Original Article

Oropharyngeal tularemia cases admitted to a military hospital in Ankara, Turkey

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Abstract

Introduction: This study aimed to review the possible sources of infection of 16 oropharyngeal tularemia hospital cases, and to document their epidemiological and demographical characteristics, laboratory findings, treatment methods, and treatment results.

Methodology: Sixteen cases from a Turkish military hospital between January 2011 and December 2012 were retrospectively evaluated. The age, sex, occupation, place of residence, symptoms, duration of symptoms, laboratory results, treatment and duration, and treatment results were recorded. Tularemia was diagnosed through tularemia-specific tests once the other conditions that may have caused lymphadenopathy were excluded.

Results: Twelve of the patients included in this study were males. The average age of the patients was 32.1±17.2 years. Sore throat, fatigue, and fever were the most frequent symptoms. The mean duration of symptoms was 21.6±6.9 days. All the patients had been treated for tonsillopharyngitis in primary healthcare institutions previously. However, despite the treatment, cervical lymphadenopathy had developed in these cases. Patients were given streptomycin, doxycycline, and ciprofloxacin monotherapy or in combination. Ten of the cases fully recovered, while five required surgical lymph node drainage. Spontaneous drainage occurred in the single remaining case.

Conclusions: Turkey is considered to be an endemic country with regards to tularemia. Prompt diagnosis and proper treatment of the disease is imperative in providing cure. Since it can be potentially confused with tuberculous lymphadenitis, differential diagnosis is vital. Patients presenting with a condition of tonsillopharyngitis in endemic areas must be carefully monitored.

Key words: *Francisella tularensis*; tularemia; oropharyngeal tularemia; cervical mass; Turkey.

J Infect Dev Ctries 2014; 8(8):994-999. doi:10.3855/jidc.4332

(Received 14 October 2013 – Accepted 01 July 2014)

Introduction

Tularemia, caused by a Gram-negative coccobacillus named *Francisella tularensis*, is an acute, febrile, granulomatous zoonotic disease. Tularemia, observed in North America, is endemic in North America, Europe, and Asia. The disease infects humans through tick bites, contact with contaminated animals, consumption of infected animals' meat, drinking contaminated water, and inhalation [1,2]. According to its virulence and certain biochemical characteristics, *F. tularensis* is studied under four separate subgroups: *F. tularensis* subspecies *tularensis* (Jellison type A or *F. tularensis* subsp. *nearctica*), *holarctica* (Jellison type B or *F. tularensis* subsp. *palaeartica*), *mediasiatica*, and *novicida*. Among these, *F. tularensis* subsp. *tularensis* is the most virulent and most common in North America. *F. tularensis* subsp. *holarctica* is responsible for mild forms of human infections and is mostly observed in waterborne outbreaks [3,4]. According to the type of bacteria and point of entry, the disease is observed in six different clinical forms, namely ulceroglandular, glandular, oculoglandular, oropharyngeal, pneumonic, and typhoidal (septicemic) tularemia. While the most frequent clinical form in North America is ulceroglandular tularemia [5,6], in Europe [7-9] and in Turkey, oropharyngeal tularemia makes up the majority of the tularemia cases [3,10,11].

This study aimed to review the possible sources of infection of 16 oropharyngeal tularemia cases at our hospital, as well as their epidemiological and demographical characteristics, laboratory findings, treatment methods, and treatment results.

Methodology

The study included 16 cases that presented at or were dispatched to our hospital, a tertiary referral medical center, between January 2011 and December

2012. The study was approved by Gulhane Military Medical Academy Ethics Committee. The demographics and clinical information about the patients were obtained from the hospital registry and patients' files. The age, sex, occupation, place of residence, symptoms, beginning day of the symptoms, laboratory results, treatment received and its duration, and treatment results were recorded on prepared forms. Tularemia was diagnosed through tularemia-specific tests once other conditions that may have caused lymphadenopathy (tuberculosis, lymphoma, and other malignant diseases) were excluded.

Case diagnosis

Tularemia was diagnosed according to the criteria previously described in the literature [2,12]. Briefly, among the patients with symptoms and findings matching tularemia (fever, acute tonsillopharyngitis, lymphadenopathy [LAP]), positive results in specific serologic tests, or positive results in polymerase chain reaction (PCR) specific to *F. tularensis* on the tissue were interpreted as probable cases. An increase in the tularemia agglutination tests by a factor of four in serum samples taken at least bi-weekly or clinical samples from which *F. tularensis* was isolated were interpreted as confirmed cases.

Diagnostic tests

In order to make a serologic diagnosis, 5 mL of venous blood was drawn from the patients and sent to the laboratory for an *F. tularensis* microagglutination test. In this method, 1:160 and above titers were recognized as positive. Real-time TaqMan PCR was performed using a set of primers and probe (forward: 5'-ACAAGAAGTCATGCTTGATTCAAC-3'; reverse: 5'-GATTACCTAAAGCATCAGTCATAGC-3'; probe sequence: ATAGCAAGAGCACAT), targeting the IS*Ftu2* gene, as described elsewhere [13]. To that end, a tissue sample extracted from the lymph node by way of fine-needle aspiration was utilized. These tests were conducted in the Turkish Public Health Institution's National Reference Laboratory. The tissue samples taken for the differential diagnosis of tuberculous lymphadenitis (TBL) were sent to pathology and microbiology laboratories for culturing, staining, and PCR.

Samples from the water supply in question could not be collected for bacteria isolation.

Treatment

Antibiotics such as streptomycin (2×15 mg/kg/day), doxycycline (2×100mg/day), and ciprofloxacin (2×750 mg/day) were used, as monotherapy or in combination. The treatment period lasted three weeks.

Statistical analysis

The continuous variables are expressed as average ± standard deviation, while the discrete variables are expressed in percentages. The data was analyzed using SPSS for Windows version 15.0 software.

Results

Twelve (75%) of the patients involved in this study were male. The average age of the patients was 32.1±17.2 (minimum: 14, maximum: 73) years. Fourteen cases came from Ankara (Figure 1), one from Tunceli (eastern part of Anatolia), and one from Istanbul. The cases from each town of Ankara, Cubuk, and Beypazari used their own public water supply. Sore throat (12 cases), fatigue (12 cases), and fever (10 cases) were the most frequent symptoms. Cervical lymphadenopathy was confirmed in all patients; it was found on the right side in 11 patients. The mean duration of symptoms was 21.6±6.9 days (minimum: 14, maximum: 35) (Table 1). Seven of the patients were military conscripts. Five of the patients had attended the hospital in December, and five of them attended in October. All the patients had been given treatment of tonsillopharyngitis in primary healthcare institutions prior to attending the hospital. However, despite the treatment, cervical LAP had developed in these cases. Tularemia was diagnosed in 15 patients via microagglutination (MA), and in one patient via PCR methods. TBL presence was not confirmed in any of the patients in the results of the tests used for differential diagnosis of tuberculous lymphadenitis.

Figure 1. Distribution of tularemia cases that occurred in Ankara, Turkey.

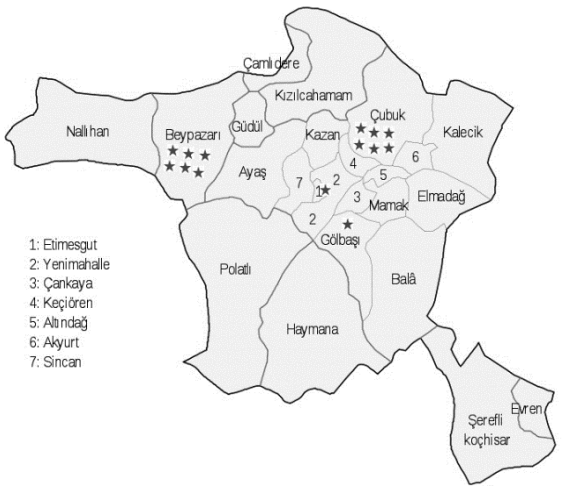

Table 1. Demographics and clinical characteristics of patients with oropharyngeal tularemia.

Case No	Sex / Age	Month of admission	Duration of symptoms* (days)	Location	Fever	Sore Throat	Fatigue	Muscle Pain	Rash	LAP	LAP localization
1	M/37	April	30	Beypazari	-	-	+	-	-	+	Right cervical
2	F/16	December	15	Golbasi	+	+	+	+	-	+	Left cervical
3	F/14	January	35	Etimesgut	+	+	+	-	-	+	Left cervical
4	M/22	October	25	Tunceli	+	+	+	-	-	+	Right cervical
5	M/38	February	20	Cubuk	+	+	+	+	-	+	Left cervical
6	F/65	February	24	Cubuk	+	+	+	-	+	+	Left cervical
7	F/73	February	19	Cubuk	+	+	+	+	-	+	Left cervical
8	M/43	October	35	Beypazari	+	+	+	+	-	+	Left cervical
9	M/21	August	24	Istanbul	-	-	+	-	-	+	Left cervical
10	M/22	October	16	Beypazari	+	-	+	-	-	+	Right cervical
11	M/21	October	20	Beypazari	-	+	+	-	-	+	Left cervical
12	M/21	January	22	Beypazari	+	+	+	+	-	+	Left cervical
13	M/42	October	15	Beypazari	-	+	-	-	-	+	Right cervical
14	M/21	February	18	Cubuk	-	+	-	-	-	+	Left cervical
15	M/22	January	14	Cubuk	+	+	+	-	-	+	Left cervical
16	M/36	February	14	Cubuk	-	-	-	-	-	+	Right cervical

*interval between onset of symptoms and initiation of treatment ; +: present; -: absent; LAP: lymphadenopathy

Table 2. Laboratory results, treatment options, and treatment results of cases with oropharyngeal tularemia.

Patient No	Sex/Age	WBC ($x10^3$/mL)	ESR (mm/h)	CRP (mg/dL)	MA titer	PCR	Treatment	Result
1	M/37	9.6	32	5.0	1:640	NA	SD	Spontaneous drainage
2	F/16	12.3	50	5.0	1:640	NA	SD	Surgical drainage
3	F/14	5.3	2	3.1	1:640	NA	SD	Recovery
4	M/22	10.0	35	12.5	1:640	NA	SD	Surgical drainage
5	M/38	7.7	3	3.0	1:1280	NA	SD	Surgical drainage
6	F/65	8.0	2	3.0	1:640	NA	D	Recovery
7	F/73	11.2	34	42.5	1:320	NA	SD	Recovery
8	M/43	7.7	52	3.0	1:2560	NA	SD	Recovery
9	M/21	13.2	50	44.0	1:1280	NA	D	Recovery
10	M/22	10.5	21	26.0	1:640	NA	DC	Surgical drainage
11	M/21	8.9	24	3.1	1:640	NA	SD	Surgical drainage
12	M/21	9.0	42	10.0	1:320	NA	C	Recovery
13	M/42	8.8	50	33.2	1:640	NA	C	Recovery
14	M/21	17.6	26	5.0	1:640	NA	SD	Recovery
15	M/22	11.0	32	5.0	1:2560	NA	SD	Recovery
16	M/36	10.2	28	5.0	1:20	Positive	SD	Recovery

MA: microagglutination; PCR: polymerase chain reaction; NA: not applied; S: streptomycin; D: doxycycline; C: ciprofloxacin

Eleven of the patients were given streptomycin + doxycycline, two of them were given doxycycline, two of them were given ciprofloxacin, and one of them was given ciprofloxacin + doxycycline. Ten of the cases fully recovered, while surgical lymph node drainage was performed on five of them. Spontaneous drainage occurred in a single remaining case (Table 1). The patients' demographic characteristics, as well as their laboratory and treatment results, are depicted in Tables 1 and 2.

Discussion

Tularemia was detected in the beginning of 20th century (1912) in the Tulare region of California, and named tularemia in reference to that region. The first case of tularemia in Turkey was diagnosed in 1936 by military doctors. From 1936 on, until 1953, cases of tularemia were reported from different parts of Turkey (Trace region, Konya, Antalya, and Tatvan), but for some unidentified reason, these reports were interrupted between 1953 and 1988 [10]. After 1988, tularemia outbreaks and cases were reported in various provinces of Turkey. The disease was incorporated into the list of notifiable diseases (category C) in 2005 in Turkey. Even though there were outbreaks and cases before, from 2005 onwards, more realistic data were gathered on the epidemiology of tularemia [10,11,14]. Between 2005 and 2008, 593 cases were observed in Turkey; however, the number increased to 1.500 in 2010, and to 2.151 in 2011 [15].

The clinical presentation of the disease varies according to the type of *F. tularensis* and the point of entry. The ulceroglandular form is the most frequently observed one. This form of disease occurs as a result of tick bite or contact with infectious game [16]. Oropharyngeal tularemia form caused by the *holarctica* subtype develops as a result of the bacteria entering the body via contaminated water and food. The disease develops with fever, sore throat, and cervical and post-auricular lymphadenomegaly [16]. In addition, in the course of tularemia, skin rashes (popular, vesiculopapular, or erythema nodosum) have been reported at rates of 5%–42% [16-18]. Vesicular types of skin rashes can be confused with varicella zoster infection [19]. Among our cases, only one (Table 2, case 6) developed a skin rash matching erythema nodosum (Figure 2).

A study carried out by Karabay *et al*. [15] in Turkey showed that because oropharyngeal tularemia progresses with similar symptoms and clinical and histopathological findings, it can be confused with TBL. In that study, tularemia antibodies were investigated in patients diagnosed with TBL. *F. tularensis* antibody positivity was confirmed in 79 (6.75%) of the patients out of 1,170 who were diagnosed with TBL. Based on this data, in countries like Turkey where tularemia is endemic, before commencing the treatment of tuberculosis in patients diagnosed with TBL, serological tests should be conducted and tularemia must be ruled out.

The strains isolated in Turkey have been *F. tularensis* subp. *holarctica* [10,20] and almost all outbreaks have been waterborne [4,10,21]. In a large study that included 205 cases, Helvacı *et al*. reported that the oropharyngeal form accounted for 83% of cases, oculoglandular for 8%, and ulceroglandular for only 1% of all the cases [16]. The disease is observed more frequently in women and in patients between the ages of 20 and 40 (50%) [7,16,17]. Our study did not contain enough cases to make an evaluation on sex and age range. However, 75% of our cases were males and 62.5% of them were between the ages of 21 and 40. The reason for the high frequency of males in this study can be due to the high number of conscripts. In rural areas, contact with non-chlorinated water is more common among women, who do the house and agricultural work; this could be the probable cause of high frequency of female cases reported in other studies.

Fourteen cases came from Ankara. Among them, six came from town of Cubuk, and six from the town of Beypazari. Cases 5, 6, and 7 (Tables 1, 2) were members of a family that had been living in the same

Figure 2. A skin rash matching erythema nodosum is shown in a case, a 65-year-old female.

village. All of them had been prescribed amoxicillin-clavulanate by their family physician for their complaints of sore throat and fever. No improvement had occurred after 10 days of treatment. When the patients attended our outpatient clinic, 20 days had passed since their first symptoms had occurred. In their medical history, the patients pointed out that a dead mouse had been sighted in the water source that supplied the village's fresh water. However, no sample was collected from the water supply in question for bacteria isolation. It was discovered that there were six more people with similar complaints and that they had attended other healthcare centers, so these cases were not presented in this report. Similarly, in October, cases 8 and 13, who were living in the same part of the town of Beypazari, Ankara, attended our center with complaints of swelling and pain in the neck. The other four cases were from different parts of the town of Beypazari.

Patients with tularemia in Turkey usually live in rural areas. The majority of the patients in our study also came from the rural areas. The tularemia cases in Turkey usually increase during winter and autumn [22]. Çelebi *et al*. reported February to be the month in which tularemia cases were observed the most frequently [23]. This was confirmed in our cases as well.

The clinical manifestations observed in the current cases appeared similar to those of the oropharyngeal form, with patients presenting with sore throat and enlarged cervical lymph nodes. In line with what Şencan *et al*. [24] concluded, left cervical region stiffness was more significant in our cases.

The gold standard diagnostic method for tularemia is culture. The culture procedures should be conducted in level-3 biosafety laboratory conditions [25,26]. Due to the difficulty of reproduction of *F. tularensis* and the hazard it bears for laboratory personnel, serologic tests should be preferred in diagnosing the disease [3,10,16,24,26]. MA has been used as the diagnostic test for a long time. Among the patients with symptoms and findings matching with tularemia, 1:160 and above positivity titer in MA is sufficient to diagnose the disease. In our cases, tularemia was diagnosed via the MA method. The MA was observed at 1:160 in 15 cases out of 16, and at 1:20 in one case. However, fine-needle lymph node aspiration of this patient revealed a positive result for *F. tularensis*-specific PCR.

Streptomycin, gentamicin, doxycycline, and quinolones are currently used in the treatment of tularemia [27,28]. Since *F. tularensis* is intrinsically resistant to beta-lactam antibiotics, this group of antibiotics is not used in treatment. A new agent named tigecycline has been found to be quite effective *in vitro* against *F. tularensis* [20]. However, there is no clinical experience on this agent as of yet. In order to achieve success in treatment, antibiotic treatment should be started within the first three weeks of diagnosis. Once the lymph node has suppurated, the effect of antibiotics in treatment is limited. Surgical drainage of suppurated lymph nodes is advised to accomplish better cosmetic results [29]. Our cases attended the hospital after 21.6 ± 6.9 days after the first symptoms were observed. Ten of the cases fully recovered, while surgical lymph node drainage was applied to five of them. Spontaneous drainage occurred in the single remaining case (Table 1).

Conclusions

Turkey is considered to be a tularemia-endemic country. After the incorporation of the disease into the list of notifiable diseases in 2005, supposedly waterborne outbreaks and cases from all corners of Turkey are being reported at an increasing rate. Diagnosis and early treatment of the disease is imperative to its cure. Since it can be potentially confused with tuberculous lymphadenitis clinically and histopathologically, differential diagnosis is imperative. Patients attending with a condition of tonsillopharyngitis in endemic areas must be carefully monitored.

Acknowledgements

We express our gratitude to Turkish Public Health Institution's Tularemia Reference Laboratory personnel.

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Conflict of interests: No conflict of interests is declared.