BRUCELLOSIS: EVALUATION OF 201 CASES IN AN ENDEMIC AREA OF MEDITERRANEAN BASIN

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ABSTRACT

Objectives: Brucellosis is a zoonotic infectious disease endemic in some countries and in the countries of the Mediterranean region, including Turkey. The aim of this study was to investigate epidemiological and clinical characteristics of brucellosis patients followed in two Southwestern Anatolian hospitals.

Patients and methods: Between January 2004 and December 2012, 201 cases of brucellosis over the age of 15 followed in Suleyman Demirel University, Faculty of Medicine and Isparta State Hospital were included to the study. The charts of these patients were retrospectively evaluated and patient complaints, physical examination findings, clinical and laboratory findings, complications and treatment results were evaluated on separate forms and recorded.

Results: There were 94 female and 107 male patients, total 201, between the age of 15 and 88. The most important route of infection was ingestion of unpasteurized milk products. Most frequent symptoms were fever, arthralgia, fatigue and hyperhidrosis, while most frequent findings were fever, splenomegaly and hepatomegaly. Most frequently involved system was the musculoskeletal. Epididymoorchitis was present in 8,9 % of the patients, haematological involvement were detected in 40% of the patients, with anaemia being most frequent. C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) levels were found to be elevated in 83.1% and in 72.6% respectively. Standard tube agglutination (STA) or Coombs tube agglutination tests were positive in 94.5% of the cases. Relapse occurred in 6.4% of the cases.

Conclusion: Brucellosis is zoonotic disease affecting different organs and systems thus presenting with various symptoms and findings. It is especially important that in endemic areas clinicians and family physicians should be informed of the epidemiological, clinical and laboratory features of brucellosis in order to prevent delayed diagnosis and complications. Furthermore, considering that the most important way of infection is still the use of unpasteurized milk products and animal farming, we believe that it is necessary to work on preventive measures.

Key words: Brucellosis, complication, zoonosis.

Introduction

Brucellosis is a zoonotic infection caused by species of Brucella bacterium. Brucella bacteria are gram negative stained, immobile coccobacilli. Infection with these bacteria occurs through ingestion of unpasteurized milk and milk products, contact with infected animals and inhalation of infected aerosols[1,2]. Brucellosis is seen in the Mediterranean region and is still endemic in the Middle East, India, Africa, Mexico, Central and South America. Annually 500.000 new cases are being reported from these countries[3,4]. The incidence of brucellosis in our country is reported to be 23/100.000[5]. The symptoms and findings are not specific to the

disease and every organ and system can be affected. Therefore, patients present to different departments with various complaints leading to delayed diagnosis and treatment[1,5]. Eventhough brucellosis has low mortality; continues to be an important public health issue because of long-term treatment, slow recovery, possible complications and sequels, and its widespread occurrence[1].

Patients and methods

Between January 2004 and December 2012, 201 cases of brucellosis over the age of 15 followed in Suleyman Demirel University, Faculty of Medicine and Isparta State Hospital were included

to the study. The charts of these patients were retrospectively evaluated and patient complaints, clinical and laboratory findings, complications and treatment results were evaluated on separate forms and recorded.

Brucellosis was diagnosed by clinical symptoms and findings, positive serum STA test (brucella STA test titer ≥1/160), and/or isolation for Brucella species from the blood or other body fluids cultures. Automatized blood culture BACTEC 9120 system (Becton-Dickinson) was used for blood culture. For STA test Brucella abortus S99 antigen (Pendik Veterinary Research Institute) was used. If complication was considered or when needed, imaging studies such as x-ray, ultrasonography, computed tomography (CT) and magnetic resonance imaging (MRI) were performed. Patients were classified to groups according to the beginning of complaints as acute if lasted less than 8 weeks, subacute if the symptoms lasted between 8 weeks and 1 year, and chronic if present for more than 1 year.

Patients were treated with dual or triple combination of doxycycline, rifampicin, streptomycin, ciprofloxacin, ceftriaxone, and trimethoprim-sulfamethoxazole. Patients were followed-up once in 2 weeks during the treatment and on the 1st, 3rd, 6th, and 12th months after the treatment period. Occurrence of similar complaints within one year after the treatment or increase in STA titers, or reproduction of Brucella species in cultures was considered as relapse of the disease.

Neurobrucellosis was diagnosed as: isolation Brucella species from cerebrospinal fluid (CSF), or isolation of Brucella species from blood cultures of patients with abnormal of CSF findings. The diagnosis of brucella pneumonia was made by Brucella species from bronchoalveolar lavage (BAL) culture in addition to compatible clinical findings of pneumonia. The diagnosis of Brucella endocarditis was made in accordance with Duke's criteria. Epididymoorchitis was diagnosed by physical examination findings and positive serology results or positive culture results and scrotal ultrasonography findings. Involvement of intraabdominal organs was considered according to US and CT findings in accordance with positive culture and serology results.

Descriptive analyses were obtained using SPSS11.0 program (median, standard deviation, frequency) and chi-square test.

Findings

Demographical findings

The median age of total 201 patient was 47.4±17.1, 94 of the patients were female (%46.7), 107 were male (53.3%), and their age was between 15 and 88 years. Of the brucellosis patients 141 (70.1%) were acute, 56 (27.8%) were subacute and 4 (2%) were in chronic phase. According to the risk factors, 78 (38.8%) patients were at high risk for brucellosis (deal with animal husbandry (n=75), veterinary (n=2) and laboratory technicians (n=1)), 140 (69.6%) patient had history of milk products ingestion and 18 (8.9%) had history of relatives with brucellosis (Table 1).

Demographical findings	n(%)
Average age (age range)	47 (15-88)
Gender	
Male	104 (53,3)
Female	97 (46,7)
Duration of symptoms	
Acute	141 (70,1)
Subacute	56 (27,8)
Chronic	4 (2)
Livestock	75 (37,3)
Consumption of unpasteurized dairy products	140 (69,6)
Healthcare workers	3 (1,4)
Family history of brucellosis	18 (8,9)

Table 1: Demographical findings.

Symptoms, physical examination and non-specific laboratory findings

The most frequently seen symptoms were fever, arthralgia, sweating, fatigue and lower back pain. The most common clinical findings were fever, splenomegaly and hepatomegaly (Table 2). C-reactive protein (CRP) levels were found to be high in 83% of the patients and the average was 48,8±45,3. Erythrocyte sedimentation rate (ESR) was found to be increased in 72.6% (ESR>20 mm/hour) and the average was 35.4±24.5. CRP and

ESR levels were found to be significantly high in patients with osteoarticular involvement compared to the patients without any complication (p<0.05). The laboratory findings are given on Table 3.

Symptoms	n (%)
Fever	143 (71,1)
Arthralgia	118 (58,7)
Sweating	105 (52,2)
Lumbago	75 (37,3)
Weakness	75 (37,3)
Myalgia	57 (28,3)
Nausea and vomiting	21 (10,4)
Stomachache	20 (10)
Scrotal pain	18 (8,9)
Clinical Findings	
Fever	122 (60,6)
Hepatomegaly	33 (16,4)
Splenomegaly	32 (15,9)
Scrotal swelling	18 (8,9)
Lymphadenopathy	7 (3,4)
Peripheral arthritis	5 (2,4)

Table 2: Symptoms and clinical findings.

Microbiological findings

STA was found to be positive in 182 (91%) of the patients (titer ≥1/160) (Table 3). Seven patients who had a negative STA were found to be positive by Coombs STA. Despite of negative STA or Coombs STA, Brucella species were isolated in the blood cultures of 9 patients, and in the bone marrow cultures of 2 patients. All of these cases were found to be subacute or chronic. Blood cultures were positive for Brucella in 69 patients, furthermore 2 patients had positive synovial fluid cultures, 2 patients positive bone marrow cultures, 1 patient positive bone biopsy culture and 1 patient positive BAL culture.

Complications

Most frequently encountered complication was osteoarticular involvement. Other complications and rare systemic involvements are presented

on Table 4. Of the patients with lower back pain 48% had spondylodiscitis.

Laboratory findings	n(%)
Anemia (male-Hb<14mg/dL and women Hb <12mg/dL)	69 (34,3)
Thrombocytopenia (platelet count <150,000 / mm3)	22 (10,9)
Leukopenia (WBC count <4000 / mm3)	30 (14,9)
Leukocytosis (WBC count> 12,000 / mm3)	9 (4,4)
Pancytopenia	9 (4,4)
ESR (ESR> 20 mm / hour)	146 (72,6)
CRP (CRP> 6 mg / L)	167 (83,1)
AST elevation (AST> 50 IU / L)	84 (41,7)
ALT elevation (ALT> 50 IU / L)	74 (36,8)
STA positivity (SAT ≥ 1/160)	183 (91)
Cooombs STA positivity (CSAT ≥ 1/160)	7 (3,4)
Culture positivity	77 (38,3)
Seronegative - culture positive cases	11 (5,4)

Table 3: Laboratory findings.
(Hb: Hemoglobin, WBC: White blood cell, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, STA: Standard tube agglutination test).

Complication	n(%)
Spondylodiscitis	26 (12,9)
Sacroileitis	19 (9,4)
Epididymoorchitis	18 (8,9)
Peripheral arthritis	5 (2,4)
Infective endocarditis	5 (2,4)
Spleen abscess	4 (1,9)
Meningitis	1 (0,5)
Pneumonia	1 (0,5)
Renal abscess	1 (0,5)
Osteomyelitis	1 (0,5)
Prosthetic joint infection	1 (0,5)
Relapse	13 (6,4)
Without complications	116 (57,7)

Table 4: Complicated cases and rare encountered forms.

Treatment and results

Most frequently used antibiotic combination was doxycycline (2x100 mg per oral) + rifampicin (2x300 mg per oral) in 40% of the patients, second frequently used combination was doxycycline (2x100 mg per oral for 42 days) + streptomycin (1x1 gr IM for 21 days) in 29% of the patients. In cases with complication (endocarditis, neurobrucellosis, sacroileitis etc.) or when the first line antibiotic was discontinued due to an adverse reaction, dual or triple treatment regimens with ciprofloxacin, trimethoprime/sulfamethoxazole and ceftriaxone were given. The average hospital stay was 8.01±4.2 days, varying between 1-23 days. The disease relapsed in 13 patients, therefore they were treated with different combination regime compared to the initial. Only one patient died due to Brucella endocarditis.

Discussion

Brucellosis is an endemic disease in the Mediterranean countries, including our country and in some other places in the world (3,4). Brucellosis may be seen in every age group. However in this study only adults were included with an average of 47.4±18.1 (15-88) years. This rate is similar to other studies in the literature[6,9]. Most of the studies show similar gender involvement in brucellosis, however only small number of studies have shown higher involvement in one of the genders[6-11]. Both genders had similar involvement rates in this study (males 53%, females 47%). The rate of chronic brucellosis was found to be lower (2%) compared to other studies[7-9]. The reason for this may be the early diagnosis of brucellosis because its endemic appearance in our country.

The main infection route is ingestion of unpasteurized milk products. In the studies performed, this rate was found to be 23.6-81%, tending to be higher in our country[6,7,9,12]. Deal with animal husbandry is also a risk factor. In some epidemiological studies these rates have been reported between 14.6 and 44.1%[7,9,11]. In our study this rate was found to be 37.3%. These rates reveal that there is insufficient animal vaccination. Again, high consumption of unpasteurized milk products in endemic areas shows the importance of public education. Also, the healthcare personnel, especially laboratory personnel and veterinarians have occupational exposure risk[7,9]. In this study 3 patients (2 veterinarians, 1 laboratory personnel) had occupational exposure.

Most frequently encountered findings are fever, sweating, arthralgia, myalgia and fatigue[1,7]. In large series, 72.2-90.5% of the patients had fever, 64.8-84.4% had sweating, 73.7-81.9% had arthralgia, 36.1%- 50.9% had myalgia[7,8,13]. In this study also fever, arthralgia, sweating and myalgia were most frequent complaints.

Brucellosis may present with various physical examination findings as it can affect many organs and systems. Most frequently encountered findings were fever, hepatomegaly and splenomegaly, found in 60.6%, 16.4% and 15.9% respectively. Similar findings are seen in other studies found in different rates[8,13,14]. Findings such as scrotal edema and rush, stiff neck, heart murmur are also detected due to the related organ involvement.

Most frequently affected system in brucellosis is the osteoarticular with 20-85% presence in cases[1,7,15]. This involvement may vary, but the most frequent types are spondylodiscitis and sacroileitis. Osteoarticular complication in our study was found to be 25%, of which 52% were spondylodiscitis and 38% sacroileitis. In some studies spondilodiscitis was more often as it was in our study, whereas some studies report sacroileitis to be more frequently encountered[8,9,11]. Especially because in most of the patients with lower back pain spondylodiscitis was present, in patients presenting with lower back pain in brucellosis endemic areas spondylodiscitis should be considered in the initial diagnosis.

Genitourinary involvement in brucellosis is being reported between 2-10%[1,20]. The most frequent involvement is unilateral epididymoorchitis. In our study epididymoorchitis was detected in 18 (8.9%) patients, while in one patient renal abscess was present, which is very rare. Given the complications in males, epididymoorchitis is on the first place with 16.8%. Especially in endemic areas these high rates brucella infection should be considered in the differential diagnosis of patients presenting with epididymoorchitis.

Endocarditis is the most frequently encountered cardiovascular complication of brucellosis and reported to be lower than 2% in various series[5,7]. In our study 5 (2.4%) patients had endocarditis with one of them being fatal. The diagnosis was late, considering the fact that the patient was lost 48 hours after the diagnosis. Various CNS complications such as meningitis, encephalitis, myelitis, brain abscess may be seen and the rate in different studies has been reported to be 1.7-17.8%[1,5,11]. In our study one case of brucellosis related meningitis

was seen and diagnosed by Brucella species isolated from CSF fluid. Especially in terms of prevention as well as for the treatment of such complications that can result in serious consequences early diagnosis of brucellosis is important.

Eventhough forms of brucellosis as pneumonia, spleen abscess, renal abscess, osteomyelitis, joint prosthesis infection are rarely seen in our study, it is important to show that brucellosis can cause a wide variety of clinical conditions. Especially renal abscess, osteomyelitis and infection of joint prosthesis are very rarely encountered forms of brucellosis[5].

Haematological complications such as anaemia, thrombocytopenia, leukopenia and coagulation disorders can be seen in brucellosis[1]. Various rates have been reported in different studies[7-13]. These haematological abnormalities are transient and improving with antimicrobial treatment[16]. The most frequent haematological findings in this study were anaemia (with 9 cases of pancytopenia). Therefore, brucellosis should be considered when investigating pancytopenia aetiology in endemic areas. Even though elevated AST-ALT levels are frequently encountered in brucellosis, clinically hepatitis is a rarely seen form and 2-3% has been reported in various series. In our study 41,7% of the patients had elevated transaminase levels but no clinical case of hepatitis has been detected. CRP may be elevated in brucellosis patients and various rates (36-93%) have been reported in different studies. ESR levels may also be elevated in 31%-80% of the patients[7,8,9,11,17]. In our study, 83.1% had elevated CRP levels and 72.6% had elevated ESR levels. ESR and CRP levels were higher and statistically significant in cases with osteoarticular, urogenital and focal involvement compared to uncomplicated cases. We believe that CRP and ESR levels may be useful in the detection of complications and in the follow-up of the response to treatment.

The most important and easily applicable diagnostic test for brucellosis is STA. Besides being easy for use, the most important advantages of this test are the high sensitivity rates (94%) and the fact that it does not require additional equipment[18]. In different series the patients had positive results to this test in 76.8-98.7%[7,8,11,17]. STA test was positive in 91% of cases and coombs STA was positive 3,4% of cases in our study . In the studies performed, about 5% of the cases were diagnosed via coombs STA[7,17].

Gold standard in the diagnosis of brucellosis is isolation of the agent from blood, bone marrow or other body fluids according to the site of localization. In the studies performed, positive blood cultures have been reported in 11-68.8% of the patients[7,8,11,12,13,19]. In our study the agent was isolated in the blood of 38.3% of the patients. In two chronic cases the agent could only be isolated from the bone marrow. In such cases if clinical suspicion is present we believe that bone marrow culture should be performed. In local organ involvement, material according to the site of involvement such as CSF, BAL, synovial fluid, bone biopsy material cultures should be performed. In fact, in this study uncommon complications of brucellosis have been diagnosed by culture of these materials.

The World Health Organization advices treatment with doxycycline (2x100 mg PO) + rifampicin (1x600 mg po) 42 days, or streptomycin (1x1 g IM) 21 days + doxycycline (2x100 mg PO)[15]. In our study doxycycline + rifampicin were most frequently used, second choice of treatment, especially in osteoarticular involvement was doxycycline + streptomycin. In 31% of the patients with focal involvement, various regimes containing ciprofloxacin, ceftriaxone and trimethoprim-sulfamethoxazole were used. The rate of recurrence in other studies was 4.7-10.1% similar to our study with 6.4%[7,8,11]. Treatment regimens and recurrence rates were not investigated as the patient groups were different and the study was not designed to compare treatment regimes.

As a result, brucellosis is still endemic in the Mediterranean countries, including Turkey, and it is a systemic infection that may present with symptoms in different systems. Especially in endemic areas clinicians and family physicians should be informed of the epidemiological, clinical and laboratory features of brucellosis in order to prevent delayed diagnosing and complications. Furthermore, considering that the most important way of infection is still the use of unpasteurized milk products and animal farming, we believe that it is necessary to work on preventive measures.

References

1) Young EJ. Brucella species. *In: Principles and Practice of Infectious Diseases*. 6th ed. Mandell GL, Bennet JE, Dolin R, Eds. Churchill Livingstone, Philadelphia (PA), 2005: *2669-2674*.

2) Yuce A, Alp-Cavus S. *Türkiye'de bruselloz: genel bakış.* Klimik Derg. 2006; 19: *87-97*.

3) Doganay M, Aygen B. *Human brucellosis: An overview*. Int J Infect Dis 2003; 7: *173-182*.

4) Pappas G, Papadimitriou P, Akriditis N, Christou L, sianos EV. *The new global map of human brucellosis*. Lancet Infect Dis 2006; 6: *91-99*.

5) Çalık Ş, Gökengin AD. *Human brucellosis in Turkey: a review of the literature between 1990 and 2009*. Turk J Med Sci 2011; 41: *549-555*.

6) Kursun E, Turunc T, Dermiroglu Y, Aslan H. *Evaluation of Four Hundred and Forty Seven Brucellosis Cases*. Intern Med 2013; 52: *745-750*.

7) Buzgan T, Karahocagil KK, Irmak H, Baran AI, Karsen H, Evirgen O, Akdeniz H. *Clinical manifestations and complications in 1028 cases of brucellosis: a retrospective evaluation and review of the literature*. Int J Infect Dis 2010; 14: *469-478*.

8) Kokoglu OF, Hosoglu S, Geyik MF, Ayaz C, Akalın S, Buyukbese MA, Cetinkaya A. *Clinical and laboratory features of brucellosis in two university hospitals in southeast Turkey*. Trop Doct 2006; 36: *49–51*.

9) Demirtürk N, Demirdal T, Erben N, Demir S, Ascı Z, Kilit TP, Kartal ED. *Brucellosis: a retrospective evaluation of 99 cases and review of brucellosis treatment*. Trop Doct 2008; 38: *59-62*.

10) Gul HC, Coskun O , Turhan V, Beşirbellioglu BA, Bilgeturk A, Erdem H. *Bruselloz: 140 olgunun geriye dönük olarak irdelenmesi*. Kor Hek 2007; 6: *249-52*.

11) Demiroğlu YZ, Turunç T, Çalışkan H, Çolakoğlu , Arslan H. *Brucellosis: retrospective evaluation of the clinical, laboratory and epidemiological features in 151 cases*. Mikrobiyol Bul 2007; 41: *517-527*.

12) Colmenero JD, Reguera JM,Martos F, Sanchez De Mora D, DelgadoM, Causse M, Martin-Fartan A, Juarez Cl. *Complications associated with B. melitensis infection: a study of 530 cases*. Medicine (Baltimore) 1996; 75: 195-211.

13) Aygen B, Doganay M, Sumerkan B, Yildiz O, Kayabas U . *Clinical manifestations, complications and treatment of brucellosis: a retrospective evaluation of 480 patients*. Med Mal Infect 2002; 32: *485-493*.

14) Mousa AR, Elhag KM, Khagali M, Marafie AA. *A nature of human brucellosis in Kuwait: study of 379 cases*. Rev Infect Dis 1988; 10: *211-217*.

15) Doganay M, Mese Alp E. In: Topcu AW, Soyletir G, Doganay M, editors. *Infeksiyon hastalıkları ve mikrobiyolojisi*. 3rd ed., Istanbul: Nobel Tıp Kitabevleri; 2008; *897-909*.

16) Turkcapar N, Kurt H. Bruselloz. Uzun O, Unal S, editörler. *Güncel Bilgiler Isığında Enfeksiyon Hastalıkları Cilt II*. 1.Baskı. Ankara: Bilimsel Tıp Yayınevi; 2002; *1015-24*.

17) Akdeniz H, Irmak H, Demiroz AP. *Evaluation of brucellosis cases in Van region of Eastern Anatolia: a-3 year experience*. Nagoya Med J 1998; 42: *101-110*.

18) Ciftci C, Oztürk F, Oztekin A Karaoglan H, Saba R, Gultekin M, Mamikoglu L. *Comparison of the serological tests used for the laboratory diagnosis of brucellosis*. Mikrobiyol Bul 2005; 39: *291-299*.

19) Kurtaran B, Candevir A, Inal AS, Komur S, Akyıldız O, Saltoglu N, Aksu HSZ, Tasova Y. *Clinical appearance of brucellosis in adults: fourteen years of experience*. Turk J Med Sci 2012; 42: *497-505*.

20) Gonen I, Umul M, Sozen H, Kaya O. *Brucellar epididymo-orchitis in Southwest Anatolia, Turkey. A retrospective study of 14 patients*. Acta Medica Mediterranea 2013; 29: *509-513*.

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