RESEARCH ARTICLE

Chinese Journal of Medical Research
Chinese J Med Res 2019; 2(2): 23-26

ISSN(e): 2618-091X
ISSN(p): 2663-8053

Designation of cut-off point rate for ELISA test in Diagnosis of Human Brucellosis

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Received: April 10, 2019; **Accepted:** June 02, 2019

Abstract

Brucellosis a world-wide disease having diverse clinical manifestations, affecting hundreds of individuals annually. Its prevalence is different in different countries and even in different regions of the same country. It can be diagnosed by different methods each one having different sensitivity and specificity and even it depends on the laboratory facilities of the concern area. Although it is usually screened by Slide Agglutination Test (SAT) and blood culture techniques, but these may produce false positive results and having extended time for test results respectively. A total of 82 patients were selected over a one-year period between March 2014 and April 2015. All patients' sera were processed for culture using BACTEC 9240 system followed by testing by ELISA. Among 82 suspected patients, only 30 (%) were positive for culture and remaining 52 (%) were negative. While ELISA IgM and IgG were 15% and 22% respectively among culture positive samples. ROC curve showed the behavior of the sensitivity and specificity of ELISA IgG and IgM by using different cut-off points. In patients with brucellosis, the mean of serum IgG was greater than IgM, significantly ($P<0.001$). ELISA IgG test was more reliable than ELISA IgM test in diagnosis of brucellosis in Iran.

Keywords: Brucellosis, ELISA IgG, ELISA IgM, Cut-off point.

INTRODUCTION

Brucellosis is a contagious bacterial disease of livestock caused by the facultative intracellular pathogen Brucella [1]. Among the Brucella spp., B. *abortus, B. canis, B. melitensis,* and *B. suis* can cause human brucellosis [1]. It is a livestock disease responsible for fetal loss due to abortions [2-4]. Worldwide, this disease has profound economic and social impact by reducing the ability of livestock producers to provide an adequate supply of disease-free meat and dairy products [5, 6]. This disease is also a true zoonosis and is actually the most common zoonotic disease worldwide causing debilitating and sometimes chronic disease in humans [7]. Human brucellosis manifests in acute form as varying, nonspecific symptoms such as undulating fever, malaise and joint pain [7]. Treatment failure, which occurs in 5–15% of cases, can result in chronic infection characterized by severe complications of the nervous system, musculoskeletal system and the heart [8, 9]. Diagnosis of brucellosis is performed by compatible clinical features and results of laboratory methods including blood culture and serologic tests [9]. 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 [10-12]. Several conventional serologic assays have been used for the diagnosis of brucellosis [12-15]. The most commonly employed method for antibody detection is standard agglutination test (SAT) [15, 16]. It is a subjective method and reporting the antibody titer could be operator dependent [16]. 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 [14-16]. The enzyme linked immunosorbent assay (ELISA) is known as a sensitive and rapid method for diagnosis of brucellosis [16]. Detection of specific immunoglobulin by a single, simple and rapid test is a

major advantage with ELISA. 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 [11-16]. ELISA can determine specific class of IgG, IgM and IgA antibodies against brucella [15, 16]. The assay is a sensitive, simple and rapid test with less limitation, and might be an acceptable alternative to SAT [15-17].

The objective of the present study was to determine an optimal cut-off point, for ELISA which would offer maximum sensitivity and specificity for the test when compared to blood culture.

MATERIALS AND METHODS

Between March 2014 and April 2015, 82 suspected cases of brucellosis were collected from people who referred to Sina and Shahid Sadoughi laboratories, Yazd, Iran. This was an experimental and cross-sectional study. The laboratory diagnosis of brucellosis was performed by blood isolation (5cc) of Brucella organism with a BACTEC 9240 system and finally, detecting Brucella IgM and IgG antibodies by ELISA test (Immuno Biological Laboratories Company, Germany). 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. The study was approved by the Ethics Committee of the Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

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 showed the behavior of the sensitivity and specificity of ELISA IgG and IgM by using different cut-off points. Tests for significance were based on the Chi-square statistics, with a significance level of $P<0.05$ chosen a priority.

RESULTS

In this study, we considered 82 suspected patients to brucellosis who referred to Sina and Shahid Sadoughi laboratories, Yazd, Iran based on blood culture results, ELISA IgG and IgM. 57.3% of the patients were male and 42.7% were females. The mean age of the patients was 38.6 ± 12.97. Mean±SD (standard deviation) of ELISA IgG, IgM were 0.59 ± 0.47 and 0.78 ± 0.49, respectively. From the 82 suspected patients of brucellosis, culture results were positive in 30 (36.6%) cases and negative in 52 (63.4%).

In interpretation of ELISA IgG, IgM results rates < 0.8, 0.8 – 1.2 and > 1.2 were considered negative, intermediate and positive, respectively. From the 82 suspected cases, ELISA IgG results were negative, intermediate and positive in 55 (67.1%), 14 (17.1%) and 13 (15.9%) cases, respectively. Also, ELISA IgM results were negative, intermediate and positive in 52 (63.4%), 8 (9.8%) and 22 (26.8%) cases, respectively.

There was a significant relationship between blood culture and ELISA IgG results in the suspected patients to brucellosis ($P\leq 0.001$) (Table 1).

Table 1: Frequency distribution of ELISA IgG results based on blood culture results in the suspected patients to brucellosis

Results		ELISA IgG			P-value
		Positive N (%)	**Intermediate N (%)**	**Negative N (%)**	
Blood culture	**Positive (n=30)**	13 (43.3)	14 (46.7)	3 (10)	≤ 0.001
	Negative (n=52)	0 (0)	0 (0)	52 (100)	
	Total (n=82)	13 (15.9)	14 (17.1)	55 (67)	

Table 2: Frequency distribution of ELISA IgM results based on blood culture results in the suspected patients to brucellosis

Results		ELISA IgM			P-value
		Positive N (%)	**Intermediate N (%)**	**Negative N (%)**	
Blood culture	**Positive (n=30)**	22 (73.3)	8 (26.7)	0 (0)	≤ 0.001
	Negative (n=52)	0 (0)	0 (0)	52 (100)	
	Total (n=82)	22 (26.9)	8 (9.7)	52 (63.4)	

Also, it was observed a significant relationship between blood culture and ELISA IgM results in the suspected patients to brucellosis (P≤ 0.001) (Table 2).

The age mean of patients with positive and negative blood culture were 39.07 ± 14.87 and 38.33±11.88 (Mean±SD), respectively. The mean of serum level of Immunoglobulins in the patients with positive and negative blood culture were 1.11 ± 0.40, 0.269 ± 0.137 for IgG and 1.34 ± 0.376, 0.466 ± 0.169 for IgM. There was no a significant relationship between age and blood culture results (P=0.073) but was observed a significant relationship between IgG and IgM means with blood culture results (P≤ 0.001), (P≤ 0.024), respectively.

The results of sensitivity and specificity of ELISA IgM and ELISA IgG, positive predictive value (PPV) and negative predictive value (NPV) in different cut-off values have shown in table 3.

Table 3: The results of sensitivity and specificity of ELISA IgM and ELISA IgG, PPV and NPV in four cut-off values

	cut-off points (IU/ml)	sensitivity	specificity	Positive predictive value	Negative predictive value
ELISA IgM	10	67.7	96.8	0.87	0.38
	25	65.5	97.2	0.94	0.84
	50	59.4	96.3	0.96	0.88
	75	55.6	100	1	0.90
ELISA IgG	10	94.8	90.2	0.85	0.89
	25	89.5	92.3	0.91	0.92
	50	80.0	100	0.98	0.91
	75	74.9	95	1	0.89

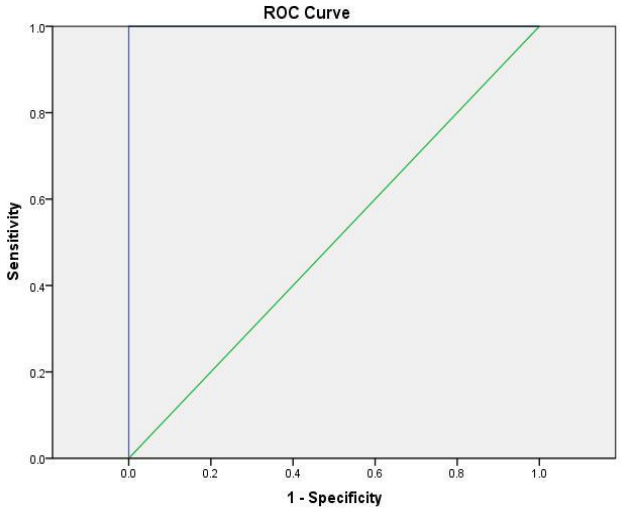

Fig 1: ROC curve (sensitivity and specificity) in ELISA IgM test

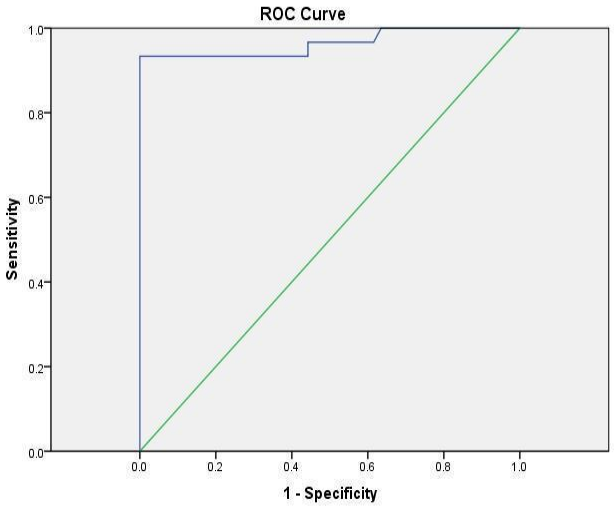

Fig 2: ROC curve (sensitivity and specificity) in ELISA IgG test

Receiver-operator characteristic (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 curves for ELISA IgM and ELISA IgG were 0.931 and 0.975, respectively (Figures 1& 2).

DISCUSSION

Brucellosis affects about 500000 individuals annually worldwide and is caused by gram-negative bacteria, Brucella spp [1-5]. Among the Brucella spp., *B. abortus, B. canis, B. melitensis*, and *B. suis* can cause human brucellosis [5, 6]. It is a common infectious disease and important public health challenge in Iran [9]. Its seroprevalence in Iran is 1-2 % [9]. 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. Clinicians are interested to find a reliable diagnostic method for brucellosis for early and correct diagnosis [10-12]. Furthermore, it has better use more than one serologic test for diagnosis of brucellosis especially in chronic and complicated cases [13, 15].

Present study showed a significant relationship between blood culture and ELISA IgG results in the suspected patients to brucellosis (P≤ 0.001). Also, it was observed a significant relationship between blood culture and ELISA IgM results in the suspected patients to brucellosis (P≤ 0.001). There was no a significant relationship between age and blood culture results (P=0.073) but was observed a significant relationship between

IgG and IgM means with blood culture results (P≤ 0.001), (P≤ 0.024), respectively.

In a study, in patients with brucellosis, the mean of serum IgG and IgM were greater than the other groups, significantly (P<0.001). Also, cut-offs of 10 IU/ml and 50 IU/ml have the most sensitivity (92.9%) and most specificity (100%) for ELISA IgG test, respectively [18]. It is similar to present study that in it the most sensitivity (94.8%) and most specificity (100%) for ELISA IgG test were related to cut-offs of 10 IU/ml and 50 IU/ml, respectively.

Another study showed that the best cut-off point of ELISA-IgG is 53 IU/ml, which yields the maximal sensitivity and specificity to diagnose acute brucellosis. At this cut off, the sensitivity, specificity, PPV, NPV, positive likelihood ratio, and negative likelihood ratio are 84.09%, 85.38%, 62.20, 94.90, 5.75, and 0.18, respectively [19].

A study compared SAT and ELISA-IgG in 56 brucellosis patients with a control group consisting of healthy individuals and patients with febrile illnesses other than brucellosis, and shwed that at the IgG level of 50 IU/ml, the sensitivity and specificity were 75 and 100%, respectively. At IgG level of 10 IU/ml the sensitivity and specificity were 92.9% and 92.1%, respectively [20].

CONCLUSION

The results of our study showed that ELISA IgG is better than ELISA IgM in diagnosis of brucellosis. Using a cut-off of 10 IU/ml and 50 IU/ml has the most sensitivity (94.8%) and most specificity (100%) for ELISA IgG test, respectively. Considering the optimal cut-off values, application of ELISA IgG could be the good way in diagnosis of human brucellosis.

Acknowledgement

The authors wish to thank the staff of Infectious Diseases Research Center of Shahid Sadoughi University of Medical Sciences, Yazd.

Conflict of Interest

We declare that we have no conflict of interest.

Financial Support

None declared.

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