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A sandfly fever virus outbreak in the East Mediterranean region of Turkey

Selma Guler [a,*], Ekrem Guler [b], Dilek Yagci Caglayik [c], Omer Faruk Kokoglu [d], Hasan Ucmak [d], Fatma Bayrakdar [c], Yavuz Uyar [c]

[a] Department of Infectious Disease, Yenisehir State Hospital, Hastane cad. No: 50, Kahramanmaras, Turkey
[b] Department of Pediatric Emergency, Faculty of Medicine, Kahramanmaras Sutcu Imam University, Kahramanmaras, Turkey
[c] Refik Saydam National Public Health Agency, Virology Reference and Research Laboratory, Ankara, Turkey
[d] Department of Infectious Disease, Faculty of Medicine, Kahramanmaras Sutcu Imam University, Kahramanmaras, Turkey

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SUMMARY

Objectives: To report a sandfly fever virus (SFV) outbreak that occurred in Kahramanmaras Province, Turkey.
Methods: We investigated the cases of 40 patients with a history of sandfly bites and with clinical findings, who were referred to our emergency service between July and August 2010. Serum samples of 19 patients were selected and analyzed using a commercial mosaic immunofluorescence test (IFT) to detect IgM and IgG antibodies against SFV.
Results: Sandfly fever was diagnosed in nine patients. All cases had a history of fly bite, and the clinical findings included fever, headache, myalgia, conjunctival hyperemia, and gastrointestinal symptoms such as diarrhea, nausea, and vomiting. In two patients, the diagnosis was confirmed by real-time PCR as sandfly Sicilian virus (SFSV). Laboratory findings in the patients included leukopenia, thrombocytopenia, and elevated levels of aspartate aminotransferase, alanine aminotransferase, creatine kinase, and C-reactive protein. All patients made a complete recovery with symptomatic treatment.
Conclusions: SFV is endemic in the Mediterranean Basin and data regarding SFV activity in Turkey are limited. This is the first report of an SFV outbreak from Kahramanmaras Province, Turkey, and provides information on epidemiological, clinical, and laboratory aspects of SFV infections.

1. Introduction

Phlebotomine sandflies are vectors of *Leishmania spp*, pathogens that cause the parasitic disease leishmaniasis in more than 80 countries in the Old and New World. Sandflies are also vectors of other human pathogens, such as the bacterium Bartonella and viruses belonging to three different genera: *Phlebovirus*, *Vesiculovirus*, and *Orbivirus*.[1]

Phleboviruses (family *Bunyaviridae*) are geographically distributed in Europe, Africa, Central Asia, and the Americas. Four serotypes that circulate in the Mediterranean area have been identified: sandfly Sicilian virus (SFSV), sandfly Cyprus virus (SFCV), sandfly Naples virus (SFNV), and Toscana virus (TOSV). SFSV, SFNV, and other related viruses cause sandfly fever, also known as 'three-day fever' or 'pappataci fever'. In endemic areas, infections occur during the summer, reaching a peak in August, in parallel with the maximum activity of the sandfly vectors.[2,3] Patients present with influenza-like symptoms and usually recover fully within a week. After a 3–6-day incubation period, high fever develops (39–40 °C) which lasts between 6 and 72 h (three-day fever). The most common symptoms include headache, anorexia, myalgia, photophobia, back pain, retro-orbital pain, and malaise. Significant leukopenia, characterized by an initial lymphopenia followed by protracted neutropenia, is present. Transient diarrhea or constipation with abdominal discomfort may also occur.[1,2] TOSV also causes neurological disorders, such as meningitis, encephalitis, and meningoencephalitis.[4–6]

The infection may be diagnosed during the early stages, based on either virus isolation or amplification of the viral genome. Antibody detection in serum samples is useful. Nevertheless, serological diagnosis is hampered by antigenic cross-reactivity between some members of the group, which may make interpretation of the antibody tests more difficult.[4] In recent years, immunofluorescence tests have been produced for the evaluation of sandfly fever virus (SFV) members. Such tests are useful and inexpensive alternative diagnostic methods.[4]

We identified the first outbreak of sandfly fever in Kahramanmaras Province, Turkey. In this study we present clinical findings and laboratory data related to this outbreak and seek to increase awareness among clinicians about areas endemic for this disease.

* Corresponding author. Tel.: +90 344 221 23 75; fax: +90 344 221 2371.
E-mail address: selmaguler38@hotmail.com (S. Guler).

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2. Materials and methods

2.1. Outbreak area and patients

Kahramanmaras is a city located in the East Mediterranean region of Turkey, with a population of one million, approximately 400 000 of whom live in the city center. Between July and August 2010, a total of 40 patients who were inhabitants of the same area of Kahramanmaras city, who had a history of sandfly bites and had clinical findings of fever, headache, myalgia, conjunctival hyperemia, and gastrointestinal symptoms such as diarrhea and nausea/vomiting, were admitted to the Infectious Disease Unit of the State Hospital. A sandfly fever outbreak in the area was considered a possibility, since the cases shared a common history of fly bite and similar clinical and laboratory features in a particular time interval. The patients were hospitalized in order to determine a possible agent of infection. Specific tests including Gruber–Widal agglutination tests and tube agglutination tests for Brucella were negative.

2.2. Laboratory analyses

Serum samples from 19 patients were selected and sent to the Arboviruses and Hemorrhagic Fevers Laboratory of Refik Saydam National Public Health Agency (RSNPHA) Virology Reference and Research Laboratory, Ankara. The serum samples were evaluated by a commercial mosaic immunofluorescence test (IFT) (Euroimmun, Germany) for the detection of specific IgM and IgG antibodies against SFSV, SFCV, SFNV, and TOSV. The slides were evaluated by immunofluorescence microscope, and graded from 0 (zero) to ++++. Those that were highly positive for one of the serotypes were considered serologically positive.[7] In accordance with the manufacturer's instructions, the IgM test was performed with a 1:10 dilution and the IgG test was performed with a 1:100 dilution, and while under the immunofluorescence microscope, the intensity of fluorescence was evaluated against positive and negative control intensities and graded from 1+ to 4+. The molecular analysis of SFV (Sicilian and Toscana virus types) was performed as previously described by Weidmann et al.[8] The commercial viral nucleic acid isolation kit (QIAamp Viral RNA Mini Kit, Qiagen) was used for RNA extraction. Thermal cycling was carried out in Rotor-Gene 6000 (Qiagen, Germany) real-time PCR machine. Plasmids containing S segments of SFV were used in the study as positive controls. This plasmid was kindly provided by Dr Manfred Weidmann, GWDG, Göttingen, Germany.

3. Results

3.1. Serologic and molecular results

According to the identification criteria for serological positivity of the commercial IFT kit used, of 19 patients, SFV-IgM and SFV-IgG antibodies were positive for SFSV serotype in four; SFV-IgM antibodies were positive for SFSV serotype in three; SFV-IgM antibodies were positive for SFSV and SFV-IgG antibodies were positive for TOSV and SFNV serotypes in one; and SFV-IgM and SFV-IgG antibodies were positive for SFCV serotype in one. Of the nine seropositive patients, five were female and four were male, and their mean age was 25.1 (range 12–38) years.

From these nine patients, the two samples that were SFSV IgM-positive but IgG-negative were found to be positive for SFSV by real-time PCR. The duration of viremia in SFV is very short, hence only those samples with a maximum of 5 days between the sampling date and the date on which complaints started were selected; therefore PCR was not applied to the remaining 10 patients.

The clinical and laboratory features of the nine patients are summarized in Table 1. All of the patients were treated symptomatically and were discharged following complete recovery.

3.2. Patient clinical and laboratory findings

Clinically, all cases had fever; myalgia/arthralgia was present in seven, headache in eight, diarrhea in three, and nausea/vomiting in three. During hospitalization, microbiological, hematological, and biochemical analyses were performed for all nine patients. Gruber–Widal agglutination tests for Salmonella and tube agglutination tests for Brucella were negative. Stool samples were obtained from the three patients suffering from diarrhea, and direct microscopic examination of these did not reveal any features that would contradict the diagnoses based on antibody analysis.

Table 1
Clinical and laboratory features of the patients

	Patient number								
	1	2	3	4	5	6	7	8	9
Gender/age (years)	F/38	F/28	F/22	M/19	M/12	M/22	F/32	F/28	M/25
Fever	+	+	+	+	+	+	+	+	+
Headache	+	+	–	+	+	+	+	+	+
Myalgia/arthralgia	+	+	+	+	+	–	+	–	+
Diarrhea	+	–	–	+	+	–	–	–	–
Nausea/vomiting	+	–	–	+	+	–	–	–	–
Conjunctival hyperemia	–	+	–	–	–	–	–	–	–
WBC count ($\times 10^9$/l)	1.590	4.250	2.270	3.070	3.800	2.500	3.409	2.300	4.890
Platelets ($\times 10^9$/l)	119	251	75	106	233	260	123	130	140
CRP (mg/dl)[a]	12	2.7	4.5	7.0	3.7	2.5	0.8	9.5	3.0
AST/ALT (mg/dl)[a]	603/330	43/43	103/88	58/42	23/13	90/87	80/60	130/142	86/86
CK (mg/dl)[a]	1560	210	127	886	18	100	21	1235	20
Probable serotype	Cyprus	Sicilian	Sicilian	Sicilian	Sicilian	Sicilian	Cyprus	Sicilian	Sicilian
SFV-IgM	SFSV (+3), SFCV (+4)	SFSV (+4), SFCV (+2)	SFSV (+3), SFCV (+)	SFSV (+2), SFCV (+)	SFSV (+)	SFSV (+3), SFCV (+)	SFSV (+), SFCV (+2)	SFSV (+3), SFCV (+)	SFSV (+3), SFCV (+)
SFV-IgG	–	SFSV (+4), SFCV (+2), SFNV (+4)	SFSV (+4), SFCV (+3)	–	SFSV (+2), SFCV (+)	SFNV (+2), TOSV (+2)	SFSV (+3), SFCV (+4)	–	SFSV (+3), SFCV (+)
PCR result	Negative	Negative	Negative	SFSV-positive	Negative	Negative	Negative	SFSV-positive	Negative

F, female; M, male; WBC, white blood cell; CRP, C-reactive protein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CK, creatine kinase; SFV, sandfly virus; SFSV, sandfly Sicilian virus; SFCV, sandfly Cyprus virus; SFNV, sandfly Naples virus; TOSV, Toscana virus.
[a] Normal values: CK 21–232 mg/dl; CRP <5 mg/dl; AST 15–37 mg/dl; ALT 30–65 mg/dl.

Laboratory findings included leukopenia in eight, thrombocytopenia in six, elevated aspartate aminotransferase (AST) in eight, elevated alanine aminotransferase (ALT) in five, elevated creatine kinase (CK) in three, and elevated C-reactive protein (CRP) in three of nine patients.

4. Discussion

Sandfly fever viruses remain a significant health problem in many areas of the world, including Africa, the Mediterranean Basin, the Middle East, and Central Asia.[2,9] Sandfly fever cases have been reported in Turkey for 3 years, along with clinical and laboratory findings. In 2007 and 2008, cases of sandfly fever were reported in Kozan, Adana, Izmir, and Mamak district of Ankara. For several of these cases, serological, molecular, and cell culture studies conducted in Turkey and Germany demonstrated the presence of virus.[10] In addition, a serological study of healthy blood donors in Ankara and Hatay provinces showed the presence of SFV antibody.[11] The present report, however, is the first report of sandfly fever virus infections in Kahramanmaras Province of Turkey.

Sandfly species of the genera *Phlebotomus* and *Sergentomyia* are common in Turkey. In 2004–2005, Simsek et al. and Tavana demonstrated the presence of *Sergentomyia* and *Phlebotomus* species in Anatolia.[12,13] The sandfly is endemic in southern Turkey, especially in the Mediterranean region, and is a vector for leishmaniasis and SFV.[12] The main vector of sandfly fever, *Phlebotomus papatasi*, is frequently encountered in Anatolia at an altitude of 0–600 meters and in hot and moderately humid areas.[12] In areas with cases of sandfly fever in the summer, these vectors have also been shown to be active.[2] Kahramanmaras Province, at an altitude of 550 meters, with a hot and moderately humid climate, is quite favorable to the survival of the sandfly.[14]

In this study in Kahramanmaras Province, we demonstrated the outbreak of Sicilian and Cyprus serotypes; epidemics of these types are located over the widest geographic area of the Old World, the same area that encompasses the geographic habitat of *P. papatasi*.[15] Outbreaks of these two serotypes, as well as the Naples serotype, have previously been reported from Turkey, in Adana, Izmir, and Mamak.[10]

The prominent symptoms reported in previous outbreaks have been high fever, headache, body pain, and nausea, and laboratory findings have included leukopenia ($1.8–4.8 \times 10^9$/l), thrombocytopenia ($50–140 \times 10^9$/l), elevation of ALT and AST levels (2.5- to 5-fold), and elevation of creatine phosphokinase (2- to 10-fold) in hospitalized and follow-up cases.[3,5,9–16] High fever, headache, and myalgia/arthralgia were the most common symptoms in our patients. Thrombocytopenia was observed in 75%, leukopenia in 55%, and elevation of transaminase enzyme levels in 78% of these cases. Similar findings have also been reported in previous studies.

The detection of IgM antibody is a reliable method for the diagnosis of an acute sandfly fever infection, although in serological tests cross-reactions can occur between SFV serotypes. In eight of our nine patients, SFSV IgM positivity and SFCV IgM positivity were found at the same time. We thought that this situation was probably related to cross-reactivity. The diagnosis can be confirmed with the plaque reduction neutralization test (PRNT) using type-specific antibodies.[4,17,18] In our patients the diagnosis was made with the immunofluorescence assay technique by seroconversion, however PRNT could not be conducted due to the requirement for live virus strains and because of technical insufficiencies.

Phlebovirus infections may be diagnosed during the early stages, based on amplification of the viral genome.[5] This method is often extremely sensitive. We confirmed infection in two patients by PCR. In addition, SFV-IgG positivity suggests active circulation of SFSV in Kahramanmaras region.

The Mediterranean region of Turkey is an area with active sandfly populations. Furthermore, sandfly fever is endemic along the rest of the Mediterranean coast, including neighbors of Turkey.[4] This disease should be considered in patients presenting with fever, headache, and myalgia/arthralgia, with laboratory findings of leukopenia, thrombocytopenia, elevated creatine kinase, and elevated liver transaminases, and with a history of fly bite, especially during the summer months.

Conflict of interest: No conflict of interest to declare.

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