Comparison of ELISA and STA tests in diagnosis of Brucellosis

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ABSTRACT

Background: Brucellosis is still a major health concern worldwide. The aim of the present study was to compare sensitivity and specificity of the STA (Standard Tube Agglutination) test and IgG ELISA (Enzyme Linked Immunosorbent Assay) in diagnosis and follow up of the brucellosis.

Materials and methods: A total of 80 patients were studied. STA test materials were prepared by Razi Institute (Tehran, Iran), while IgG ELISA kit were manufactured by GmbH (IBL, Germany). Meanwhile, blood culture and liver function tests were requested for all patients.

Results: Totally, 26 cases were positive in both STA and ELISA evaluation, of whom 8 cases showed high titers in both tests. ELISA was demonstrated to be more sensitive when compared with STA.

Conclusion: Although STA is a widely applied test, it cannot differentiate acute and chronic states of brucellosis. Our data suggest that IgG ELISA may be a suitable test for diagnosis and follow up of brucellosis.

Keywords: Brucellosis, ELISA, Standard Tube Agglutination.

INTRODUCTION

Brucellosis is an important public health concern in developing countries. The disease is found globally, however, is more common in the Mediterranean countries, the Arabian Peninsula, the Indian subcontinent and in parts of Mexico and Central and South America (1). In the Islamic Republic of Iran, brucellosis represents a major health problem and continuously reported with increasing frequency from various parts of the country. It is quite a prevalent disease all around the country (2). Diagnosis is occasionally confounded because of non-specific clinical manifestations, and is confirmed only if brucella species are recovered from blood, bone marrow or other sites (1,3,4). Brucellosis is usually associated with an intense humoral response (5,6). Isolation of the microorganism is possible only in a minority of the infected patients in the acute phase of the disease (7). Although most laboratories are now employing rapid isolation techniques (BACTEC, Dupont isolator, PCR methods and so on), these techniques are not available in most developing countries and conventional methods of isolation are too slow to use routinely for diagnosis (1,3,8). Therefore, in the absence of bacteriological confirmation, a presumptive diagnosis can be made on the basis of a single high rising titer of specific antibodies (1,3). Among a variety of serological tests, STA (Standard Tube Agglutination) is the most widely used (5,7). Evaluation of various ELISA assays for IgG and IgM have shown that...
these techniques are generally more sensitive and specific than conventional tests, while they are able to distinguish specific antibodies of IgM and IgG classes associated with acute and chronic brucellosis (9,10). The obtained results are always easily interpreted, since they are specific for single immunoglobulin classes. On the other hand, they are not routinely available in developing countries, especially in rural areas. Hence, attempts should be applied to increase the sensitivity of available tests. In the present study, IgG ELISA specific brucella melitensis was used as antigen and compared with classical Wright tube agglutination test.

PATIENTS and METHODS

Totally, 80 patients including 74 hospitalized patients (40 males and 34 females) at Sina and Imam Khomeini hospitals in Tabriz and 6 other outpatient cases were studied. The diagnosis was verified on clinical and laboratory findings. Simultaneously, blood culture, liver function tests, STA and IgG ELISA tests were requested for all patients. STA test materials were prepared by Razi Institute (Tehran, Iran), while IgG ELISA kit were manufactured by GmbH (IBL, Germany). Briefly, the procedures of these tests are described bellow:

Wright agglutination test
A- Preparation of two folds serial dilution of serum samples (starting dilution of 1/20).
B- 0.5cc of antigen solution (brucella abortus) was added to each tube.
C- Shake gently the tubes then incubate in 37°C for overnight.
D- The latest tube that shows agglutination will be considered the titer of serum antibody.

ME (2- Mercapthoetanol) Wright
This test was performed like Wright agglutination but the solution of antigen was mixed with a reductive chemical agent (2-ME) that reduce the S=S bonds in IgM molecules. Thus, ultimately acute, subacute and chronic states of brucellosis could be distinguished.

Coombs Wright
This test was also performed like Wright agglutination, however, antihuman globulin (AHG) was added to each tube to appear incomplete or blocking antibodies in serum samples following three times washing and centrifugation.

IgG ELISA test
A- 100UL of prediluted serum samples were added to each of wells that coated with brucella melitensis.
B- Incubate the plate at room temperature for an hour.
C- Wash the wells three times with washing solution.
D- 100UL of conjugate was added to each of the wells.
E- Incubate the plate for 30 minutes.
F- Wash again as described in stage C.
G- Substrate and chromogen solution was added.
H- After incubation (10 minutes), the stopping solution was added, then absorbencies were measured at 415-620 nm.

RESULTS

Totally, 26 patients were positive according to both tests (STA and IgG ELISA), of whom 8 cases showed high titers in both tests (table 1).

<table>
<thead>
<tr>
<th>No.</th>
<th>IgG ELISA</th>
<th>Agglutination</th>
<th>Coomb's Wright</th>
<th>ESR</th>
<th>CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32</td>
<td>1/80</td>
<td>1/320</td>
<td>38</td>
<td>+++</td>
</tr>
<tr>
<td>2</td>
<td>49</td>
<td>1/160</td>
<td>1/160</td>
<td>40</td>
<td>++</td>
</tr>
<tr>
<td>3</td>
<td>284</td>
<td>1/320</td>
<td>1/320</td>
<td>65</td>
<td>+++</td>
</tr>
<tr>
<td>4</td>
<td>254</td>
<td>1/640</td>
<td>1/640</td>
<td>45</td>
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<tr>
<td>5</td>
<td>269</td>
<td>1/640</td>
<td>1/640</td>
<td>70</td>
<td>++</td>
</tr>
<tr>
<td>6</td>
<td>318</td>
<td>1/640</td>
<td>1/1280</td>
<td>60</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>29</td>
<td>1/1280</td>
<td>1/1280</td>
<td>122</td>
<td>++</td>
</tr>
<tr>
<td>8</td>
<td>293</td>
<td>1/1280</td>
<td>1/1280</td>
<td>134</td>
<td>++</td>
</tr>
</tbody>
</table>
Among the 18 remaining cases, 15 revealed low titers with STA (≥1/160), while a variety of positive titers have been recorded with IgG ELISA. Meanwhile, 3 cases were positive with STA but negative with IgG ELISA test. Table 1 represents STA and IgG ELISA results of 8 high-titer group patients as well as their ESR, CRP and liver function test results.

DISCUSSION

Brucellosis, a zoonotic infection, is still a major health concern among Iranian population. Vaccination of ruminants (especially sheep and cows) against brucella and use of pasteurized milk or milk products could decrease the rate of infection in the human societies (11). On the other hand, prompt diagnosis and treatment of infection is another efficient strategy (12).

Isolation of microorganism from blood culture is a qualified monitoring technique, but requires relatively long time or experienced Lab technician (13,14).

In the present study, none of the patients revealed to be blood culture-positive. However, we have encountered the following limitations: the cross-reaction between brucella and other microorganisms, the presence of blocking or excessive level of antibodies that ensue false negative reactions.

ELISA was demonstrated to be more sensitive when compared with STA, indeed, 15 cases that were negative with STA were revealed to be positive with ELISA, however, 3 cases that were positive with STA, revealed to be negative with IgG ELISA test, therefore, we concluded that it is advisable to perform both IgG and IgM ELISA technique in order to achieve higher accuracy.

REFERENCES