrogen, Carlsbad, CA) and they were visualized on a UV light transilluminator. The DNA products were purified directly from the gel using the QIAquick Spin Kit (Qiagen) and underwent molecular cloning with the Qiagen PCR Cloning^{Plus} kit, following the manufacturer's instructions. The positive clones underwent automatic sequencing for both strands at the McLab company (San Francisco, CA). The sequences

* Address correspondence to Uriel A. López-Lemus, School of Medicine, University of Colima, Avenida Universidad 333, Las Víboras, Colima, México 28040. E-mail: lemus_a83@yahoo.com

obtained were compared with those published in the NCBI using the Nucleotide Blast software.

HIV-1 viral load determination was carried out at the Institute for Epidemiological Diagnosis and Reference (Department of Health, Mexico) with the COBAS AmpliPrep/COBAS TaqMan HIV-1 Test (Roche; Basel, Switzerland) and the COBAS Taqman 48 Analyzer, following the manufacturer's instructions. The HIV viral load results prior to DENV infection were obtained from the medical records of all the patients.

This study was approved by the Ethics Committee of the Biomedical Research Center of the University of Colima and the Institutional Review Board of the Department of Health (State of Colima, Mexico). All the patients signed statements of informed consent.

RESULTS

A total of six HIV-1-infected patients coinfecting with DENV were analyzed. The molecular tests showed the presence of two strains of DENV-1 during these coinfections. Patients 1 and 6 presented with DENV-1 strain DENV-1/MX/BID-V3664/2006 (GenBank GQ868499) infection, whereas patients 2, 3, 4, and 5 had DENV-1 strain DENV-1/MX/BID-V3744/2008 (GenBank GQ868529) infection. Clinical diagnosis and laboratory analysis results during the study follow-up are shown in Tables 1 and 2.

As can be seen in Table 2, patients 2, 4, and 6 presented with an undetectable HIV-1 viral load (< 40 HIV-1 RNA copies/mL) before the DENV infection and it remained undetectable during and after coinfection. Patient 3 presented with a slight decrease in the HIV-1 viral load during DENV infection and it was registered as undetectable 6 months after coinfection. Patient 5 presented with an increase in the HIV-1 viral load during DENV infection and showed a viral load decrease 6 months after coinfection. In contrast, patient 6 showed a slight increase in the viral load during DENV infection and at 6 months after coinfection. None of the patients experienced severe dengue symptoms, despite having shown the alarm symptoms of vomiting, abdominal pain, positive tourniquet test, leukopenia, or thrombocytopenia on their hospital admission. Likewise, there were no clinical complications attributable to HIV-1 infection during the follow-up period.

DISCUSSION

There is little existing information on HIV and DENV coinfection in tropical regions. Our study reports on six HIV-1-infected patients that presented with DENV-1 infection in the State of Colima, Mexico, an endemic area for DENV transmission. To the best of our knowledge, this is the first follow-up period analysis of the intermediate-term progression of HIV viral loads in six HIV-1-positive patients coinfecting with DENV-1 in a tropical region of Mexico. No severe complications from either the HIV-1 or DENV infection were observed. This behavior is possibly because of the fact that all patients were treated with highly active antiretroviral therapy (HAART),¹⁵ and that severe forms of dengue are attributed to the excessive activity of certain cytokines, such as interleukin (IL)-10 and tumor necrosis factor (TNF)- α , that are usually attenuated in HIV-infected patients.¹⁶ Our study results are consistent with those of previous reports,^{8,9} but those studies did not carry out a case follow-up.

The identification of two different variants of DENV-1 in these coinfections is of particular interest because of the epidemiological and clinical impact they could have, especially on HIV-infected patients, whose clinical course could be unpredictable. A previous report from Oaxaca, Mexico, has shown multiple recombinations in the DENV-2 genome that could lead to more severe clinical complications.¹⁷ In addition, specific recombinations in the DENV-1 NS5 gene leading to a more severe clinical course in HIV-uninfected patients in the State of Colima, Mexico, have been found (U. A. Lopez-Lemus et al., unpublished data). The diagnosis of dengue in HIV-infected patients is a clinical challenge. The natural course of the disease can be a source of confusion due to immunologic modifications caused by HIV-1 or to the coexistence of other opportunistic infections.^{3,4} The relatively few prior studies in regard to HIV/DENV coinfection is not only the result of the low prevalence of this viral coinfection, but also of the difficulty in opportunely diagnosing DENV infection in HIV-infected patients in tropical regions.

The HIV-1 viral load was undetectable in the majority of the patients from the beginning of the study to the end of the follow-up, and it was reduced after DENV infection in two cases. Only one patient showed a discrete elevation in the HIV-1 viral load after DENV coinfection. These findings

TABLE 1
Clinical diagnosis of dengue in HIV-1 positive patients

Patient	Age	Sex	Temperature* (°C)	Days of progression†	Abdominal pain	Vomiting	Headache	Tourniquet test	Gingivorrhagia/capillary leak	HAART	NS1/IgM/IgG‡
1	36	Female	38.0	3	+	+	–	–	+	ATV/RTV, TDF, FTC	+/-/-
2	41	Male	39.5	2	–	–	–	–	+	EFV, TDF, FTC	+/-/+
3	57	Male	37.5	4	–	+	–	+	–	SQV/RTV, TDF, FTC	+/-/-
4	32	Male	39.0	2	+	+	+	+	–	ZDV, 3TC, QV/RTV	+/-/+
5	47	Female	38.0	3	+	+	+	+	–	SQV/RTV, TDF, FTC	+/-/-
6	38	Male	38.5	2	+	+	+	+	+	LPV/RTV, ZDV, 3TC	+/-/-

ATV = atazanavir; EFV = efavirenz; FTC = emtricitabine; HAART = highly active antiretroviral therapy; HIV = human immunodeficiency virus; LPV = lopinavir; RTV = ritonavir; SQV = saquinavir; 3TC = lamivudine; TDF = tenofovir; ZDV = zidovudine.

* Temperature at the time of the medical evaluation.

† Days of progression at the time of the clinical analysis and the blood, HIV viral burden, and serologic tests for dengue virus.

‡ Confirmation result by means of SD BIOLINE Dengue Duo rapid test (Yongin-si, Gyeonggi-do, Republic of Korea).

TABLE 2
Laboratory results of the HIV-1-infected patients during DENV infection and HIV viral loads during the study follow-up

Patient No.	CD4+ (cells/ μ L)	Platelets ($\times 10^3$)	Leukocytes ($10^3/\mu$ L)	Neutrophils ($10^3/\mu$ L)	Lymphocytes ($10^3/\mu$ L)	ALT (IU/L)	AST (IU/L)	Albumin (g/dL)	HIV-1 viral load (HIV-1 RNA copies/mL)		
									R1	R2	R3
1	349	92	1,200	738	96	159	248	3.1	62	40	Undetectable
2	584	107	2,300	1,242	644	56	89	4.2	Undetectable	Undetectable	Undetectable
3	222	44	1,200	888	140	109	101	3.7	66	102	42
4	365	62	1,400	546	490	103	109	4.0	Undetectable	Undetectable	Undetectable
5	445	172	4,300	2,303	860	43	51	4.1	2,890	3,490	6,140
6	375	61	1,100	620	480	102	105	4.0	Undetectable	Undetectable	Undetectable

ALT = alanine aminotransferase; AST = aspartate aminotransferase; DENV = dengue virus; HIV = human immunodeficiency virus; R1 = HIV-1 viral loads 6 months before DENV infection; R2 = HIV-1 viral loads during DENV infection; R3 = HIV-1 viral load 6 months after DENV infection.

suggest that there was no significant HIV viral load behavior attributable to DENV infection during the study follow-up. Our results coincide with a previous report¹¹ in which HIV viremia remained at normal levels in a patient with a multiple HIV, DENV-1, and hepatitis B virus infection. Interestingly, a previous report suggests that HIV replication is transiently inhibited when there is a DENV-1 coinfection in peripheral blood mononuclear cells with no antiviral treatment.⁶ We cannot explain this phenomenon due to the lack of evidence of this viral coinfection; however, some reports have shown an inhibitory effect on HIV-1 replication during the expression of the NS5 protein of DENV-1 and DENV-2 in CD4+ T cells.^{7,18,19} This reduction has been associated with overexpression of the SDF-1 cytokine and reduced CD4 receptor and CXCR4 coreceptor expression.^{7,19} Likewise, GBV-C NS5A and NS3 protein expression have shown suppressed HIV-1 replication in T lymphocytes.^{20,21} Interestingly, recent studies at our laboratory have shown that the expression of the non-structural replicative proteins, NS1 and NS3 of the DENV-2 New Guinea C strain, through a lentiviral-based vector, suppressed HIV replication > 90% in Jurkat CD4+ T cells on the third day, compared with the control cells (U. A. Lopez-Lemus, unpublished data).

In conclusion, the results presented herein suggest that DENV infection did not cause severe clinical complications in HIV-1-infected patients treated with HAART. No severe complications attributable to HIV infection or significant behavior of the HIV viral loads were observed in any of the patients studied. Additional studies are needed to understand any interactions between DENV and HIV, as well as the HAART effect on dengue disease progression.

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Authors' addresses: Francisco Espinoza-Gómez and Iván Delgado-Enciso, Department of Public Health, University of Colima, Colima, Mexico, E-mails: fespino@uclm.mx and ivan_delgado_enciso@uclm.mx. Salvador Valle-Reyes, Rodolfo Ochoa-Jiménez, and Uriel A. López-Lemus, Department of Public Health, University of Colima, School of Medicine, Colima, Mexico, E-mails: juanchavador@hotmail.

com, doccochoa@hotmail.com, and lemus_a83@yahoo.com. Conrado Arechiga-Ramírez and José L. Gámez-Arroyo, Hospital Regional Universitario de Colima, Internal Medicine, Colima, Mexico, E-mails: drpepearechiga@latinmail.com and gamezajl@hotmail.com. Roberto Vázquez-Campuzano and Carmen Guzmán-Bracho, Department of Emerging Diseases and Emergencies, Institute for Epidemiological Diagnosis and Reference, Mexico City, Mexico, E-mails: roberto.vazquez@salud.gob.mx and carmen.guzman@salud.gob.mx. Clemente Vázquez, Department of Biomedical Sciences, University of Colima, Colima, Mexico, E-mail: clemvas@uclm.mx.

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