Original article

Unusual presentation of Brucellosis: Afebrile, culture positive Brucellosis and culture positive, seronegative Brucellosis

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Abstract

Objective: To investigate the unusual presentation of brucellosis. **Methods**: This prospective study was carried out on 46 patients suspected to brucellosis. The diagnosis was made with isolation of brucella species by Bone Marrow culture. **Results**: Among 40 culture positive patients, there were two unusual presentations of brucellosis; Afebrile culture positive and culture positive seronegative brucellosis. **Conclusion**: Some brucellosis patients would not match with criteria for diagnosis of brucellosis. Although it is needed to have positive serology or culture for diagnosis of brucellosis but sometimes, it is the clinical experiences, which help to diagnose and treat these kinds of patients.

Keywords: Afebrile; Brucellosis; Culture positive; Seronegative

INTRODUCTION

Brucellosis is a systemic infectious disease that its clinical features are diverse. In Iran the rate of annual report of brucellosis reach to more than 17 000 cases[1]. Several infectious and non-infectious diseases are considered as differential diagnosis. In practice we confront with some seropositive persons for example ranchers [2], farm and veterinary service long standing workers[3], old brucellosis infection[4] who have been still appearing with clinical symptoms resembling brucellosis.

Differentiation and diagnosis of brucellosis can be made by both culture and serological tests. The efficacy of blood cultures decreased significantly with chronic and sub-acute forms of infection, whereas that of bone marrow culture (BMC) decreased only in chronic forms[5]. Because bacterial isolation techniques are time consuming, the conventional serological methods, the tube and slide agglutination tests (SAT) [6] and an enzyme linked immunosorbent assay (ELISA) [3] are the most widely used methods by which the diagnosis is made by a demonstration of high antibodies titer in the presence of compatible clinical symptoms[7].

Based on historical, epidemiological, clinical, and serologic or bacteriologic data, four groups or types of patients are identified: Group Ⅰ who have clinical symptoms and positive agglutination reactions (active brucellosis), Group Ⅱ who have merely positive agglutination reactions (by history of prior infection, inactive brucellosis is likely) and Group Ⅲ who have positive agglutination reactions, but in-

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sufficient data are available for further classification[8], and chronic patients [4] which classified as group Ⅳ.

One of the main reasons for this perplexing issue of various groups is SAT titers could remain high for a prolonged period of time [9], with no specific clinical correlations[10]. On the other side, clinical symptoms are also misleading. There is no fever or other laboratory abnormalities in some clinically active brucellosis (for example leucocytosis, relative lymphocytosis, erythrocyte sedimentation rate (ESR) more than 25 mm/ 1st hour)[11].

Based on these various features of clinical and laboratory challenge of brucellosis, group Ⅱ (inactive Brucellosis), Ⅲ (incomplete data) and Ⅳ (chronic form) are the innocent groups who may be treated for several times in endemic area. In this area brucellosis diagnosis is a practical challenge for General Practitioners, so that over diagnosis or for instances under diagnosis may be a problem.

The main aim of this study is to present some unusual presentation of brucellosis which were confirmed by culture and must not be missed or neglected. We hope this case series could alert healthy responsible for challenge of diagnosis of brucellosis in endemic area.

MATERIALS AND METHODS

The study group comprised 46 patients suspected to brucellosis. These patients were referred to infectious ward of Shahr-e-kord University of Medical Sciences, from January 2003 till December 2006. Based on some clinical (Fever, myalgia, arthelagia, sweating), epidemiological (animal contact) and/or laboratory evidence, [anemia, SAT 1: 160 (Brucella abortus plain antigen; Iranian serum Razi Institute, Karaj, Tehran, Iran), positive coomb's wright test 1: 40 (only for SAT negative cases), 2-mercaptoethanol (2ME) 1/80, positive CRP, elevated ESR more than 30 mm, relative lymphocytosis], a case of brucellosis was identified. BMC was considered as gold standard test for diagnosis of brucellosis. After getting consent for bone marrow aspiration and shave the sternum, we aspirated bone marrow. After withdrawing syringe contained 5 - 10 mL of bone marrow aspirate, the sample was left in refrigerator for a while to be freezed, then was left in room temperature for thawing, damaging the cells, and to release brucella microbe from cells. Then, the thawed sample was inoculated aseptically into broth phase of Castaneda's biphasic medium consisting of brain heart infusion agar and broth (High media, Mumbai, India). The media were incubated at 35℃ for 7 days (in some of them for 14 and 21 days) and the bottles were observed daily to see appearance of colony. Because there is not needed to see turbidity in bottle, so we cultured all of them on chocolate agar and sheep blood agar plates in duplicate on 7th day, (in some on 14th or 21st days). It must be noted that each sample has been inoculated into 2 plates, one of them has been left in the candle jar (with a 5 % - 10 % CO_2 atmosphere), and the other one has been left into the incubator up to 72 hours, but negatives ones have been kept for maximum of 21 days.

The plates were observed daily. We were waiting to see visible brucella colony. Brucella colonies are shiny yellow soft ones that resemble fine grains of sand. Brucellae are small, gram-negative, oxidase- and urease-positive coccobacilli.

Brucella isolates were identified with the help of gram staining, urease testing, and monitoring of H_2S production (4 days) and sensitivity to dye; thionin (1: 25 000; 1: 50 000; and 1: 100 000), motility test, oxidase and nitrate tests. Catalase tests, which can have positive results for brucella, have not been performed because the technique can cause the nebulization of particles. Species identification is performed on the basis of these particular characteristics. All steps have been done with standard precaution of biosafety class 2 under microbiological hood.

RESULTS

Of 46 blood specimens drawn, 40 showed growth of *B. melitensis* by the Castaneda technique. The results of culture were positive mainly on 7th day, but there were some ones that were positive on14th - 21st day.

This is a case series study which included 46 patients suspected to brucellosis. Serologic diagnosis of brucellosis is easy when brucellosis is suspected, but

in this study the diagnosis was established in 40 patients by isolating brucella melitensis (Table 1).

Among 46 patients, 6 have been excluded by negative BMC (Group A, Table 1). They are patients who had positive serology, fever, resident of endemic area and based on epidemiologic, clinical, laboratory data, the diagnosis of brucellosis was probable or suspected, but final diagnosis was ruled out by negative BMC.

Group B (Table 1) included 5 patients who were presented by positive serology of SAT and 2ME, mild to moderate increased ESR and positive C-reative protein (CRP), but no fever. They could be accounted for people having positive seroloprevalence of brucellosis (group Ⅱ or Ⅲ) and no need to treatment, but finally BMC approved the diagnosis and they were classified as group Ⅰ (Table 2).

There were 10 patients in groups C (Table 1) who had previous history of brucellosis, and constitutional symptoms but no positive serologic results (SAT and coomb´s wright). They may be ruled out for Brucellosis, but they have been approved by BMC as active Brucellosis (Table 3). And finally the last group, group D which includes 25 patients who have acute brucellosis and are not the subject of this article (Table 1).

Table 1 The results of bone marrow aspiration, clinical (fever) and serology of patients.

Classification	Number	Serology	Fever	Bone marrow culture *
Group A Mimicking but no Brucellosis	6	positive	positive	negative
Group B Afebrile culture positive Brucellosisp	5	positive	negative	positive
Group C Seronegative, culture positive Brucellosis	10	negative	positive	positive
Group D Classic Brucellosis	25	positive	positive	positive

* Isolating brucella melitensis

Table 2 Group B, afebrile, culture positive brucellosis.

Gender	Age	Constitutional symptoms *	CRP, ESR	SAT	2ME	CBC, U/A	Previous history
Female	19	No constitutional symptoms Sever arthritis of hip	CRP positive ESR <30mm	SAT =1/160	2ME =1/80	N/L = CBC N/L U/A	No
Female	40	Constitutional symptoms No localization	CRP + ESR =49mm	SAT =1/320	2ME =1/160	N/L	No
Female	30	Constitutional symptoms No localization	CRP + ESR =5mm	SAT =1/80	2ME =1/40	Anemia Speelenomegaly Leucopenia	No
Female	45	Constitutional symptoms No localization	CRP + ESR =31mm	SAT =1/320	2ME =1/160	Leucopenia	No
Male	20	Constitutional symptoms No localization	CRP + ESR =16mm	SAT =1/320	2ME =1/160	Leukocytosis	Yes

* = Constitutional symptoms: chili sensation, LBP, weight loss (Less than 5 kg), loss of appetite.

Table 3 Group C, seronegative, culture positive Brucellosis.

Gender	Age	Constitutional symptoms *	Previous history	Animal contacts	CRP, ESR	CBC, UA	Drugs	Date of defeverecence after treatment
F	35	Positive/sever tenosynovitis	Positive	Positive	CRP + ESR = 39mm	Leukocytosis	SM + Tetracycline	7
M	21	Positive/epididimo -orchitis	Negative	Positive	CRP + ESR = 23mm	Leucopenia	RMP + SM/TMP	2
M	39	Positive	Negative	Positive	CRP + ESR = 5mm	N/L CBC	SM + Tetracycline	4
M	20	Positive	Negative	Positive	CRP + ESR = 20mm	N/L CBC	SM + Tetracycline	3
M	11	Positive	Negative	Positive	CRP + ESR = 42mm	Leukocytosis	RMP + SM/TMP	4
M	56	Positive	Negative	Positive	CRP + ESR = 21mm	Leukocytosis	SM + Tetracycline	7
M	35	Positive	Positive	Positive	CRP + ESR = 35mm	Leucopenia	RMP + Doxicycline	3
M	22	Positive	Negative	Positive	CRP + ESR = 14mm	N/L CBC	SM + Tetracycline	5
F	29	Positive	Positive	negative	CRP + ESR = 4mm	N/L CBC	SM + Tetracycline	6
F	55	Positive	Negative	negative	CRP + ESR = 16mm	N/L CBC	SM + Tetracycline	3

* = Constitutional Symptoms: chili sensation, LBP, weight loss (Less than 5 kg), loss of appetite.

DISCUSSION

Among 46 patients of this study, 6 have been excluded by negative BMC for brucellosis. Five patients of group B were presented by positive laboratory data, but no fever, in which the diagnosis of inactive brucellosis or seroloprevalence positive of brucellosis in endemic area, was probable [8]. But BMC approved the diagnosis of active brucellosis. The group C, included 10 patients who had previous history of brucellosis, and constitutional symptoms but no positive serologic results, which hardly consider the diagnosis of brucellosis, but they were approved by BMC as active brucellosis.

In endemic area there is always a challenge for diagnosis of brucellosis. This will be more complicated when there is not a unique guideline for cut off for titer of STA or 2 ME in issue of diagnosis[6,8,12,13].

The first and screening test for diagnosis of brucellosis is Rose Bengal plate test for which the high specificity and diagnostic sensitivity was reported[4,14]. Both Rose-Bengal and SAT (≥1/160) are usually positive in patients with brucellosis and currently used in the diagnosis of brucellosis[15]. Its value is well established in the acute forms of the disease[16]; for the diagnosis of subacute or chronic forms, however, a Coombs test must be performed to show incomplete antibodies. The agglutination carried out with serum treated with 2ME allows the determination of the type of immunoglobulins present[16].

A presumptive diagnosis of brucellosis can be made by demonstrating high or rising titers of antibodies to Brucella antigens but isolation of the organism from blood, bone marrow, or tissue is the only irrefutable proof of the disease. The techniques introduced by Ruiz-Castaneda improved the chance of culturing Brucella spp[17].

The percentage of cases with positive cultures ranges from 15 to 70 percent[18]. Based on the important role of culture for diagnosis of brucellosis, in this study, we used BMC as a golden test for diagnosis to discuss around the challenge of diagnosis, which its positive rate is reported to be 87 % and higher than previous report[18].

Group B-Afebrile culture positive Brucellosis

In endemic area, asymptomatic seropositivity is not unusual[4]. One study indicated that in endemic area, 53 % of seropositive persons have no any previous history of brucellosis and 47 % of them were asymptomatic patients with past history of brucellosis. The titer of SAT and coombs wright varies (1/80 up to 1/640) and (1/160 up to 1/640) respective-

ly[4]. In other words, fever, which is one of the main word of (Malta fever) and clinical feature of brucellosis, in some patients, may not be as a clinical feature[11]. These two issues can complicate diagnosis of brucellosis. These 5 patients of group B are also afebrile but serologic positive patients which could easily be classified as asymptomatic brucellosis[4,8,11].

In these cases, 2ME could be a good diagnostic test. One seroprevalnce study indicated positive titer of SAT and coombs tests but negative 2ME could be due to previous and inactive infection of at least more than one year ago[4], also another study indicated that the 2ME and CRP tests were useful in checking the brucellosis activity[19]. Based on finding of previous studies (without considering 2ME test), these patients can be classified as asymptomatic seropositive brucellosis[4,8,11], but based on finding about 2ME [3,4,20] (positive 2ME, CRP[19], and elevated ESR), they are classified as acute brucellosis, the diagnosis which was confirmed by BMC too. The lack of fever in this group may be related to the character of brucellosis which is named as undulant fever[11].

Group C-Seronegative culture positive Brucellosis

This group included 10 patients of seronegative brucellosis (Table 3). In some cases of brucellosis, seroconversion does not occur[1]. This seronegativity can be attributed to the performance of tests early in the course of infection, the presence of blocking antibodies, or the so-called "prozone" phenomenon (i.e., the inhibition of agglutination at low dilutions due to an excess of antibodies or to nonspecific serum factors)[8].

One study showed 35 % false negative reactions for SAT which is higher than 25 % rate of our study[21]. Another study indicated, 11 cases out of 30 cases (36.7 %) gave negative results by the slide-agglutination screening test used at the recommended single serum dilution of 1∶ 80 (prozone phenomenon) [22].

But the comparison of conventional tests of SAT or 2ME with others, like ELISA or enzyme immune assay (EIA) could give better concepts of seronegative brucellosis. Incomplete IgG and IgA are formed in chronic brucellosis and these antibodies do not detect by classical SAT. However these antibodies could be measured with indirect Coombs and EIA techniques. Thirty-seven (84.08 %) of the 44 patients whose SAT negative and indirect Coombs positive were found positive for EIA IgG. Sixteen (34.70 %) of the 46 patients whose SAT and indirect Coombs negative were found positive EIA IgG[23]. On the other hand, titers measured by the 2ME test were low or negative in 10 patients who had positive blood cultures[24]. By similar finding, our 10 patients had negative titer for SAT, 2ME and Coombs test, but were confirmed by BMC.

Assays for IgG and IgM in 30 culture-positive cases gave significant ELISA values. By SAT test, 10% of these cases gave readings less than 1∶ 160. It may be due to that in few patients with acute disease, only IgM was detected. Beside, the ELISA test, in addition to measuring antibody classes directly, also detects incomplete antibodies. By this, ELISA can efficiently replace 2ME and the Coomb's antihuman-globulin test[25]. ELISA overcomes some of the shortcomings of SAT test. A comparison between ELISA and SAT tests yields higher sensitivity and specificity of ELISA test[7]. So this test could be another resolution to overcome seronegativity issue.

Without BMC recommendation for wok up of Brucellosis whose serology remain negative, many of them are categorized as FUO. BMC are recommended for patients of fever of unknown origin (FUO) for whom the routine workup turns out to be negative[19]. The efficacy of cultures of blood decreased significantly with chronic and subacute forms of infection, whereas that of BMC decreased only in chronic forms. Prior use of antibiotics reduced the positivity of cultures of blood but did not affect BMC[26]. On the base of results of this study BMC strongly is recommended for patients with FUO, negative serology, and unexplained articular or hematologic involvement, and patients in whom brucellosis is suspected.

Our study revealed identification of brucella melitensis by BMC in 40 persons out of 46 patients suspected to brucellosis (87 %). 10 patients (25%) out of these 40 showed serology negative tests, but positive BMC. 25 cases (62.5 %) had matched positive BMC and serologic results. And 5 out of these 40 patients (12.5 %) had positive serology tests but not fever. In one word, in our study, 75 % of culture positive proven brucellosis patients

were diagnosed by serology alone. The similar findings could be found in other studies. Serological diagnosis was established in up to 86 % of the patients[5,26,27].

In endemic area diversity of clinical or serologic presentation of brucellosis is common and by results of this study it could conclude that it must be aware of over or under diagnosis of brucellsis. Other word neither any seropositive patient is affected to brucellosis nor any seronegative patients is brucellosis free. Just must be aware and alert to not miss any diagnosis.

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