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Comparison of PCR, Wright Agglutination Test and Blood Culture for Diagnosis of Brucellosis in Suspected Patients

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Abstract: Brucellosis has long been prevalent in Iran, with considerable medical and economic importance. Timely diagnosis is needed for early management and effective prevention of its consequences in human beings and animals. Current diagnostic methods impose peculiar challenges in terms of analytical method performance. This study compares diagnostic sensitivity, specificity, predictive Value of Positive (PVP) and Predictive Value of Negative (PVN) for Polymerase Chain Reaction (PCR), Wright agglutination test and blood culture used for patients suspected of brucellosis. In 120 patients clinically suspected of brucellosis and referred by physicians to the Yazd central Medical Laboratory, some relevant demographic, occupational, nutritional and clinical data were collected. Also, venous blood samples were drawn for diagnosis of brucellosis using PCR, Wright agglutination test and blood culture techniques. The most frequent symptom of patients was arthralgia (82 cases, 68.3%). PCR was positive in 25 cases (20.8%), wright test in 21 patients (17.5%) and blood culture in 6 cases (5%). In 20 out of 21 wright-positive cases, PCR was positive and all of the culture-positive patients had positive PCR. Sensitivity, specificity, PVP and PVN of blood culture compared to PCR (as the gold standard test) were 24, 100, 100 and 86%, respectively, but the above parameters when PCR is compared with blood culture (as gold standard) were 100, 83, 24 and 95%, respectively. PCR has better analytical performances than blood culture for diagnosis of brucellosis and is suitable for confirmation of Wright-positive cases.

Key words: Brucellosis, diagnosis, Wright agglutination test, PCR, blood culture

INTRODUCTION

Brucellosis (undulant fever) is an emerging zoonotic disease caused by bacteria of the genus *Brucella*, which exists in various animals and the humans are mainly accidentally infected through contact with those animals or by consumption of contaminated dairy products. Each of the *Brucella* species is more prevalent in some parts of the world. Human brucellosis is now frequently reported from Russia, Africa, India, Europe and America. According to the world health organization, its annual incidence is about 500000, the *B. melitensis* being the most prevalent species (Seleem *et al.*, 2010; Swai and Schoonman, 2012).

Brucellosis has long been prevalent in Iran, with considerable medical and economic importance (Alavi and Alavi, 2010). Timely diagnosis is needed for early management and effective prevention of its consequences in human beings and animals. Current diagnostic methods impose peculiar challenges in terms of analytical method performance. Routine laboratory tests for diagnosis of brucellosis include culture, Polymerase Chain Reaction (PCR) and serologic tests based on detection of anti-*Brucella* antibodies, the most prevalent of them being wright agglutination test. However, definite diagnosis of brucellosis is traditionally based on culture of blood, bone marrow, tissues or body fluids like joint fluid, cerebrospinal fluid, urine, wound, pus, etc. Cultures do not have high sensitivity (around 40-70%) and require long incubation up to 6 weeks, although it is regarded by many as the gold standard (Christopher *et al.*, 2010).

The objective of the study was to compare diagnostic sensitivity, specificity, Predictive Value of Positive (PVP) and Predictive Value of Negative (PVN) for PCR, wright agglutination test and blood culture used for patients suspected of brucellosis. Clinical signs and symptoms of patients are also described.

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MATERIALS AND METHODS

Study area and sample population: In this study, 120 patients clinically suspected of brucellosis and referred by physicians to the Yazd Central Medical Laboratory were enrolled. Yazd province is a central region in Iran with endemic brucellosis (Ghilian *et al*., 2011). A physician collected some relevant demographic, occupational, nutritional and clinical data.

Tests procedure: Fifteen milliliter of venous blood were drawn for diagnosis of brucellosis using PCR, serologic test (both Rose Bengal test and rapid Wright agglutination test, followed by titration in positive cases) and blood culture (on biphasic Castaneda medium), carried out in accordance with the relevant brochures and also previous described diagnostic tests techniques (WHO, 2006; Godfroid *et al*., 2010; Yu and Nielsen, 2010). We tried to draw samples at the time of fever, but if impossible, a longer (more than the usual 28 days) incubation was applied for blood culture. A blind subculture at each week was done, using *Brucella* agar medium containing sheep blood. Grown bacteria were attested with oxidase test, urease test and Gram staining. For the PCR test, EDTA-blood was kept at -20°C until the time of White Blood Cell (WBC) lysis using Miller technique, in which 0.5 mL of sodium citrate is mixed with 1 mL of ready solution containing 230 mM of sucrose, 5 mM of $MgCl_2$, 1% Triton X-100 and 10 mM of Tris-HCl at pH of 7.5. The blood-lysate mixture was centrifuged at 1500 g for 2 min, supernatant discarded and the sediment washed 2 times with 1 mL of Milli Q water. When WBCs were devoid of the red color of erythrocytes, DNA extraction was performed by a certified PCR kit (Pouya Zist Tech, Iran). The primers used were B4 (5-TGG CTC GGT TGC CAA TAT CAA-3) and B5 (5- CGC GCT TGC CTT TCA GGT CTG-3) which amplify a 223 bp fragment on a gene that encodes a 31 kDa *B. abortus* antigen (MWG-biotech, Germany), as attested previously.

Statistical analyses: Data were analyzed using SPSS software ver. 16 and McNemar's test. $p<0.05$ was considered as the level of statistical significance. The following formulas were used:

$$\text{Sensitivity} = \frac{\text{True positive}}{\text{True positive+false negative}}$$

$$\text{Specificity} = \frac{\text{True negative}}{\text{True negative+false positive}}$$

$$\text{PVP} = \frac{\text{True positive}}{\text{True positive+false positive}}$$

$$\text{PVN} = \frac{\text{True negative}}{\text{True negative+false negative}}$$

RESULTS

The most frequent symptom of patients was arthralgia (82 cases, 68.3%, Table 1). There was no significant difference between the sexes and two age groups (age<35, age = 35) regarding the positivity rate) (Table 2 and 3). PCR was positive in 25 cases (20.8%), Wright test in 21 patients (17.5%) and blood culture in 6 cases (5%). In 20 out of 21 wright-positive cases, PCR was positive and all of the culture-positive patients had positive PCR (Table 4). Comparison of Wright test, PCR and culture in terms of sensitivity, specificity, PVP and PVN are summarized in Table 5.

DISCUSSION

Clinical manifestations and complications of patients in present study is in accordance with previous reports (Alavi and Alavi, 2010; Buzgan *et al*., 2010).

In our study, the sensitivity, specificity, PVP and PVN of blood culture compared to PCR (as the gold

Table 1: Symptoms of the cases according to the result of PCR

	PCR positive		PCR negative	
Symptom (p-value = 0.140)	No.	%	No.	%
Fever	7	28	22	23.2
Arthralgia	14	56	68	71.6
Both of them	4	16	5	5.3
Total	25		95	

Table 2: Results of PCR, culture and serologic tests in patients according to age

Result if PCR is considered as gold standard (p-value = 0.293)	Age<35		Age≥35	
	No.	%	No.	%
Positive	14	25	11	17.2
Negative	42	75	53	82.8
Total	56		64	

Table 3: Results of PCR, culture and serologic tests in patients according to sex

Result if PCR is considered as gold standard (p-value = 0.181)	Male		Female	
	No.	%	No.	%
Positive	11	16.4	14	26.4
Negative	56	83.6	39	73.6
Total	67		53	

Table 4: Findings by 3 diagnostic methods

		PCR		Culture		Wright	
		+	-	+	-	+	-
PCR	+	-	-	6	19	20	5
	-	-	-	0	95	1	94
Culture	+	6	0	-	-	6	0
	-	19	95	-	-	15	99
Wright	+	20	1	6	15	-	-
	-	5	94	0	99	-	-

Table 5: Comparison of wright test, PCR and culture in terms of sensitivity, specificity, PVP and PVN

	If PCR is considered as gold standard (%)	If culture is considered as gold standard (%)
Sen. of wright	80	100
Spec. of wright	98.9	86.8
PVP of wright	95.23	28.57
PVN of wright	94.94	100
Sen. of culture	24	-
Spec. of culture	100	-
PVP of culture	100	-
PVN of culture	83.33	-
Sen. of PCR	-	100
Spec. of PCR	-	83.3
PVP of PCR	-	24
PVN of PCR	-	100

standard test) were 24, 100, 100 and 86%, respectively, but the above parameters when PCR is compared with blood culture (as gold standard) were 100, 83, 24 and 95%, respectively, which are very close to those of Kazemi *et al.* (2008).

In a recent study from Yazd, Iran, 19 (6.3%) out of 300 blood donors had a Wright agglutination tube test titer of 1/80 or higher (5.7% of men and 4.3% of women). There were 17 cases (5.7%) positive for *Brucella* IgG antibody by enzyme-linked immunosorbent assay (ELISA). The sensitivity, specificity, PVP and PVN for ELISA when compared with Wright agglutination test were 5.3, 94.7, 6.3 and 93.7%, respectively. The authors concluded that if a 1/80 titer in the Wright agglutination tube test is considered as diagnostic for brucellosis, the frequency of the disease in blood donors (which are generally a healthy population) is not negligible (Ghilian *et al.*, 2011).

Maleknejad *et al.* (2007) studied 102 brucellosis-suspected patients by PCR and culture with blind subculture of all culture-negative cases at 7, 14, 21 and 28 days, in whom 41 cases (40.2%) had bacteremia. They stated that the BACTEC 9120 system was able to detect all of the traditional culture-positive cases.

There have been many studies on the diagnostic methods for brucellosis, but comparison of the routine tests in terms of sensitivity, specificity, PVP and PVN are rare, likely because various settings cause different rates of positivity in each test and also due to the old belief that culture should be considered as the reference method or gold standard in every bacterial infection. PVP and PVN are mostly interested by clinicians who want to decide upon reliability of a lab result and are met by our study which shows that a negative *Brucella* PCR result is much more dependable than a positive result, but the inverse is true for blood culture of *Brucella*. We believe that the PVP and PVN of a Wright test (95.23 and 94.94%, respectively) are better evaluated when contrasted with PCR as a gold standard and accordingly, a positive or negative Wright test is equally and highly reliable.

In a study on 263 patients with brucellosis in Kuwait it was found that 89 cases had positive result in all of the blood culture, Wright agglutination tube test and PCR; 110 cases had positive Wright agglutination tube test and negative blood culture, from which 104 cases showed positive PCR. They concluded that Wright agglutination tube test has some false positive results, or it may be that PCR failed to diagnose brucellosis in 6 cases (Al-Nakkas *et al.*, 2005). However, without assuming a method as gold standard, one cannot determine the sensitivity or specificity of other tests.

Another study on 17 acute brucellosis patients showed that all blood samples contain high titers of anti-*Brucella* antibodies, but PCR was positive in 14 and blood culture in only 8 cases. In the 33 individuals in the healthy control group only one had a high anti-*Brucella* antibody titer. The authors suggested that PCR is a useful diagnostic test for brucellosis due to its high speed, sensitivity and specificity, especially as a complementary test to serologic tests and culture in those who do not have characteristic clinical findings (Al-Attas *et al.*, 2000).

A study done by Navarro *et al.* (2004) concluded that PCR is the best method for diagnosis of PCR because serologic tests and culture have low sensitivity and specificity. However, they mentioned that sensitivity and specificity of different PCR kits used in laboratories vary and no standardization is yet achieved regarding sample preparation, target genes and procedure. They tried to introduce a suitable PCR method.

Morata *et al.* (2001) evaluated 34 human brucellosis cases, in whom 33 (97%) had positive PCR test in non-blood specimens, but only 29.4% showed positive culture. They found that 11.4% of the patients have negative serologic test or low antibody titer. So, the authors recommend PCR as a helpful detecting method for brucellosis based on its high sensitivity accompanied by high speed and low contamination risk.

CONCLUSION

Since the PCR had the highest detection rate, is much more rapid than culture (hours vs. weeks) and now is easily available in almost every modern laboratory, we suggest that peripheral blood PCR should be considered as the gold standard for diagnosis of brucellosis, both as confirmation of diagnosis in suspected cases and also for epidemiologic surveillance studies, if the costs could be justified. In conclusion, PCR has better analytical performances than blood culture for diagnosis of brucellosis and is suitable for confirmation of wright-positive cases.

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