Evaluation of Different Serologic Laboratory Tests Used For Diagnosis of Brucella Antibodies among Patients in Azadi Teaching Hospital in Kirkuk City

Hadi H. Mohammad Ali* ***Ali H. Mohammad Ameen*****
Collage of Medicine - Kirkuk University
****Collage of Nursing - Kirkuk University***

Received: 3/2/2010, Accepted:1/6/2010

Abstract

Brucellosis is a zoonotic disease, endemic in Iraq, and transmitted through dairy products. Several serological tests have already been used for *Brucella* infection diagnosis. Sera from a total 182 suspected patients having brucellosis attended Azadi General Hospital during the period between June – September 2009 had been screened by different serological tests, Rose Bengal agglutination test, *B.abortus* antigen agglutination test, *B.melitensis* antigen agglutination test as well as ELISA IgM, IgG have been used in this investigation to determine the type of infection and to evaluate the best serologic tests . By comparing the results obtained from these serologic tests and by using statistical methods (PPV, NPV and F-measure) it was found that the Rose Bengal test is most useful and more sensitive than other two tests *B.abortus* antigen test and *B.melitensis* antigen test. There have been no significant differences between the rate of acute and chronic infection. The result also suggested that the male and female have the same susceptibility for infection with *Brucella*.

Introduction

Brucellosis results from infection by various species of *Brucella*, a Gram negative, facultative intracellular coccobacillus or short rod in the family Brucellaceae. Six named species occur in animals: *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis* and *B. neotomae* (Young, 1995).

Brucellosis is a zoonosis, and virtually all infections derive directly or indirectly from exposure to animals and their products. The disease is distributed throughout the world, especially in the Mediterranean basin, the Arabian Peninsula, the Indian subcontinent, Mexico and Central South America (Mandell et al., 2005).

Brucellosis generally presents as an acute or subacute febrile illness with protean clinical manifestations. To the unaware patient, the acute phase of the disease may be experienced as an innocent febrile illness that does not need consultation with a physician. However, brucellosis should be treated promptly because the infection may persist, and the patient may develop severe complications (Corbel, 1997)

Diagnoses of brucellosis have investigated to be demands on epidemiology, clinical, and laboratory information. At present, laboratories have found to be not able to diagnose the infection with more confidence, although isolation and identification are the more assured methods for the diagnoses. However, many difficulties have reduced the sensitivity of these methods and they are being unusable in several laboratories (Al Dahouk, et al, 2003 ; Gall & Nielsen, 2004).

Studies have shown that a good chance of isolation of bacteria from blood cultures is less than 3% (Hajia & Rahbar, 2006). Therefore, laboratory diagnosis of brucellosis very often relies on detecting specific serum antibodies. Several serological tests (Rose-bengal, 2-Mercaptoethanol, Wright, Coombs, Complement fixation and ELISA test) have been used for the diagnosis of human brucellosis with different specificity and sensitivity. Among serologic tests ELISA is the most sensitive and specific of the *Brucella* serologic routine tests and is useful to monitor antibodies in patients undergoing treatment, isotype determining and phase of disease, and it may be positive when other tests are negative (Esmaeilzadeh, 2004). Therefore, in this study ELISA technique has been used to evaluate conventional tests (slide agglutination tests) that applied in the most laboratories to detect antibodies against *Brucella* species and considers as a diagnostic procedures.

Aims of study

1- Evaluation the commercial serologic tests that are available in most laboratories for *Brucella* diagnosis.
2- To determine the incidence of *Brucella* infection and the phase (stage) of disease (chronic or acute) in Azadi Teaching Hospital in Kirkuk city.

Materials and methods

One hundred eighty two patients were included in this study; 46 were male and 136 were female, and their ages ranged from 12 to 77 years (mode; 25 years). Serum samples were collected from patients attended to Azadi Teaching Hospital from June to September 2009 and were screened by three types of antigen kits (Rose Bengal test kit from PLASMATIC U.K., *B. abortus* antigen kit from BIOTEC U.K. and *B. melitensis* antigen kit from BIOTEC U.K.) to detect antibodies specific for *Brucella* species. All serum samples were also tested by (ELISA IgM kit and ELISA IgG kit from DRG, GmbH, Germany) to evaluate serologic tests and to determine incidence and the phase (stage) of infection.

Statistical analysis

For comparison between the results of several tests to determine the true and false result, the statistic analysis applied in this study consists of:

1- Positive predictive value (PPV): is the proportion of patients with positive test results who are correctly diagnosed.
The Positive Predictive Value can be calculated as:

$$PPV = \frac{\text{number of True Positives}}{\text{number of True Positives} + \text{number of False Positives}}$$

2- Negative predictive value (NPV): is the proportion of patients with negative test results who are correctly diagnosed.

The Negative Predictive Value can be defined as:

$$NPV = \frac{\text{number of True Negatives}}{\text{number of True Negatives} + \text{number of False Negatives}}$$

3-Sensitivity: measures the proportion of actual positives which are correctly identified. Calculate by:
Sensitivity = True Positive / (True Positive +False Negative)
4-Specificity: measures the proportion of negatives which are correctly identified.
Specificity =TN / (TN+FP)
5-Accuracy: accuracy of a measurement system is the degree of closeness of measurements of a quantity to its actual (true) value.
ACC = (TP + TN) / (TP + TN + FP + FN)
6-F-measure: can be used as a single measure of performance of the test.
F-measure = 2 x [(PPV x sensitivity) / (PPV + sensitivity)
7-False positive rate (α) = FP / (FP + TN)
8-False negative rate (β) = FN / (TP + FN)

Results and Discussion

Incidence of *Brucella* infection according to routine serologic tests that have been used (Rose Bengal, *B. abortus*, and *B. melitensis*) were (62.6%, 68.2%, and 39%) respectively.(Table:1). These results are not precision due to inability of these tests to recognize between acute and chronic infection as well as false positive since *Brucella* antigens share with other microorganisms antigens. It has been proved that the presence of 4-amino,4,6 dideoxymannose in the Lps is responsible for the antigenic cross-reactions with certain other gram-negative bacteria, such as *Vibrio cholerae* O1 and *Yersinia enterocolitica* O9(Perry & Bundle,1990).

In addition of these three tests using, ELISA test had been used to determine the stage of infection and to evaluate routine serologic tests. The incidence of acute and chronic infection recorded by ELISA IgM test, and ELISA IgG were 53.8% and 48.3% respectively. Slight difference between acute and chronic infection may be due to insufficient eradication of the infection as a result of imperfect treatments in patients included in this study. The lowest rate of infection recorded by *B. melitensis* test was 39.5% may be due to *B. melitensis* require higher infectious doses to obtain infection rates in animals similar to those of *B. abortus* (Kahl-McDonagh, et al, 2007).

Table: (1) Prevalence of brucellosis according to the serologic tests

Tests	No. of sample examined	Positive No.	Positive %	Negative No.	Negative %
Rose Bengal test	182	114	62.6	68	37.4
B. abortus test	182	124	68.2	58	31.8
B. melitensis test	182	72	39.5	110	60.5
ELISA IgM	182	98	53.8	84	46.2
ELISA IgG	182	88	48.3	94	51.6

The infection rate and determination of the phase (stage) of infection in male and female (Table:2). Using ELISA IgM test it was found to be 52% and 54.4% for male and female respectively, while it was found to be 47.8% and 48.5% for male and female respectively by using ELISA IgG test. There was a slight difference between two values and not significant ($P > 0.05$). These results conclude that there is a same susceptibility for infection between male and female. Other study proved this fact (Güneş et al, 2009).

Table :(2) Phase determination of *Brucella* infection according to ELISA test

Gender	Examined No.	ELISA IgM positive	ELISA IgM %(acute)	ELISA IgG positive	ELISA IgG %(chronic)
Male	46	24	52	22	47.8
Female	136	74	54.4	66	48.5
Total	182	98	53.8	88	48.3

To evaluate the agglutination test (Rose Bengal test B. abortus, B. melitensis) results of these tests compare with the results of ELISA test to determine true positive , true negative, false positive and false negative , for example, if the result of agglutination test matches up with the result of ELISA test, the result consider as a true result, if don't matches up with ELISA test, the result consider as a false result, because ELISA test is more sensitive and specific than other routine tests (Esmeilzadeh,2004). Table- 3 shows true and false (positive and negative) results for each of these tests: Rose Bengal, *B. abortus*, and *B.melitensis*. The number of positive sera recorded by Rose Bengal test, *B. abortus* test and *B. melitensis* were 114, 124 and 72 respectively (Table-1), but after the comparison with ELISA test had been done, the number of true positive results were: 76, 60 and 42 respectively (Table:3). False positive occurred in almost diagnostic tests and this phenomenon may be due to cross-reaction between *Brucella* and other microorganisms such as *Vibrio cholerae* O1 and *Yersinia enterocolitica* O9 as mention above. False negative may occur either due to low sensitivity and specificity of the tests or due to prozones phenomena which occur in serologic tests (Perry & Bundle, 1990).Less false positive percentage (38/114; 33%) and less false negative percentage (22/66; 32%) recorded by Rose Bengal test.

Table (3) True and False results for routine serologic tests

Test	Total positive	TP	FP	FP%	Total negative	TN	FN	FN %	Total
Rose Bengal test	114	76	38	33	68	46	22	32	182
B. *abortus* test	124	60	64	٥١	58	20	38	65	182
B. *melitensis* test	72	42	30	41	110	54	56	60	182
TP = True positive , FP = False positive , TN = True negative , FN = False negative									

Predictive values are often used in medical researches to evaluate the usefulness of a diagnostic test. Hence the PPV (Positive Predictive Value) is used to indicate the probability that in case of a positive test, that the patient really has the specified disease. The F-measure can be used as a single measure of performance of the test (Altman & Bland, 1994). Therefore to evaluate routine serologic tests by compare with ELISA test, data statistically analyzed using PPV, NPV and F- measure in this study.
PPV , F-measure and Sensitivity for Rose Bengal test were (67% , 72%, and 77.5%) respectively, and for *B. abortus* test were (48% , 53%, and 61%) respectively, and for *B. melitensis* test were (58% , 49%, and 43%), (Table:4).

Table: (4) Evaluation of serologic tests

Test	PPV	NPV	SEN.	SPEC.	FPR	FNR	ACC.	F. M.
Rose Bengal test	67%	68%	77.5%	55%	45%	22%	67%	72%
B. abortus test	48%	34%	61%	24%	76%	39%	44%	53%
B. melitensis test	58%	49%	43%	64%	36%	57%	53%	49%

PPV= positive predictive value
NPV = Negative predictive value
SEN. = sensitivity
SPEC.= specificity
FPR = false positive rate
FNR = false negative rate
ACC = accuracy
F. M. = F- measure

From these results extrapolate that Rose Bengal test has the more PPV, F-measure and sensitivity and these make this test more useful for *Brucella* diagnosis than the other two tests. These differences between kits may be due to different companies that supplied kits, Rose Bengal test from PLASMA UK, *B.abortus* test and *B. melitensis* test from Biotic UK, and these kits are available in Iraq hospitals for *Brucella* diagnosis, the laboratory staff in Azadi General Hospital were suffering from misdiagnosing when they used these three tests together for this reason this study was achieved.

Conclusion

1- Rose Bengal test is the most sensitive and more specific for diagnosis of brucellosis. Overall extrapolation of data from our study indicates that the ranking of tests according to their reliability of diagnosing human brucellosis is as follows: Rose Bengal test > *B. abortus* test > *B. melitensis* test.

2- Incidence of *Brucella* infection and phase (stage) of disease determined by ELISA test, approximately fifty percentages of patients have acute infection (IgM antibody) and there is no significant difference of incidence between acute and chronic infection ($P>0.05$).

References

- Al Dahouk, S. Tomaso, H. Nockler, K. Neubauer, H. and Frangoulidis, D.(2003): Laboratory-based diagnosis of brucellosis-a review of the literature. Part I: Techniques for direct detection and identification of Brucella spp. Clinical Laboratory.Vol.49, pp. 487-505.
- Altman DG, Bland JM (9 Jul 1994): Diagnostic tests 2: Predictive values,. British of Medical Journal. Vol.309, No. 102, 6947p.
- Corbel MJ, (1997): Brucellosis: an overview. Emergency Infectious Disease. Vol.3, pp. 213–221
- Esmaeilzadeh A.,(2004): ELISA vs. routine tests in the diagnosis of patients with brucellosis. 14th European Congress of Clinical Microbiology and Infectious Disease. Prague / Czech Republic, Abstract number: 903_r2158, May 1–4.
- Gall, D. and Nielsen, K., (23 Dec 2004): Serological diagnosis of bovine brucellosis: a review of test performance and cost comparison. Rev Sci Tech., Vol. 3, pp. 989-1002.
- Güneş ,T., Alim, A. Kaya, S.and Poyraz, Ö.(2009): Seroprevalence of brucellosis in high-risk groups in central Anatolia. Cumhuriyet Medical Journal (CMJ), Vol. 31, No. 2.pp.104-110.
- Hajia, M. and Rahbar, M., (2006): Isolation of Brucella from blood culture of hospitalized brucellosis patients. Iranian Journal of Clinical Infectious Disease. Vol.1, pp.5-8.
- Kahl-McDonagh, M. M., Arenas-Gamboa, A. M., and Ficht, T. A. (2007): Aerosol Infection of BALB/c Mice with Brucella melitensis and Brucella abortus and Protective Efficacy against Aerosol Challenge. Infection and Immunity, October, Vol. 75, No. 10, pp. 4923-4932.
- Mandell Gerald L, Dolin Raphael and Bennett John E., (2005): Principles and Practice of Infectious Diseases, sixth Edition, part III, ELSEVIER Churchill Livingstone, United State of America, 2670 p.
- Perry, MB. and Bundle, DR., (1990): Lipopolysaccharide antigens and carbohydrates of Brucella. In: Adams LG, ed. Advances in Brucellosis Research. Austin, Tex: Texas A&M University Press; pp. 76–80.
- Young EJ, (1995): An overview of human brucellosis. Clinical of Infectious Disease, Vol. 21,pp. 283–289.

تقييم اختبارات مصلية مختبرية مختلفة المستخدمة في تشخيص الأجسام المضادة للبروسيللا بين المرضى في مستشفي آزادي التعليمي في مدينة كركوك

هادي حسين محمد علي* علي حسن محمد أمين**

***كلية الطب ــ جامعة كركوك**

****كلية التمريض ــ جامعة كركوك**

تاريخ الاستلام:٢٠١٠/٢/٣، تاريخ القبول: ٢٠١٠/٦/١

الخلاصة

يعتبر مرض البروسيلا من الإمراض المشتركة وهو مرض مستوطن في العراق ينتقل للإنسان عن طريق منتجات الألبان وتوجد عدة أنواع من الإختبارات المصلية لتشخيص الإصابة بمرض حمى مالطا. أجريت الدراسة الحالية على ١٨٢ مريض راجعوا مستشفى أزادي العام في كركوك للفترة من حزيران إلى أيلول ٢٠٠٩ ويتوقع سريرياً اصابتهم بهذا المرض، تم جمع عينة المصل منهم وأجريت عليها أربعة اختبارات مصلية وهي اختبار التلازن الزهري، اختبار التلازن الخاص بالبروسيلا المجهضة ،اختبار التلازن الخاص بالبروسيلا المالطية وكذلك استخدام تقنية الـــــ ELISA لتحديد مستوى الكلوبيولين المناعي (IgM, IgG) وذلك لمعرفة نوع الإصابة وتقييم الأختبارات المصلية الثلاثة ، بعد مقارنة النتائج التي تم الحصول عليها وباستخدام طريقة الإحصاء (PPV, NPV and F-measure) أظهرت النتائج أن طريقة التلازن الزهري هي الطريقة الأكثر حساسية مقارنة باختباري التلازن الخاص بالبروسيلا المجهضة واختبار التلازن الخاص بالبروسيلا المالطية. وأظهر ت الدراسة ايضاً عدم وجود فروق معنوية بين الإصابة الحادة والمزمنة وكانت استعداد الإصابة بين الذكور والإناث متساوية تقريباً.