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Original article

A comparative study of agglutination tests, blood culture & ELISA in the laboratory diagnosis of human brucellosis

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ABSTRACT

Introduction: Brucellosis is one of the important causes of Pyrexia of Unknown Origin (PUO) in endemic areas. The present study was carried out to compare the diagnostic efficacy of Agglutination tests, Blood culture & ELISA in the diagnosis of human brucellosis in cases of Pyrexia of Unknown Origin. Materials & Methods: This descriptive study included 42 patients clinically diagnosed with PUO & were investigated for brucellosis and 42 age and sex matched healthy controls. Statistical analysis was done to determine and compare the sensitivity, specificity, positive predictive value & negative predictive value of the test methods. Results: Among the 42 cases, none of the blood cultures grew any isolate of Brucella. A total of 7 cases were serologically positive by agglutination tests. 13 of the 42 cases of clinically suspected brucellosis yielded positive results with ELISA (8 were positive for IgM antibodies alone by ELISA, 3 cases exhibited IgM and IgG classes of antibodies together and 2 were positive for IgG class of antibodies alone). None of the samples in the control group had any positive observations. Conclusion: ELISA is the most reliable and rapid method for the diagnosis of brucellosis.

1. Introduction

Brucellosis is a zoonosis caused by bacteria of the genus Brucella, which affect both humans and animals such as cattle, sheep, goats, and swine. Many names have been applied to it, often relating to localities in which it was particularly prevalent; Malta fever, Mediterranean fever, Gibraltar or Rock fever, and undulant fever are probably the best known [1,2,3]. Brucellosis manifests with varying symptoms and signs and may mimic typhoid. It is endemic in various countries across the globe, including India [4,5,6]. The incidence in humans ranges widely between different regions, with values of up to 200 cases per 100,000 population. It is one of the causes of fever of prolonged duration in endemic areas and one of the important causes of pyrexia of unknown origin [7, 8]. Despite being endemic in many developing countries, brucellosis remains under-diagnosed and under-reported. Furthermore, since]. brucellosis is an important cause of veterinary morbidity and mortality, the disease can also cause important economic losses in developing countries. Therefore, the diagnosis must be confirmed by isolation of Brucella, mostly from blood culture or by the detection of an immune response to its antigens [9,10]. Though Brucellosis is diagnosed in the laboratory by using various techniques like culture, serological tests including agglutination tests, Rose Bengal card tests, IgM and IgG Enzyme Linked Immunosorbent Assay (ELISA), Polymerase chain reaction (PCR) etc, the timely and accurate diagnosis of human brucellosis continues to challenge clinicians because of its non-specific clinical features, slow growth rate in blood cultures, and the complexity of its serodiagnosis [2,11]

Hence, the present study was carried out to compare the diagnostic efficacy of agglutination tests, blood culture & ELISA in the diagnosis of human brucellosis in cases of Pyrexia of Unknown Origin.

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2.Materials and Methods

This descriptive study was conducted over a period of one year from August 2003 to July 2004. All patients presenting to the VIMS Hospital, Bellary and clinically diagnosed with PUO were investigated in the department of microbiology for brucellosis. 42 clinically suspected cases of brucellosis and 42 serum samples from healthy individuals (voluntary blood donors) were included in this study. Control group age ranged from 18 to 65 years (median, 32.5 years); 19 were females, and all of them lived in the same area. Among the cases, 25 were males and 17 were females, and their ages ranged from 12 to 80 years (median, 41 years). 22 patients lived in a rural habitat, and 20 patients reported exposure to animals or their products. In 40 cases (96%) it was possible to identify at least one risk factor for brucellosis: close contact with animals (96%), ingestion of raw milk (4%), or work in an abattoir (64%). Fever was present in 78% of patients, and 42% presented signs of focal infection.

Informed consent was taken from all subjects investigated under the study. Guidelines stated by Alton G.G et al were followed while performing the investigations. The diagnosis of brucellosis was based on clinical findings and on either positive blood cultures for Brucella or the presence of serum antibodies (SAT titer $\geq$160). At least three blood cultures were drawn from each patient at diagnosis. Follow-up cultures were drawn at the end of the treatment and 3, 6, and 12 months later. Blood samples collected for blood culture by adopting aseptic precautions from the subjects included under the study were processed in the microbiology laboratory using Castaneda's biphasic media, prepared using Brucella selective agar (M822- Batch no. YA022) with Brucella selective supplement, modified (FD161) and Brucella selective broth (M 348- Batch no. 3E160) with Brucella selective supplement (FD005) (Hi-Media). Serum separated from clotted blood of the subjects was used for serological investigations.

The serological tests performed were Slide agglutination test, Tube agglutination test, IgM Enzyme linked immunosorbent assay (IgM ELISA) and IgG Enzyme linked immunosorbent assay (IgG ELISA).

The agglutination tests were performed using commercial febrile antigen kits containing stained Brucella abortus (Batch nos. 103902 and 280904) and Brucella melitensis (Batch nos. 104902 and 281904) antigen suspensions procured from Swemed diagnostics, according to the manufacturer's guidelines. A titre of 1:80 or more was considered significant for agglutination tests. IgM & IgG ELISA were performed using commercial kits procured from NOVATEC Immundiagnostica GmbH, Germany (Product codes- BRUG0050 & BRUM0050 respectively). In order to evaluate ELISA antibody concentration, a highly specific standard curve as well as a highly specific evaluation table included in each test kit was used. Results are shown in arbitrary units per milliliter of serum by extrapolating the absorbance values by means of a standard curve established with an internal reference sample. Titres over 30 U for IgG, over 20 U for IgM, and over 15 U for IgA were considered positive by the manufacturer.

2.1. Statistical analysis

Sensitivity, specificity, positive predictive value & negative predictive value of the techniques performed were calculated using appropriate formulae. SPSS software (version 13.5; SPSS Inc., Chicago, IL) was used for the statistical management of the data and tests for significance like Fisher's exact probability test and chi-square test were applied wherever necessary. All statistical analysis were carried out at 5% level of significance.

3. Results

None of the blood cultures by Castaneda method yielded any isolate of Brucella spp. in both the study and the control groups.

A total of 13 cases in the study group were found to be serologically positive for brucellosis. 5 cases were positive by Slide agglutination and an additional 2 cases were found to have significant titres by Tube agglutination test (table 1). Among the 7 cases yielding significant titres against Brucella, three sera had agglutinin levels of 1:80, three had titres of 1:160 and one sample had a titre of 1:320 (table 2).

Table 1. Tests Performed And Cases of Brucellosis Detected

Test performed	No. of cases of brucellosis detected Total no. of brucellosis cases: 13 (Percentage of positives)
Blood culture	0 (0.00)
Slide agglutination test	5 (38.46%)
Tube agglutination test	7 (53.84%)
IgM ELISA	11 (84.61%)
IgG ELISA	5 (38.46%)

Table 2. Break-up of The Agglutination Titre And The Number of Cases In Each Group

Agglutination titre	No. of cases (%)
Negative	25 (59.52)
1:20	1 (2.38)
1:40	9 (21.42)
1:80	3 (7.14)
1:160	3 (7.14)
1:320	1 (2.38)
1:640	0 (0.00)

13 of the 42 cases of clinically suspected brucellosis yielded positive results with ELISA (8 of those tested were positive for IgM antibodies alone by ELISA, 3 cases exhibited IgM and IgG classes of antibodies together and 2 cases were positive for IgG class of antibodies alone). The ELISA tests also gave positive results in all these cases. ELISA tests were also positive in 4 cases (44.44%) where the agglutination titres were 1:40 and in 2 cases (8%), where the agglutination tests were negative (table 3).

Table 3. Comparison of Agglutination Titre And Elisa Results

Agglutination titre (No of cases)	Positive by ELISA (IgM and IgG)	Percentage positivity by ELISA
Negative (25)	2	8
1:20 (1)	0	0
1:40 (9)	4	44.44
1:80 (3)	3	100
1:160 (3)	3	100
1:320 (1)	1	100

17 samples (40.47%) of the patients exhibited agglutinins against Brucella in the present study. Taking into consideration the results of ELISA and agglutination tests together, 19 sera samples (45.23%) showed the presence of agglutinins against Brucella (Table 3).

For comparison purposes, the sera of 42 healthy blood donors were also analyzed. Sera from these healthy individuals were uniformly negative for IgG and IgM by ELISA. No serum sample in the control group demonstrated significant titres, though 6 of the 42 controls (14.28%) showed low agglutinin titres.

ELISA was more sensitive, specific, accurate and reliable compared to agglutination tests and culture (table 4). Further, it can be observed that the IgM and IgG ELISA tests, when used in conjunction, are the most reliable tests currently available for the diagnosis.

Table 4. Parameters Compared In Various Techniques

Parameters assessed	Blood culture	Slide agglutination test	Tube agglutination test	ELISA (IgM and IgG)
No. of True positives	00	05	07	13
No. of True negatives	29	29	29	29
No. of False positives	00	00	00	00
No. of False negatives	13	08	06	00
Sensitivity (%)	00	38.46	53.84	100
Specificity (%)	69.04	100	100	100
Positive predictive value	00	100	100	100
Negative predictive value	69.04	78.37	82.85	100
Accuracy	69.04	80.95	85.71	100
Reliability	-0.3096	0.3846	0.5384	1.0

4. Discussion

Brucellosis is a disease caused by the Gram-negative aerobic coccobacillus, Brucella. Four species have been recognized as causing human infection: Brucella abortus, Brucella melitensis, Brucella suis and Brucella canis. The cause of this disease was obscure until 1887 when Bruce reported numerous small coccal organisms in stained sections of spleen from a fatally infected soldier and isolated an identical organism in culture from spleen tissue of 4 other soldiers [12,13]. This bacterium, which he named Micrococcus melitensis, produced a remittent fever in inoculated monkeys.

Bellary district has a predominantly agriculture based economy. Milk production, cattle, sheep & goat rearing practices are associated activities among the farmers. Data pertaining to the prevalence of human brucellosis, a zoonotic disease, in this region is currently not available. Brucellosis has been reported from Belgaum, Bijapur and Manipal in Karnataka state [14,15,16]. The combination of potential exposure, consistent clinical features and raised levels of Brucella agglutinin (with or without positive cultures of blood or tissues) confirms the diagnosis of Brucellosis [17].

A strong clinical suspicion supported by a positive serological test is usually diagnostic of the illness. However, isolation of the causative organism from blood or bone marrow specimens is the confirmatory evidence in cases of brucellosis. Unfortunately, the isolation rates are very low, necessitating more reliance on the serological tests. In our study, none of the blood cultures by Castaneda technique yielded any isolate. Similar findings have been reported by Joshi D.V and Prakash O (1971) from Delhi and Stephen S, et al from Manipal (1978) [18,16]. This has been attributed to the empirical antibiotic treatment given to the patients, which could have suppressed the bacteriological evidence. Blood cultures yielding B. abortus are rare in brucellosis; hence, the difficulty in establishing the diagnosis of brucellosis in a patient not demonstrating antibodies [19].

None of the serological techniques used in the diagnosis of brucellosis are 100% sensitive and specific. Further, the serological tests are confounded with the problem of false positive and false negative results in many instances [20,21,22]. Serology remains the mainstay of laboratory diagnosis, but the interpretation of results is fraught with difficulties. The large number of techniques in use is evidence of the problems.

The results of the present study can be compared to the results of a study conducted by Mathai E, et al [23]. They reported 9 samples of the 23 (39.1%) cases with clinically suspected brucellosis to be positive for IgM and 3 for IgG by ELISA and 5 (21.7%) by the SAT; all the controls tested for IgM and IgG by ELISA were negative. The authors concluded that ELISA is more sensitive and rapid for the diagnosis of brucellosis.

5. Conclusion

A high percentage of clinically suspected cases of brucellosis in the present study had agglutinins against Brucella (30.95%). Serological tests are more sensitive as compared to blood culture. ELISA is most sensitive and rapid for the diagnosis of brucellosis. A combination of IgM and IgG ELISA was found to be most efficient for detecting brucellosis, among the techniques evaluated.

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