

Phlebovirus infection in Greece: a case report

Papa A¹, Kesisidou Ch², Kontana A¹, Arapidou Z², Petropoulou D²

¹Department of Microbiology, Medical School, Aristotle University of Thessaloniki, Thessaloniki

²Internal Medicine Clinic, General Hospital of Florina, Florina
Greece

Abstract

Background: Sandfly-transmitted phleboviruses cause to humans an asymptomatic or mild infection to severe meningitis or encephalitis. Phlebovirus infections are common in the Mediterranean countries during the summertime when sandflies are active. It is estimated that several cases remain undiagnosed.

Description of case: In July 2013, a 45-year-old Greek male, was admitted to the General Hospital of Florina in north-western Greece with two-day history of fever, myalgia, arthralgia, mild headache, and hemorrhagic exanthema. A serum sample obtained from the patient on the 6th day of illness was tested negative for West Nile virus infection. Instead, high titers of IgM and IgG antibodies against Toscana virus (TOSV) were detected, with low titers against sandfly fever Naples virus and no reactivity against sandfly fever Sicilian and Cyprus viruses, suggesting that the causative agent was TOSV or other phlebovirus with antigenic similarity to TOSV.

Conclusion: Phleboviruses have to be included in the differential diagnosis of acute summer febrile cases, accompanied or not by neurological symptoms. Effort has to be paid to test clinical samples during the first days of the disease when the virus is detectable by molecular or isolation methods, in order to elucidate the complex epidemiology of phleboviruses in the Mediterranean area. Hippokratia 2015; 19 (2):189-191.

Keywords: Toscana virus, phlebovirus, Greece

Corresponding author: Prof. Anna Papa, MD, PhD, A' Dept. of Microbiology, Medical School, Aristotle University of Thessaloniki, 54124, Thessaloniki, Greece, tel. +302310999020, +302310999006, fax +302310999151, e-mail: annap@med.auth.gr

Introduction

Sandfly-transmitted phleboviruses (genus *Phlebovirus*, family *Bunyaviridae*) are endemic in the Mediterranean countries causing to humans an asymptomatic or mild infection with flu-like symptoms, like fever, myalgia, arthralgia, and malaise. The disease is known as sandfly fever or phlebotomus fever. Toscana phlebovirus (TOSV) has the potential for neuroinvasion, causing meningitis or meningoencephalitis¹. Although the outcome is usually favorable, a few severe and even fatal cases have been reported^{2,3}.

A high prevalence (up to 50%) of IgG antibodies against TOSV (or other phleboviruses with antigenic similarities to TOSV) has been reported in Greece, with the highest rates being detected in the islands and the coastal areas⁴⁻⁶. However, the number of reported cases of phlebovirus infection is limited: one case with TOSV seroconversion was described in a German traveler returning from Greece⁷, and four cases were diagnosed in Greece (in Thessaloniki, Serres and Trikala prefectures). The first Greek case was an 8-year-old boy with meningoencephalitis in whom TOSV IgG seroconversion was observed⁸. The second, was a febrile child in whom a novel phlebovirus (Adria virus) was detected⁹, while the

third case was a patient with fever and lethargy in whom IgM and IgG antibodies against TOSV were detected in serum and cerebrospinal fluid¹⁰. The fourth was a severe encephalitis case caused by TOSV lineage C¹¹. Here we report a case of phleboviral infection observed in Florina prefecture, northwestern Greece.

Case presentation

In July 2013, a previously healthy 45-year-old male, resident of Agios Germanos village, near the Prespes Lake in Florina prefecture (Figure 1), was admitted to the General Hospital of Florina with two-day history of fever (38°C), myalgia, arthralgia, mild headache, and hemorrhagic exanthema. The patient did not present any focal neurological signs or neck stiffness. He reported that five days before onset of the symptoms, he travelled from Germany back to Greece, driving through Austria, Hungary, Serbia and FYROM, and that he stayed overnight in Serbia.

Main laboratory findings were elevated levels of alanine transaminase (46.4 IU/l, normal <40 IU/l), γ -glutamyl transferase (114 IU/l, normal 8-61 IU/l), C-reactive protein (2.65 mg/dl, normal <0.5 mg/dl), and erythrocyte sedimentation rate (39 mm/1h). White and red blood cell



Figure 1: Map of Greece: Florina prefecture is marked. The location of Greece in Europe is seen in the inset.

and platelet counts were at normal levels. The patient was hospitalized for nine days and was discharged without any sequelae.

Given that an outbreak of West Nile virus (WNV) infections was ongoing in northern Greece (although not in Florina prefecture), patient's serum sample obtained on the 6th day of illness was sent to the National Reference Centre for Arboviruses in Thessaloniki for testing for probable WNV infection. Since there was no laboratory evidence of WNV infection, the sample was further tested by indirect immunofluorescent assay (IFA) for the detection of IgM and IgG antibodies against four phleboviruses: TOSV, sandfly fever Naples virus (SFNV), sandfly fever Sicilian virus (SFSV) and sandfly fever Cyprus virus (SFCV). A commercially available kit was used (Sandfly fever virus Mosaic 1, Euroimmun, Lübeck, Germany). Viral RNA was extracted from patient's sample, and a reverse transcriptase (RT)-nested polymerase chain reaction (PCR) was applied using phlebovirus generic primers¹².

High titers (serum dilution 1:1000) of IgM and IgG antibodies against TOSV were detected in patient's serum; the respective titers against SFNV were 10-fold lower (Figure 2), while no reactivity was detected against SFSV and SFCV, suggesting that the causative virus was TOSV or other phlebovirus with antigenic similarity to TOSV. Due to the negative PCR result, the exact phlebovirus strain could not be identified. This was most probably due to the low and short viremia seen in phleboviral infections (RNA is usually detectable during the first 1-2 days after symptoms' onset).

Discussion

Phleboviruses, including TOSV, are endemic in the Mediterranean countries where sporadic cases, or even outbreaks, are reported every year¹. Also, several cases have been reported in travelers returning from Mediterranean endemic areas¹³⁻¹⁶. In the present case, it cannot be concluded whether the phlebovirus infection was acquired

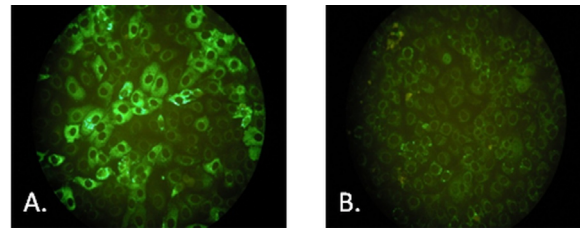


Figure 2: Indirect fluorescent assay visualized with the fluorescence microscope. Patient's IgM antibodies are stained with fluorescein-labelled anti-human antibodies (original magnification x400). The IgM antibodies are against A: Toscana virus; B: Sandfly fever Sicilian virus. No fluorescence was seen in the slides covered with Sandfly fever Naples virus and Sandfly fever Cyprus virus (not shown). The commercial kit Sandfly fever virus Mosaic 1 (Euroimmun, Lübeck, Germany) was used.

during the patient's travel through the Balkans or upon his arrival in Greece, since the incubation period of phleboviral infections is 3-6 days, and the patient presented the first symptoms four days after his return to Greece.

Previous studies showed that although the seroprevalence is high in the islands and coastal regions of Greece, it is low in the mountainous areas of the mainland, and specifically in Florina prefecture the TOSV seroprevalence is 0%⁴⁻⁶. However, the area near the Prespes Lake was not checked. Phleboviruses are endemic also in the Balkan countries¹⁷⁻²². Actually, the role of phlebotomine sandflies as vectors of phleboviruses was first demonstrated in 1909 in Dalmatia by an Austrian military commission²³, while large outbreaks with several thousands of cases were observed after World War II in Serbia²⁴.

The patient of the present report had highest titers of IgM and IgG antibodies against TOSV, and lower titers against SFNV (which belongs to the same serocomplex), with no reactivity against SFSV and CYPV (which belong to a different serocomplex). Phleboviruses in the same serocomplex exhibit close antigenic relationships resulting in cross-reactivity in serology. Neutralization assays enable the discrimination among phleboviruses within a serocomplex; however, for accurate results, a panel of phleboviruses (including several novel strains) has to be used. In the present case, based on the IFA results it can be suggested that a phlebovirus of the SFNV serocomplex, most probably TOSV, was the causative agent of the disease.

Regardless whether the infection was acquired in Greece or any other Balkan country, the present study suggests that phleboviruses have to be included in the differential diagnosis of acute summer febrile cases, accompanied or not by neurological symptoms. Testing of clinical samples obtained during the first days of the disease will enable the molecular detection, the genetic characterization and the isolation of the virus in cell culture. These data, combined with field studies on sandfly collections, will elucidate the complex epidemiology of phleboviruses in the Mediterranean area.

Conflict of interest

None reported by authors.

References

1. Charrel RN, Gallian P, Navarro-Mari JM, Nicoletti L, Papa A, Sanchez-Seco MP, et al. Emergence of Toscana virus in Europe. *Emerg Infect Dis.* 2005; 11: 1657-1663.
2. Baldelli F, Ciufolini MG, Francisci D, Marchi A, Venturi G, Fiorentini C, et al. Unusual presentation of life-threatening Toscana virus meningoencephalitis. *Clin Infect Dis.* 2004; 38: 515-520.
3. Bartels S, de Boni L, Kretzschmar HA, Heckmann JG. Lethal encephalitis caused by the Toscana virus in an elderly patient. *J Neurol.* 2012; 259: 175-177.
4. Anagnostou V, Papa A. Prevalence of antibodies to phleboviruses within the sand fly fever Naples virus species in humans, northern Greece. *Clin Microbiol Infect.* 2013; 19: 566-570.
5. Anagnostou V, Papa A. Seroprevalence of Toscana virus among residents of Aegean Sea islands, Greece. *Travel Med Infect Dis.* 2013; 11: 98-102.
6. Papa A, Andriotis V, Tzilianos M. Prevalence of Toscana virus antibodies in residents of two Ionian islands, Greece. *Travel Med Infect Dis.* 2010; 8: 302-304.
7. Dobler G, Treib J, Haass A, Frosner G, Woesner R, Schimrigk K. Toscana virus infection in German travellers returning from the Mediterranean. *Infection.* 1997; 25: 325.
8. Anagnostou V, Sdouga M, Volakli H, Violaki A, Papa A. Phlebovirus meningoencephalitis complicated by *Pseudomonas aeruginosa* pneumonia: a case report. *Vector Borne Zoonotic Dis.* 2011; 11: 595-596.
9. Anagnostou V, Pardalos G, Athanasiou-Metaxa M, Papa A. Novel phlebovirus in febrile child, Greece. *Emerg Infect Dis.* 2011; 17: 940-941.
10. Papa A, Mallias J, Tsergouli K, Markou F, Poulou A, Milidis T. Neuroinvasive phlebovirus infection in Greece: a case report. *Intervirology.* 2014; 57: 393-395.
11. Papa A, Paraforou T, Papakonstantinou I, Pagdatoglou K, Kontana A, Koukoubani T. Severe encephalitis caused by toscana virus, Greece. *Emerg Infect Dis.* 2014; 20: 1417-1419.
12. Sanchez-Seco MP, Echevarria JM, Hernandez L, Estevez D, Navarro-Mari JM, Tenorio A. Detection and identification of Toscana and other phleboviruses by RT-nested-PCR assays with degenerated primers. *J Med Virol.* 2003; 71: 140-149.
13. Defuentes G, Rapp C, Imbert P, Durand JP, Debord T. Acute meningitis owing to phlebotomus fever Toscana virus imported to France. *J Travel Med.* 2005; 12: 295-296.
14. Epelboin L, Hausfater P, Schuffenecker I, Riou B, Zeller H, Bricaire F, et al. Meningoencephalitis due to Toscana virus in a French traveler returning from central Italy. *J Travel Med.* 2008; 15: 361-363.
15. Gabriel M, Resch C, Gunther S, Schmidt-Chanasit J. Toscana virus infection imported from Elba into Switzerland. *Emerg Infect Dis.* 2010; 16: 1034-1036.
16. Sonderegger B, Hachler H, Dobler G, Frei M. Imported aseptic meningitis due to Toscana virus acquired on the island of Elba, Italy, August 2008. *Euro Surveill.* 2009; 14: pii: 19079.
17. Papa A, Velo E, Bino S. A novel phlebovirus in Albanian sandflies. *Clin Microbiol Infect.* 2011; 17: 585-587.
18. Punda-Polic V, Jeronic A, Mohar B, Sisko Kraljevic K. Prevalence of Toscana virus antibodies in residents of Croatia. *Clin Microbiol Infect.* 2012; 18: E200-E203.
19. Punda-Polic V, Mohar B, Duh D, Bradaric N, Korva M, Fajs L, et al. Evidence of an autochthonous Toscana virus strain in Croatia. *J Clin Virol.* 2012; 55: 4-7.
20. Venturi G, Marchi A, Fiorentini C, Ramadan N, Quaglio G, Kalaveshi A, et al. Prevalence of antibodies to phleboviruses and flaviviruses in Peja, Kosovo. *Clin Microbiol Infect.* 2011; 17: 1180-1182.
21. Hukic M, Salimovic-Besic I. Sandfly - Pappataci fever in Bosnia and Herzegovina: the new-old disease. *Bosn J Basic Med Sci.* 2009; 9: 39-43.
22. Gligic A, Miscovic Z, Tech RB, Travassos da Rosa A, Zivkovic V. First isolation of Naples sandfly fever virus in Yugoslavia. *Mikrobiologija.* 1982; 19: 167-175.
23. Doerr R, Franz K, Taussig S. Das Pappataciefieber: ein endemisches Drei-Tage-Fieber im Adriatischen Kustengebiet Oesterreich-Ungarns. *Deuticke Franz, Leipzig und Wien,* 1909, 1-166.
24. Karakašević B. O prvoj epidemiji papatačijeve groznice na teritoriji NR Srbije. *Vojno sanitetski pregled.* 1947; IV: 224-228.