Acta Medica Alanya

RESEARCH ARTICLE

ARAŞTIRMA

Acta Medica Alanya

2020;4(1): 56-60.

DOI:10.30565/medalanya.646672

A comparison of a new diagnostic test of the human Brucellosis, the Brucella Coombs Gel Test, with other methods

Brusellozun Tanısında Yeni Bir Metot Olan Brucella Coombs Gel Testin Diğer Yöntemlerle Karşılaştırılması

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ABSTRACT

Aim: The aim of this study was to compare the efficiency of the Brucella Coombs Gel Test (BCGT), a new serological diagnosis test, with the methods used in routine laboratory such as Brucella immuncapture test (BCAP), Standard Tube Agglutination (STA), Rose Bengal (RB) and ELISA for the diagnosis of brucellosis.
Patients and Method: The serum samples of 107 patients with a presumptive diagnosis of brucellosis sent from three different clinics (internal medicine, infectious disease and pediatric clinics) were subjected to four different diagnostic methods (BCAP, RB, STA, and ELISA). The correlations between these diagnostic tests were analyzed using the Cohen's Kappa test. Additionally, sensitivity, specificity, positive and negative predictive values, and accuracy of BCGT were measured.
Results: According to the obtained data, the positivity of different Brucella tests (BCAP, RB, STA, BCGT, ELISA IgG and IgM) were 102 (95.3%), 96 (89.7%), 80 (74.8%), 100 (93.5%), 104 (97.2%) and 101 (94.3%), respectively. According to the Kappa test results, there was strong agreement between BCAP and BCGT (K=0.824). Furthermore, the sensitivity and specificity values of BCGT in our study were 98.08% and 71.43%, respectively.
Conclusion: BCGT is a rapid, cost-effective and highly sensitive test, which appears to be a promising technique for the diagnosis of human brucellosis; however, further scientific studies are needed to support the applicability of this test in routine laboratories.

Keywords: Brucella, Coombs Gel test, Rose Bengal, Standard tube agglutination

ÖZ

Amaç: Bu çalışmada, rutin laboratuvarlarda brusellozun tanısında kullanılan Brucella immuncapture test (BCAP), Rose Bengal (RB), Standart Tüp Aglütinasyon (STA) ve ELISA testleri ile yeni bir serolojik test olan Brucella Coombs Gel Test (BCGT)'in etkinliğinin karşılaştırılması amaçlanmıştır.
Hastalar ve Yöntem: Dâhiliye, enfeksiyon hastalıkları ve pediatri kliniklerinden bruselloz ön tanısı ile laboratuvarımıza gönderilen 107 hastaya ait serum örneklerinde BCAP, RB, STA, ELISA IgG/IgM ve BCGT testleri uygulanmıştır. Cohen's Kappa testi ile tanı testleri arasındaki uyum istatistiksel olarak analiz edilmiştir. Ayrıca, BCGT testinin duyarlılık, özgüllük, pozitif ve negatif prediktif değerleri saptanmıştır.
Bulgular: Elde edilen verilere göre; 107 hastanın 102'si (95.3%) BCAP testi ile 96'sı (%89.7) RB testi ile 80'i (%74.8) STA testi ile 100'ü (%93.5) BCGT ile 104'ü (%97.2) ELISA IgG testi ile ve 101'i (94.3%) ELISA IgM testi ile pozitif olarak tespit edilmiştir. Yapılan istatistiksel analizler sonucunda (Cohen's Kappa Test), BCAP ile BCGT (K=0.824) arasında güçlü uyum saptanmıştır. Ayrıca BCGT testinin duyarlılık ve özgüllüğü sırasıyla %98.08 ve %71.43 olarak saptanmıştır.
Sonuç: BCGT insan brusellozunun tanısında kullanılabilecek umut vadeden bir teknik gibi görünen, hızlı, az maliyetli ve yüksek duyarlılığa sahip bir testtir. Ancak, bu testin rutin laboratuvarlarında kullanılabilirliğini destekleyecek daha fazla bilimsel çalışmaya ihtiyaç vardır.

Anahtar kelimeler: Brusella, Coombs Gel test, Rose Bengal, Standart tüp aglütinasyon

Received 14.11.2019 Accepted: 04.01.2020 Published (Online):02.03.2020

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To cited: Karamese M, Acar O. A comparison of a new diagnostic test of the human Brucellosis, the Brucella Coombs Gel Test, with other methods. Acta Med. Alanya 2020;4(1):56-60. doi:10.30565/medalanya.646672

INTRODUCTION

Brucellosis is a zoonotic infection transmitted from animals to humans by ingestion of infected food products, direct contact with infected animals or inhalation of aerosols [1, 2]. Serious difficulties are still encountered in understanding the pathogenic mechanisms of human brucellosis, identifying the markers that indicate the severity, progression of disease, and developing treatment methods [3, 4].

Diagnosis of human brucellosis is quite important because of the long treatment period. Despite the long incubation period (minimum 7 days), blood culture is the gold standard method for brucellosis, however, serological methods have been used more frequently due to the difficulties in the growth and identification of microorganisms [5]. The Rose Bengal (RB) is a rapid, high-sensitivity, cost-effective plaque agglutination test. Furthermore, it is frequently used as a screening test in human brucellosis; however, it should be evaluated together with other verification methods [6]. Standard tube agglutination (STA) test is also an easy, inexpensive and reliable test in the diagnosis of brucellosis, when evaluated together with the clinical data, and it is the most commonly used method in the serologic diagnosis of brucellosis in the world [7], as total antibodies against S-lipopolysaccharide on the bacterial surface are detected. It should be noted, however, that this test has some disadvantages such as being time-consuming and laborious and leading to false negative results because of the blocking of antibodies [8]. On the other hand, the ELISA method enabled us to detect more positivity as well as different classes of antibodies than other agglutination methods. In this method, different results can be obtained depending on the structure of the solid phase and anti-globulin, which affects the sensitivity, specificity and applicability of the method, the antibody profile may not always be clinically compatible and titers may remain positive for a long time. Furthermore, ELISA tests are more expensive than other agglutination methods, requiring experienced personnel [5]. In recent years, the Brucella immuncapture test (BCAP), which is based on sandwich ELISA, has been used to detect blocking antibodies. In the BCAP test, the wells were coated with Coombs antibodies developed against human IgG, IgM, IgA, and three antibodies can be detected in the serum of the patient. Additionally, these tests have been defined as a useful methods for both diagnosis and follow-up the disease [7, 9, 10].

In the laboratory diagnosis of brucellosis, new studies are needed to establish new diagnostic tests with high sensitivity/specificity, are cost-effective and provide reliable results in a short period [11]. The Brucella Coombs Gel Test (BCGT) is developed in our country and is a new method that is being used in the serological diagnosis of brucellosis. In this test, tube agglutination and the coombs method are performed together in gel wells and its most important advantage is that it provides results in 30 minutes and does not require incubation [12].

The aim of this study was to compare the efficiency of BCGT, a new serological diagnosis test, with other diagnostic methods used in routine laboratory such as BCAP, STA, RB and ELISA.

PATIENTS AND METHOD

The serum samples of 107 patients with a presumptive diagnosis of brucellosis sent from three different clinics (internal medicine, infectious disease and pediatric clinics) to Kafkas University, Health Research and Application Hospital, Microbiology Laboratory between January 2015-2016 were included in the study. The local ethics committee of Kafkas University, Faculty of Medicine approved the study (Approval number: 218).

In our microbiology laboratory, BCAP, RB, and STA tests were routinely performed to suspected patients' sera. BCGT and Brucella ELISA IgM/IgG tests were then performed to our experimental group's sera. The Brucella IgG/IgM antibody test kits were used in accordance with the manufacturer's protocols.

The Brucella Coombs test antigen, which is routinely used in our laboratory, has a smooth lipopolysaccharide (LPS) structure. The Brucella immuncapture test (Metser Lab, Istanbul, Turkey) was also used in accordance with manufacturer's protocols. For evaluation, the blue/purple dot shape was evaluated as negative and homogeneous

turbidity above 1/160 was evaluated as positive.

The procedure of studying of BCGT: The first well of plate was filled with 5-µL serum+100-µL diluent, and other wells were filled with only 50-µL diluent. Brucella antibody was added to the serum samples, which were diluted on 96-well plates. The samples were transferred to the 12x8 gel matrix microtubes including antihuman IgG gel matrix (Coombs antibodies). The microtubes were centrifuged for 20 minutes at 3,000 rpm. Then, the results were evaluated visually (negative if pink colored was seen at the bottom of microtubes, and positive if pink colored was seen on the top of the microtubes).

The Brucella IgG and IgM ELISA tests were performed and interpreted according to the manufacturer's instructions (Vircell, S.L., Spain). Briefly, 96-well microplate were coated with 100 µl of Brucella antigen. After the steps of incubation and washing 100 µl of 1:1000 dilution of serum was added and microplates were then incubated. The following processes were completed and the reaction was finally stopped. The results were read on a MultiSkan GO spectrophotometer (Thermo Fisher Scientific) at 450 nm absorbance. The results obtained via the five (BCAP, RB, STA, BCGT, ELISA) methods were recorded.

The results of the BCGT were compared with the Brucella immuncapture test, and the sensitivity, specificity, positive and negative predictive values of the BCGT were calculated. The total number of cases examined (TN), true positive (TP), falsa positive (FP), true negative (TN), and false negative (FN) results were used for calculation. The sensitivity and specificity are equal to TP/(TP+FN), and TN/(TN+FP), respectively. Accuracy was calculated as the proportion of the true results (both true positives and true negatives) among the total number of cases examined [13]. All obtained data were analyzed by using the Statistical Package for the Social Sciences (SPSS) version 22.0 software (SPSS Inc., Chicago, IL, USA). The "number (n)," "percentage (%)," "mean," "standard deviation (SD)," median, minimum and maximum values were given for the descriptive statistics. The independent samples t-test or Mann-Whitney U test were used to compare numerical variables. Agreements of the diagnostic tests of Brucella were calculated by Cohen's Kappa coefficient. The kappa values between 0.21 and 0.40 were interpreted as fair, 0.41–0.60 as moderate, 0.61–0.80 as good, and >0.80 as almost perfect agreement [14].

RESULTS

Of the 107 patients who attended three different clinics (infectious disease, internal medicine and pediatric), 57 were men (53.3%) and 50 were women (46.7%). The mean age was 40.29±13.64. There was no statistically significant difference between age and gender (Z=-1.625, p=0.104). The distribution of patients according to departments was internal medicine clinic (n=62, 57.9%), infectious disease clinic (n=35, 32.7%) and pediatric clinic (n=10, 9.3%), respectively. The characteristics of the patients are reported in Table 1.

Table 1: Demographic data of patients

Patient characteristics	Male (n, %)	Female (n, %)	Total
Age	42.72±12.61	37.52±14.35	40.29±13.64
Gender	57 (53.5%)	50 (46.7%)	107
Clinics			
Internal medicine	32 (56.1%)	30 (60.0%)	62
Infectious disease	21 (36.8)	14 (28.0%)	35
Pediatric	4 (7.0%)	6 (12.0%)	10

According to the obtained data, the positivity of different Brucella tests (BCAP, RB, STA, BCGT, ELISA IgG and IgM) was 102 (95.3%), 96 (89.7%), 80 (74.8%), 100 (93.5%), 104 (97.2%) and 101 (94.3%), respectively. The detailed positivity and negativity rates are shown in Table 2.

Table 2: Distribution of positive and negative results of Brucella diagnostic tests

		Positive (n, %)	Negative (n, %)
RB		96 (89.7)	11 (10.3)
STA		80 (74.8)	27 (25.2)
BCGT		100 (93.5)	7
ELISA	IgG	104 (97.2)	3 (2.8)
	IgM	101 (93.5)	6 (6.5)
BCAP		102	5

RB: Rose Bengal, STA: Standard Tube Agglutination, BCGT: Brucella Coombs Gel Test, BCAP: Brucella Capture Test

Furthermore, the agreements among Brucella diagnostic tests were analyzed by Cohen's Kappa test. According to the Kappa test results, there were an excellent/almost perfect agreement between BCAP and BCGT (K=0.824). A moderate agreement was detected between BCAP and ELISA test (K=0.482). In contrast, there were no significant agreement by Kappa test between other Brucella diagnosis methods. The Cohen's Kappa test results are shown in Table 3.

Table 3: The Kappa test results of Brucella diagnostic tests

	STA	RB	BCGT	ELISA	BCAP
STA		κ= -0,171	κ= 0,344	κ= 0,157	κ= 0,254
RB	κ= -0,171		κ= -0,087	κ= -0,046	κ= -0,069
BCGT	κ= 0,344	κ= -0,087		κ= 0,375	κ= 0,824
ELISA	κ= 0,157	κ= -0,046	κ= 0,375		κ= 0,482
BCAP	κ= 0,254	κ= -0,069	κ= 0,824	κ= 0,482	

RB: Rose Bengal, STA: Standard Tube Agglutination, BCGT: Brucella Coombs Gel Test, BCAP: Brucella Capture Test

On the other hand, the sensitivity and specificity of BCGT compared to BCAP is reported in Table 4. After the calculations, the sensitivity and specificity of BCGT were 98.08% [95% CI: 93.23-99.77%] and 71.43% [95% CI: 29.04-96.33%], respectively. The accuracy was calculated according to the formula given in the statistical analysis part of this study and it was detected as 96.4% [95% CI: 91.03-99.01%].

Table 4: The sensitivity and specificity values of Brucella Coombs Gel Test

	BCGT	CI 95%
Sensitivity	98.08%	93.23% - 99.77%
Specificity	71.43%	29.04% - 96.33%
Positive Likelihood Rate	3.43	1.06 – 11.07
Negative Likelihood Rate	0.03	0.01 – 0.13
Positive Predictive Value	98.08%	87.44% - 97.43%
Negative Predictive Value	71.43%	36.96% - 91.42%
Accuracy	96.4%	91.03% - 99.01%

BCGT: Brucella Coombs Gel Test. The calculations were performed by using Brucella Capture test as a gold standard test

DISCUSSION

The diagnosis of brucellosis in clinic is quite difficult especially in the absence of specific clinical features. At that point, specific microbiological tests should be used to diagnose the probable brucellosis in order to prevent the problems associated with the treatment processes and responses [15-17]. These diagnostic tests are the Rose Bengal test [18], the standard tube agglutination test [10], the immuncapture test [19] and the ELISA test [20]. In our study, the results have shown that the BCGT test is a promising technique for the diagnosis of human brucellosis, is in agreement with gold standard test and has high sensitivity (98.08%).

A literature check has revealed similar results. A study (n=117) reported positive results in 81 (95.3%) patients with Rose Bengal, 53 (62.3%) patients with STA and 64 (75.3%) patients with Coombs test [21]. Another study from Turkey (n=71) reported positive results in 56 (%78.8) patients with Rose Bengal, 30 (42.2%) patients with STA and 52 (73.2%) patients with BCAP [5]. We found 89.7% positivity with RB, 74.87% positivity with STA, 93.5% positivity with BCGT, and 97.2% positivity with ELISA IgG. On the other hand, a study that compared the diagnosis methods (Brucella Coombs Gel test and STA) reported 100% positivity rate in BCGT [10].

The sensitivity rates of BCGT compared to the Brucella immuncapture test reported in scientific studies from Turkey are between 94-100%, and the specificity rates are between 82-100% [10, 21]. Kalem et al. [22] performed a study that compared both ELISA and Coombs Gel tests in a similar fashion as in our study. They reported that BCGT and ELISA tests had high sensitivity (100% and 92.8%, respectively) and specificity (100% and 79.7%, respectively) when compared to the immuncapture test. Another study reported that the sensitivity, specificity, positive and negative predictive values of the Coombs gel test were 100%, 82.2%, 84.3%, and 86%, respectively [23]. The results were closely similar; however, only a study reported [16] that the sensitivity of BCGT was 78.8%, if the titer was above 1/160, and the accuracy of this test was 88.7%. In our study, we compared the positivity results of BCGT to BCAP. The sensitivity and specificity were 98.08%, and 71.43%, respectively and in addition, the accuracy was determined to be 96.4%.

On the other hand, the kappa test results supported

the sensitivity and specificity rates of BCGT results. A study performed in 2015 [24] reported that BCGT was in excellent agreement with the Brucella immuncapture test (K=0.979). Another study [5] stated similar findings as with others, that BCGT was in almost perfect agreement with classic coombs test (K=0.846), though Koçman et al. [16] detected that the BCGT (above 1/160) was in good agreement with Brucella immuncapture test (K=0.724). In our study, we detected that there was an excellent agreement between the BCGT and BCAP tests with the following Kappa test findings: K=0.824. Eventually, this high sensitivity/specificity rates and high agreement results of the BCGT compared with other diagnosis test makes it more preferable. Most of the related studies reported similar percentages and Kappa results with the BCGT and advised to use it in the diagnosis of brucellosis [10, 17, 23].

Study Limitations:

Our study had some limitations, namely that the study population should be extended to get better results about the sensitivity and specificity of diagnostic tests. Secondly, the blood culture results and follow-up of the patients should be obtained and included in further studies.

CONCLUSION

As a result, it is now well established that the Brucella Coombs Gel is a high sensitive, cost-effective and rapid test compared to the ELISA and PCR methods; however, more comprehensive studies should be performed in both control groups and patients, in order to show the realistic efficiency of the BCGT and to support the applicability of this test in routine laboratories.

Acknowledgement: This study was supported by Kafkas University, Kars, Turkey; Scientific Research Project Council (project no.: 2015-TS-16).

Conflict of interests: The authors declare that there is no conflict of interests.

Funding sources: There is no source of funding or financial interest in this study.

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