Cervical lymphadenitis: tuberculosis or tularaemia?†

O. Karabay[1], S. Kilic[2], S. Gurcan[3], T. Pelitli[4], A. Karadenizli[5], H. Bozkurt[6] and S. Bostanci[5]

1) *Department of Infectious Diseases, Sakarya University Medical Faculty, Sakarya,* 2) *Refik Saydam National Public Agency, National Tularemia Reference Laboratory, Ankara,* 3) *Department of Medical Microbiology, Trakya University Medical Faculty, Edirne,* 4) *Primary Health General Directorate, Department of Zoonotic Diseases, Turkish Ministry of Health, Ankara,* 5) *Department of Medical Microbiology, Kocaeli University Medical Faculty, Kocaeli and* 6) *Tuberculosis Control Department, Turkish Ministry of Health, Ankara, Turkey*

Abstract

Both tuberculosis cervical lymphadenitis (TCL) and oropharyngeal tularaemia (OT) have similar signs, symptoms and pathological findings. We aimed to investigate the frequency of tularaemia antibodies in patients diagnosed with TCL. Using data from the Tuberculosis Control Dispensaries between the years of 2008 and 2011 in Turkey, all patients diagnosed with TCL were informed about and included in the study. Control group subjects were selected from healthy blood donors who lived in the same region. After informed consent was obtained, the sera obtained from volunteer TCL patients and the control group were tested with a microagglutination technique for *Francisella tularensis*. Antibodies to *Brucella* were also investigated with a tube agglutination test for cross-reactivity in sera that were seropositive for tularaemia. Sera were obtained from a total of 1170 individuals in the TCL group and 596 in the control group from 67 of 81 provinces in Turkey. *Francisella tularensis*-positive antibodies were found in 79 (6.75%) cases in the TCL group and two (0.33%) cases in the control group with a titre of $\geq$ 1:80 ($p < 0.01$). When the presence of antibody of any titre was considered, the ratio became 8.2% (96/1170) in the TCL group and 0.67% (4/596) in the control group ($p < 0.001$). For the first time, with this study, tularaemia serology was found to be positive in a significant portion (6.75%) of diagnosed cases of TCL. In tularaemia endemic regions, it was concluded that tularaemia serology should be investigated in patients suspected of having TCL.

Keywords: Cervical lymphadenitis, *Francisella tularensis*, lymphadenitis, oropharyngeal tularaemia, tuberculosis, microagglutination test
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Corresponding author: O. Karabay, Department of Infectious Diseases, Sakarya University Medical Faculty, 54100 Korucuk-Sakarya, Turkey
E-mail: drkarabay@yahoo.com

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Introduction

Francisella tularensis can remain viable within macrophages [1]. Regional lymphadenopathy is one of the most typical findings of tularaemia [2,3]. *Francisella tularensis* is transmitted by environmental exposure and is also defined as a potential bioterrorism agent [4,5].

While ulceroglandular tularaemia is the most common clinical form in a significant part of the world, oropharyngeal tularaemia (OT) is the most common clinical presentation in Turkey [6]. OT generally presents with pharyngitis and lymphadenopathy after consuming contaminated water and/or food [7]. Cervical lymphadenopathy (CL) generally progresses to a pathology with granulomatous inflammation and/or caseification necrosis in many OT cases who are treated with inappropriate antibiotics (e.g. beta-lactams) [8,9].

Tuberculosis is an important public health problem in Turkey and throughout the world [10,11].Tuberculosis may be present as extrapulmonary tuberculosis [12,13]. In Turkey, approximately 1600–2000 tuberculosis lymphadenitis cases are detected annually [14]. Tuberculosis cervical lymphadenopathy (TCL) and some bacterial diseases (such as cat scratch disease, actinomycosis, non-tuberculosis mycobacterial diseases, etc.) may cause suppurative lymphadenopathy. OT has very similar findings as well. Both have symptoms and findings such as fever, chills and CL and granulomatous inflammation in biopsy

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specimens. Our hypothesis is that OT may be misdiagnosed as TCL in tularaemia endemic areas [15,16]. To our knowledge, there is no study in the English literature concerning tularaemia prevalence in patients diagnosed with TCL. For the first time with this study, we aimed to investigate the frequency of tularaemia antibodies in patients diagnosed with TCL.

Methods

This study was planned by the Tularaemia Study Group of the Turkish Microbiology Society and was supported by the Turkish Ministry of Health.

Approvals

This study has been approved by the local ethics board (Ethics Board of Duzce University, Turkey). Institutional approvals were obtained from the Turkish Ministry of Health.

Cases

In Turkey, there were 229 tuberculosis control dispensaries. For this study, all of the recorded cases who were followed and treated as extrathoracic tuberculosis lymphadenitis (ETTL) were invited into the study. Of voluntary patients with TCL, 1170 were included in this study.

In Turkey, most TCL cases were diagnosed on clinical and/or pathological findings without definite microbiological diagnostic confirmation. So some patients were probably infected with *F. tularensis* rather than *M. tuberculosis*.

Control group

Healthy blood donors living in the patient regions were selected as a control group according to suitable age and sex. The case–control ratio was intended to be 1:1. Each member of the control group was informed about the purpose of the study. Their blood was taken after informed consent.

Samples

Blood samples were taken from cases living in 67 out of 81 cities in Turkey. All of the sera were transported to the Refik Saydam Hıfzıssıhha Institute (Ankara, Turkey) under appropriate conditions. Sera were stored at −80°C until the study day.

Tests

Specific agglutinins to *F. tularensis* were investigated by microagglutination tests using the *F. tularensis* LVS (NCTC 10857) strain in the Department of National Tularemia Diagnosis Reference Laboratory of the Refik Saydam Hifzissihha Institute. Two-fold dilutions of patient sera were prepared in U base plates from 1/10 to 1/1280 titre and final dilutions were obtained with the addition of antigen in an equal volume. A $\geq$ 1/80 titre was accepted as significant. All microagglutination positive sera obtained from subjects were also examined by serial dilution (from 1:10 to 1:1280) using *Brucella* antigen.

Brucella antibodies showing cross-reactivity with *F. tularensis* were investigated with *Brucella* tube agglutination tests.

Recall of seropositive cases

Seropositive patients were phoned and recalled for investigation of epidemiological and clinical features of tularaemia. Data such as drinking water characteristics environmental exposure (contact with hunting animals or ticks, etc.) and clinical findings (fever, sore throat or swallowing difficulty) were recorded for the patients who responded to this call (Table 1).

Statistical analysis

Statistical analysis was conducted using SPSS, 15.0 (SPSS, Chicago, IL, USA). The data of the patients were grouped. The Mann–Whitney *U*-test was used to compare continuous variables; qualitative variables were compared using the chi-square test and Fisher's exact tests. A p value < 0.05 was accepted as significant.

Results

Demographic data

A total of 1170 patients with TCL and 596 control group subjects from 67 cities in Turkey were included in the study.

TABLE 1. Demographic and clinical properties of tularaemia agglutination positive cases

Risk factors	Query	Yes n %	No n %
Epidemiological	Living in rural areas	38 (63)	22 (37)
	History of cattle feed?	36 (60)	24 (40)
	Hunting	12 (20)	48 (80)
	Contact with stream of water wells	11 (18)	49 (82)
	Is drinking water chlorinated?	38 (63)	22 (37)
	Contact with lake water?	13 (22)	47 (78)
	An increase in the population of mice in their habitat	33 (55)	27 (45)
Clinical	Sore throat	27 (45)	33 (55)
	Fever	31 (52)	29 (48)
	Lymphadenomegaly	60 (100)	
	Single lymphadenomegaly	15 (25)	
	Lymphadenomegaly no more than two places	8 (13)	
	Lymphadenomegaly with more than two places	9 (15)	
	No information about lymphadenomegaly	28 (46)	
	Cough	15 (25)	45 (75)
	Haemoptysis	5 (8)	55 (92)
	Lung tuberculosis history	3 (5)	57 (95)
	Tuberculosis in the family	8 (13)	52 (87)
	Lymphadenomegaly in family members	3 (5)	57 (95)
	Pharyngitis	60 (100)	0 (0)
	Lesion in the mouth	12 (20)	48 (80)
	Conjunctivitis	14 (23)	46 (77)
	Rash	8 (13)	52 (87)
	Splenomegaly	4 (7)	56 (93)
	Hepatomegaly	4 (7)	56 (93)
	Suppuration	20 (33)	40 (77)

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The median age of the TCL subjects was 44.1 years (± 19.2), and 847 (72.4%) of them were female. Examination of at least 1170 control group subjects was planned within the scope of the study. However, only 596 control subjects, 280 (47%) male and 316 (53%) female), were included. The average age of control group subjects was 39.2 years (± 17.1).

Tularaemia seropositivities

Significant titres for tularaemia antibody seropositivity were detected in 79 of the patients (6.75%) while two subjects of the control group were found to be positive for significant antibody titres ($p < 0.01$) (Table 2). The seropositivity rate increased up to a level of 8.2% (96/1170) for the study group when positivities at lower titres were added. The median age of the tularaemia seropositive subjects was 41.2 years (± 19.1) and 50 of them (63.3%) were female. *Francisella tularensis*-positive antibodies were found in two subjects (0.33%) in the control group with a titre of ≥1:80 ($p < 0.01$).

Use of diagnostic tests in patients with TCL

We also investigated from the archives how to diagnose TCL in the 1170 patients. Histopathological examinations were used for 926 patients (79.1%) and 190 of them were also tested by mycobacteriological methods (acid fast staining and/or culture), in addition to histopathological examination. A total of 24 patients were tested only with mycobacteriological methods, while neither histopathological examination nor mycobacteriological methods were used for 220 patients. Of 1170 patients, 214 (18.2%) were tested by mycobacteriological methods and 13 of them were positive for tularaemia antibodies. Nine hundred and fifty-six TCL (81.8%) patients were diagnosed by clinical and/or histopathological findings and 65 (6.9%) of them were positive for tularaemia agglutination (Table 3).

Brucella serology results

Tularaemia seropositive subjects were investigated for *Brucella* serology and only in two cases were significant (≥1/160) *Brucella* agglutination titres found.

TABLE 2. Comparison of controls and cases

Parameters	TCL diagnosed group *N* (%)	Controls *N* (%)	p
N (1766)	1170	596	
Tularaemia positivity (any titre)	96 (8.2%)	4 (0.7%)	< 0.001
Tularaemia positivity (>1/80)	79 (6.7%)	2 (0.3%)	< 0.001

TCL, cervical tuberculosis lymphadenopathy.

Epidemiological and clinical characteristics of seropositive patients

A total of 79 tularaemia seropositive patients were recalled to the dispensaries where they lived. Feedback was obtained for 60 (76%) of 79 patients. Various epidemiological and clinical data were recorded. The majority of the patients diagnosed with tularaemia lived in rural areas and 37% of the patients were using non-chlorinated water. Among the cases, 60% were feeding cattle in a barn, and 52% had fever and pharyngitis anamnesis (Table 1).

Data on tuberculosis and TCL in Turkey

According to official records of the years 2005–2009, the total number of tuberculosis cases varied between 20535 and 17402 annually. Within the same period, while the rate of ETTL was 7.9% (1622 cases) in 2005, it progressively rose and reached a level of 11.7% (2036 cases) in 2009 [14]. Similarly, while the numbers of recorded tularaemia cases were 431 in 2005, this increased to approximately 1500 in 2010.

Discussion

There are only a limited number of case reports indicating that OT was misdiagnosed as TCL [17,18]. However, there is no available study concerning the misdiagnosis rate in a wide-scale case series. The fact that OT cases were highly misdiagnosed as TCL (6.75%) has been reported for the first time in this study in the English literature.

Tularaemia has been known in Turkey since 1936. Four tularaemia outbreaks occurred in Turkey up until 1988, and 77% of these cases were reported as OT [19,20]. The number

TABLE 3. Using of diagnostic tests for clinically compatible cases with tuberculosis cervical lymphadenopathy

	Tularaemia seropositivity		
Using of diagnostic tests	Positive	Negative	Total
Mycobacterial analysis (microscopy and/or culture)			
Available	14	200	214
Not available	65	891	956
Microscopy (EZN staining)			
Positive	2	56	58
Negative	12	143	155
Mycobacterial culture			
Positive	0	2*	2[a]
Negative	0	0	0
Histopathological analysis			
Available	65	861	926
Not available	14	230	244
Both mycobacterial and histopathological analysis			
Available	13	177	190
Not available	13	207	220

*A case had culture positivity but EZN staining negativity and the other one had only culture request but no EZN staining.

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of endemic regions with reported cases has increased in the last 10 years and the disease has become widespread in all regions of Turkey. The annual number of tularaemia cases between 2005 and 2008 was approximately 166. This increased to 428 in 2009 and exceeded 1500 in 2010 [21].

Tuberculosis lymphadenitis is the most frequently occurring form of extrapulmonary tuberculosis [17]. It was reported that tuberculosis is responsible for 43% of peripheral lymphadenitis in developing countries [22]. Differential diagnosis of TCL is difficult. Similar clinical findings may be seen in TCL and OT. It is not possible to distinguish the diseases with only clinical signs because they have the same clinical symptoms. The most common clinical findings in patients with TCL are neck mass, fever, chills, malaise, weight loss and night sweats. History and physical examination, radiological and laboratory evaluation, PPD test and fine needle aspiration biopsy are usually insufficient for the diagnosis [23]. Culture positivity of TCL is diagnostic for 10–77% of cases [24]. In Turkey, histopathological evaluation is the most important criterion for clinicians to make the decision to initiate antituberculosis therapy due to the diagnosis of tuberculosis lymphadenitis. When we consider the increasing trend of both tularaemia and tuberculosis lymphadenitis in recent years, probable misdiagnosis should be kept in mind. We suggest that some tularaemia cases might be diagnosed as tuberculosis lymphadenitis. Turhan *et al.* [25] reported a great outbreak in our country in which all cases were oropharyngeal tularaemia. They reported a frequency of fever of 91% and of sore throat of 74%. They encountered fever and malaise in all subjects. Lymph node suppuration was seen in 1/3 of cases. Similarly, in this study, we encountered a history of fever and pharyngitis in our tularaemia seropositive cases of 60% and 100%, respectively. Whereas pharyngitis complaints are not the usual complaints of TCL patients, we detected it in all our tularaemia seropositive patients. A history of haemoptysis was determined in only eight cases. We believe this situation supports our hypothesis.

Granulomatous inflammation and caseification necrosis are the most common histopathological findings in biopsy specimens of TCL cases. Many pathologists reported possible tuberculosis lymphadenitis when they determined caseification necrosis and granulomatosis inflammation in lymphadenopathy biopsy specimens. However, similar caseification necrosis and granulomatous inflammation are also seen in OT [26]. Clinicians and pathologists not experienced with tularaemia may easily misdiagnose it as TCL in patients with CL and granulomatous inflammation. Without bacteriological confirmation, diagnostic errors are more likely. We think that if the diagnosis is not confirmed through mycobacteriological methods (Ehrlich Ziehl Nelson staining and *Mycobacterium* culture, PCR, etc.), a tularaemia serology should be applied before tuberculosis treatment is started for patients suspected of having TCL in tularaemia endemic regions.

In this study, 63% of tularaemia seropositive cases were female. Of the cases 46.6% were housewives according to occupation. [27]. In our country, tularaemia is usually transmitted by a water-borne route. In Turkey, housewives are exposed to water contact during housework and land work in rural areas [21].The vast majority of female OT patients may frequently be in contact with non-chlorinated water. There were histories of non-chlorinated drinking water in 37% of tularaemia seropositive patients (Table 2).

This study has some limitations. Only 67 of 81 cities (83%) could be included in our study. The control group remained below the targeted number due to a wide geographical study area and low rate of voluntary attendance. Further limitations of the study are the low number of subjects and the high number of women in the control group. Still, the large numbers of cases and controls provide a significant contribution to the literature on the subject.

In summary, tularaemia antibodies can be found in a considerable proportion of cases diagnosed as TCL. In addition, both tularaemia and tuberculosis can cause granulomatous inflammation and caseification necrosis in the lymph nodes. We conclude that tularaemia serology should be tested in patients suspected of having TCL in tularaemia endemic regions.

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Transparency Declaration

Nothing to declare.

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