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

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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.

\[
\text{ACC} = \frac{(TP + TN)}{(TP + TN + FP + FN)}
\]

6- **F-measure**: can be used as a single measure of performance of the test. \n
F-measure = \[2 \times \left(\frac{\text{PPV} \times \text{sensitivity}}{\text{PPV} + \text{sensitivity}}\right) / (\text{PPV} + \text{sensitivity})\]

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

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

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

<table>
<thead>
<tr>
<th>Gender</th>
<th>Examined No.</th>
<th>ELISA IgM positive</th>
<th>ELISA IgM % (acute)</th>
<th>ELISA IgG positive</th>
<th>ELISA IgG % (chronic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>46</td>
<td>24</td>
<td>52</td>
<td>22</td>
<td>47.8</td>
</tr>
<tr>
<td>Female</td>
<td>136</td>
<td>74</td>
<td>54.4</td>
<td>66</td>
<td>48.5</td>
</tr>
<tr>
<td>Total</td>
<td>182</td>
<td>98</td>
<td>53.8</td>
<td>88</td>
<td>48.3</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Test</th>
<th>Total positive</th>
<th>TP</th>
<th>FP</th>
<th>FP%</th>
<th>Total negative</th>
<th>TN</th>
<th>FN</th>
<th>FN%</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rose Bengal test</td>
<td>114</td>
<td>76</td>
<td>38</td>
<td>33</td>
<td>68</td>
<td>46</td>
<td>22</td>
<td>32</td>
<td>182</td>
</tr>
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<td>B. abortus test</td>
<td>124</td>
<td>60</td>
<td>64</td>
<td>58</td>
<td>54</td>
<td>20</td>
<td>38</td>
<td>65</td>
<td>182</td>
</tr>
<tr>
<td>B. melitensis test</td>
<td>72</td>
<td>42</td>
<td>30</td>
<td>41</td>
<td>110</td>
<td>54</td>
<td>56</td>
<td>60</td>
<td>182</td>
</tr>
</tbody>
</table>

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

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

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


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

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الخلاصة

يعتبر مرض البروسيلا من الإغراض المشتركة وهو مرض مستوطن في العراق ينتقل لننسل لن طريق منتج الألبان وتوجد عدة أنواع من الاختبارات المصلية لتشخيص الإصابة بمرض حمى مطع. أجريت الدراسة الحالية على 182 مريض راجعوا مستشفى آزادي العام في كركوك للمرة من حزيران إلى أيلول 2006 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. وتم استخدام تقنية ELISA لمعرفة نوع الإصابة وتقييم الاختبارات 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 باختبار التلازن الخاص بالبروسيلا المجهضة، اختبار التلازن الخاص بالبروسيلا المالطية. وأظهرت الدراسة أيضاً عدم وجود فروق معنوية بين الإصابة الحادة والمزمنة وكانت استعداد الإصابة بين الذكور والإناث متساوية تقريباً.