ORIGINAL ARTICLE

Diagnostic Efficacy of Modified Coagglutination Test in the Diagnosis of Human Brucellosis

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Abstract:

Background: Laboratory help is must for the diagnosis of human brucellosis due to protean clinical manifestations. As culture is hazardous, time consuming and less sensitive, serological tests are preferred for the diagnosis. Agglutination tests like Rose Bengal Plate Test (RBPT), Serum Agglutination tests (SAT), 2-Mercaptoethanol test (2-ME) that are commonly employed for the diagnosis either lack sensitivity or specificity. Coombs test and Brucellacapt though are sensitive and specific, workout costly. Therefore, modified coagglutination test was developed and its diagnostic efficacy was evaluated. *Aims and Objectives:* To develop modified coagglutination test for the diagnosis of human brucellosis and compare it with Coombs test. *Materials and Methods:* Serum samples collected from 191 brucellosis patients and 100 controls were subjected to RBPT, SAT, 2-ME, Coombs test and modified coagglutination test (MCOAG). Blood culture was performed by Castaneda's method in all the patients. *Results:* Significant difference in the positivity rate was seen between MCOAG and 2-ME. The results of MCOAG were comparable with Coombs test. *Conclusions:* Modified coagglutination test is a better option to Coombs test for the serodiagnosis of brucellosis in resource constrained countries as it is sensitive, specific & cost effective.

Key words: Brucellosis, RBPT, SAT, 2-ME, Modified Coagglutination Test, Coombs Test.

Introduction

Brucellosis is primarily a disease of domesticated animals such as cattle, goat and sheep. Human infection can occur through exposure to infected animals; consumption of contaminated, unpasteurized animal products; direct contact with infected animal parts and inhalation of infected aerosolized particles [1, 2]. In humans it presents with non-specific symptoms that resemble enteric fever /malaria / tuberculosis / rheumatoid arthritis, hence laboratory help is necessary for the diagnosis [3-5]. Isolation of *Brucella* organisms from the clinical specimens is the "Gold standard" for diagnosis. However it is not routinely performed since it is difficult, hazardous, time-consuming, lacks sensitivity in treated patients and requires highly skilled personnel and special level III bio safety cabinets [3, 6].

The development of a specific PCR has overcome some problems with culture. PCR is sensitive, rapid, provides results in less than 24 hours and reduces the hazards of handling the organism in the laboratory [7, 8]. In spite of all these advantages, PCR is yet out of reach of common laboratories in developing countries.

In addition, its specificity in the clinical practice especially among high-risk group subjects and in treated cases wherein the DNA load persists for years after clinical cure remains uncertain [9]. Hence serological tests still remain the mainstay in diagnosis of brucellosis. Currently though an array of serological tests is available for the diagnosis of human brucellosis, agglutination tests are most commonly used. Among the agglutination tests Coombs test and Brucellacapt are reported to be more sensitive as well as specific [3, 10, 11,12]. Both these tests are expensive and are out of reach for smaller laboratories in India. Coagglutination test has been found useful for detection of incomplete antibodies in brucellosis by Ansorg [13]. Keeping this in mind, modification of coagglutination test was developed and its efficacy was compared with Coombs test.

Material and Methods:

Serum samples were collected from individuals of the following groups.

Group I: Fifty healthy high-risk group subjects who had regular contact with animals (25 practicing veterinarians and 25 farmers).

Group II: Fifty patients, suffering from diseases manifesting fever, other than brucellosis. These patients were laboratory confirmed cases of Enteric fever (*N= 23*), Malaria (*N= 21*), Dengue fever (*N=03*), Tuberculosis (*N=03*).

Group III: Included 191 patients with clinical symptoms and epidemiological history compatible to brucellosis and antibody titers ≥160 IU by the Serum Agglutination Test (SAT).

The study was approved by Institutional Ethical Committee and written informed consent was taken from all the subjects.

All the serum samples were subjected to RBPT, SAT, 2-ME, MCOAG and Coombs test.

The antigens for RBPT and SAT were procured from The Division of Biological Products, Indian Veterinary Research Institute (I.V.R.I.); Izatnagar, Uttar Pradesh, India. The procedures were carried out according to the manufacturer's guidelines. For 2-ME test, 0.1 M 2-Mercaptoethanol was used in place of phenol saline and further procedure was carried out as in SAT.

The MCOAG test was performed according to the procedure given by Ansorg et al. [13] with some modifications. In this test serum was treated with 2-ME instead of $MnCl_2$ before testing. Stable *Staphylococcus aureus* Cowan's I serotype (ATCC 12598) reagent (5% v/v) in PBS with 0.01% thiomersal was prepared and stored as stock suspension at 4^0C until use. First 2-ME test was performed by standard protocol and the tubes were incubated at 37°C for 2 hours. After incubation the tubes were centrifuged at 2000 rpm for 10 min. The supernatant was discarded and the pellet was washed twice in normal saline. The pellet was re-suspended in 0.5 ml of normal saline and 0.5 ml Staphylococcal working reagent was added. The working reagent was prepared by diluting the stock to get 0.01% cell suspension. The tubes were then incubated overnight at 37°C and results were recorded as in SAT.

The procedure for Coombs test was similar to MCOAG test, the only difference was 0.5 ml of antihuman globulin was used in place of Staphylococcal working reagent.

Statistical Analysis:

Statistical analysis of the data was done using Grap Pad InStat software.

Results:

In group I, of the 50 subjects 4 showed positive reaction by RBPT and also significant SAT titers (≥160 IU). In group II, two patients, one with enteric fever (culture proven) and one with malaria (positive peripheral smear for *M. falciparum*) were positive by RBPT. Their SAT titres were 80 IU and 40 IU respectively. None of group I and group II sera showed significant (≥80 IU) titres by 2-ME, MCOAG or Coombs tests.

In group III, of the 191 cases with SAT titers ≥ 160 IU, significant 2-ME titers were seen in 145. Coombs test and Modified coagglutination test showed positive results in 151 patients. Six patients who had insignificant 2-ME titers, initially labelled as inactive brucellosis cases were picked up by MCOAG and Coombs test (which detect both complete and incomplete antibodies) wherein the titers showed significant rise. If only 2-ME titers were considered these cases would not have been treated for brucellosis. Results of various serological tests performed are given in (Table 1).

There was almost total correlation between MCOAG and Coombs test titers, same titers were obtained in 149 out of 191 sera with significant SAT titers. In 4 cases rise in titer by MCOAG was one dilution greater than Coombs

Table 1-Antibrucellar Antibodies in Different Groups by RBPT, SAT, 2-ME Coombs and MCOAG Tests

Groups	Screened (Number)	Positive (Number)	Significant Titers (IU)			
		RBPT	SAT	2-ME	Coombs	MCOAG
Group-I	50	04	04	00	00	00
Group-II	50	03	00	00	00	00
Group-III	191	191	191	145	151	151
Total	291	198	195	145	151	151

Significant titres for SAT: ≥ 160 IU, 2-ME/Coombs /MCOAG: ≥ 80 IU

SAT: Serum Agglutination Test, 2-ME: 2-Mercaptoethanol, MCOAG: Modified Co-Agglutination test

Table 2. SAT, 2-ME, Coombs and MCOAG Titres of 195 Suspected Cases of Brucellosis

Tests	Titre IU/ml									
	Nil	20	40	80	160	320	640	1280	2560	5120
SAT	0	0	0	0	45	71	34	31	7	7
2-ME	46	0	4	25	44	43	18	11	4	0
Coombs	15	0	29	5	52	52	25	12	5	0
MCOAG	13	2	29	5	49	54	26	12	5	0

SAT: Serum Agglutination Test, 2-ME: 2-Mercaptoethanol, MCOAG: Modified Co-Agglutination test

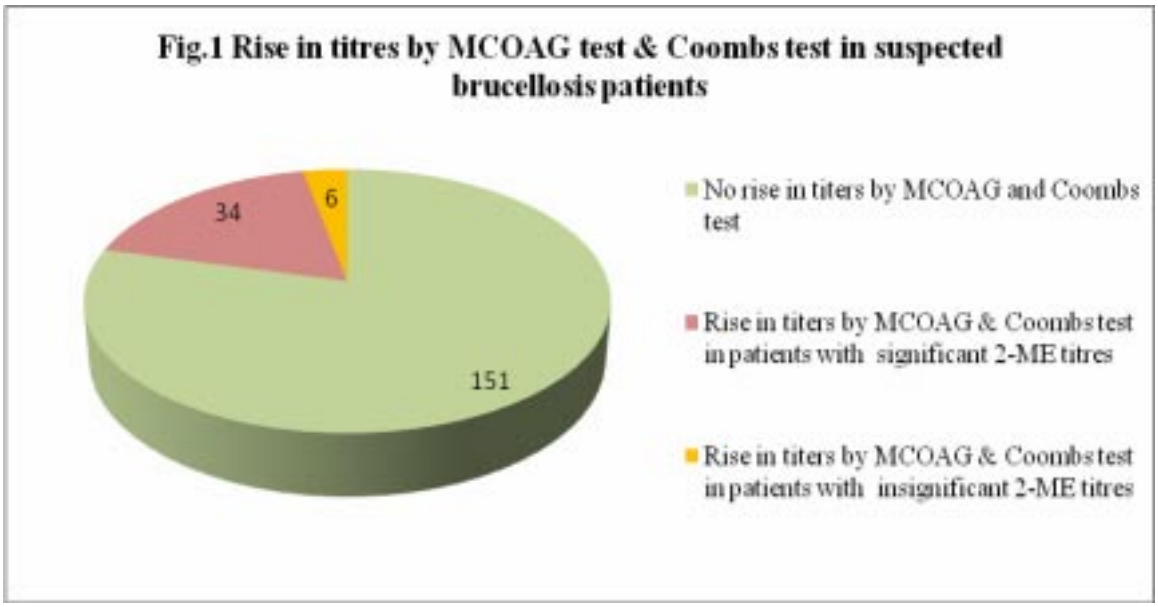
Table 3-Comparison of Sensitivity & Specificity of 2-ME Test & MCOAG Test in with Coombs Test

Test	Compared with	Sensitivity (%)	Specificity (%)
2-ME	Coombs test	96	100
MCOAG		100	100

2-ME: 2-Mercaptoethanol, MCOAG: Modified Co-Agglutination test

abdomen, nausea, night sweats, fatigue were the noticeable symptoms.

In this study 4 control group subjects and 46 patients showed significant SAT titres but had insignificant 2-ME titers. Of the 46 patients, 8 had 2-ME titers of 20 IU and remaining 38 had < 20 IU. Repeat serology after 2 and 4 weeks in these cases did not show any rise by SAT or 2-ME tests hence these cases were considered as cases of inactive brucellosis.



test and in 1 it was two dilutions greater. The agglutination and clarity of supernatant was better with MCOAG than Coombs test.

None of the control samples showed any titers by MCOAG test thus giving the specificity of 100%. Sensitivity and specificity of MCOAG was better than 2-ME test and was equivalent to Coombs test.

Blood culture was performed in all the patients. *Brucella spp.* could be isolated from 46 with the isolation rate of 24.08%.

Fever, joint pain, low backache, headache, pain

Discussion:

Brucellosis is endemic in India. Due to protean clinical manifestations, diagnosis requires high suspicion and laboratory aid [3-5]. Agglutination tests like RBPT and SAT have a significant role. RBPT is used as a screening test and the results are confirmed by SAT. But some studies suggest that significant SAT titers may be noted in individuals with repeated subclinical exposures as in high-risk occupations and also in treated cases [14, 15]. In this study we have come across 4 control individuals and 46 pa-

tients with high SAT titers and insignificant 2-ME titers. According to Young EJ, Buchanan and Faber the diagnosis of brucellosis should rely upon results of 2-ME tests that detect IgG antibodies. [16, 17] However this test also suffers from the disadvantage of giving false negative results due to blocking antibodies. The blocking antibodies can be detected by Coombs test and Brucellacapt [11, 12]. However these tests workout costly and hence are not performed in small laboratories. Hence an attempt has been made to use modified coagglutination test to detect non agglutinating blocking antibodies in human brucellosis and compare its efficacy with 2-ME test and Coombs tests.

When compared with 2-ME test titers, modified coagglutination has shown rise in titre in 40 patients, among which 6 have had insignificant 2-ME test titres. All of them showed MCOAG and Coombs test titers ≥320 IU & hence have been considered as cases of active brucellosis and have been advised to take treatment. Sensitivity of culture was too low probably due to prior antibiotic treatment. All the patients had been already treated by local doctors. None of them was earlier diagnosed as a case of brucellosis.

When compared to Coombs test, Modified Coagglutination test is cost effective, reagents are stable hence this test can be used in place of Coombs test for the detection of incomplete antibodies in human brucellosis. Further studies are needed to substantiate these results.

Conclusions:

In light of this study, we conclude that, positive RBPT and significant SAT titres are not indicative of an active brucellosis as these can be positive in healthy occupationally exposed individuals. High 2-ME titres could be considered diagnostic for therapeutic purpose. Due to false negative 2-ME test some patients with long standing brucellosis may be missed who can be detected by Coombs / MCOAG tests. Coombs test can be replaced by MCOAG in resource constrained countries as it is cost-effective and equally specific.

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