Original Research Article

Brucellosis among patients with pyrexia of unknown origin

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Abstract

Background: Brucellosis is one of the most common zoonotic infections globally. The laboratory confirmation of human Brucellosis is based on microbiological, serological or molecular methods, each having its own advantages and disadvantages. **Aim:** To study Brucellosis among patients with pyrexia of unknown origin. **Materials and Methods**: Blood samples from 111 patients with PUO were taken and blood from 50 healthy controls were also collected. All the samples were tested by IgG and IgM ELISA and Standard Agglutination Test (SAT). IgG ELISA results were compared with SAT for Brucellosis. **Results:** Anti Brucella IgM antibodies were found in 2 (1.80%) and IgG in 4 (3.60%). 3 patients (2.7%) out of 111 patients were positive by SAT.Sensitivity and specificity of IgG and SAT were 100% and 99.07% respectively. PPV (positive predictive value) and NPV (negative predictive value) were 95% and 100% respectively and diagnostic accuracy was 99.09%.**Conclusion:**Standard Agglutination Test being cumbersome, time consuming and showing false negativity due to blocking antibodies, IgG ELISA and IgM ELISA can be used for diagnosis of Brucellosis.

Key Words: Pyrexia of unknown origin, brucellosis, IgG ELISA,Standard agglutination test

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INTRODUCTION

Brucellosis is one of the most common zoonotic infections globally. It is a significant and increasing veterinary and public health problem in India. In India 80% of the population live in approximately 5,75,000 villages and thousands of small towns and have close contact with domestic/ wild animal population owing to their occupation. Hence, human population stands at a greater risk of acquiring zoonotic diseases including Brucellosis. The disease has an added importance in countries like India, where conditions are conducive for wide-spread human infection on account of unhygienic conditions and poverty.[1]The laboratory confirmation of human Brucellosis is based on microbiological, serological or molecular methods, each having its own advantages and disadvantages. The Enzyme Linked Immunosorbent Assay (ELISA) is known as a sensitive and rapid method for diagnosis of Brucellosis. Detection of specific immunoglobulin by a single, simple and rapid test is a major advantage with ELISA. In addition to the benefit of ELISA in diagnosis of Brucellosis in endemic areas, it could be useful as a screening test in areas with low incidence of the disease.[2]The objective of the present study was to study Brucellosis among patients with pyrexia of unknown origin.

MATERIAL AND METHODS

A total of 111 patients' histories were taken and blood was collected. Serum was separated and stored at -20°C till tested. Blood from 50 healthy controls was collected and tested.

Inclusion criteria

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Samples were collected from patients with symptoms of fever for more than 8 days with joint pain, arthritis, backache and shoulder pain. The history of animal contact along with occupational history was also noted.

Exclusion criteria

Samples were not collected from patients suffering from fever for less than 8 days and having fever without joint pain, arthritis, backache.

METHODOLOGY

Collection of sample: Patients were selected based on inclusion criteria. Blood was collected from patients attending medicine OPD (outpatient department) and IPD (Indoor patient department). After informed consent, 5 ml of venous blood was drawn from each of the above patients and delivered in vacutainer tubes. Blood was allowed to clot in vacutainer. Serum was separated after centrifugation at 2200-2500 rpm for 15 minutes and stored at -20°C in aliquots for further testing.

IgM ELISA for Brucella: IgM ELISA for Brucella was done by IgM Brucella NovaTEC. For this, reagents were kept at room temperature before commencing assay.The test sera were diluted in test tubes by adding 10µL of test serum along with 1000µL of diluents and vortexed.Well "A1" of the microtiter plate was labelled as blank. 100µL of diluted patient samples and controls were delivered into respective microwells.The plates were covered and kept for 1 hour at 37±1°C.Microwells were washed 3 times with diluted wash buffer. 100µl Brucella anti-IgM conjugate was added to each microwell.The plates were covered and kept for 30 minutes at 37±1°C.Microwells were washed 3 times with diluted wash buffer. 100µl TMB solution was added in each microwell. The microwells were incubated for 15 minutes at room temperature in the dark. 100µl stop solution were added in the wells.Microwell plate was read by using Microwell ELISA reader at 450 nm.As per kit instruction >11 NTU was considered as positive.

IgG ELISA for Brucella: IgG ELISA for Brucella was done by using ELISA IgG Brucella NovaTEC.The test sera were diluted 10/1000µl in test tube by adding 10µl of test serum along with 1000µl of diluents and vortex was done.Well "A1" was labelled as blank. 100 µl of diluted patient samples and controls were delivered into respective microwells.The plates were covered and kept for 1 hour at 37±1°C.Microwells were washed 3 times with diluted wash buffer. 100µl Brucella anti-IgG conjugate was added to each microwell.The plates were covered and kept for 30 minutes at 37±1°C.Microwells were washed 3 times with diluted wash buffer. 100µl TMB solution was added in each microwell. The microwells were incubated for 15 minutes at room temperature in the dark. 100µl stop solution were added in the wells.Microwell plate was read by Microwell ELISA reader at wavelength of 450 nm.As per the kit literature >11 NTU were considered as positive.

Standard Agglutination Test for Brucella: Brucella abortus plain antigen (Phenol killed Brucella abortus S99) was procured from IVRI, Izatnagar, India. Eight test tubes were placed in a rack for each sample.0.8 ml of 5% NaCl solution was added to the first tube and 0.5 ml into each of the remaining seven tubes.0.2 ml of positive and negative control were added to the first tube of 1st and 2nd row. 0.2 ml of test serum was added to the first tube of 3rd to 8th row.Two-fold serial dilution was done by transferring 0.5ml of the mixture from 1st to 8th tube.0.5 ml of mixture was discarded from 8th tube of each row.0.5 ml of antigen was added in each of the test tubes.Final dilutions were from 1:20 in first tube to 1:2560 in eighth tube for each sample.The tubes were incubated at 37°C for 24 hours.Test result was read by examining the tubes against a black background with light coming from behind the tubes.A positive reaction was seen as agglutinate appearing at the bottom of the tube leaving the upper portion of the mixture clear.

RESULTS

Majority of patients belonged in the age group of 21-40years (67.57%). Anti – Brucella antibodies were detected in the age group of 21-40years (66.6%).

Table 1: Age wise distribution of patients

Age group (in years)	No. Of patients	IgM Brucella positive	IgG Brucella positive
<20	11 (9.91%)	0	1*
21-40	75 (67.6%)	2	2(1*/2)
41-60	14 (12.6%)	0	0
>60	11 (9.91%)	0	1*
Total	111 (100%)	2	4

*=positive by SAT for Brucella

Majority of patients were males (51.35%). Principal presentation was fever (100%) followed by arthralgia and backache.50% of the patients presented with headache and myalgia along with fever.

Table 2: Sex wise distribution of febrile patients

	Study group	IgM Brucella positive	IgG Brucella positive
Males	57 (51.3%)	1	3*
Females	54 (48.6%)	1	1
Total	111 (100%)	2	4(3*/4)

*=positive by SAT for Brucella

All the patients included in the study had history of animal contact as per inclusion criteria. Occupation having risk of animal contact was observed in around 50% of patients.

Table 3: Risk factors

Risk factors	No. of patients	Percentage (%)
Animal contact history-direct or indirect	111	100%
Farmer/laborer/Vegetable vendors	54	48.65%
Travel history to an area of flood	19	17.12%
History of consumption of unpasteurized milk	6	5.41%
Dairy worker	01	0.90%
Veterinarian	01	0.90%

IgG testing was positive in 4(3.60%) patients out of 111. SAT was positive in 3 patients (2.7%) out of 111 tested.

Table 4: Comparison of IgG ELISA with SAT

	SAT positive	SAT negative	Total
IgG ELISA positive	3	1	4
IgG ELISA negative	0	107	107
Total	3	108	111

Sensitivity and specificity of ELISA were 100% and 99.07% respectively. PPV (positive predictive value) and NPV (negative predictive value) were 95% and 100% respectively. Kappa coefficient was 0.853 and diagnostic accuracy 99.09%.

Table 5: Result of IgM, IgG, SAT with reported relevant history

	IgM for Brucella	IgG for Brucella	SAT for Brucella	Animal contact history	Occupational history	Clinical findings
Patient 1	positive	negative	negative	Yes	Field worker	Fever with malaise + headache
Patient 2	positive	negative	negative	Yes	Laborer	Fever with malaise + headache
Patient 3	negative	positive	positive	Yes	Field worker	Fever with arthralgia
Patient 4	negative	positive	positive	Yes	Works at meat shop	Fever with headache
Patient 5	negative	positive	positive	Yes	Veterinary doctor	Fever with arthralgia
Patient 6	negative	positive	negative	Yes	Vegetable vendor	Fever with arthralgia

Patients seropositive for IgM antibodies showed fever with malaise and headache as major clinical symptoms while patients showing IgG antibodies presented with fever and arthralgia.

DISCUSSION

Majority of patients were found in the age group of 21-40 years and maximum percent positivity of 67% (4 out of 6 Brucella antibody positive patients) was seen in the age group of 21-40 years. Goel *et al*, Mangalgi *et al* and Yohannes *et al* noted similar findings.[3-5] In the present study, 6 out of 111 patients showed serological evidence of Brucella. 4 out of 6 were males and 2 were females. Thus, male: female ratio in the present study was 2:1. Goel *et al* reported male: female ratio as 2.33:1 in seropositive cases.[3]Mantur *et al* reported male to female ratio as 3:1.[6] The explanation for this preponderance is that males indulge more commonly in outdoor activities. In present study, we have included patients with the history of animal contact directly or indirectly. We found that farmers and laborers were affected mostly. Similar findings were reported by Goel *et al* with highest prevalence reported in agricultural workers (60%) followed by dairy workers (30%).[3] In the present study, consumption of unpasteurized dairy products was not a significant risk factor . As patients in the present study were from suburban areas, drinking pasteurised milk is not a routine practice. To investigate the role of consumption of unpasteurized dairy products, a study should be performed that covers the entire population including urban,rural and suburban areas.Antibodies against Brucella were found in 6 patients of PUO out of 111 patients in the present study (5.4%). In the region of rural western Maharashtra Goel et al reported prevalence of Brucellosis in PUO by serology is 2%.Basvaraj et al

found prevalence of Brucellosis among general population in Davangere to be 2.4% by serology.7Thakur and Thapliyal et al reported a seroprevalence of 17.39% in field veterinarians and abattoir workers for Brucella.8 In Kashmir, a study done by Kedari et al found 28 of 3532 (0.8%) patients of pyrexia of unknown origin to be positive for Brucellosis.9 In Karnataka Mantur et al reported seroprevalence of Brucella to be 1.6%.6 In the present study, 2 out of 111 patients were IgM Brucella positive (1.8%) and 4 were IgG Brucella positive (3.60%). Pathak et al reported seropositivity in 3.54% and 4.96% samples respectively detected by SAT and IgG ELISA.10Mantur et al studied 92 provisionally diagnosed Brucella cases in which IgM and IgG together were positive in 56 (60.9%) patients.6 Low positivity in the present study could be due to less sample size or actual low prevalence of Brucella infection. Study group in the present study was patients attending routine OPD of a tertiary care hospital and not specific occupation group (high risk group for Brucellosis) which may have resulted in low seropositivity. In the present study, SAT was performed on all samples and 3 (2.7%) were found to be positive. Asaad et al reported 2.6% SAT positivity in their study.11 Agasthya et al reported 2.26% samples positive by SAT among high risk population.12Nawihi et al tested 304 serum samples to detect Brucella antibodies of which 87 (28.6%) were positive by SAT.[13] All these studies showing high percentage of SAT positive results were done in target population such as farmers, dairy workers or veterinarians and were from rural area. Present study was conducted in patients from urban and suburban areas attending OPD with complaint of fever. Also, blocking antibodies in the patients' sample can result in false negative result, which may also be the reason for low positive percentage of SAT in the present study. When SAT was compared with IgG ELISA, sensitivity and specificity obtained were 100% and 90% respectively. PPV and NPV were 75% and 100% respectively. The accuracy of a test is its ability to differentiate the patients and healthy cases correctly. To estimate the accuracy of a test, we calculated the proportion of true positive and true negative in all evaluated cases. In the present study, diagnostic accuracy obtained was 99.09%. Kappa coefficient is considered as a measure of agreement between two tests. In the present study, kappa coefficient, when IgG ELISA and SAT were compared, was found to be 0.853. This indicates good agreement between the two tests. Asaad *et al* studied 54 Brucella confirmed cases and found sensitivity and specificity of IgG ELISA as 96.3% and 100% respectively.[11] In the present study, 2 samples were IgM ELISA positive and SAT and IgG ELISA negative while 3 samples were IgM ELISA negative and SAT and IgG ELISA positive. With respect to 2 only IgM ELISA positive samples, being an acute infection, IgG may be in low titres showing IgG ELISA negative and SAT negativity could be due to incomplete antibodies. IgM ELISA negative samples (3 samples) were found to be positive by SAT as well as IgG ELISA. IgM ELISA negative in these 3 samples may be because the infection was chronic with no or very low titre of IgM antibodies not picked up by IgM ELISA.[14] 2 (1.8%) patients were positive for Brucella IgM antibodies which were negative by SAT and IgG both were having history of animal contact. Both these patients had fever with malaise and headache. Presence of IgM antibodies indicates acute infection in these patients.4 patients (3.6%) showed presence of IgG antibodies by ELISA. All of them had history of animal contact and presented with complaint of fever with headache and arthralgia.. Mantur et al studied 92 Brucella positive cases and found that fever, joint pain, low backache and headache were the main symptoms. Maria Jesus et al studied chronic course of the disease.6 They found that patients presented with focal manifestations such as spondylitis and non-focal symptoms of fever, arthralgia, malaise and headache. Specific symptoms of acute and chronic Brucellosis were not mentioned in the literature and by the study groups. In the present study, acute infection with only IgM ELISA positivity showed different clinical symptoms than patients showing only IgG ELISA positivity indicating chronic infection. As the sample size was very less in the present study, we need to extend the study to large population to comment and confirm clinical symptoms in acute and chronic Brucellosis.

CONCLUSION

The clinical symptoms of zoonotic infections such as Brucellosis are not specific and these present as PUO, therefore it is beneficial to include diagnostic test for Brucellosis in the battery of tests for PUO. This will help in diagnosis of these infections early to start the therapy resulting in decreased morbidity and mortality. Also, SAT being cumbersome, time consuming and showing false negativity due to blocking antibodies, IgG ELISA and IgM ELISA can be used for diagnosis of Brucellosis.

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