

BRUCELLOSIS IS A MAJOR PROBLEM: A FIVE YEARS EXPERIENCE

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ABSTRACT

Brucellosis is a chronic granulomatous infection which is endemic in Mediterranean countries and Turkey. The aim of this study is to analyze the clinical, laboratory findings and therapeutic features in patients with brucellosis.

A retrospective study was conducted with 91 patients who developed brucellosis between 2005 to 2009. The diagnosis was based on clinical findings compatible with brucellosis, serological tests positive, and/or isolation of Brucella species from blood, or other tissues.

The mean age was 33 years (16-67 years). Sixty-threes of patients (69.2%) were male. Forty (44%) cases had an occupational history relevant for Brucella exposure and 85 (93%) cases consumption with contaminated animal product. The mean diagnostic delay was 15 days, much longer in focal brucellosis. A total of 77 (85 %) cases had acute brucellosis. The focal brucellosis complications were observed in 39 (42.8%) cases: osteoarticular involvement 32 (82%), epididymo-orchitis 4 (10%), and central nervous system involvement 3 (8%). Chronic brucellosis occurs in 3 (3.3%) cases. Clinical manifestations included non-specific symptoms such as fever (95%), sweats (90%), arthralgia and lower back pain (63%). Of the patients 84 (92%) had serological titre =1/160 and 28 (31%) blood cultures were positive. All of the patients were cured by antibiotic therapy (Doxycycline+rifampicin/streptomycine, streptomycine+rifampicin/Doxycycline, ceftiraxone/rifampicin). Relapse was observed in 5 (5.4%) patients.

Brucellosis is an infection with multiple presentations. Its early diagnosis was mandatory to avoid severe complications.

Key words: brucellosis, relapses, Mediterranean.

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Introduction

Brucellosis is one of the most widespread zoonosis and causes more than 500 000 human infections per year worldwide^(1,2). Brucellosis is caused by Brucella species which are small, non-motile, non-spore-forming, encapsulated Gram-negative coccobacilli. Four species are responsible from human brucellosis: Brucella abortus, Brucella melitensis, Brucella suis, and Brucella canis. Brucellosis is transmitted to human generally after consumption of unpasteurized milk and milk products. Sometimes, it may occur in human as a result of direct contact with animals. Brucella species ini-

tially localize in the regional lymph nodes, and then disseminate hematogenously to the reticuloendothelial system organs and then multiply within phagocytic cells. The release of bacterial endotoxin from phagocytic cells lead to symptoms and signs in host. Brucellosis can mimic many various multisystem diseases and generally presented with high fever, myalgia and arthralgia of the large joints. Bacteriological or serological tests are routinely used to confirm this infectious disease. However showing wide clinical polymorphism, which frequently leads to misdiagnosis and treatment delays, and complications. Brucellosis are seen generally in spring and summer but it may be encountered in all

seasons. This disease occurs in all age groups, more common in adults⁽³⁻⁷⁾.

Turkey is a large country in the eastern Mediterranean region and brucellosis is an endemic disease in Turkey. Especially in Southeastern Anatolia of Turkey, many people of all ages suffer from brucellosis and it is a still major public-health problem. For this reason, we aimed to analyze the clinical, laboratory findings and therapeutic features in patients with brucellosis.

Materials and methods

Patients, study design

We conducted a retrospective study of patients who developed brucellosis between 2005 and 2009 and were admitted to the Department of Infectious Diseases of Dicle University Hospital, Diyarbakir. A total of 91 patients with brucellosis (16-67 years) were included in this study. The diagnosis was based on clinical findings compatible with brucellosis, serological tests positive, and/or isolation of *Brucella* species from blood, or other tissues. In this study, demographic characteristics, patients and family history, area of residence, history of raw milk or milk products consumption or work in animal breeding, and clinical and laboratory findings, as well as clinical outcomes and complications were recorded and evaluated.

Diagnosis of brucellosis was performed according to clinical and laboratory findings of the patients: isolation of microorganisms in blood, other body fluids or tissue samples, or the presence of compatible clinical symptoms such as arthralgia, fever, sweating, chills, headache, myalgia, and malaise combined with a serum antibody titer $\geq 1/160$ or at least a four-fold increase in this titer by the standard tube agglutination (STA) test in a two- or three-week interval, and the patients with symptoms persist more than 1 year were defined as having chronic brucellosis⁽³⁾.

Automated blood culture system (the BacT/ALERT 3D system, bioMérieux, Durham, NC, USA) was used for isolation of *Brucella* spp. from blood and bone marrow specimens. Identification and antibiotic susceptibility of the isolates were performed by the BD Phoenix (BD Diagnostics, Sparks, MD) automated system. Routine laboratory tests, including complete blood count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), aspartate aminotransferase (AST) and alanine transaminase (ALT), renal func-

tion profiles, and urine examination were also performed.

Focal form or complication was considered as the presence of symptoms or physical signs of infection at a particular anatomic site in a patient with active brucellosis. Osteoarticular involvement was considered when there were inflammatory signs such as heat, redness, pain, swelling, or functional disability in any peripheral joint, or when there was unrelieved pain at rest together with radiological alterations and/or radionuclide uptake in any deep joint, evaluated independently by both the clinician and the radiologist. Neurobrucellosis was considered as: isolation of *Brucella* species from Cerebrospinal fluid (CSF) of patients with suspected findings for brucellosis, or isolation of *Brucella* species from bone marrow or blood cultures of patients with abnormal CSF findings, with or without STA positivity of any titer in CSF with abnormal findings. Hematologic involvement was considered as hematologic abnormalities in laboratory and clinical findings (epistaxis, bleeding, petechiae, purpura, disseminated intravascular coagulation (DIC), and thrombophlebitis), excluding asymptomatic or poorly symptomatic cytopenias or coagulation disturbances. Hepatic involvement was defined as a five-fold increase (>200 IU/l) in AST and ALT levels without any other etiologic explanation, and/or total bilirubin levels of over 2.5 g/dl. Relapse was defined as the reappearance of symptoms or a positive blood culture after the treatment⁽⁸⁾.

Treatment and follow-up

The patients were received various antibiotics and combinations such as Doxycycline plus rifampicin/streptomycin, streptomycin plus rifampicin/Doxycycline, ceftriaxone/rifampicin. The antibiotic regimens as follows oral Doxycycline (100 mg every 12 h), oral rifampicin (300 or 600 mg every 24 h), intramuscular streptomycin (1 g every 24 h), ceftriaxone (2 g per day). During hospitalization the patients were followed up for at least 2–3 weeks. Outpatients were called for control visits at 2-week intervals. Complete blood count, CRP, ESR, and AST, ALT results were evaluated at control visits. STA was performed after a one-month interval. After completion of therapy all patients were followed up for at least 6 weeks. After the treatment period, patients were recalled for the first control visit two weeks later and then at 6, 10 and 14 weeks, 6 months, and 1 year later.

Complete blood count, CRP, ESR, and liver enzymes were examined and STA performed at the first control visit and at each further control visit. During the follow-up period, blood cultures were only performed in patients who were considered to be relapse.

Statistical analysis

The obtained data were analyzed using the Statistical Package for the Social Sciences (SPSS version 11.0 for Microsoft Windows). A p value of < 0.05 was considered significant.

Results

Among 91 patients included in this study, 63 cases (69.2%) were male and 28 (30.8%) cases were female. The mean age was 33 years (16-67 years). An occupational history relevant for Brucella exposure was present in 40 (44%) cases and consumed contaminated animal product (eating fresh cheese, yogurt and drinking milk) was noted in 85 (93%) cases according to history.

The mean diagnostic delay was 15 days. In focal brucellosis the mean diagnostic delay was 19 days. Acute brucellosis was predominant, in 77 (85%) cases (Table 1).

Features of brucella cases	Number of patients (%)
Male	63 (69.2%)
Occupational history	40 (44%)
Consumption of contaminated animal product	85(93%)
Acute brucellosis	77 (85%)
Chronic brucellosis	3 (3.3%)
Focal brucellosis complications	39 (42.8)
osteoarticular involvement	32 (82%)
epididimo-orchitis	4 (10%)
central nervous system involvement	3 (8%)
Fever	86 (95%)
Sweats	82 (90%)
Arthralgia and lower back pain	57 (63%)
Serological titre =1/160	84 (92%)
Blood culture positive	28 (31%)
Relapse	5 (5.4%)

Table 1: Features of 91 cases with brucellosis in a 5-year period (2005-2009) in Dicle University Hospital.

The focal brucellosis complications were seen in 39 (42.8%) cases: osteoarticular involvement in 32 (82%) cases, epididimo-orchitis in 4 (10%) cases, and central nervous system involvement in 3 (8%) cases (Table 1). Hematologic involvement and hepatic involvement were not detected in any cases.

Chronic brucellosis occurs in 3 (3.3%) cases. Clinical manifestations include non-specific symptoms such as fever were observed in 86 (95%) cases, sweats 82 (90%) cases, arthralgia and lower back pain 57 (63%) cases (Table 1).

According to results of standard tube agglutination (STA) tests, of the patients 84 (92%) had serological titer =1/160 and 28 (31%) blood cultures were positive. All of the patients were cured by antibiotic therapy. Relapse in follow-up period was observed in 5 (5.4%) patients (Table 1).

Discussion

Human brucellosis is the most common zoonosis worldwide. It is estimated that the number of individuals with brucellosis may be up to 26 times higher than the 500,000 cases, annually⁽⁸⁾. Mediterranean basin, Persian Gulf, the Indian sub-continent, and parts of Mexico and Central and South America are endemic areas. The Balkan Peninsula and many of the former Soviet Union Asian Republics are also endemic for Brucellosis^(9,10).

Brucellosis is endemic, and approximately 10,000 human brucellosis cases are reported annually in Turkey. According to literature incidence of brucellosis is 150 cases per 1 million inhabitants^(11,12). The South-eastern Anatolia region of Turkey is a region with higher population and most of the people live in rural areas. Dicle University Hospital is located in Diyarbakir which is a province located in the South-eastern Anatolian with more than 2 million population and serves the big majority of South-eastern Anatolian population. Prevalence of Brucellosis varies widely from region to region due to several factors, including food habits, milk processing methods, social customs, climatic conditions, socioeconomic status, and environmental conditions. In Turkey, an increase of reported human cases were seen from 1986 (3.03/100,000 population) until 2004 (25.65/100,000).

However with the infection-control policies implemented in Turkey such as livestock vaccination, elimination of infected animals, control of ani-

mal movements, and education of people especially living in rural areas lead to decline in the number of human cases, from 18,563 cases in 2004 to 9,818 cases in 2008^(11,13). The number of reported patients with Brucellosis varies according to the geographic regions of Turkey. For example, it was reported to be as high as 27.2% among high-risk patients in Eastern Anatolia⁽¹⁴⁾. With the new developments in recent years in molecular methods, it was able to determine the epidemiological relationship between *Brucella* isolates of Turkey. In a Turkey study of Kilic et al., multiple-locus variable-number tandem-repeat analysis (MLVA) was performed to analyze the epidemiological relationship and genetic diversity among 162 human *Brucella* isolates collected from all geographic regions of Turkey. They found that the most prevalent MLVA genotypes found in Turkey are from the East Mediterranean region. They detected that more than half of the human brucellosis cases resulted from either very close cross-transmission in a location or persistent and ongoing transmission among the different regions⁽¹¹⁾.

The primary transmission route of brucellosis is the consumption of unpasteurized milk or milk products and direct contact with animals. According to epidemiologic studies in Turkey, a history of raw dairy product consumption has been reported in 60% to 90% of cases⁽¹⁴⁻¹⁷⁾. In Eastern Anatolia region of Turkey it was found that eating traditionally prepared soft cheeses was an important transmission route. In an Eastern Anatolia study, Aypek et al. indicated that 34.1% of persons gained brucellosis from livestock and seroprevalence of brucellosis in human was 8.8%. They suggested that due to animals were kept closely together in small stables, infections can spread easily among animals and are easily transmitted to humans⁽¹⁴⁾. In addition they found that a family history of eating unpasteurized milk and milk products (65.9%) was a risk factor for brucellosis⁽¹⁴⁾. In our study consumed contaminated animal product (eating fresh cheese, yogurt and drinking milk) was noted in 93% cases according to history of the patients, and occupational history relevant for *Brucella* exposure was present in 44% of the cases, in accordance with the literature. These studies indicated that vaccination of livestock and prevention of feeding unpasteurized milk and milk products is necessary to control brucellosis in Turkey.

According to world literature brucellosis is common among the young and middle-aged popu-

lations in endemic countries^(18,19). In a Turkey study, it was found that average age was 40 ± 17 years in patient with brucellosis and in only 11% of the cases were ≥ 65 years old⁽²⁰⁾. In our study the mean age was 33 years (16-67 years) and our result was compatible with the other studies. Studies also showed that brucellosis was common among males but the incidence of the disease was not directly related to gender⁽²⁰⁻²²⁾. The reason of the frequent occurrence of male population may be dealing with animal husbandry is more common in males in our region.

Brucellosis may presented with acute, subacute, and chronic forms. Some studies demonstrated that the acute form was seen in 25%-77% of the patients, the subacute form in 12.5%-59%, and the chronic form in 5%-27.5%^(23,24). In a Turkey study it was indicated that 61% of patients with brucellosis were diagnosed as having the acute form, 35% the subacute form, and 4% the chronic form⁽²⁴⁾. The acute form was the most common form due to the symptoms were more remarkable in acute period⁽²⁰⁾. Brucellosis can mimic many multisystem diseases and generally presented with high fever, myalgia and arthralgia of the large joints. Fever was found to be more common in the acute disease⁽¹⁶⁾. In our study acute brucellosis was also predominant (85%) and the rates of clinical manifestations (fever (95%), sweats (90%), arthralgia and lower back pain (63%)) were compatible with literature data.

Osteoarticular involvement was the most common focal complication of brucellosis and morbidity⁽²⁵⁾. In some studies osteoarticular involvement was observed in 20%-85% of brucellosis cases^(20, 26). In male, various genitourinary infections including epididymo-orchitis, prostatitis, cystitis, pyelonephritis, interstitial nephritis, exudative glomerulonephritis, renal and testicular abscess, and seminal vasculitis have been seen in brucellosis. According to literature the most frequent genitourinary complication of brucellosis is epididymo-orchitis, affecting 2-20% of males with brucellosis⁽²²⁾. In a study from the South-eastern Anatolia region of Turkey, Çelen et. al found that 18.8% of the males with brucellosis suffered from epididymo-orchitis⁽²²⁾. In an another Turkey study Buzgan et al. found that genitourinary system involvement was present in 3.7% of the patients, mainly presenting as epididymo-orchitis (3.4%)⁽²¹⁾. The same investigators reported that central nervous system involvement was present in 5.6% of the cases⁽²¹⁾. In our study the focal brucellosis complications were

consistent with other studies. In addition the mean diagnostic delay was much longer in focal brucellosis. The reason of this may be misdiagnosis of the big majority of patients with focal brucellosis in health centers.

The diagnosis of brucellosis can be made serologically and clinically. In addition culture methods can be used in the diagnosis of *Brucella* infection⁽²⁶⁾. Serologic tests are the methods of choice for diagnosing brucellosis when bacterial isolation is not possible⁽¹⁴⁾. Culture positivity in brucellosis cases may be low. In a Turkey study 21% of the patients with brucellosis was blood culture positive for *Brucella* species⁽²⁰⁾. In our study 31% of blood cultures were positive. We thought that the lower rates of culture positivity was due to slow growing of *Brucella* species and failure of blood culture systems.

Brucella is an intracellular bacterial pathogen that infects host macrophage cells. In 1986, the WHO (World Health Organization) recommended doxycycline, combined with either rifampicin or streptomycin for treating human brucellosis⁽²⁷⁾. Antibiotic susceptibilities changed over time. Fluoroquinolones, macrolides and tigecycline were alternative drug choices⁽²⁷⁾. In Turkey there were limited studies investigating the antibiotic susceptibility of *Brucella* species isolated from human. In a Southeastern Anatolian study, MIC90 (Minimum Inhibitory Concentration required to inhibit the growth of 90% of organisms) values of doxycycline, streptomycin, rifampin, trimethoprim-sulfamethoxazole and tigecycline were 0.064 mg/L, 1 mg/L, 2 mg/L, 0.125 mg/L and 0.094 mg/L, respectively. It was suggested that tigecycline can be an alternative choice for the treatment of brucellosis⁽²⁸⁾. In our study, all of the patients were cured by antibiotic therapy and relapse in follow-up period was observed in 5 patients.

In a Turkey study, rifampicin plus doxycycline was the most common regimen administered in brucellosis cases (54.5%), the overall relapse rate was 9.09%. In Turkey study the highest relapse rate of 12.5% was seen in patients received doxycycline plus rifampin⁽¹⁴⁾. According to the literature, despite the treatment, acute brucellosis relapses in 5%-40% of patients⁽¹⁴⁾. In our cases the reason of the relapses may be patient's non-compliance of drug, inadequate doses of treatment, and drug resistance. In the light of our data we can say that large multicenter studies are necessary for determining the most appropriate treatment options in brucellosis.

In conclusion, brucellosis is an infection with multiple presentations. Its early diagnosis is mandatory to avoid severe complications. Brucellosis can be eradicated in humans by education programs about risk factors for brucellosis transmission, in rural areas, where human contact with domestic animals is widespread. In addition vaccination of all livestock is essential for controlling the disease in animals.

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