Le Infezioni in Medicina, n. 1, 98-103, 2020

Dengue fever complicated by liver dysfunction due to possible co-infection with hepatitis E in a returning traveller from Cuba

Spinello Antinori[1,2], Valentina Morena[1,2], Gabriele Pagani[1,2], Giulietta Venturi[3], Andrea Giacomelli[1,2], Laura Milazzo[2], Anna Lisa Ridolfo[2], Nadia Zanchetta[4]

[1]Department of Biomedical and Clinical Sciences "Luigi Sacco", University of Milan, Italy;
[2]III Division of Infectious Diseases, ASST Fatebenefratelli Sacco, Luigi Sacco Hospital, Milan, Italy;
[3]National Reference Laboratory for Arboviruses, Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy;
[4]Clinical Microbiology, Virology and Bioemergency, ASST Fatebenefratelli Sacco, Luigi Sacco Hospital Italy

SUMMARY

Dengue fever is a mosquito-borne infection that co-circulates with Chikungunya and Zika virus infection in many parts of the world. Dengue virus (DENV) is occasionally responsible for acute hepatitis and a few cases of acute hepatitis due to co-infection with DENV and hepatitis E virus have been described in India. A 37-year-old Cuban woman living in Italy was admitted to our hospital with a presumed arboviral infection upon her return to Italy short after a 15-day trip to her home-country to visit relatives. An acute infection due to DENV serotype 1 was initially diagnosed, following a clinical course characterized by signs of liver dysfunction that were possibly due to co-infection with hepatitis E virus.

Keywords: Dengue virus, Hepatitis E, serological cross-reactivity, Chikungunya virus, Zika virus.

INTRODUCTION

Arboviral infections caused by dengue virus (DENV), Chikungunya virus (CHIKV) and Zika virus (ZIKV) are endemic in the Caribbean Islands, Central and South America where they share common vectors (*Aedes* mosquitoes) [1]. Although DENV, CHIKV and ZIKV infections each have some unique characteristics, their clinical presentation is usually characterized by highly similar febrile illnesses that may include fever, headache, myalgia, arthralgia, and skin rash. Consequently, when making a diagnosis, all three viruses should be screened for using appropriate tests, given that sporadic cases of arbovirus co-infection (DENV/CHIKV and DENV/CHIKV/ZIKV) have been described in people living in endemic countries and returning travellers [2-5].

The clinical picture of arboviral infections may include signs of variably severe liver dysfunction, and cases suggestive of acute hepatitis due to concurrent dengue fever and hepatitis A, or hepatitis E viruses' infection which have been reported both from India and Pakistan [6, 7].

Herein, we report a case of dengue infection associated with a probable hepatitis E virus (HEV) infection in a traveller returning from Cuba.

Corresponding author
Spinello Antinori
e-mail: spinello.antinori@unimi.it

CASE REPORT

A 37-year-old Cuban woman who had been living in Italy for five years was admitted to our Infectious Disease Department in November 2018 be-

cause of a suspected arboviral infection. She had started complaining of high fever, chills, severe headache and asthenia upon her return to Italy from a 15-day trip to her country of origin (1-15 November 2018) to visit friends and relatives. Four days after her return, a general practitioner, who prescribed amoxicillin-clavulanate, visited her. However, because symptoms persisted with a new onset rash on November 20, she attended the nearest Emergency Department, which documented fever (38.1°C) and a maculopapular rash involving her face, trunk and legs. Laboratory tests revealed leukopenia (1.65 10^9/L), thrombocytopenia (101x10^9/L), increased C reactive protein values (252 mg/L) and a slight increase in aspartate aminotransferase (AST) levels (64 U/L). A chest X-ray was negative. The antibiotic therapy was discontinued, and fluid hydration was prescribed.

The patient was subsequently transferred to our ward, where she promptly underwent various microbiological diagnostic tests for DENV, CHKV, and ZIKV infection. Workup included, serological tests (NovaLisa Dengue virus IgM/IgG ELISA, NovaTec, Germany; NovaLisa Chikungunya IgM/IgG ELISA, NovaTec, Germany; Zika virus IgM/IgG, Dia.Pro Diagnostic, Italy; Platelia Dengue NS1 Ag ELISAtest, Biorad, France), as well as molecular testing [Multiplex real-time polymerase chain reaction (PCR) for DENV and CHKV: FTD Dengue/Chik, Fast Track Diagnostic, Luxenbourg; and reverse transcription PCR for ZKV: RealStar Zika Virus RT-PCR Altona Diagnostics, Germany]. Serum samples were also sent to the National Reference Laboratory for Arboviruses for plaque reduction neutralization testing (PRNT) for DENV, CHKV and ZKV [8].
Table 1 shows the results of the laboratory exam-

Table 1 - Laboratory test results during hospitalisation for dengue fever and hepatitis E co-infection.

Parameter	*Reference values*	*ED*	*Hospitalisation*						*Follow up*
		20/11/2018	*21/11/2018 (Day 1)*	*23/11/2018 (Day 3)*	*24/11/2018 (Day 4)*	*25/11/2018 (day 5)*	*26/11/2018 (day 6)*	*28/11/2018 (day 8)*	*10/12/2018*
C-reactive protein (mg/L)	<1.0	252	129	58	ND	ND	2.2	1	0.9
Hemoglobin (g/dL)	125-155	130	120	118	122	121	123	114	110
Hematocrit (%)	38-46	39	35	35	36	36	37	34	34
RBC (1012/L)	4.13-5.15	4.83	4.44	4.35	4.57	4.53	4.62	4.23	4.05
MCV (fL)	81.8-95.3	81.6	78.7	81.0	80.0	80.4	80.0	81.0	82.7
WBC (109/L)	4.19-9.35	1.65	1.48	1.09	1.78	2.46	2.88	3.29	5.73
Neutrophils (%)	40.1-72.5	68.0	58.0	75.0	31.0	42.0	36.0	42.0	63.0
Lymphocytes (%)	19.6-46.5	19.4	24.0	17.0	52.0	41.0	46.0	37.0	25.0
Monocytes (%)	5.3-11.6	14.5	18.0	8.0	12.0	14.0	15.0	18.0	11.0
Platelets (109/L)	169-359	101	102	53	63	92	131	164	300
AST (U/L)	11-34	64	53	236	523	447	394	87	22
ALT (U/L)	8-41	31	29	114	271	286	325	184	24
PT (INR)	0.85-1.18	–	1.14	1.10	1.05	1.12	1.07	ND	1.09
aPTT (ratio)	0.85-1.20	–	1.65	1.16	1.18	0.97	0.98	ND	1.01
LDH (U/L)	125-220	320	293	583	895	635	532	292	226
Total bilirubin (mg/dL)	<1.20	–	0.42	<1.20	< 1.20	< 1.20	0.65	–	1.12
Dengue IgM (Index)	<1 Negative >1 Positive	–	1.9	–	–	–	–	8.9	10.5
Dengue IgG (Index)	<1 Negative >1 Positive	–	0.1	–	–	–	–	1.4	3.1
Dengue NS1 Ag	Negative	–	Positive	–	–	–	–	–	–
Dengue RNA, (blood)	Negative	–	Positive (serotype 1)	–	–	–	–	–	Negative

>>> *Segue*

Parameter	Reference values	ED	Hospitalisation						Follow up
		20/11/2018	21/11/2018 (Day 1)	23/11/2018 (Day 3)	24/11/2018 (Day 4)	25/11/2018 (day 5)	26/11/2018 (day 6)	28/11/2018 (day 8)	10/12/2018
Dengue RNA (urine)	Negative	–	Negative	–	–	–	–	–	Negative
Dengue PRNT	PRNT80*≥10 Positive PRNT50**≥10 Borderline	–	Negative	–	–	–	–	Positive	–
Chikungunya IgM (Index)	<9 Negative >11 Positive	–	16.92	–	–	–	–	–	–
Chikungunya IgG (Index)	<9 Negative >11 Positive	–	4.56	–	–	–	–	–	–
Chikungunya RNA (blood)	Negative	–	Negative	–	–	–	–	–	–
Chikungunya RNA (urine)	Negative	–	Negative	–	–	–	–	–	–
Chikungunya PRNT	PRNT80*≥10 Positive PRNT50**≥10 Borderline	–	Negative	–	–	–	–	Negative	–
Zika virus IgM (Index)	<0.8 Negative >1.1 Positive	–	0.4	–	–	–	–	–	–
Zika virus IgG (Index)	<0.8 Negative >1.1 Positive	–	0.1	–	–	–	–	–	–
Zika PRNT	PRNT80*≥10 Positive PRNT50**≥10 Border line	–	–	–	–	–	–	Negative	–
HbsAg	Negative	–	Negative	–	–	–	Negative	–	–
HBs Ab (mUI/mL)	<10 Negative >10 Positive	–	Negative	–	–	–	Negative	–	–
HBc IgM	Negative	–	–	–	–	–	Negative	–	–
HBc IgG	<1.0 Negative >1.0 Positive	–	Negative	–	–	–	Negative	–	–
HCVab	Negative	–	Negative	–	–	–	–	–	–
HAV IgM	Negative	–	–	–	–	–	Negative	–	–
HAV IgG	Negative	–	–	–	–	–	Positive	–	–
HEV IgM	<1 Negative >1.2 Positive	–	–	–	–	–	1.4	–	1.2
HEV IgG	<0.9 Negative >1.1 Positive	–	–	–	–	–	9.9	–	8.5
HEV RNA (blood)	Negative	–	–	–	–	–	-	–	Negative
HIV 1/2 Ag-Ab	Negative	–	Negative	–	–	–	-	–	–
CMV IgG (U/mL)	<12 Negative >14 Positive	–	–	–	–	–	136	–	–
CMV IgM (U/mL)	<18 Negative >22 Positive	–	–	–	–	–	20.5	–	–
CMV avidity test (Index)	<0.15 low 0.15-0.25 medium >0.25 high	–	–	–	–	–	0.52	–	–

*Serum dilution leading to an 80% reduction in viral infectious units. **Serum dilution leading to a 50% reduction in viral infectious units.

inations. The patient was positive for anti-DENV IgM, DENV- NS1 antigen, and blood RT-PCR revealed the presence of DENV; she was also positive for anti-CHKV IgM, but blood and urine PCR were CHKV negative, and she was also negative for ZIKV IgG and IgM.
PRNT for DENV and CHKV resulted negative on the first day of hospitalization converting positively eight days later for DENV but negative for CHKV.
During the first four days of hospitalization, the patient remained febrile, and her rash became generalized (face, trunk, abdomen and legs) along with pruritus; she also complained of nausea prompting administration of metoclopramide. Over the same period, her AST and alanine aminotransferase (ALT) levels increased, respectively reaching peaks of 523 U/L and 325 U/L. Serological tests for viral hepatitis (A, B, C and E) and cytomegalovirus (CMV) were performed on the sixth day of hospitalization. CMV serology was equivocal (borderline IgM positive, IgG positive), but an avidity test ruled out an acute CMV infection. Serology for hepatitis A, B, C viruses was negative, whereas anti-hepatitis E virus (HEV) IgG and IgM were positive.
The course of hospitalization was uneventful, and the patient was discharged after eight days with only slightly high liver enzyme values. A follow-up examination twelve days later showed that her liver enzyme levels had normalized; she was still positive for anti-HEV IgM and IgG but an HEV-RNA test that could only be performed at this time proved negative.

DISCUSSION

We describe a case of imported dengue fever with acute anicteric hepatitis and probable HEV co-infection. An increase in liver enzyme levels is frequently observed during dengue fever, being reported in 26-85% of the cases diagnosed in endemic countries such as India, and in 34% of the cases of imported dengue [9-11]. However, the liver dysfunction observed during DENV infection is generally mild. One study conducted in India found that only 11.4% of the patients with dengue fever had liver enzyme levels of >10 times above normal limits; while another study, found that only 13% of the patients had ALT levels >200 IU/L [9, 10]. Nevertheless, liver failure with a marked increase in AST levels (up to >1000 IU/L) has been observed in 3.8% of the patients dying from dengue fever in India, and there is even one case report of a patient with dengue fever-related acute liver failure receiving a liver transplantation [10, 12]. The peak AST and ALT levels of our patient were respectively 15 and 8 times higher than normal, which is consistent with the greater increase in AST than in ALT described in a large series of patients with dengue fever [10].
Interestingly, in a recent retrospective study conducted in Taiwan looking at over 4000 patients who were hospitalized with dengue fever, values of AST >203 UI/L, ALT >55 UI/L, AST2/ALT criteria >337,35 and AST/platelet count ratio index >19,18 on day 3 of illness resulted in a true positive rate (TPR) for identifying early mortality respectively of 90%, 78%, 100%, 100% [13]. In our patient, on day 3 of hospitalization, three out of the four mentioned variables were consistent with those identified with high TPR of early mortality. Although the clinical picture of acute hepatitis presented by our patient could have been exclusively attributed to dengue fever, we searched for other possible concomitant causes and found a serological pattern that was suggestive of concomitant acute hepatitis E. Three cases in India with concomitant diagnoses of dengue fever, hepatitis E and leptospirosis presenting with jaundice, acute renal failure and encephalopathy, in which the diagnosis of HEV infection was based on a single positive anti-HEV IgM test have been reported in the literature to date [6, 14, 15].
However, according to the guidelines of the European Association for the Study of the Liver (EASL), HEV RNA testing (alone or together with IgM and IgG testing) is recommended to ensure an accurate diagnosis of hepatitis E, although it is worth noting that HEV-RNA can be detected only in 25-44% of patients with acute hepatitis, even when it is sought early in the course of infection [16-18]. In our case, we followed an algorithm in which HEV-RNA in blood and stools are required to confirm a positive anti-HEV IgM serological test. Unfortunately, our patient had already been discharged when the results of the anti-HEV antibody test became available, and this prevented us from immediately searching for HEV-RNA. The duration of HEV-RNA in blood and stools is very short during the symptomatic phase of hepatitis E, which

may explain the negative result of the HEV-RNA test that could only be performed several days after the onset of acute hepatitis. We conclude that the prompt use of both serological and molecular methods would have allowed a more accurate diagnosis of HEV infection.

Finally, the finding that our patient was positive for anti-CHIKV IgM further confirms the existence of previously described serological cross-reactions between *Flavivirus*es (DENV, ZIKV) and between them and *Alphavirus* (CHIKV) that can complicate the interpretation of serological tests [8, 19-22]. Molecular tests are highly sensitive and specific for Arboviruses, and allow the specific agent involved to be identified. In our case, the prompt use of RT-PCR solved the question of serological cross-reactions between DENV and CHIKV by indicating DENV as the cause of the patient's infection. However, it should be considered that the short duration of viremia during acute arboviral infection is a limitation of molecular tests, whereas PRNT (which allows virus-specific neutralizing antibodies to be measured) has proved to be highly specific in identifying the agents involved in primary arboviral infections. Studies suggest that this test should be included in the routine diagnostic algorithm of arboviral infections, especially in the case of patients presenting some time after the acute onset of symptoms [8, 22].

In conclusion, although arboviral infections are frequently associated with some degree of liver dysfunction, careful consideration should always be given to the possible contribution of liver damage caused by co-infection with the various agents that are prevalent in areas in which arboviruses are endemic.

Acknowledgments

We thank Kevin Smart for English assistance.

Conflict of interest

All the authors declare no conflict of interest.

REFERENCES

[1] Mercado-Reys M, Acosta-Reyes J, Navarro-Lechuga E, et al. Dengue, chikungunya and zika virus coinfection. Results of the national surveillance during the zika epidemic in Columbia. *Epidemiol Infect.* 2019; 147, e77, 1-7.

[2] Farrell DF, Lupone CD, Kenneson A, et al. Case report: An acute chikungunya infection and a recent secondary infection in a peripartum case in Ecuador. *Am J Trop Med Hyg*. 2018; 98, 838-40.

[3] Raut CG, Rao NM, Sinha DP, et al. Chikungunya, dengue, and malaria coi-infection after travel to Nigeria, India. *Emerg Infect Dis.* 2015; 21, 908-9.

[4] Villamil-Gomez WE, Gonzalez-Camargo O, Rodriguez-Ayubi J, et al. Dengue, Chikungunya and Zika infection in a patient from Colombia. *J Infect Public Health.* 2016; 9, 684-6.

[5] Schilling S, Emmerich P, Gunther S, Schmidt-Chanasit J. Dengue and Chikungunya virus coinfection in a German traveller. *J Clin Virol.* 2009; 45, 163-4.

[6] Singh RK, Ghatak T, Saigal S, Baronia AK. Comparison between three rare cases of coinfection with dengue, leptospira and hepatitis E: is early endothelial involvement the culprit in mortality? *Ann Med Health Sciences Res.* 2014; 4, 32-4.

[7] Yakoob J, Jafri W, Siddiqui S, Riaz M. Dengue fever with hepatitis E and hepatitis A infection. *J Pak Med Assoc.* 2009; 59: 176-7.

[8] Fortuna C, Remoli ME, Rizzo C, et al. Imported arboviral infections in Italy, July 2014-October 2015: a National Reference Laboratory report. *BMC Infect Dis.* 2017; 17, 216.

[9] Tavakolipoor P, Schmidt-Chanasit J, Burchard GD, Jordan S. Clinical features and laboratory findings of dengue fever in German travellers: a single-centre, retrospective analysis. *Travel Med Infect Dis.* 2016; 14, 39-44.

[10] Bhushan D, Kumar R. Clinical profile, hepatic dysfunctions, and outcome of dengue patients in a tertiary care hospital of Eastern India. *J Assoc Phys India.* 2018; 66, 47-9.

[11] Rabbani MU, Aslam M, Zaheer MS, Ashraf MU. Clinical and laboratory profile of dengue fever in a North Indian tertiary hospital. *J Assoc Phys India*. 2018; 66, 37-9.

[12] Galante A, Adey O, Lau L, et al. Liver transplantation for acute liver failure due to Dengue fever: first successful reported case worldwide. *Hepatology.* 2019; 70, 1863-5.

[13] Yeh C-H, Lu B-Z, Liang W-J, et al. Trajectories of hepatic and coagulation dysfunctions related to a rapidly fatal outcome among hospitalized patients with dengue fever in Tainan, 2015. *Plos Negl Trop Dis.* 2019; 13 (12), e0007817.

[14] Behera B, Cahudry R, Pandey A, et al. Co-infections due to leptospira, dengue and hepatitis E: a diagnostic challenge. *J Infect Dev Ctries.* 2010; 4, 48-50.

[15] Chaudry R, Pandey A, Das A, Broor S. Infection potpourri: are we watching? *Indian J Pathol Microbiol.* 2009; 52, 125.

[16] EASL. EASL clinical practice guidelines on hepatitis E virus infection. *J Hepatol.* 2018; 68, 1256-71.

[17] Marrone G., Biolato M., Mercurio G., et al. Acute

HEV hepatitis: clinical and laboratory diagnosis. *Eur Rev Med Pharmacol Sci.* 2019; 23: 764-70.
[18] Davern TJ, Chalasani N, Fontana RJ, et al. Drug-Induced Liver Injury Network (DILIN). Acute hepatitis E infection accounts for some cases of suspected drug-induced liver injury. *Gastroenterology.* 2011; 141, 1665-72.
[19] Hoz JM, Bayona B, Viloria S, Accini et al. Fatal cases of Chikungunya virus infection in Colombia: diagnostic and treatment challenges. *J Clin Virol.* 2015; 69, 27-9.
[20] Faccini-Martinez AA, Botero-Garcia CA, et al. With regard about the case of Dengue, Chikungunya and Zika coinfection in a patient from Colombia. *J Infect Public Health.* 2016; 9, 687-88.
[21] Villamil-Gomez WE, Rodriguez-Morales A.J. Reply: Dengue RT-PCR-positive, Chikungunya IgM-positive and Zika RT-PCR-positive co-infection in a patient from Colombia. *J Infect Public Health.* 2017; 10, 133-04.
[22] Kayiwa JT, Nankya AM, Ataliba IJ, et al. Lutwarma JJ. Confirmation of Zika virus infection through hospital-based sentinel surveillance of acute febrile illness in Uganda, 2014-2017. *J Gen Virol.* 2018; 99, 1248-52.