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MUSTAFA ERTEK

HALİL YAZGI

ZÜLAL ÖZKURT

AHMET AYYILDIZ

MEHMET PARLAK

TÜBİTAK

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E-mail: medsci@tubitak.gov.tr

Comparison of the Diagnostic Value of the Standard Tube Agglutination Test and the ELISA IgG and IgM in Patients with Brucellosis

Mustafa ERTEK[1]
Halil YAZGI[2]
Zülal ÖZKURT[1]
Ahmet AYYILDIZ[2]
Mehmet PARLAK[1]

[1] Department of Clinical Bacteriology and Infectious Diseases, Atatürk University, Medical School, Erzurum - TURKEY

[2] Department of Microbiology and Clinical Microbiology, Atatürk University, Medical School, Erzurum - TURKEY

Background and Aims: Brucellosis is endemic in Turkey. Since it affects many organs and the symptoms are non-specific, the diagnosis by clinical findings is difficult and may be easily missed. Many serological tests have been used for the diagnosis of human brucellosis. This study compared the diagnostic value of the Brucella standard tube agglutination test (SAT) with that of ELISA (Brucella specific IgG and IgM) tests in patients with Brucella bacteremia.

Patients and Methods: Thirty-two patients with brucellosis who had positive blood and/or bone-marrow cultures for Brucella species, and 20 healthy individuals as controls were included in the study.

Results: At the end of the study SAT was positive in 30 of the 32 patients, ELISA IgG was positive in 26 and ELISA IgM was positive in 32. Of the 20 control sera, all were negative in SAT, 1 was positive in ELISA IgG, and 3 were positive in ELISA IgM. The positive predictive value of SAT was 100.0% and the negative value was 90.9%. The positive and negative predictive values for ELISA IgG were 96.3% and 76.0%, and for ELISA IgM were 90.9% and 89.5%, respectively.

Conclusions: SAT may be preferred to ELISA in acute brucellosis because it is cheap and easily applicable.

Key Words: Brucellosis, ELISA, Standard tube agglutination test

Brusellozis Hastalarda ELISA IgG-IgM ve Standart Tüp Agglütinasyon Testlerinin Tanısal Değerlerinin Karşılaştırılması

Giriş ve Amaç: Brusellozis Türkiye'de endemik bir hastalıktır. Birçok organı etkilediği için semptomlar nonspesifiktir. Klinik bulgulara göre tanı koymak oldukça zordur ve teşhis kolayca atlanabilir. İnsan brusellozis olgularının teşhisinde birçok serolojik test kullanılmaktadır. Brucella bakteriyemisi saptanan hastalarda Brucella standart tüp aglütinasyon testi (SAT) ile ELISA'nın (Brucella IgG ve IgM) tanı değerini karşılaştırmayı amaçladık.

Hastalar ve Yöntem: Kemik iliği ve/veya kan kültüründe Brucella spp üremesi olan 32 hasta ve kontrol grubu olarak 20 sağlıklı gönüllü çalışma kapsamına alındı.

Bulgular: Çalışma sonunda 32 hastanın 30'unda SAT, 26'sında ELISA IgG ve 32'sinde IgM pozitif bulundu. SAT için pozitif prediktif değer %100, negatif prediktif değer %90.9 olarak saptandı. ELISA IgG ve IgM için pozitif ve negatif prediktif değerler sırasıyla %96.3 ve %76; %90.9 ve %86.5 olarak saptandı.

Sonuç: Daha ucuz ve kolay uygulanabilir olması nedeniyle akut brusellozis de "SAT" ELISA'ya tercih edilebilir.

Anahtar Sözcükler: Brusellozis, ELISA, Stadard Tüp Aglütinasyon Testi

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Correspondence
Mustafa ERTEK
Department of Clinical Bacteriology and Infectious Diseases,
Atatürk University,
Medical School,
TR-25240 Erzurum - Turkey

ertekmus@atauni.edu.tr

Introduction

Brucellosis is a zoonosis caused by bacteria of the genus Brucella, which affect both humans and animals such as the cow, sheep, goat, camel and pig. Bacteria enter hosts through the digestive tract via contaminated dairy products and animal feed, the respiratory tract via aerosols, or the skin via contact with infected animals on farms or in slaughterhouses. Since the symptoms of brucellosis are non-specific, the clinical diagnosis of the disease is difficult. Therefore, the diagnosis must be supported and confirmed by the isolation of the agent, mostly from blood culture or by the detection of antibodies against bacterial antigens (1,2).

The gold standard in the diagnosis of brucellosis is the isolation of Brucella bacteria. The isolation rate of the bacteria from blood cultures ranges from 47.1% to 94.1%,

depending on the methods used and the period of incubation (3,4). In the absence of bacteriologic confirmation, a presumptive diagnosis can be made on the basis of high or rising titers of specific antibodies. A variety of serologic tests have been applied to brucellosis, of which the standard agglutination test (SAT) is the most widely used. More recently, the Brucella enzyme-linked immunosorbent assay (ELISA) test was introduced into clinical laboratories. The purpose of this study was to compare the diagnostic value of SAT with that of Brucella ELISA tests in patients with Brucella bacteremia.

Materials and Methods

The subjects of this study were 32 patients with brucellosis who had positive blood and/or bone-marrow cultures for Brucella species, and 20 healthy individuals as controls. Both patients and controls were from the same epidemiological area.

Patients were selected according to their clinical symptoms and laboratory findings. Two blood samples (10 ml each) and one bone marrow sample (sternal aspirate, 1 ml) from patients were obtained aseptically for culture. They were inoculated into BACTEC bottles separately and incubated in the BACTEC 9240 system (Becton-Dickinson, Maryland, USA) for 21 days. Bottles giving a positive growth index during the incubation period were Gram stained and then subcultured to both chocolate agar and blood agar plates and incubated at 37 °C in a 5%-10% CO_2 atmosphere. When this was not the case, Gram staining and a blind subculture were performed after 21 days. The isolates of Gram-negative cocco-bacilli were identified by conventional methods (e.g., motility; oxidase, catalase and urease activity; glucose fermentation; and production of H_2S). Brucella spp. suspected isolates were confirmed by slide agglutination using type-specific antisera (Murex Diagnostics Dartford, United Kingdom).

Fifty-two sera samples from both groups were tested for Brucella specific IgG and IgM antibodies by ELISA using a commercial kit (Novum, Germany). The test was performed and evaluated according to the kit procedure. The same samples were also tested by SAT using *B. abortus* antigen (Pendik Veterinary Institute, İstanbul, Turkey). Sample dilutions started from 1:20 for SAT. Samples with an antibody titer of 1:160 or greater were considered positive.

Results

In the patients, 30 of 32 samples gave positive results with SAT (titer >1:160). In the same group, Brucella IgG and IgM antibodies with ELISA were positive in 26 and 32 patients, respectively. In 24 of the patients both IgG and IgM antibodies were detected. From 20 control sera, all were negative (titer<1/80) in SAT, 1 was positive in ELISA IgG and 3 were positive in ELISA IgM. The positive predictive value of SAT was 100.0% and the negative value was 90.9%. The positive and negative predictive values for ELISA IgG were 96.3% and 76.0%, and for ELISA IgM were 90.9% and 89.5%, respectively. The sensitivity and specificity rates of SAT, ELISA IgG and ELISA IgM tests were 93.7% and 100.0%, 81.3% and 95.0%, and 93.8% and 85.0%, respectively (Table).

Discussion

In the absence of a positive culture, the diagnosis of brucellosis rests on the demonstration of specific antibodies. A variety of serologic tests have been applied to brucellosis, of which SAT is the most widely used (5). In the SAT test a single serum titer of 1/160 or greater is considered significant (6). However, early in infection lower titers may be present; therefore, it is important to obtain both acute and convalescent-phase sera. Sometimes agglutination can be masked at low dilutions of serum (zone of antibody excess or prozone), especially when the serum contains a high titer of antibodies. Prozone was seen in 6% of positive sera, occurring most often at the lowest dilution (1:20 and only rarely at a dilution of 1:80 or greater). The prozone phenomenon is of little practical importance as long as serum samples are routinely diluted beyond 1:320. The other false negative situations are being blocking antibodies—that some patients have a factor in the serum that blocked the agglutination reaction. On the other hand, false-positive reactions can also be seen in the SAT test and they occasionally result from cross-reactions with antibodies to Salmonella spp., Yersinia spp., *Vibrio cholera, Francisella tularemia* or *Escherichia coli* O:157. False positive and false negative reactions can be avoided by routinely diluting the serum beyond 1/320 (1,6,7). In the present study the prezone phenomenon was not detected in any of the sera tested.

If the diagnosis of brucellosis cannot be achieved by SAT because of the low titer of antibodies and the

Table. Results of SAT and ELISA IgG / IgM tests in patients with Brucellosis and in controls.

	ELISA							
	SAT		IgG		IgM		IgG and IgM	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Patients (n = 32)	30	2	26	6	32	0	24	8
Control (n = 20)	0	20	1	19	3	17	1	17
Sensitivity	93.7		81.3		93.8		75.0	
Specificity	100.0		95.0		85.0		94.4	
PPV	100.0		96.3		90.9		96.0	
NPV	90.9		76.0		89.5		68.0	

PPV = Positive predictive value; NPV = Negative predictive value

presence of blocking antibodies, Brucella IgG-specific and IgM-specific ELISA test systems have been shown to be an acceptable alternative to SAT for the diagnosis of subacute and chronic brucellosis. The detection of specific immunoglobulins by a single, simple and rapid test is a major advantage of ELISA (8). However, occasionally false positive results may occur in ELISA because of nonspecific binding of bovine IgM by smooth LPS from *B. abortus* when the latter is used as the solid-phase antigen.

Kostoula et al. reported that ELISA appears to be more sensitive than the tube agglutination test for the diagnosis of human brucellosis, because this method detects specific IgG, IgM and IgA antibodies (9). Gazapo et al. stated that ELISA IgG and IgM positivity are helpful for epidemiological evaluations, whereas some false positive results can be obtained in classical tube agglutination tests due to the cross reactivity between Brucella spp. and Salmonella spp., *Vibrio cholera* and Yersinia bacteria (8). As is well known, incomplete antibodies are commonly seen in subacute and chronic brucellosis, and so ELISA is recommended by some authors as a susceptible test for the diagnosis of such cases and it was asserted that ELISA could detect incomplete antibodies (10,11).

In the present study, in which the diagnostic values of SAT and ELISA IgM and ELISA IgG tests were compared in Brucella bacteremia cases, 30 of the 32 patients' sera gave positive results in the SAT test (93.8%). ELISA IgM and ELISA IgG tests were positive in 32 (100%) and in 26 (81.3%) of the patients, respectively. Both ELISA IgG and IgM positivity was 75% (in 24 of 32). These results indicated that ELISA IgM and ELISA IgG tests had low specificity (85%, 95%) compared to SAT (100%). Early in infection, antibodies of the IgM class predominate, followed shortly by a switch to IgG antibodies. In the patients, 24 (75%) sera gave positive results with both ELISA IgG and IgM tests. The reason why the positivity of ELISA IgM is higher than that of ELISA IgG is that the cases were in acute phases. In many studies performed with ELISA, it was determined that IgG positivity and the increasing of the antibody titers were considerably valuable in relapsed cases and in patients with chronic infections (12,13).

Previous studies report that the IgM antibody may be detected after the first week following the entry of the organism. The peak level is reached 4 weeks later (14). The IgG antibody has a delayed appearance, although it is found together with IgM 4 weeks after the initial antigenic stimulus; the IgM antibody level always exceeds the IgG antibody level in the acute stage of the disease. In the study by Memish et al., where all the patients had acute cases of brucellosis with bacteremia, the IgM test was more sensitive than the IgG (5). Similarly, Gad El-Rab detected only IgM antibodies in few patients with acute disease as well (15).

Memish et al. reported that in patients with Brucella bacteremia, the sensitivity of either ELISA IgM or IgG was lower than that of SAT; however, combining IgM and IgG had similar sensitivity and specificity to SAT (5).

Güneri and Öğütman, who studied 29 patients with brucellosis, compared the ELISA and SAT tests, and found positive results in 80% of the patients by SAT and 72% by ELISA (16).

Sirmatel et al. found that the SAT positivity rate were significantly higher than the ELISA IgG and IgM rates in patients with acute brucellosis (17).

In a study conducted using ELISA in Kuwait, Brucella IgG had a sensitivity and specificity of 98% for patients with acute or chronic brucellosis, while Brucella IgM had a sensitivity of 98% and a specificity of 98% for patients with acute brucellosis. However, in patients with chronic brucellosis the Brucella IgM was very low. The authors of the study reported that Brucella ELISA is a rapid, sensitive and specific assay providing a profile of immunoglobulin classes in the diagnosis of acute and chronic brucellosis; therefore, it is useful for mass screening and could be considered the method of choice for the serological diagnosis of the named disease (18). Similarly, in the study by Prado et al., the sensitivities were 65.8%, 92.6% and 85.3% for SAT, ELISA IgG and ELISA IgM, respectively (19).

In contrast to our findings, several studies have demonstrated that ELISA is more sensitive and rapid than SAT and other conventional tests used in the diagnosis of brucellosis. It is said that the detection of specific immunoglobulins by a single, simple and rapid test is a major advantage of ELISA (12,20,21).

Conclusion

The overall data in the present study showed that the sensitivity of SAT and ELISA IgM tests were nearly equal, but the sensitivity of ELISA IgG was lower than that of the other two. On the other hand, the specificity of SAT was higher than that of both ELISA IgG and IgM. According to the results of our study, SAT may be preferred to ELISA in acute brucellosis because it is cheap and easily applicable.

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