Efficacy of immunological tests in diagnosis of acute human brucellosis

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Abstract:

The study was carried out in Al-Hashimyia hospital, Babylon governorate for the period from May, 2005 to May ,2006 to evaluate the results of some immunological tests in diagnosis of acute phase of brucellosis in human populations. A total of 410 patients with suspected brucellosis were admitted to the hospital with age-group ranged from 18 to 65 years. 150 patients were studied, as documented cases with acute brucellosis , and 150 healthy persons were involved as control group. Numerous immunological tests like ELISA test , Rose- Bengal test , standard tube agglutination test and E-rosette test were used to demonstrate cellular and humoral immune response of infected host . ELISA test was the best and accurate test in diagnosis of the acute infection, followed by standard tube agglutination test . All cases of acute brucellosis showed a high number of active rosette –forming cells.

Key words: Human brucellosis , Acute infection, Immunological tests.

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Introduction:

Human brucellosis, mainly caused by *Brucella melitensis* and *B.abortus,* is prevalent on the Southern and Eastern edges of the Mediterranean basin , particularly in Tunisia, Libya, Egypt, Israel, Lebanon and Syria , and in the Arabian peninsula and Iran(1). In Iraq , brucellosis is a common disease among human and animal populations(2,3).

Brucellosis continues to be a major zoonosis worldwide. Animal reservoirs for the disease include a variety of domesticated animals: cattle, goats , sheep , pigs and dogs(4). Infection in humans result from direct or indirect contact with animal sources(5). Human brucellosis is usually associated with occupational or domestic exposure to infected animals or their products; farmers, shepherds, goatherds, veterinarians and workers in slaughterhouses and meat-processing plants(6). Brucellosis has been arbitrarily classified into acute and subacute , chronic , bacteraemia , localized or mixed types(7).

Because the brucella organism grows slowly, the cultures should be maintained for at least four weeks to increase recovery. The diagnosis depends on clinical features , thus clinical diagnosis must usually be supported by results of bacteriology and / or serology(6). Numerous serological procedures have been tried in the diagnosis of human brucellosis , the most widely used was the standard tube agglutination test , Rose -Bengal test and enzyme-linked immunosorbent assay (8).

Aim of this study: Evaluation of results of some immunological tests that used widely for diagnosis of human infection with acute brucellosis among populations of Al-Hashimyia district / Babylon governorate.

Materials and methods:

Study design:
The study was conducted at AL-Hashimyia hospital, Babylon governorate during the period from May,2005 to May, 2006 . Four hundred and ten patients were admitted to the hospital with suspected brucellosis. The range of age-group occurred within (18-65 years old). One hundred and fifty patients were diagnosed with acute brucellosis and they involved in this study as experimental group ,and 150 healthy persons were used as control group.

Bacteriological examinations:
Blood specimens were collected on basis of clinical indication by standard procedure (9). Culture for isolation of Brucella was carried out by standard technique, including use of selective media , brucella agar (Oxoid /UK) . Brucella isolates were phenotypically identified with colonial morphology, Gram stain , and final identification with a series of conventional biochemical tests(10). Bacteriological diagnosis was confirmed by immunological tests as below.

Immunological tests:

ELISA test :

Indirect-ELISA test kit (IDEXX-laboratories, Italia) involved in the study. Lipopolysaccharide-coated wells were used . ELISA test was performed according to procedure of Edward *et al.*(11).The results were reported as negative , suspected , or positive when the ratio of sample optical density to the positive –control optical density (S/P ratio) was less than 70% , 70%to 100% , more than 100% , respectively.

Standard Agglutination test(SAT) :
Standard tube brucella agglutination test (Welcome research laboratories / UK) was used as described by Diaz *et al*(12) .In briefly, Serum of patient was inactivated at 56C for 30min. Equal volume of serial dilutions of serum and standardized antigen of *Brucella abortus* (strain S99) were mixed , and incubated at 37C for 48hr. The test was used to determine the titer of antibodies in serum against brucella organism.

Rose-Bengal test (RBT) :
Two types of brucella antigen kits were used, Spain kit(Intervet company) and Iraqi kit(Institute of sera and vaccines, Baghdad).The test was performed according to Diaz *et al.*(12). The results were recorded as positive or negative agglutination .Control sera were used for each batch of the test .

E-rosette test (ERT):
Peripheral blood was collected from patient group and healthy group. The lymphocytes was separated by Ficol-Hypaque(Pharmacia, France)according to procedure of Boyun *et al.*(13). E-rosette forming was noted and used for counting of T- lymphocytes according to Jondal *et al* (14).

Statistics:
The results of serologic tests were analyzed by using the computerized SAS system, 2002 . The results were considered significant when $p<0.01$.

Results:

A total four hundred and ten patients with suspected brucellosis were admitted to Al-Hashimyia hospital. One hundred and fifty patients were diagnosed with acute brucellosis. Of which, one hundred and three patients (68.6%) were positive for blood culture. After that, these cases were documented as brucellosis by bacteriological examinations . All 150 patients with acute brucellosis were positive(100%) for serological tests, see table-1.

Table-1: Diagnosis of acute brucellosis by blood culture and serological tests.

Brucella spp.	Blood culture(+ve) No.(%)	Serological tests(+ve) No.(%)
B.melitensis(80 cases)	63 (42.0)	80(53.3)
B.abortus (70 cases)	40 (26.6)	70(46
Total (150 cases)	103(68.6)	150(100)

Immunological tests in this study, ELISA test was positive in 150 cases(100%) of acute infection with brucella organism. Tube Agglutination test was positive in 130 cases(86.6%) .The titer of agglutinins ranged from 1:160-1:320 in positive cases, comparing with normal values of control group. Rose-Bengal test was positive in 115 cases (76.6%)

E-rosette test showed elevated lymphocytes level in all of the 150 cases(100%) with acute infection (table-2).

Table-2: Ratios of positive cases of brucellosis detected by several Immunological tests that commonly used in the study.

Immunological tests	No. positive cases (%)
Tube agglutination test	130(86.6)
Rose- Bengal test	115(76.6)
ELISA test	150(100)
E-rosette assay	150(100)
Total cases	150(100)

One of the objectives of this study was to evaluate the detection of antibodies for diagnosis of acute brucellosis by local Rose-Bengal kit which produced in an institute of sera and vaccines when compared with imported kit(Spain origin).

Results of table-3 showed that Rose- Bengal test was positive in 115 cases (76.6%) by intervet kit compared with 113 caese (75.3%) by Iraqi kit. There was non–significant differences between them at significant level (p<0.01).

Table-3: Comparison of Rose-Bengal test values (between imported and local kits) in diagnosis   of Brucella infection.

Kit type	No. Positive cases (%)
Imported kit	115(76.6)
Local kit	113(75.3)
Total	150(100)

Discussion:

 Diagnosis of brucellosis depends on clinical features with positive blood culture and raised brucella agglutinins(7).

In this study, Blood cultures were positive in 68.6% of cases , whereas serological test showed 100% cases were positive .These results of blood culture may be due to the fact that brucellae were intracellular organisms and difficult to cultivate , and most patients were taken antibiotic treatment before bacterial culture(15,16). Delayed hospital admission may have contributed to low brucella isolation rate from blood(3).

 Because the cultures should be maintained for at least four weeks as incubation period for diagnosis of acute brucellosis(7,8). The cultures of brucella organisms were potentially hazardous to laboratory personnel(7,16).Therefore, most cases of brucellosis should be diagnosed by serological tests(1,5,6).

Most patients with acute brucellosis produce antibodies of immunoglobulin M (IgM) within few days of onset of the disease(5). To assess the humeral immune response of the patient to brucella antigen , the agglutination test and ELISA test were used(1).

In our study, tube agglutination test was positive in 86.6% . The titer of agglutinins was ranged from 1:160-1:320, whereas the control group was appeared normal values of antibodies.

The most frequently used test in diagnosis of brucella is standard agglutination test measuring antibodies to *Brucella* antigens. A four fold or greater rise in titer to 1:160 or higher is considered significant (6). Most patients with acute brucellosis develop agglutinin titers. Risining titers indicate recent active infection(16,17). While some patients have low agglutinin titer .The interpretation of stable titers in low or intermediate range 20-160 is difficult . In symptomless patients they probably indicate past or latent infection .In patients with symptoms suggestive of brucellosis they may indicate active infection (1).

The standard agglutination test is still the main stay of serological diagnosis of acute brucellosis(18). But Prozone phenomenon sometimes occur in agglutination test. The immunoglobulin M(IgM) is major agglutinating antibody formed especially in first week , followed by IgG and IgA antibodies in chronic infection(6).All these antibodies are active in agglutination test. Prozone phenomenon due to IgG and IgA can give false–nagative agglutination test in low dilution . This can be overcomed by testing sera at high dilution (>1:320). Also fales–positive test due to immunological cross-reactivity have been associated with brucella skin testing , Cholera vaccination , or infection with *Vibrio cholera, Francisella tularensis , Yersinia enterocolitica*(6,7).

In our study, prozone was not occurring because the study involve patients with acute infection only .Non false-positive was occurring in our results because the patients have not history with tulereamia , Cholera vaccination or cholera infection, or yersinia infection..

In present study , Rose-Bengal test was positive in 115 cases (76.6%) by Spain kit , and in 113 cases (75.3%) with Iraqi kit. There was non–significant differences between them at significant level (p<0.01) .

The rapid slide or plate agglutination test for brucella antibodies. The most effective of these tests is Rose-Bengal plate or card test which used as antigen suspension of

smooth brucella cells stained with Rose Bengal and suspended in an acid buffer. The test discriminated against agglutinins of low avidity and is not subject to prozones (1). some investigators rely on the Rose-Bengal test , which has not been fully validated for human diagnostic use(6).

Results of this study showed that ELISA test was the best method in diagnosis of acute infection of brucella organism . These results explain that the test was more sensitive than agglutination test. Because the ELISA test was very sensitive and could as easily be made specific for antibodies (1,19). Investigation from other studies of patients with acute brucellosis showed that the ELISA was the most sensitive diagnosis test(20).

The E-rosette test (ERT) was used to assess the cellular immune response of the patient to brucella antigen (1) . The test showed raised number of active rosette-forming lymphocytes cells in all cases with acute infection , In range (46-85%) mean(65.5 ± 7.6),while healthy persons as control group have normal values of lymphocytes cells(15-40%) mean(22.6 ± 1.4), with highly significant differences at $P<0.01$. This result due to the brucella organism were intracellular parasite within infected cell which lead to activation of CD4 and CD8 and forming E-rosette.(16).

Finally, the study shows that the ELISA was the best and accurate test in diagnosis of the disease , followed by standard tube agglutination test for diagnosis of acute infection. All cases of acute brucellosis have been a high number of active rosette–forming cells.

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