

Original Article



BrucellaCapt test, Wright and Coombs wright in diagnosis of Brucellosis

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Abstract

Introduction: Rapid and accurate diagnosis of the disease can have many effects on patients' healing and recovery, so we decided to investigate the accuracy of brucella capture test with Coombs Wright in patients with brucellosis.

Methods: The present study was a descriptive study performed on patients referred to clinics with brucellosis symptoms. Blood samples were taken from all patients. Patients' information, including age, sex, response to Coombs, Coombs Wright, and Brucella capture tests, as well as patients' response to treatment, were entered into a pre-prepared checklist. After obtaining laboratory tests for serological assessment, patients' information including demographic information and laboratory tests were obtained and analyzed using SPSS v. 16.

Results: This study was performed on 91 patients with brucellosis. The mean age was 34.6 years, and 75.8% of the patients were male. In this study, 84.6% of patients showed a positive Coombs test, and 26.4% of patients showed a titer of 1:80, 85.7% of the patients showed positive Coombs Wright test, and 29.7% of the patients had a titer of 1:160. In the study of Brucella capture tests in patients, 98.9% of patients showed a positive test, of which 34.1% had a titer of 1:160 of the test.

Conclusion: The results of this study showed that the Brucella capture test is a powerful test for the diagnosis of brucellosis, and in the same condition, patients are more likely to be diagnosed with two Wright and Coombs Wright tests.

Introduction

Brucellosis is considered one of the most important and common diseases of humans and animals.¹ The disease-causing Brucella bacterium infects a wide range of domestic and wild mammals.²

The disease is caused by different species of Brucella micro-organism. Malta fever is transmitted from infected cattle, pigs, sheep, or goats to humans. Malta fever affects the body's hematopoietic organs such as bone marrow, lymph nodes, liver, and spleen. Malta fever is found in both acute and chronic types. The incubation period of this disease can be 5 to 60 days (several months have also been seen). Malta fever is more common in men in their 20s and 60s.³

Consumption of milk, dairy products (cheese), or meat products of infected animals and other ways can spread the disease to humans. It is more common in people who come into contact with a lot of animals (farmers, ranchers, butchers, veterinarians), and people who travel to contaminated areas.⁴

The definitive diagnosis of brucellosis is made by a blood test, and with the complete cure, it usually improves in 3 to 4 weeks. The surrounding and family members of the sick person who may have eaten the same contaminated food should be examined and tested.⁵

Types of brucellosis serological tests include standard tube agglutination test (STA) or Wright Test, which assesses IgM and IgG; 2ME agglutination test that tests for IgG; Coombs Wright test, which mainly shows IgG class antibodies; Complement fixation test that shows IgG class antibodies; Radioimmunoassay and ELISA tests, which are more sensitive and specific than the standard test and complement fixation, and show both M and G immunoglobulins. However, they can also be adjusted to evaluate a particular class of immunoglobulins, so these tests can easily distinguish acute from chronic brucellosis, as well as an acute attack in the chronic context. In other words, IgM, IgG, and IgA specific anti-brucellosis antibodies can be tested by radioimmunoassay.⁶ However, there are no problems with blocking and non-agglutinating antibodies in these tests, and in the acute or chronic stage of the disease, specific antibodies can be examined separately, and when the interpretation of agglutination tests is ambiguous, an ELISA test can be performed. Acute and chronic brucellosis can also be differentiated by IgM or IgG test, but this test also cross-reacts with yersiniosis. Rose Bengal test, ring test, and agglutination on a slide which are rapid agglutination methods.⁷

Rapid and accurate diagnosis of the disease can have many natures on the course of treatment and recovery

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of patients, so we decided to investigate the diagnostic accuracy of Brucella cap with Combs Wright in patients with brucellosis.

Materials and Methods

Type of study

This study is a cross-sectional descriptive-analytical study.

Population

Patients with brucellosis-compatible symptoms who tested positive for Wright, Combs Wright, and Brucella Capture were referred to infectious disease clinics during the three months of spring. Patients with brucella or recurrence of the disease in the past six months and dissatisfaction with participation in the study were excluded from the study.

Using Dr. Lin Naing software, calculation of error coefficient of 0.05 and considering power 80% for the present study and referring to the results of the previous study comparing the sensitivity and specificity of Brucella Capture and Combs Wright, 98 people were calculated.

Study design

In this study, patients who were referred to the clinics within a year and showed symptoms of brucellosis (fever, sweating, arthralgia, hepatomegaly, splenomegaly, or symptoms of focal disease) were included, and Wright and Combs Wright tests $\geq 1:160$ and Brucella capture test was performed. The criterion for confirmation and correctness of the above tests is based on the patients' clinical response to drug treatment. The patients who did not respond clinically to the treatment were excluded from the study. Randomization was performed based on RandList.1.2 software, and based on this samples were included in the study. Conscious consent was obtained from all patients regarding the need for blood sampling to prepare additional blood samples. Patients' information, including age, sex, Combs test, Combs Wright and Brucella capture tests, as well as patients' response to treatment, were entered in a pre-prepared checklist.

Patients who were examined one year before the onset of the disease were considered to have chronic brucellosis.

After diagnosis, patients were treated with doxycycline and streptomycin or gentamicin. Brucellosis was detected according to standard microbiological techniques. Serological methods of standard tube agglutination test (SAT), Combs Wright, and brucella capture test were performed on several serum samples. SAT and Combs Wright was performed on a U-shaped microtiter plate instead of a tube, and serums were diluted twice with normal saline between 1:20 and 1:40 up to 960. SAT showed the highest dilution of agglutination titer during 24 hours and 37°C. Combs Wright was washed with SAT microtiter plate, which had been washed three times with saline phosphate buffer, and centrifuged in 3000 g for 20 minutes, after which 15 μ L of anti-human globulin was added to each after the third wash. The results were read

at 37°C after 30 minutes.

Brucellosis agglutination was performed similarly to Combs Wright, and all brucellosis-specific antibodies were identified. Brucella capture was provided by the manufacturer to specify that 50 μ L of the diluted serum sample was placed in a U-shaped microtiter plate to which anti total human immunoglobulin was added, then 50 μ L of the antigen suspension added (*Brucella melitensis* killed by *Brucella melitensis*). Formaldehyde was added to all liquids. The plates are covered with adhesive tape and left in a dark, damp room at 24°C for 24 hours. The positive reaction at the bottom of the liquids showed agglutination, and the negative reaction was indicated by a plate at the center and bottom of the liquid.

Data analysis and statistical analysis

Data obtained from the study using descriptive statistical methods (frequency, percentage), and chi-square test or Fisher's exact test, diagnostic values (sensitivity, specificity, positive and negative predictive, and positive and negative likelihood ratio) were analyzed using SPSS v. 16 software. In this study, a P value less than 0.05 was considered statistically significant.

Results

The present study was performed on 98 patients with suspected symptoms of Malta fever. It was observed that 7 patients (14.7%) did not respond to drug treatment, so these 7 patients were excluded from the study, and the study continued on 91 patients. The mean age of patients was 34.6 ± 13.13 years. Also, 32 patients (35.2%) were in the age range of 21 to 30 years (Figure 1). Sixty-nine patients (75.8%) of this study are male.

The Wright test was performed in all patients. Among these patients with brucella, it was observed that this test was positive in 77 patients (84.6%) (titer above 1:80)

In the study of Wright test titer, it was observed that 1:80 titer with 24 cases (26.4%) was the most common antibody titer in the diagnosis of this disease (Figure 2). The Combs Wright test also showed that 78 patients (85.7%) had a positive Combs Wright test. The Combs Wright test titer was also evaluated, and the results showed that 1:160

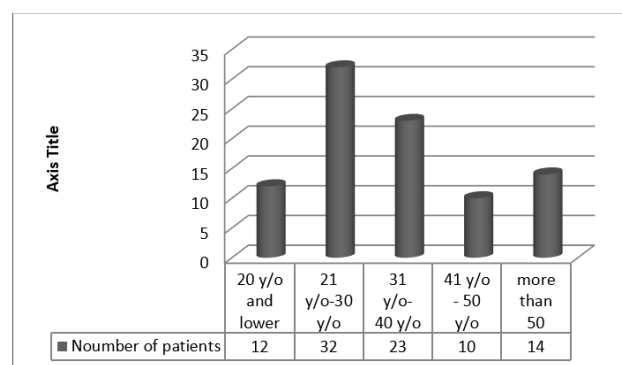


Figure 1. Frequency of patients according to age

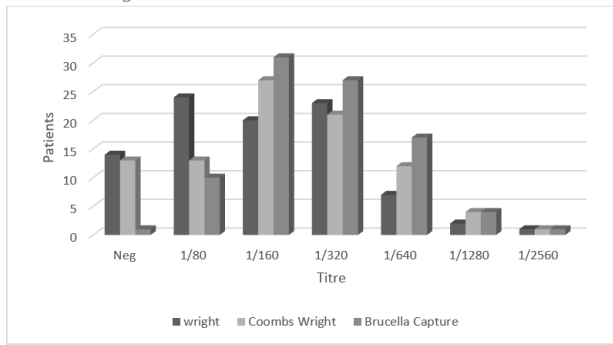


Figure 2. Frequency of patients according to titer

titer with 27 cases (29.7%) was the most common titer observed in patients (Figure 2). The third and final test evaluated among patients was the Brucella Capture test. In this study, it was observed that 90 patients (98.9%) have a positive Brucella capture test. The Brucella capture test titer was also evaluated, and it was observed that 1:160 titer with 31 cases (34.1%) is the most common repeated titer among patients (Figure 2).

The positive and negative cases of Brucella capture and

Coombs Wright test were analyzed together, and it was observed that in 2 subjects, both Coombs Wright test and negative capture were reported, but in 13 patients, despite the negative Coombs Wright test, but in the test, capture was reported positively (Table 1).

The positive and negative cases of Brucella capture and Wright test were also analyzed together, and it was observed that in 2 patients, both Wright test and Capture test were reported negatively, but in 15 patients, even though the Wright test was negative, a positive test was reported (Table 2).

In the study of the relationship between patients' sex and the results of the Brucella test, it was observed that only in the Coombs Wright test, the percentage of a positive test in men was significantly higher than in women. In the other two tests, no significant discrepancies were observed (Table 3).

The correlation between Brucella diagnostic tests showed a positive and significant correlation between all Brucella diagnostic tests, including Coombs, Coombs Wright, and Brucella Capture (Table 4).

Table 1. Relation between coombs wright and brucella capture

Brucella Capture	Positive coombs wright test		Negative coombs wright test		P value
	Frequency	Percent	Frequency	Percent	
Positive	83	86.5	13	13.5	0.576
Negative	2	100	0	0	

Table 2. Relation between wright and brucella capture

Brucella Capture	Positive wright test		Negative wright test		P value
	Frequency	Percent	Frequency	Percent	
Positive	81	84.4	15	15.6	0.544
Negative	2	100	0	0	

Table 3. Relation between patients sex and brucella tests

	Test result	Male		Female		P value
		Frequency	Percent	Frequency	Percent	
Wright	Positive	61	88.4	16	72.2	0.076
	Negative	8	11.6	6	27.3	
Coombs wright	Positive	62	89.9	16	72.7	0.046
	Negative	7	10.1	6	27.3	
Brucella Capture	Positive	68	98.6	22	100	0.570
	Negative	1	1.4	0	0	

Table 4: Correlation between brucella detection tests

		Wright test	Coombs wright test	Brucella capture test
Wright test	Correlation	----	0.942	0.908
	P value	----	<0.001	<0.001
Coombs wright test	Correlation	0.942	----	0.946
	P value	<0.001	----	<0.001
Brucella capture test	Correlation	0.908	0.946	----
	P value	<0.001	<0.001	----

Discussion

This study was performed on 98 patients with brucellosis with a mean age of 34.6 years and 75.8% male. In a study by Seyednozadi et al, 53.8% of patients were male, and 46.2% were female⁸. In the study of Hashemi et al, it was observed that 70.47% of patients were male and 29.53% were female, and the mean age of patients was 41.1%.⁹ In a study conducted by Esalatmanesh et al, 31 patients with brucellosis had a mean age of 41.8 years, and 58% of patients were male, and 42% were female.¹⁰ In the study of Casanova et al, it was reported that out of 48 patients with brucellosis, 77% of them died, and the average age of these patients was 40.83 years.¹¹ Studies show that the dominant gender in all studies is male. Due to the occupational aspect of this patient and the greater relationship between males and animals carrying the disease, the incidence of this disease in men can be justified. It was also observed that the fourth decade of life was the most common time of contracting this disease, which can be explained by the greater exposure of people of this age to livestock and disease transmission.

In this study, it was observed that 84.6% of patients showed a positive burn test, and 26.4% of patients showed a titer of 1:80. In the study of Seyednozadi et al of the total Wright tests related to patients with brucellosis, 8.2% less than 1:80 and 11.2% equal to 1:80 and 80.6% equal to or more than 1:160. The sensitivity of the Wright test was calculated based on titers of 1:80 and 1:160% and 80.6%, respectively.⁸ In the study of Hashemi et al, it was observed that 88.6% of patients with brucellosis had a positive burn test. It was also observed that 11.4% of patients had a titer less than 1:80, 20.9% had a titer of 1:80, and 67.7% had a titer of more than 1:160.⁹ In another study conducted by Kazemi et al on 104 patients in four provinces of Mazandaran, Kermanshah, Kurdistan, and Hormozgan, 80.7% of patients had a positive burn test, and 14.4% had a positive culture.¹² Another study by Sarigüzel et al in Turkey in 2011 on 21 patients with positively cultured brucellosis had a positive burn test of 71.4%.¹³ In another study by Marei et al in Egypt on 50 patients, 90% of brucellosis-positive samples had a positive burn test.¹⁴ In the study of Esalatmanesh et al, it was observed that 64.5% of patients with brucellosis had a positive burn test.¹⁰ Because healthy individuals were not evaluated in the present study, sensitivity and specificity were not evaluated in this study.

In this study, it was observed that 85.7% of patients showed a positive Combs Wright test, and 29.7% of patients showed a titer of 1:160. In the study of Hashemi et al, 87.5% of patients had a positive Combs Wright test.⁸ It was also reported that 12.5% of patients had a titer less than 1:80, 22.1% had a titer of 1:80, and 65.4% had a titer of more than 1:160. The originality of Esalatmanesh, et al in their study reported a positive rate of Coombs test in patients with brucellosis 64.5%.¹⁰ In the study of Orduña et al, it was observed on 82 patients with brucellosis that it

is positive in 91.4% and among the patients with Coombs Wright test positive titers of 1:640 and 1:1280 each with 29.2 percentage of the most common repeated headings among patients.¹⁵ The Combs Wright test was also approved in the majority of studies as a very valuable test in the diagnosis of brucellosis. In most studies, this test, like the present study, was observed to be positive in more than 85% of patients.

In the study of Brucella capture test in patients, it was observed that 98.9% of patients showed a positive test, and among these patients, 34.1% had a titer of 1:160 of this test. In the study of Orduña et al, it was reported that among the sick thigh with brucellosis 95.12% of patients had Brucella capture test positive, and among these, titer 2560 with 19.5% was the most common repeated titer among patients.¹⁵ The results showed that this test had a sensitivity of 95.1% and specificity of 81.5% in the titer of 1:160. In the study, Casanova et al reported that brucellosis had a titer of more than 1:160% in 87.9% of patients with brucellosis and a negative titer was reported in 12.1% of patients.¹¹ Titer 1:640 was the most common titer among patients. Sensitivity and specificity, positive predictive value (PPV) and negative predictive value (NPV) were 91.6%, 95.9%, 99.2% and 67.1%, respectively. In a study conducted by Mantur et al, it was also reported that the sensitivity and specificity of immunocapsulation tests in patients with brucellosis are 97.29% and 97.08%, respectively, and between its sensitivity and specificity to the test.¹⁶ Agglutination increased significantly but was not significantly different from Wright Coombs. Mantecón et al stated in another study that 98% of immunocapsulation tests could diagnose patients with brucellosis.¹⁷ In the study of Peeridogaheh et al, it was also stated that among patients with blood culture positive for brucellosis, immunocapsulation test was positive in all patients, among 46 patients with suspected brucellosis, 80.4% of this test was positive and in healthy individuals This test was positive in 3.2%, so for this test, sensitivity and specificity were 80.4% and 96.8%, respectively.¹⁸ In the study of Alişkan et al, which was performed on patients with brucellosis, by setting a threshold of 1:160 for the brucellosis test, it was observed that 92% of patients had a positive test.¹⁹ Also, none of the patients without this disease reported a positive Brucella capture test. Therefore, it was observed that the Brucella capture test has a sensitivity of 92% and a specificity of 100%. Immunocapsulation test is a test with high specificity and sensitivity in the diagnosis of brucellosis. Since in the present study, the specificity and sensitivity of this test were not evaluated in this study, but in comparison with other diagnostic tests for brucellosis, it was observed that it has higher diagnostic power and perform this test along with one of the two burn tests or Combs Wright can diagnose brucellosis in patients with very high potency.

Study Highlights

What is current knowledge?

- Wright and coombs wright are powerful test to diagnose Brucella

What is new here?

- Brucella Capture test is more powerful than others in diagnosis of Brucella

Conclusion

The results of this study showed that the Brucella capture test is a powerful test in the diagnosis of brucellosis, and in the same conditions, it detects more patients than the two Wright and Combs Wright tests.

Limitation

The limitation of this study was the small number of patients due to the limited study time. In this study, healthy patients were not evaluated. It is recommended that another study be performed with a larger volume of patients. It is suggested to perform further studies, in which healthy patients are also evaluated to obtain the sensitivity and specificity of these tests.

Conflict of Interest

Authors declare no conflict of interest in this study.

Ethical Approval

This manuscript was approved by the regional ethic committee of Tabriz University of medical sciences with no.: IR.TBZMED.REC.1397.319. All patient information was confidential as indicated in the checklist. Patients were not charged for the study. The informed consent form was provided for all patients

Author's Contribution

Study design and supervision, MH; study conduct, HOO; data gathering and writing, LA.

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