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ORIGINAL ARTICLE

The false sero-negativity of brucella standard agglutination test: Prozone phenomenon

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ABSTRACT

Objectives: We aimed to assess prozone phenomenon that is quite rare and causes false negativity in serological diagnosis of brucellosis with standard dilution titers.

Materials and methods: In this study the tests of four cases that have false negative serological results were evaluated. Blood cultures were obtained from all cases while cerebrospinal fluid cultures were studied in the two cases. Standard agglutination test (SAT) and Coombs test were performed to all patients.

Results: SAT and Coombs test was negative in titers up to 1/640 in all cases. The SAT and Coombs tests in cerebrospinal fluid (CSF) of the two cases with neurobrucellosis diagnosis were negative, as well. Since the clinical and laboratory findings suggested the brucellosis, the serums were restudied by diluting up to 1/10240 titer and we saw that the first 3 cases became positive at a titer of 1/1280. The fourth case remained negative and therefore, we applied high dilution Coombs test. This time the test gave a positive result at 1/10240 titer beginning from 1/2560 titer. *B.melitensis* was isolated from two cases.

Conclusion: SAT and Coombs' test must be diluted to titers 1/2560 or more in order to exclude false sero-negativity in cases with clinical and laboratory findings suggesting brucellosis. *J Microbiol Infect Dis 2011; 1(3):110-113*

Key words: Brucellosis; prozone phenomenon; standard agglutination test; Coombs test

Yanlış negatiflik veren brusella standart aglütinasyon testi: Prozon fenomeni

ÖZET

Amaç: Bu çalışmada, brusella standart serolojik dilüsyon titrelerinde yanlış negatif sonuç veren ve oldukça nadir görülen prozon fenomenini araştırmayı amaçladık.

Gereç ve yöntem: Burada serolojik olarak yalancı negatif olan dört hastanın test sonuçları değerlendirildi. Olguların tümünden kan kültürü ve iki olgudan da beyin omurilik sıvısı (BOS) kültürü alındı. Bütün hastalara standart aglütinasyon test (SAT) ve Coombs testi çalışıldı.

Bulgular: Bütün olgularda SAT ve Coombs testi 1/640 titreye kadar negatif idi. Keza nörobruselloz tanısı alan iki olgunun BOS SAT ve Coombs testi negatif idi. Hastaların klinik ve laboratuar bulguları brusellozu düşündürdüğünden serumlar 1/10240 titreye kadar dilüe edilerek yeniden çalışıldı. Üç olgu 1/1280 titreden itibaren pozitifleşmeye başlarken dördüncü olgu yine negatif kaldı. Bu olgunun serumunu da 1/10240 titreye kadar dilüe ederek Coombs testi çalıştık. Bu kez 1/2560 titreden başlayarak 1/10240 titreye kadar pozitif sonuçlandı. İki olgunun kültüründen *B.melitensis* izole edildi.

Sonuç: Klinik ve laboratuar bulguları brusellozu düşündüren olgularda yanlış negatifliği ekarte etmek için SAT ve Coombs testi 1/2560 ve üzeri titrelere kadar dilüe edilerek çalışılmalıdır.

Anahtar kelimeler: Bruselloz, prozon fenomeni, standard aglütinasyon test, Coombs testi

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INTRODUCTION

Brucellosis is seen widely throughout the world and it is hyperendemic in the Mediterranean Basin and Arabian Peninsula, India, Mexico, and Central and South America.[1] In Turkey, brucellosis is common, especially in the Middle, East and Southeast Anatolia regions.[3,4] It is a multisystem disease that may present with a broad spectrum of clinical manifestations and complications.[5] Today there are still problems about the pathogenesis, treatment and diagnostic tools of brucellosis.[6] The most frequently used diagnostic tool is the standard agglutination test. However, this test may sometimes cause false negativity.

In tubes in which agglutination has occurred before dilution because of the optimal antibody-antigen ratio, agglutination may not be observed because of the relative excess of antibodies against antigens. Therefore, the test becomes positive while it is negative in the first tubes.[6] Prozone phenomenon is false standard agglutination test negativity seen due to high antibody presence in the serum. In this study, we aimed to assess prozone phenomenon.

MATERIALS AND METHODS

This study was performed in Harran University Hospital. In this study, the test results of four cases were evaluated. Three of the patients were women and one was man; their ages ranged from 17 to 44 years old. Blood cultures were studied for all patients while cerebrospinal fluid (CSF) cultures were studied only for two cases. Rose Bengal (RB) test, Standard agglutination test (SAT) and Coombs test were applied to all patients. SAT was performed using a commercial kit (Cromatest, Knickerbocker Laboratories, Barcelona, Spain). Coombs test using antihuman gammaglobulin sera (Ortho diagnostic systems, Madrid, Spain). Brucella culture was performed using BACTEC 9050 blood culture system (Becton, Dickinson and Company, USA). Gram, India ink and Ziehl-Neelsen stains were routinely carried out on the CSF of two cases. In addition, blood and CSF were cultured for conventional bacteria, tuberculosis and fungi for two cases. CSF was also analyzed for cells, glucose, and protein content. Tests for typhoid fever, toxoplasmosis, infectious mononucleosis, herpes virus antibodies, blood smears for malaria and tuberculin test were performed in all cases. A chest radiograph was taken in every case for tuberculosis. Cranial MR scanning was undertaken in two neurobrucellosis cases.

RESULTS

The symptoms and findings of the cases were listed in Table 1. The symptoms of the first and fourth cases suspected meningitis, therefore CSF analysis were done and neurobrucellosis (NB) diagnosis was made. The CSF analysis of the first case; increased pressure, leukocyte count of 430/mm^3 (>70% mononuclear), protein 515 mg/dL and glucose 44 mg/dL (serum glucose: 104 mg/dL). The CSF analysis of the fourth case; increased pressure, leukocyte count of 300/mm^3 (>60% mononuclear), protein 52 mg/dL and glucose 55 mg/dL (serum glucose: 136 mg/dL). RB, SAT and Coombs tests were negative in all cases up to 1/640 titer. The CSF RB, SAT and Coombs test of NB cases were also negative. Since the clinical and laboratory findings suggested brucellosis, the serums were restudied by diluting up to a titer of 1/10240. The first three cases began to be positive at a titer of 1/1280. However, the fourth case was still negative and therefore high dilution Coombs test was studied at that case. This time, the test positively resulted at the titer of 1/10240 starting from a titer of 1/2560. B. mellitensis was isolated from CSF of the first and fourth cases on 7th and 6th days, respectively. The remaining tests of the cases were normal. The culture and serological test results were listed in table 2. The patients were treated with specific brucellosis treatment without sequel. The clinical and laboratory findings were normal in follow-up controls.

Table 1. Clinical and laboratory findings

Case Age/sex	Symptoms and findings	Hb g/dL	Platelet /mm³	WBC /mm³	CRP mg/dL	ESR mm/h
Case-1 17/F	Fever, diplopia, headache, emesis, starting 6 weeks before	12.8	170000	5470	2.7	40
Case-2 36/F	Fever, sweating, fatigue, diffuse joint pains, weight loss starting 7 weeks before	10.2	161000	3200	8.5	30
Case-3 42/M	Arthralgia for 4 weeks, fever, fatigue, diffuse maculopapullar lesions added on the last week.	13.2	227.000	11600	15.5	70
Case-4 44/F	Rare headache for 5 months, fever, chilling and shivering and swelling of knee and wrist joints at the last one month.	13	350000	17500	27.6	94

Hb: hemoglobin, WBC: white blood cell, ESR: erythrocyte sedimentation rate

CRP: C-reactive protein (normal value: 0.1-0.5 mg/dL

Table 2. The culture and serological test results of the cases

Case	Rose Bengal	SAT positive titer	Coombs' test positive titer	SAT result	Coombs' result	Culture result
1	Negative	1/1280	1/1280	1/2560	1/2560	*B.melitensis*
2	Negative	1/1280	1/1280	1/5120	1/5120	Negative
3	Negative	1/1280	1/1280	1/2560	1/2560	Negative
4	Negative	Negative	1/2560	Negative	10240	*B.melitensis*

SAT: standard agglutination test

DISCUSSION

Fever, extreme sweating, headache, fatigue, weight loss, waist pain and diffuse body pain are the most frequently seen symptoms of brucellosis.[1] Headache, fever and neurological symptoms are commonly seen in NB, as well.[2] Similarly, fever, sweating, fatigue, diffuse joint pains and weight loss were present in our cases. The NB cases had fever, headache and diplopia. Although culture positivity is the most significant diagnostic method in brucellosis, the frequent usage of antibiotics before diagnosis, late growth of the pathogen in culture and chronic cases cause problems in culture results.[3-6] Cultures were positive in two of our cases; one of them had systemic brucellosis and the other had NB.

Standard agglutination test and RB test are the most frequently used routine tests for brucellosis diagnosis.[6,8] RB test is easy, rapid and has low false positivity rates and so commonly used especially for acute brucellosis diagnosis.[7,8] However, this test lost its importance in case of low pH since IgM and IgG lost their agglutination capacities, chronic patients with low antibody levels and patients with occupational exposure. In patients with a history of chronic illness, RB test is unreliable, therefore tests such as standard tube agglutination tests (Wright agglutination, SAT) and coombs' test should be done.[9,10]

Standard agglutination test, a reliable test, has been commonly used for diagnosis. SAT may give false negative results due to blocking antibodies in serums with high IgG antibodies and due to non-agglutinated IgG in chronic cases.[10,11] In the case of presence of immunoglobulin defined as blocking antibodies, the antigen- antibody combining occurs but the agglutination does not occur. Since the antibodies in this case are in IgG form, Coombs test is applied. The Coombs reactive added in the medium provides establishment of real seropositivity by making bridges between antibodies.[12-14]

Prozone phenomenon is called as false SAT negativity seen due to high antibody presence in the serum. In tubes in which agglutination has occurred before dilution because of the optimal antibody-antigen ratio, agglutination may not be observed because of the relative excess of antibodies against antigens. Therefore, the test becomes positive while it is negative in the first tubes.[6,9,11]

Rose Bengal, SAT and Coombs test were negative up to 1/640 titer in our first three cases. When the serums were studied with higher dilutions, SAT began to become positive beginning from a titer of 1/1280 and resulted positively at titers of 1/2560, 1/5120 and 1/2560, respectively. However, in our fourth case, SAT was still negative although it had been diluted up to a titer of 1/10240. It was negative when studied with Coombs test titrated to 1/1280, as well. This time coombs test was restudied by diluting up to a titer of 1/10240, it began to become positive at a titer of 1/2560 and resulted positively at 1/10240 titer. While prozone phenomenon was observed in all of our cases, it was understood that both prozone phenomenon and blocking antibodies had played role in the fourth case. We searched the literature in English, and see that Prozone Phenomenon is a rarely reported condition. Those studies were generally case reports.[15-17]

According to our knowledge, prozone phenomenon and blocking antibody presence have not been reported to be in the same case, yet. Our 4th case is the first such case. Hendricks et al. reported 6 cases with prozone phenomenon. But they also reported that the Coombs test were positive in those serums.[14] In fact it is understood that the false negativity was not due to prozone phenomenon but due to blocking antibodies. Because of both SAT and Coombs tests results were negative up to 1/640 titer in our cases. The beginning and the last positive titers of the serums were same in both SAT and Coombs test. Therefore, in contrary to Hendricks et al, we do not think that false negativity due to prozone phenomenon can be overcome by Coombs test. Because, antibody excessiveness is present in prozone phenomenon and so high dilution test should be studied in order to establish the real positivity.

In the laboratories, brucellosis is misdiagnosed since SAT is studied at titers of 1/320-1/640 to save time and antibody. Delay or oversight of the diagnosis would cause high sequel rates. Although the clinic and CSF findings of NB are similar to other meningitides such as tuberculosis meningitides, its treatment is completely different. The culture positivity is lower than 50% in NB, so serological test have been used for diagnosis of it.13 The false negativity in tests, cases may result with mortality when it causes oversight of the diagnosis. In order to prevent sequels and mortality, prozone phenomenon must be considered in seronegative cases.

In conclusion, in cases with clinical and laboratory findings suggesting brucellosis, SAT and Coombs' test must be diluted to titers 1/2560 or more in order to exclude false sero-negativity. This could be useful approach in endemic area when patients have diagnostic difficulties.

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