Prevalence of Toscana sandfly fever virus antibodies in neurological patients and control subjects in Sicily

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SUMMARY

Toscana sandfly fever virus (TOSV) is an arthropod-borne virus transmitted to humans by sandfly vectors. It has been associated with human cases of meningitis and meningo-encephalitis mainly occurring during the warm season. We performed a retrospective serological study to evaluate TOSV circulation in Palermo, Sicily, and to compare TOSV seroprevalence in patients with neurological symptoms and in a control group of patients without neurological symptoms. When sera from 155 patients with and without neurological symptoms were evaluated, the rate of overall TOSV IgG reactivity was 17.4%. Patients with neurological symptoms showed a higher percentage of TOSV IgG positivity than control patients (25% versus 10.8%). TOSV exposure was confirmed by virus neutralization tests which also detected a Naples virus (SFNV) infection. TOSV should be considered as an etiologic agent in the differential diagnosis of fever and meningo-encephalitis in Sicily.

KEY WORDS: Toscana virus, Seroprevalence, Viral encephalitis, Sandfly Naples virus, Sicily, Italy

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INTRODUCTION

Toscana virus (TOSV) is an arthropod-borne RNA virus transmitted to humans by insects of *Phlebotomus* genus (Ciufolini *et al*., 1985; Verani *et al*., 1982; Verani *et al*., 1984). TOSV belongs to the genus *Phlebovirus*, family *Bunyaviridae* and it was isolated for the first time in 1971 from the sandfly *Phlebotomus perniciosus* in Tuscany, central Italy (Verani *et al*., 1982). In the sandfly fever group, the Sandfly Sicilian (SFSV) and Sandfly Naples (SFNV) viruses induce phlebotomus (sandfly) fever, a non-fatal mild febrile disease associated with malaise, and influenza-like symptoms lasting 2-4 days. TOSV has been associated with human cases of meningitis and meningo-encephalitis especially occurring during the warm season and in some endemic areas it represents the most frequent cause of meningitis from May to October (Charrel *et al*., 2005). The virus has been detected in Italy and Spain and recently spread to many other Mediterranean countries (Amaro *et al*., 2011; Brisbarre *et al*., 2011; Depaquit *et al*., 2010). Most cases of the disease have been reported in residents or travellers to the Mediterranean area. In Italy, clinical and epidemiological studies have demonstrated human infection by TOSV in Tuscany, Piedmont, Umbria, Marches, Emilia Romagna, Lazio and Sardinia (Cusi *et al*., 2010). TOSV has already been detected in sand flies in Sicily (Venturi *et al.,* 2007). Recently, TOSV was isolated from an American tourist who travelled to Sicily (Kay *et al*., 2010) and TOSV specific antibodies have been documented in Kaposi sarcoma patients and control subjects in this region (Amodio *et al*., 2011).
We performed a retrospective study to compare TOSV seroprevalence in patients with neurolog-

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ical symptoms and in a control group of patients without neurological symptoms.

PATIENTS AND METHODS

Anti-TOSV specific IgG and IgM antibodies were evaluated in serum samples collected from 155 patients admitted to the University Hospital of Palermo between 1999 and 2007, including 72 sera from patients with neurological symptoms and 83 from patients without neurological diseases, hospitalized for acute or chronic liver disease or sampled during pregnancy. All patients were residents of Palermo province, including the city and the surrounding towns. Median age of the neurological patients group was 44 years (range, 0-88), with a female percentage of 38.9%. Clinical history of the patients was retrieved from medical records in most cases. Median age of the non-neurological patients was 30 years (range, 24-40), with a female percentage of 50.6%.
Serum samples, stored at -20°C, were tested by commercial enzyme-linked immunosorbent assay (ELISA) tests for TOSV IgG and IgM specific antibodies (Enzywell Toscana virus – DIESSE Siena, Italy). The virus neutralization for SFNV/TOSV was performed on Vero cell monolayers by plaque reduction neutralization tests (PRNT) (Nicoletti *et al.,* 1996) using virus strains ISS.Phl.3 and SFN M52. Neutralizing antibody titres were calculated as the reciprocal of the serum dilution that gave an 80% reduction of the number of plaques (NT80), as compared to the virus control. Titres ≥10 were considered positive.

RESULTS

Among 155 serum samples tested, 27 (17.4%) were positive for TOSV-specific IgG antibodies. The presence of anti-TOSV IgG was detected in 18 (25%) of 72 patients with neurological symptoms, 14 male and 4 female, whose ages ranged from 27 to 88 years. All samples from neurological patients were also searched for TOSV-specific IgM antibodies but none of them was positive. Clinical manifestations of these patients are shown in the table 1. In the control group of 83 subjects without neurological symptoms TOSV-specific IgG antibodies were detected in 9 patients (10.8%), 5 male and 4 female, age range 24 to 39 years. Specific IgM reactivity was tested in 43 samples from control patients but none was found to be positive. For 18 IgG-positive samples sufficient amounts of serum were available for PRNT evaluation to detect and discriminate neutralizing antibodies against TOSV and SFNV. Exposure to TOSV was confirmed by the PRNT in four samples, while a neutralizing antibody titre ≥10 against SFNV was found in a single sample.

DISCUSSION

In recent years, TOSV has gained much interest as an emerging pathogen. TOSV geographical distribution has been demonstrated to be wider than previously indicated, including an increasing number of countries in the Mediterranean basin, and imported cases have been reported involving tourists visiting these areas (Amaro *et al*., 2011; Brisbarre *et al*., 2011; Charrel *et al*., 2005; Cusi *et al*., 2010; Depaquit *et al*., 2010; Kayi *et al*., 2010). Also a wider clinical spectrum of TOSV-associated diseases is now documented, including asymptomatic or mild diseases without central nervous system involvement, such as febrile erythema or influenza-like illness, as well as unusual clinical manifestations or severe sequelae of the neurological infection (Bartels *et al*., 2011; Brisbarre *et al*., 2011; Serata *et al*., 2011). Genetic heterogeneity has been demonstrated within TOSVs with two genotypes (A and B) being distinguished and corresponding to different geographic circulation, defining an Italian and a Spanish lineage, respectively (Sanbonmatsu-Gámez *et al*., 2005). Anti-TOSV specific IgG reactivity has also been detected in ovines, horses, cats and dogs, indicating that a large percentage of domestic animals can be infected by TOSV and eventually act as amplifying hosts for the virus (Ciluna *et al*., 2007; Navarro-Marí *et al*., 2011).
Our results show that the overall percentage of antibody positivity (18%) is similar to that found in asymptomatic subjects in other Italian regions, such as Tuscany (19.8%) (Terrosi *et al*., 2009) and Umbria (16%) (Francisci *et al*., 2003). The prevalence of TOSV-specific IgG antibodies was higher among patients with neurological symptoms

TABLE 1 - *Clinical symptoms and laboratory results of TOSV serology positive cases. The cut-off OD/CO ratio for positive IgG antibodies result was considered to be 1.1. In the virus neutralization test for SFNV/TOSV titres ≥10 were considered positive.*

No.	*Year of sampling/ age/gender*	*Neurological symptoms*	*Diagnosis at sampling*	*TOSV IgG (OD/CO ratio)*	*TOSV IgM*	*PRNT (titre)*
1	2000/28/female	No	Pregnancy	1.978	neg	neg
2	2001/32/male	No	Liver disease	1.732	neg	neg
3	2003/38/male	No	Liver disease	3.659	neg	neg
4	2003/27/male	No	Liver disease	1.343	neg	n. d.
5	2004/28/male	No	Liver disease	1.978	neg	TOSV (320)
6	2004/29/female	No	Pregnancy	1.670	n. d.	neg
7	2005/37/male	No	Liver disease	1.452	neg	neg
8	2005/39/female	No	Pregnancy	1.917	neg	neg
9	2006/24/female	No	Pregnancy	1.118	neg	neg
10	2000/66/male	Yes	Unspecified	1.632	neg	n. d.
11	2001/n.a./male	Yes	Meningoencephalitis	1.750	neg	neg
12	2001/62/male	Yes	Meningoencephalitis	1.539	neg	n. d.
13	2002/79/male	Yes	Peripheral polyneuropathy	3.307	neg	SFNV (10)
14	2002/52/male	Yes	Motor and sensory neuropathy	2.402	neg	neg
15	2002/54/male	Yes	Encephalitis	2.374	neg	neg
16	2002/75/male	Yes	Myelitis	1.867	neg	n. d.
17	2003/60/male	Yes	Unspecified	2.317	neg	n. d.
18	2003/n.a./male	Yes	Myelitis	1.113	neg	TOSV (320)
19	2003/70/male	Yes	Unspecified	1.137	neg	n. d.
20	2004/66/male	Yes	Altered consciousness	1.662	neg	n. d.
21	2004/88/female	Yes	Myelitis	2.025	neg	neg
22	2005/85/male	Yes	Peripheral polyneuropathy	1.271	neg	n. d.
23	2006/80/male	Yes	Unspecified	1.474	neg	neg
24	2006/49/female	Yes	Unspecified	5.689	neg	TOSV (160)
25	2006/70/female	Yes	Headache	3.330	neg	n. d.
26	2006/27/male	Yes	Meningoencephalitis	2.789	neg	TOSV (320)
27	2007/65/female	Yes	Meningitis	1.691	neg	neg

PRNT: virus neutralization test for SFNV/TOSV; n d.: not determined.

compared to those without neurological disease (25% versus 10.8%). However, the different prevalence of antibody positivity could simply be related to the median age of the two groups of patients, being higher in the first. In fact, previous epidemiological studies demonstrated an age-dependent increase in TOSV specific immunity (Terrosi *et al*., 2009), which is also observed for SFSV and SFNV (Cohen *et al*., 1999). Acute TOSV infections could be ruled out as demonstrated by the absence of IgM antibodies at the time of blood sampling. Only four ELISA-positive sera could be confirmed for TOSV infections by PRNT. This result could depend on an insufficient level of neutralizing antibodies in samples which were reactive in ELISA but with low optical density/cut-off (OD/CO) ratio. Cross-reactions between sandfly virus serotypes should also be taken into consideration, as an ELISA false-positivity to TOSV was demonstrated by the detection of an SFNV-neutralizing sample. This sample belonged to a 79-year-old patient. The finding is in agreement with previous serological studies which have detected SFNV specific antibodies only in persons born before the 1940s. In fact, malaria eradication campaigns conducted in Italy in that period were probably responsible for the disappearance or decreased circulation of the SFNV sandfly vector *Phlebotomus papatasi* (Nicoletti *et al.,* 1996).

Our results highlight the need to include diagnostic testing for TOSV among those routinely carried out for central nervous system infections diagnosis. At present, this agent is not frequently taken into consideration as an etiological agent in cases of meningitis or meningo-encephalitis in our region. Moreover, the local climate allows the flebotomine sandfly to be active for a long period of the year in Sicily. Therefore, TOSV aetiology should be considered also in cases of meningitis occurring in spring and autumn. Finally, tourists visiting the region could be at higher risk of clinically expressed infection due to the lack of a pre-existing immunity, which seems to play a role in limiting the disease in the areas of current TOSV prevalence (Cusi *et al*., 2010). Even though, to our present knowledge, TOSV is the only sandfly-transmitted phlebovirus with neurotropic activity, infections with SFNV can be highly incapacitating and their correct diagnosis is essential (Dionisio *et al*., 2003).

Sicily has to be included among Mediterranean areas at risk for acquiring TOSV infection. Further studies would be interesting to clarify the clinical role of TOSV in neurological syndromes as well as in diseases with mild clinical signs in our region.

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