

Serologically Confirmed Cases of Mediterranean Spotted Fever in the Trakya Region of Turkey

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SUMMARY: We have described the epidemiological, clinical and laboratory features of 13 cases of Mediterranean spotted fever in the Trakya Region of Turkey. Patients with high fever and maculopapular rash were admitted to the Trakya University Hospital between April and September in 2001-2002. Diagnostic titers were detected in 12 (92%) out of the 13 patients by IFA and in 6 (46%) by the Weil-Felix Proteus agglutination assay. Most of the patients (69%) were farmers, living in rural areas of three cities (Edirne, Tekirdağ, Kırklareli) with a total population of one million. The average age of 13 patients (7 males and 6 females) was 60.1±14.1 (mean ± standard deviation). All the patients had high fever (39°C) and a maculopapular rash. A rash on the palms or on the soles was observed in nine (69%) of the patients. Two cases (15%) had an eschar. The region around Trakya in Turkey is an endemic area for tick-borne diseases including rickettsioses.

Key Words: Mediterranean spotted fever, rickettsioses, Trakya region, tick borne disease, Turkey

Trakya Bölgesi'nde Serolojik Olarak Doğrulanmış Akdeniz Benekli Ateşi Olguları

ÖZET: Bu çalışmada Trakya Bölgesi'nde saptanan ve serolojik olarak doğrulanmış 13 Akdeniz Benekli Ateşi olgusunun epidemiyolojik, klinik ve laboratuvar özellikleri tanımlanmıştır. Trakya Üniversitesi Tıp Fakültesi, Eğitim ve Araştırma Hastanesi' ne 2001-2002 yılları Nisan-Eylül ayları arasında yüksek ateş ve makulopapüler döküntüsü olan hastalar başvurmıştır. IFA testi ile on üç hastanın on ikisinde (%92), Weil-Felix Proteus aglütinasyonu ile altısında (%46) tanınan titrelere saptanmıştır. Hastaların çoğunluğu (%69) toplam nüfusu bir milyon olan üç şehrin (Edirne, Tekirdağ, Kırklareli) kırsal kesimlerinde yaşayan tarımla uğraşan kişilerdir. On üç hastanın yaş ortalaması (7 erkek, 6 kadın) 60.1±14.1 (ortalama ± standart sapma)'dır. Bütün hastalarda yüksek ateş ve makulopapüler döküntü vardı. Dokuz hastada (%69) döküntünün avuç içi ve ayak tabanını da tuttuğu saptandı. İki hastada eskar saptandı. Trakya Bölgesi riketsiyozlar gibi kenelerle bulaşan hastalıklar için endemik bir alandır.

Anahtar Sözcükler: Akdeniz Benekli Ateşi, Riketsiyoz, Trakya Bölgesi, kene kaynaklı hastalık, Türkiye

GİRİŞ

In the Mediterranean area and southern Europe including Italy, Spain, Portugal, Greece, Turkey, Cyprus, Israel, Romania and Bulgaria, spotted fever is primarily caused by *Rickettsia conorii* (Mediterranean spotted fever) which is transmitted through the bite of the brown dog tick *Rhipicephalus sanguineus* (17). However, since 1991, five newly recognized tick-borne rickettsioses have been described throughout Europe: *Rickettsia conorii Astrakhan*, *Rickettsia mongolotimonae*, *Rickettsia slovaca*, *Rickettsia helvetica*, *Rickettsia conorii Israel* (14, 15, 17).

Patients with high fever and maculopapular rash were admitted to the Trakya University Hospital between April and September in 2001-2002. A beta-lactam antibiotic was

administered to most patients for common febrile infections in different clinics but they were not effective. However, with onset of rash accompanying high fever, the patients were admitted to the Trakya University Hospital, where they were diagnosed as Mediterranean spotted fever (MSF) and treated with doxycycline. In this retrospective study, we described the symptoms and clinical findings of 13 MSF cases diagnosed and serologically confirmed in the Trakya Region of Turkey. These cases were also the first confirmed occurrence of MSF in Turkey.

PATIENTS AND METHODS

Patients: MSF was diagnosed clinically according to the clinical signs such as high fever, maculopapular rash (may include palms or soles), headache, myalgia, arthralgia and/or a unique eschar (the "tache noire") at the tick bite site (2, 8, 20, 21). They filled out questionnaires about tick exposure and the presence of symptoms or signs consistent with acute tick-

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borne rickettsioses. Three blood samples were taken from each patient on admission to the hospital for total blood cell count, biochemistry analysis and serologic diagnosis. Two weeks later another sample was taken to obtain convalescent phase serum.

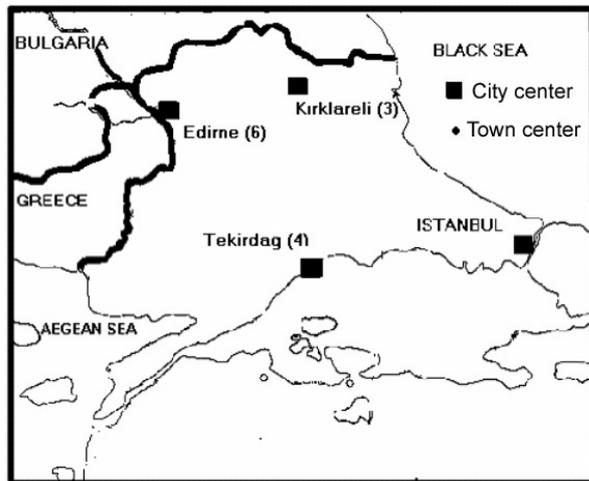


Figure 1. The localization of the patients in Trakya Region of Turkey

Serology: Two serological tests were used to confirm infection. The Weil-Felix *Proteus* agglutination assay (*P. vulgaris* OX-19, OX-2 strain agglutination), (Micropath antigens, Omega Diagnostics Limited, UK) was performed on each sample according to the manufacturer's instructions by diluting each serum 1/20 to 1/640. A fourfold or greater rise in titer between the acute and the convalescent sera using the Weil-Felix *Proteus* agglutination assay, or a single Weil-Felix titer ≥ 320 were accepted as positive result (2, 8, 20).

The IFA test (*Rickettsia conorii*- Spot IF, BioMerieux, Marcy L'Etoile, France) used contained *Rickettsia conorii* antigen that had been grown in Vero cells and fixed on the slides. All samples were tested in a 1/40 initial dilution. Slides were incubated in a moist chamber at 37°C for 30 minutes and washed three times with phosphate-buffered saline. After adding anti-human immunoglobulin G sheep globulin labeled with fluorescein (Sanofi Diagnostics, Pasteur), slides were incubated in a moist chamber at 37°C for 30 minutes and washed as previously described. Intense fluorescence of the *Rickettsiae* situated in or outside the cells was considered to be a positive reaction. End-point titers were obtained by serial dilutions on positive specimens. Negative and positive serum samples from our laboratory collection were used in each test as negative and positive controls.

A seroconversion, a fourfold or greater rise in titers between the acute and the convalescent sera using the IFA assay, or a single IFA titer ≥ 128 were accepted as positive results (2, 8, 20).

RESULTS

Thirteen patients were diagnosed with MSF (Table 1). Diagnostic titers were detected in 12 (92%) out of the 13 patients by IFA and in 6 (46%) by Weil-Felix *Proteus* agglutination assay. One patient had negative serology results, although he had the characteristic clinical findings including an eschar.

Most of the patients (9/13) were farmers, living in rural areas of three cities (Edirne, Tekirdağ, Kırklareli) with a total population of one million. All had contact with dogs but only two patients remembered being bitten by a tick. The average age of 13 patients (7 male and 6 female) were 60.1 ± 14.1 (34-57-80) (mean \pm standard deviation (minimum-median-maximum)). Six (46%) of the patients were over the age of 60.

All the patients had high fever (39-40°C) and maculopapular rash which usually appeared three days (mean, range 1-6 days) after the onset of fever. The rash in the palms or on the soles was observed in nine (69%) of the patients. Two cases (15%) had eschar. Some patients also had headache (six [46%] of 13 patients), myalgia (five [38%] of patients), petechial rash (three [23%]). An old man with thrombocytopenia had haemorrhagic bullae on both of his heels and simultaneous gastrointestinal bleeding. Two patients (15%) had stupor but one (8%) had meningismus. One patient (8%) had hepatomegaly; nine (69%) had elevated AST levels. Four patients (31%) had leucocytosis and three (23%) had leucopenia. Two (15%) had thrombocytopenia. Three (23%) had elevated blood urea nitrogen levels. All the patients had strongly positive CRP reactions.

Four patients (31%) had cardiac insufficiency and one (8%) had diabetes mellitus.

All the patients were treated with doxycycline (200 mg/day, 10 days) and their temperature returned to normal three days (mean, range 1-5 days) after therapy. No treatment failure or relapse occurred.

DISCUSSION

MSF is a tick-borne rickettsioses prevalent in the Mediterranean countries (17). In Turkey, *R. conorii* is the most probable agent of spotted fever with the combination of epidemiologic properties, characteristic clinical findings of the patients and the results of serological tests.

Spotted fever group rickettsioses share most of their clinical features except the frequency of the inoculation eschar which varies with the causative species (17). *R. conorii* Israel is also transmitted by *Rhipicephalus sanguineus*; the inoculation eschar is usually lacking and the disease may be severe (17). *R. conorii* Astrakhan is transmitted by *Rhipicephalus pumilio*; the presence of eschar was reported in 20% of patients (17). The IFA assay is the "gold standard" for the diagnosis of

rickettsial diseases but IFA provides group reactive serologic test results so can not differentiate among spotted fever rickettsia (8, 17, 20).

Although *R. conorii* has always been considered to produce a less severe disease than *R. rickettsii*, severe forms of MSF have been reported in 6% of the patients and the mortality rate may reach 2, 5% (16). For the patients with life-threatening, acutely incapacitating rickettsial disease, early diagnosis is a serious problem because IFA provides confirmation of the diagnosis in convalescence so it is not helpful in making critical therapeutic decisions during the acute illness (8, 17). Six of our patients (6/13) were over the age of 60, with underlying diseases (four patients had cardiac insufficiency and one had diabetes mellitus). When we observed the characteristic clinical findings of rickettsial disease, we preferred to start treatment immediately with doxycycline before we obtained the results of laboratory assays. None of our patients died. No therapy failure or relapse occurred.

A presumed case of *R. conorii* infection with bilateral multiple retinal hyperfluorescent dots was reported from the Trakya

Region before (6). In the Turkish literature, forty eight presumed cases of MSF have been reported from various hospitals in Istanbul since 1992. Cases were diagnosed according to clinical findings and the Weil-Felix *Proteus* agglutination; only two of the cases were confirmed by IFA (1, 3, 5, 10, 11, 12, 13, 18).

In northern Greece, including the west of the Trakya Region, the prevalence of antibodies to *R. conorii* and *R. typhi* were determined by IFA in the general population. Antibodies to *R. conorii* and *R. typhi* were found to be 7, 9 % and 2 %, respectively (4). The prevalence of antibodies to *R. conorii* correlated with increasing age and was statistically higher in men (4). Farmers and residents of rural areas had significantly higher prevalence of antibodies to *R. conorii* in comparison to urban residents. The prevalence of antibodies to *R. conorii* in our region was not studied but Vural et al investigated *R. conorii* antibodies in the Antalya area of Turkey and detected *R. conorii* IgG antibodies in 13% of serum samples (19).

In Turkey, Merdivenci and Karaer *et al* demonstrated that *Ixodes ricinus*, *Hyalomma species*, *Dermacentor marginatus*,

Table 1. Clinical and laboratory data of patients with rickettsioses

Patient no	Date of onset Month/Year	Age/Gender	City	A. bite	Eschar	Other	Weil-Felix Early/Late	IFA Early/Late
1	July/2001	56/M	Tekirdağ	NN	No	headache, myalgia, ↑AST, ↑CRP	0/0	40/160
2	August/2001	70/F	Edirne	NN	No	↑CRP	0/0	40/160
3	August/2001	80/F	Kırklareli	Yes	Yes (arm)	myalgia, ↑AST, ↑CRP	0/0	0/0
4	September/2001	73/F	Edirne	Yes	Yes (knee)	stupor, ↑CRP	0/0	40/160
5	July/2002	40/M	Edirne	NN	No	↑AST, ↑CRP	0/320	0/160
6	August/2002	57/F	Kırklareli	NN	No	headache, ↑AST, ↑CRP	0/0	0/80
7	August/2002	65/F	Tekirdağ	NN	No	petechial rash, ↑AST, ↑CRP, leukopenia, thrombocytopenia	0/0	160/160
8	August/2002	69/M	Tekirdağ	NN	No	headache, ↑AST, ↑BUN, ↑CRP, leukocytosis	320/320	0/80
9	August/2002	50/M	Kırklareli	NN	No	↑AST, ↑CRP, leukopenia, thrombocytopenia	320/320	0/80
10	June/2002	34/F	Edirne	NN	No	headache, myalgia, ↑CRP, leukopenia	320/320	160/160
11	June/2002	45/M	Edirne	NN	No	headache, myalgia, petechial rash, hepatomegaly, ↑CRP, leukocytosis	320/320	160/160
12	June/2002	56/M	Tekirdağ	NN	No	headache, myalgia, ↑AST, ↑BUN, ↑CRP, leukocytosis, thrombocytopenia	0/0	160/160
13	June/2002	69/M	Edirne	NN	No	petechial rash, stupor, meningismus, ↑AST, ↑BUN, ↑CRP, leukocytosis	320/320	160/160

Haemaphysalis species, *Rhipicephalus species* were the most commonly encountered tick species throughout the country and also in the Trakya Region (7, 9).

In conclusion, Trakya Region of Turkey is a new endemic area for tick-borne diseases including rickettsioses. The thirteen cases of MSF described in this paper are the first confirmed occurrence of this disease in Turkey and further clinical studies are needed to isolate and to characterize the agent by molecular methods.

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