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Comparing of Routine 2 Mercaptoethanol (2ME) and Coombs Wright Plus 2ME

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Abstract: Problem statement: Serologic tests like Wright, Wright containing Anti-human globulin (Coombs Wright) and 2ME are the main methods of diagnosing brucellosis. The routine method of using Wright test and then performing 2ME is not enough sensitive to diagnose brucellosis. The goal of this study is to compare the results of routine 2ME with 2ME on serum containing antihuman globulin (Coombs Wright+2ME). **Approach:** In this study 100 patients with brucellosis were evaluated. The serums of these patients were tested using routine 2ME and Coombs Wright with adding 2ME. Then the results of these tests were compared. Sensitivity and Specificity of these two methods were also calculated. **Results:** The sensitivity of routine 2ME was 52%. The sensitivity of 2ME Plus Coombs Wright was calculated as 97%. Sensitivity and Specificity of routine 2ME method against Coombs Wright plus 2ME method were respectively 53% (54-51: CI) and 75% (95-31: CI). **Conclusion:** According to the results, Coombs Wright plus 2ME can be used for negative 2ME test patients in order to follow up their response to treatment. In addition, it is not necessary to do Wright test and routine 2ME and instead of them, Coombs Wright plus 2ME can be used.

Key words: Brucellosis, wright test, coombs test, 2-Mercaptoethanol (2ME), susceptible patients

INTRODUCTION

Brucellosis is one of zoonoses which are still highly prevalent in Iran (Hatami *et al*., 2010; Roushan *et al*., 2004; Pappas *et al*., 2005; Moradi *et al*., 2006). According to WHO report, the number of diagnosed and reported patients may be 10 to 25 times fewer than real number of infected people in the society; one of the main reasons may be the difficulty of diagnosing the disease and especially chronic brucellosis (Wise, 1980; Roth *et al*., 2003).

The only precise method of diagnosis is culture of brucella Spp; however the sensitivity of the culture is related to accuracy of the laboratory and other conditions. The results of positive culture vary from 15-90% (Wise, 1980; Gotuzzo *et al*., 1986; Yagupsky, 1999; Memish *et al*., 2000; Roushan *et al*., 2004; Pappas *et al*., 2005) and of course it is not always possible to culture blood. Recently, PCR methods are developed but they are not accepted as the routine method, hence serologic tests like Wright and Coombs Wright are the most practical methods (Young, 1991; Serra and Vinas, 2004; Yu and Nielsen, 2010). Sensitivity and specificity of Wright test are different (Serra and Vinas, 2004; Surucuoglu *et al*., 2009; Yu and Nielsen, 2010). As sometimes their result is false negative (Bettelheim *et al*., 1983; Surucuoglu *et al*., 2009), negative Wright test can not reject the probability of brucellosis in endemic regions (Serra and Vinas, 2004).

After a positive Wright, 2-Mercaptoethanol (2ME) is used as a complementary test in order to distinct Active brucellosis from non active brucellosis and to detect previous contacts with brucellosis Antigen and for follow up of treatment. However in patients with negative Wright test we cannot perform 2ME test. In such situation Coombs test that contains anti-human globulin is suitable (Coombs Wright plus 2ME), as it reduces the number of false negative results (Bettelheim *et al*., 1983; Dahouk *et al*., 2003; Mohsenpour *et al*., 2011). Therefore, it seems using 2ME test together with Coombs test can be more accurate in confirming active chronic brucellosis than 2ME with Wright test. Nevertheless, the sensitivity and specificity of this test in people with positive Coombs test are unknown and there is no study regarding this subject.

Because of high prevalence of brucellosis in Iran and the importance of quick diagnosis and treatment of this disease and in order to follow up treatment responses, it is necessary to develop an especial, sensitive and accessible laboratory method (Serra and

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Vinas, 2004). Thus, this study was conducted in order to compare the results of routine 2ME test in positive Wright test patients with Coombs Wright plus 2ME.

MATERIALS AND METHODS

This was a cross sectional, prospective study. Sample Population included brucellosis patients who referred to infections ward in Tohid Hospital in Sanandaj. One hundred patients were involved in the study through convenience sampling.

The brucellosis diagnosis was based on clinical futures, high titres of antibrucella antibodies. Titres >=1/160 or a fourfold or greater increase in the initial titres in two paired serum samples drawn 2-3 weeks for Wright's or >=1/40 for Coomb's antibrucella test and for Coombs Wright plus 2ME were defined as positive. All serologic tests including Wright test, Coombs Wright test, routine 2ME test and Coombs Wright plus 2ME were done for all patients. Data were gathered from documents and were recorded in questionnaires.

The sensitivity and specificity of routine 2ME test (standard) and Coombs Wright plus 2ME test were calculated in patients with negative Wright and positive Coombs. The results of Coombs test were considered as the gold standard.

After collecting data, they were analyzed using SPSS 11.5 software. Then sensitivity, Specificity, Positive and Negative Predictive value was calculated. The confidence intervals of indicators were calculated as well. The Mann-Whitney U test was used for comparing median in two groups.

The calculations were done as it is shown below Table 1.

Table 1: Diagnostic indicators formula

		Coombs Wright plus 2ME		
		Negative	Positive	Total
Routine 2ME	Positive	A	B	a + b
	Negative	C	D	c + d
	Total	a + c	b + d	a + b + c + d

a: True positive (TP); b: False Positive (FP) ، c : False Negative (FN) ، d: True Negative(TN) Sensitivity = a/ (a + c), Specificity = d/ (b + d) Positive Predictive Value (PPV) = a/ (a + b) Negative Predictive Value (NPV) = d/(c + d)

Table 2: Evaluation of Routine 2ME test and Coombs Wright plus 2ME test as the gold standard

		Coombs Wright plus 2ME	
		Positive	Negative
Routine 2ME	Positive	51	1
	Negative	45	3

Sensitivity = 53% ($CI_{95\%}$: 51-54) Specificity = 75% ($CI_{95\%}$: 31-95) Positive Predictive Value (PPV) = 98% ($CI_{95\%}$: 95-99) Negative Predictive Value (NPV) = 0.06% ($CI_{95\%}$:0.03-0.08)

Table 3: Comparing median of Coombs, routine 2ME and Coombs Wright plus 2ME

Variables	Wright positive (n = 52)	Wright negative (n = 48)	P-value*
Human globulin Serum test (Coombs')	1/320 (1/40-1/320) †	1/80 (1/20-1/320)	<0.001
Routine 2ME	1/160 (1/40-1/160)	1/9 (1/9-1/20)	<0.001
Coombs Wright plus 2ME	1/320 (1/30-1/320)	1/60 (1/20-1/320)	<0.001

†: Median (Minimum-Maximum) *: Mann-Whitney U test was used

RESULTS

From 100 patients 40 (40%) were male and 60 (60%) were female. The average of the patient's age was 46.1 (±11). The median of patient's Coombs titre was 1/160 (minimum of 1/40 and maximum of 1/320). All 100 brucellosis infected patients had positive Coombs test. 48 people (48%) had negative Wright test and 52 patients (52%) had positive Wright test. The result of routine 2ME test was negative in 48 persons (48%) and positive in 52 persons (52%). Thus, sensitivity of routine 2ME was calculated as 52%. 2ME test was done in specimen that Coombs Wright was done on it and 97% of the cases were positive and 3% were negative.

Based on these results, the sensitivity of Coombs Wright plus 2ME was done on it was calculated as 97%. Sensitivity and specificity of routine 2ME and Coombs Wright plus 2ME were respectively 53% (CI: 51-54) and 75% (CI: 31-95).

In addition n, the positive and negative predictive values were respectively 98% (CI: 95-99) and 0.06% (CI: 0.03-0.08) (Table 2).

Comparing routine 2ME and Coombs Wright plus 2ME, there was a significant statistical difference in Coombs titer ($p<0.001$) and all titers in people with positive Wright were higher (Table 3). 23 people (57.5%) among males and 29 people (48.3%) among females were positive in Routine 2ME tests and there was no significant difference.

DISCUSSION

The goal of this study was to compare the results of routine 2ME and Coombs Wright plus 2ME, in order to assess and evaluate the diagnostic value of these methods and to follow up the medical response of patients with negative Wright. The hypothesis of the study was that the results of Coombs Wright plus 2ME are more valuable and favorable than the results of routine 2ME test. The sensitivity of Coombs Wright plus 2ME for diagnosing brucellosis was 97%, while it was only 52% in routine 2ME test. Furthermore,

Coombs Wright plus 2ME test had higher sensitivity and specificity comparing with routine 2ME which had 47% of false negative. It seems it is not necessary to do Wright test and routine 2ME test in suspected patients. Routine 2ME had high positive predictive value, but it had low negative predictive value, so its positive result is valuable in diagnosing brucellosis while its negative result dose not role out the infection. Therefore, in susceptible patients, Coombs test can be used as a first step and then Coombs Wright plus 2ME can be applied. In addition, Coombs Wright plus 2ME can be used for following the medical response in people with negative Wright test. In this study it was not possible to assess the specificity of 2ME in both methods because healthy people were not involved in the study.

Serra and Vinas (2004) study the sensitivity of Coombs test in acute patients and previously infected people was reported as 100%, whereas Wright test had a sensitivity of 67%. They concluded in endemic regions and among people with chronic disease, Wright test is not appropriate because it demonstrates lots of patients as negative. The sensitivity of Wright test is low because in a long lasting disease IgG3 and IgG4 increases and it do not have the agglutination capability or it is blocked by other antibodies. Therefore it is not diagnosed by Wright test and routine 2ME can not be used. Whereas, based on the results of this study this problem can be solved via performing Coombs Wright plus 2ME. Coombs test has several advantages: first of all, it differentiates acute brucellosis from chronic cases and it solves the problem of blocking antibodies (Hall and Manion, 1953). Orduna *et al*. (2000) study Coombs test had more sensitivity and less specificity than Otero *et al*. (1982) defined modified Coombs test as valuable in diagnosing patients with low titer in Wright test. Besides, this method may be more preferable than Eliza method as in Gomez *et al*. (2008) study all patients had positive Wright and Coombs test results while Eliza test was not able to diagnose some patients.

One of the limitations of this study was that the specificity of Coombs Wright plus 2ME was not defined because only brucellosis infected people were assessed, so it is suggested to do similar studies involving all brucellosis susceptible patients in order to assess the sensitivity and specificity of this test again.

CONCLUSION

According to the results, it is not necessary to perform Wright and routine 2ME test for diagnosis of brucellosis and instead of those tests, Coombs test and then anti-human globulin serum 2ME test can be sued (Coombs Wright plus 2ME). In addition, Coombs Wright plus 2ME may be sued for following up the medical response in people with negative Wright test.

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