Determination of the Accuracy and Optimal Cut-off Point for ELISA Test in Diagnosis of Human Brucellosis in Iran

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Abstract- In endemic area the most challenging problem for brucellosis is to find a reliable diagnostic method. In this case-control study, we investigated the accuracy of ELISA test for diagnosis of human brucellosis and determined the optimal cut-off value for ELISA results in Iran. The laboratory diagnosis of brucellosis was performed by blood isolation of *Brucella* organism with a BACTEC 9240 system and/or detection of *Brucella* antibodies by standard agglutination test (titer ≥ 1:160). Serum level of ELISA IgG and ELISA IgM from 56 confirmed cases of brucellosis and 126 controls were compared with each other by Box plot graph and Receiver Operating Characteristic (ROC) curve. Box plot graphs showed the high degree of dispersion for IgG and IgM data in patients compared with all controls. We observed partially overlapping for IgM data (not for IgG) between cases and controls in graphs. The area under ROC curve for distinguishing between cases and controls was larger for IgG compared to IgM. Based on results of this study, ELISA IgG test was more reliable than ELISA IgM test in diagnosis of human brucellosis in Iran. Using a cut-off of 10 IU/ml and 50 IU/ml had most sensitivity (92.9%) and most specificity (100%) for ELISA IgG test, respectively.

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Keywords: Brucellosis; Brucellosis diagnosis; Elisa; Sensitivity; Specificity

Introduction

Human brucellosis is a common infectious disease and important public health challenge in Iran. It has a seroprevalence of 1-2 % in this country (1). Brucellosis involves several organs and has variable complications. The different clinical manifestations may lead to misdiagnosis.

Diagnosis of brucellosis is performed by compatible clinical features and results of laboratory methods including blood culture and serologic tests.

The gold standard of diagnosis is isolation of organism from blood, bone marrow and other body fluids, but blood culture yield varies widely and may be as low as 15 % based on different culture techniques (2). This would make a point towards importance of serologic tests and need to explore more into this domain. Several conventional serologic assays have been used for the diagnosis of brucellosis. The most commonly employed method for antibody detection is standard agglutination test (SAT). It is a subjective method and reporting the antibody titer could be operator dependent. It may be associated with false positive and false negative results. The lack of seroconversion could be attributable to the presence of blocking antibodies or inhibition of agglutination at low dilution due to an excess of antibodies (3). Because of the importance of early diagnosis in suspected clinical cases and for lowering the misdiagnosis, it is necessary to use other diagnostic serologic methods.

The enzyme linked immunosorbent assay (ELISA) is known as a sensitive and rapid method for diagnosis of brucellosis. Detection of specific immunoglobulin by a single, simple and rapid test is a major advantage with ELISA (4-6). In addition to benefit of ELISA in diagnosis of brucellosis in endemic area, it could be useful as a screening test in areas with low incidence of disease (7).

In one study the sensitivity of SAT for diagnosis of brucellosis was similar to combination of IgM and IgG

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ELISA test (8). In another study, Ciffici *et al.* found the sensitivity 94.3%, 97.1%, and 71.4% for SAT, ELISA IgG and ELISA IgM, respectively (9). In spite of high sensitivity of ELISA test in diagnosis of brucellosis, the definite specificity and fixed cut-off point has not been determined and different reports have been published with varying results (2,10,11). Hence, we need to determine the optimal cut-off point of ELISA test for decreasing false positive results.

In this case-control study, we investigated the accuracy of ELISA IgM and ELISA IgG for the diagnosis of human brucellosis and determined the optimal cut-off value for ELISA results in Iran.

Materials and Methods

From Oct 2005 to Feb 2009, fifty-six confirmed cases of brucellosis were collected from Department of Infectious Diseases of Imam Khomeini Hospital in Tehran. The laboratory diagnosis of brucellosis was performed by blood isolation of *Brucella* organism with a BACTEC 9240 system and/or detection of *Brucella* antibodies by SAT (titer ≥ 1:160). All patients had complete response to anti-brucellosis drugs in follow up. We considered two control groups including 73 healthy controls and 53 non-brucellosis febrile patients. The patients and controls that had previous history of brucellosis or usage of anti-brucellosis drugs within previous year were excluded from the study. Blood samples were obtained from all patients and controls and checked for *Brucella* IgM and IgG antibodies by ELISA test (Immuno Biological Laboratories Company, Germany). IgM and IgG serum levels of patients were compared with controls by means of Box plot graph. To determine the optimal cut-off point for ELISA results the Receiver Operating Characteristic (ROC) curve was drown and the IgM and IgG levels yielding maximal sensitivity and maximal specificity were selected.

Statistical analysis

Sensitivity and specificity ELISA test for detecting brucellosis, with 95% confidence intervals (CIs), were calculated for each of cut of points of serum level of IgG and IgM. ROC curves were constructed using different cut off levels for normal. The level providing optimum discrimination was then used to dichotomize the variable. Tests for significance were based on the Chi-square statistics for the 2-by-2 tables, with a significance level of $P<0.05$ chosen a priority.

Results

Nineteen of 56 confirmed cases of brucellosis had positive blood culture for *Brucella melitensis*. The standard agglutination test results were 1/160 or more in 54 patients. There was no significant difference between three groups according to age and sex, statistically ($P>0.05$). Mean±SD (standard deviation) of ELISA IgM was 102.4±128.5, 4.0±2.6 and 1.5±2.5 IU/ml in brucellosis patients, healthy controls and non-brucellosis febrile controls, respectively. Mean±SD of ELISA IgG was 160.4±80.5, 4.0±2.6 and 4.9±8.1 IU/ml in brucellosis patients, healthy controls and non-brucellosis febrile controls, respectively. In patients with brucellosis, the mean of serum IgG and IgM were greater than the other groups, significantly ($P<0.001$). The distribution of ELISA IgM and ELISA IgG data was shown in each group by Box plot graph (Figures 1 and 2).

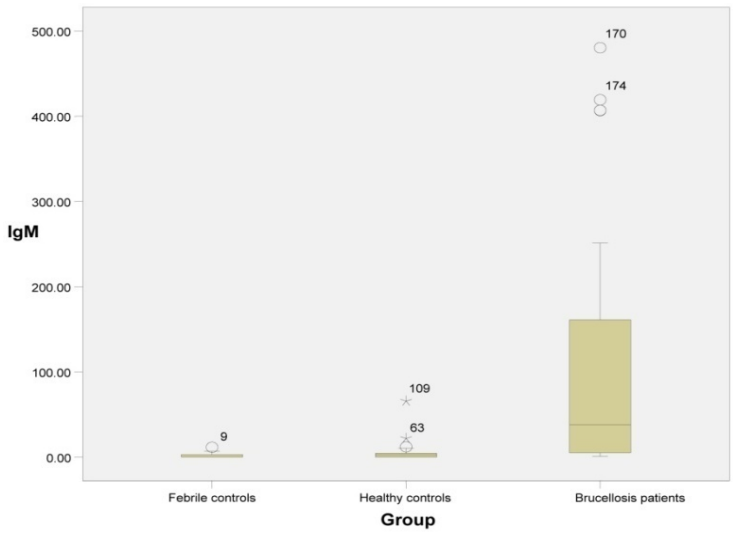


Figure1. Dispersion of IgM in patients and control groups.

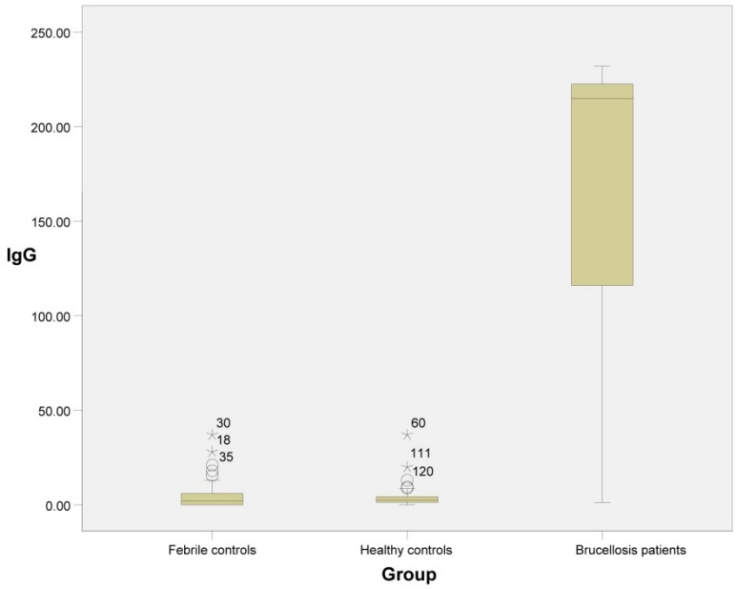


Figure 2. Dispersion of IgG in patients and control groups.

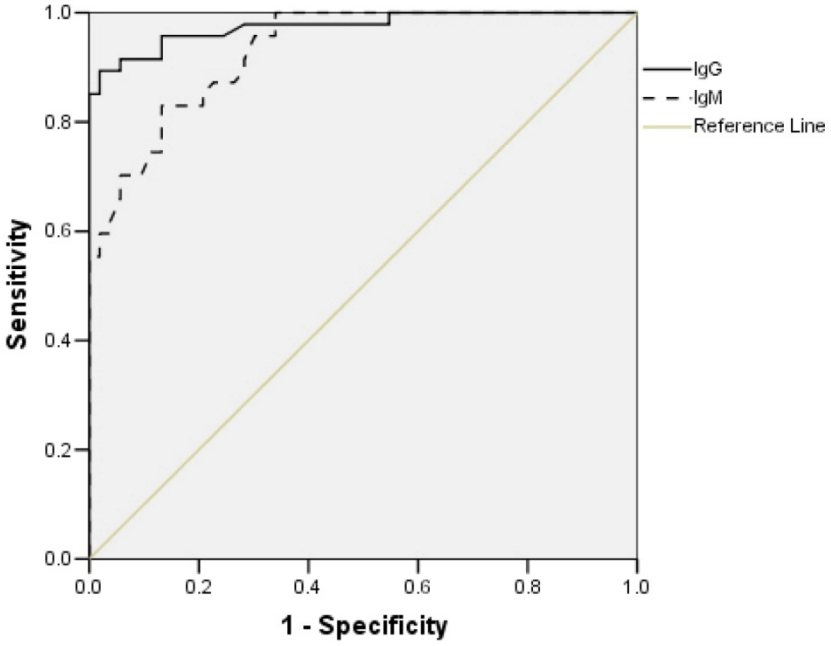
The Box plot graph indicated the high degree of dispersion for IgG and IgM data in brucellosis patients compared with non-brucellosis febrile patients and healthy controls. It was also observed partially overlapping of IgM data (not for IgG data) within the interquartile range (25 to 75 percentile) in patients and controls.

The ROC curve showed the behavior of the sensitivity and specificity of ELISA IgG and IgM by using different cut-off points. The area under ROC curve for discrimination cases and healthy controls were 0.978 and 0.854 for ELISA IgG and IgM, respectively (Figure 3). In this manner the area under ROC curve for discrimination cases and non-brucellosis febrile patients were 0.975 and 0.931 for ELISA IgG and IgM, respectively (Figure 4). All of the areas in figures 3, 4 were significantly different from 0.5 (P<0001).

The results of sensitivity and specificity ELISA IgM and ELISA IgG, positive predictive value (PPV) and negative predictive value (NPV) in different cut-off values have shown in table.1.

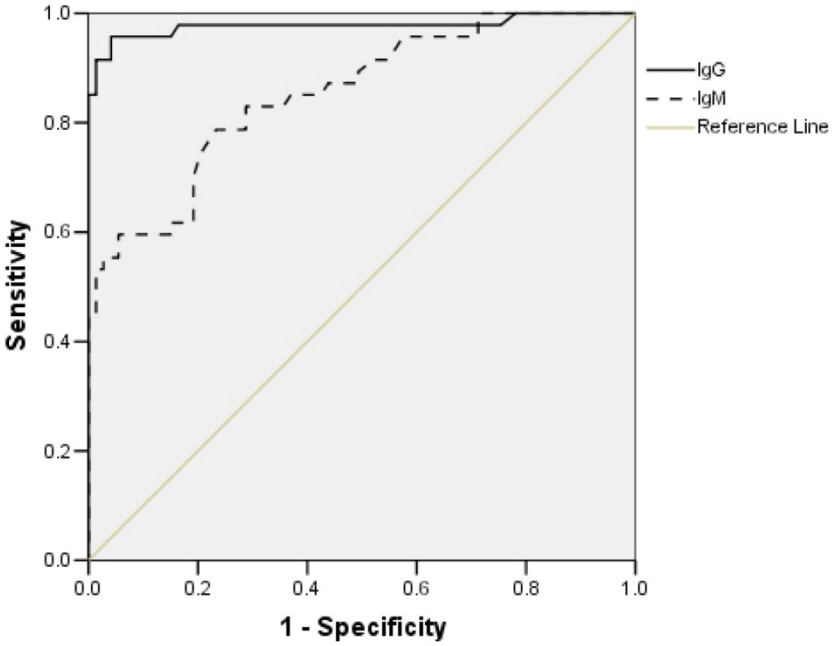
Table 1. Diagnostic Performance of serum IgG and IgM in detecting of brucellosis.

	Cut-offs points (IU/ml)	Sensitivity (95% CI)	Specificity (95% CI)	Positive Predictive Value	Negative Predictive Value
IgG					
	10	92.9 (81.9-97.7)	92.1 (85.5-95.9)	0.83	0.98
	25	87.5 (75.3-94.4)	96.8 (91.6-99.0)	0.93	0.95
	50	75.0 (61.4-85.2)	100 (96.3-100)	1	0.90
	75	69.6 (55.7-80.8)	100 (96.3-100)	1	0.88
IgM					
	10	17.7 (12.1-25.0)	84.0 (63.1-94.7)	0.87	0.15
	25	46.8 (32.4-61.8)	99.2 (65.0-100)	0.96	0.83
	50	46.8 (32.4-61.8)	99.2 (65.0-100)	0.96	0.83
	75	44.7 (30.5-59.8)	100 (96.3-100)	1	0.83



(Brucellosis vs. healthy subjects)

Figure 3. Receiver-operator characteristic (ROC) curve for diagnosis of brucellosis by serum IgG and IgM. The areas under the curves were very similar: 0.975 for the IgG and 0.931 for IgM.



(Brucellosis vs. non-brucellosis febrile patients)

Figure 4. Receiver-operator characteristic (ROC) curve for diagnosis of brucellosis by serum IgG and IgM. The areas under the curves were similar: 0.978 for the IgG and 0.854 for IgM.

Discussion

Brucellosis is known as a prevalent infectious disease in Middle East region (12). In Iran the incidence of brucellosis has increased in recent years and has been associated with a numerous economic and health problems (13). Because of delay in diagnosis, some patients with brucellosis refer to hospital with longstanding disease and some complications. Clinicians are interested to find a reliable diagnostic method for

brucellosis for early and correct diagnosis. Furthermore, it had better use more than one serologic test for diagnosis of brucellosis especially in chronic and complicated cases.

IgM and IgG anti-brucella antibodies could be easily detected by ELISA method and this helps to determine the stage and activity of the disease (14-16). The results of ELISA may be positive when other tests are negative. There are regional differences in the prevalence of antibodies to *Brucella* in countries in which the disease is endemic. So it is necessary to establish a "normal range" for healthy people in high incidence area (17). The lack of definite cut-off value is main problem with widespread use of ELISA for diagnosis of brucellosis.

In this present study we determined the appropriate cut-off value for ELISA test in Iran. We used two control groups (healthy controls and non-brucellosis febrile controls) in order to increase accuracy of results. Base on results of this study, we found a significant difference of mean levels of IgM and IgG between patients and controls. Box plot graph showed the high degree of dispersion of IgM and IgG data in patients compared with all controls (Figures 1 and 2). It can prove the high sensitivity of ELISA test for diagnosis of brucellosis in our patients. Furthermore, the presence of partially overlapping of IgM data (not in case of IgG) within the interquartile range confirms that ELISA IgG is more reliable than ELISA IgM for diagnosis of brucellosis. This is compatible with results of another study which showed high sensitivity of ELISA IgG and low sensitivity ELISA IgM in diagnosis of brucellosis (17).

To determine the optimal cut-off point for ELISA results, ROC curve was drown and the IgM and IgG levels yielding maximal sensitivity and maximal specificity were selected.

It is observed that the areas under ROC curve for distinguishing between cases and controls were significantly different from 0.5 (P<0001) for ELISA IgG and IgM (Figures 3, 4). Furthermore, these areas were larger for IgG compared with IgM. These findings demonstrate that ELISA is useful test for discrimination between cases and controls and in compared to ELISA IgM, the ELISA IgG has more accuracy in diagnosis of brucellosis. These results are more promising than those obtained in earlier studies. As brucellosis is endemic in Iran, low titers of ELISA IgM and ELISA IgG may be reported in healthy people and non-brucellosis patients. We chose cut-offs 10, 25, 50 and 75 arbitrarily. After calculation of sensitivity and specificity ELISA IgG and IgM with above cut-off values, maximal sensitivity (92.9 %) and maximal specificity (100%) for ELISA IgG were observed by cut-offs of 10 IU/ml and 50 IU/ml, respectively (Table 1).

The results of our study showed that ELISA IgG is more reliable test than ELISA IgM in diagnosis of brucellosis. Using a cut-offs of 10 IU/ml and 50 IU/ml has the most sensitivity (92.9%) and most specificity (100%) for ELISA IgG test, respectively. Considering the optimal cut-off values, application of ELISA IgG could be helpful in diagnosis of human brucellosis.

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