Original Research Article

Brucellosis and leptospirosis among patients with PUO: A study at tertiary care hospital

Tanushree Zirwar[1], Sae Pol[2*], Renu Bhardhwaj[3]

[1]Resident, [2]Associate Professor, [3]HOD, Department of Microbiology, B J Government Medical College and Sassoon General Hospital, Pune, Maharashtra, INDIA.
Email: tzee1227@gmail.com

Abstract

Background: Diagnosis of zoonotic infection is important when history of patient indicating animal contact is obvious especially with respect to Leptospirosis and Brucellosis. The present study is necessary to increase our knowledge and help the society to make necessary interventions which will help to control the morbidity and mortality in the region. **Aim:** To study brucellosis and leptospirosis among patients with pyrexia of unknown origin. **Materials and Methods:** A total of 111 patients diagnosed as PUO were studied. Leptospirosis was diagnosed with Anti-leptospiral IgM antibody ELISA, IgG and IgM ELISA were used to detect Brucellosis. All samples were tested with standard agglutination test for Brucellosis. **Results:** 2 out of 111 patients, i.e. 1.80% showed the presence of Anti Leptospiral antibodies. Samples from 111 patients enrolled in present study and 50 from the control group where subjected to IgG and IgM ELISA for Brucellosis. Anti-Brucella IgM antibodies were found in 2 (1.80%) and IgG in 4 (3.60%). Samples from patients in the study group were subjected to standard agglutination test along with control group samples. 3 patients (2.7%) out of 111 patients were positive by SAT. **Conclusion:** As clinical symptoms of zoonotic infections such as Brucellosis and Leptospirosis are not specific and are present as PUO, it is beneficial to include diagnostic test such as IgG ELISA and IgM ELISA for Brucellosis and Leptospirosis in the battery of test for PUO.

Key Words: Pyrexia of unknown origin, brucellosis, leptospirosis, ELISA, standard agglutination test

[*]**Address for Correspondence:**
Dr. Tanushree Zirwar, Resident, Department of Microbiology, B J Government Medical College and Sassoon Hospital, Pune, Maharashtra, INDIA.
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INTRODUCTION

Pyrexia of unknown origin (PUO) is a grouping of many unrelated medical conditions that share the feature of persistent unexplained fever despite basic investigation. In spite of extensive medical experience and the development of new technologies, this condition remains as difficult for physicians as it was when first described. In India 80% of the population live in approximately 575000 villages and thousands of small towns; 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 and Leptospirosis. Human Brucellosis is a major bacterial zoonosis reported worldwide. Brucellosis is a disease of protean manifestation. It affects various body organs, systems and tissues. Any specific clinical, haematological, biochemical or imaging feature of its own is not present so that it can be distinguished from other febrile illnesses.[1] In India, leptospirosis is a major endemic disease of zoonotic importance. Climatic conditions, occupational risk factors, socioeconomic conditions, interdependence with animals are determinants of the incidence and prevalence of the disease in India.[2]The diverse spectrum of Leptospirosis makes it difficult to confirm a diagnosis if adequate laboratory support is not present. In such situation, clinical, epidemiological and biochemical findings can be of great help in diagnosis of disease as well as its

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severity. It requires a high degree of clinical suspicion based on epidemiological information. This allows the physician to recommend specific diagnostic tests which facilitate early diagnosis.[3]In present study Brucellosis and Leptospirosis were studied among patients with PUO to increase our knowledge and help the society make necessary interventions which will help to control the morbidity and mortality of the disease in the region.

MATERIAL AND METHODS

The present study was conducted in a tertiary care hospital. A total of 111 patients diagnosed as PUO were studied. Blood from 50 healthy controls were collected and tested.

INCLUSION CRITERIA

a. Brucellosis: 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.

b. Leptospirosis: Samples were collected from patients with symptoms of fever for more than 8 days with myalgia, conjunctival suffusion, anuria or oliguria and /or proteinuria, jaundice with a history of exposure to flood water or mud along with occupational history.

EXCLUSION CRITERIA

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

b. Leptospirosis: Samples were not collected from patients suffering from fever for less than 8 days and fever without myalgia, conjunctival suffusion, anuria.

METHODOLOGY

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 Leptospira: IgM ELISA was done using ELISA kit from Pan Bio. For this, reagents were kept at room temperature before commencing assay. The test sera were diluted in a microwell plate by adding 10 µl of test serum along with 990 µl of diluent gives 1:100 dilution. Well "A1" was labelled as blank. 100:l of diluted patient samples and controls were delivered into respective microwells. The plates were covered and kept for 30 minutes at 37±1°C.Microwells were washed 6 times with diluted wash buffer.100µl HRP Conjugate anti-human IgM was added to each micro well. The plates were covered and kept for 30 minutes at 37±1°C.Microwells were washed 6 times with diluted wash buffer.100µl TMB solution was added in each microwells. The microwells were incubated for 10 minutes at room temperature in dark. 100µl of stop solution were added in the microwells. Microwell plate was read by using microwell ELISA reader at 450nm. As per the kit literature PANBIO>11 units were considered as positive.

IgM ELISA for Brucella: IgM ELISA for Brucella was done by IgM Brucella Nova TEC. For this, reagents were kept at room temperature before commencing assay. The test sera were diluted in the test tube by adding 10µl of test serum along with 1000µl of diluent, vortex was done. Well "A1"was labelled as blank. 100µl of diluted patient samples were extracted 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. (repeat step 5). 100µl TMB solution was added in each microwell. The microwells were incubated for 15 minutes at room temperature in dark. 100µl stop solution were added in the wells. Micro well plate was read by using microwell ELISA reader at 450nm. As per the kit literature >11 NTU was considered as positive.

IgG ELISA for Brucella: IgG ELISA for Brucella was done by using ELISA IgG Brucella Nova TEC. The test sera were diluted 10/1000µl in test tube by adding 10µl of test serum along with 1000µl of diluent, vortex was done. Well "A1" was labelled as blank. 100µl of diluted patient samples were extracted 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 dark. 100:l stop solution were added in the wells. Microwell plate was read by microwell ELISA reader at wavelength of 450nm. As per the kit literature >11 NTU were considered as positive.

Standard Test Agglutination 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 were added to the first tube of 3rd to 8th row. Two-fold serial dilution was done by transferring 0.5ml of the mixture starting from the 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 dilution ranged from 1:20 first tube to, 1:2,560 in 8th tube. 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 observed, when agglutination was observed at the bottom of the tube leaving the upper part clear.

RESULTS

Majority of patients were belonged in the age group of 21-40years (67.57%). Antibrucella antibodies were detected in the age group of 21-40 years(66.6%)and antileptospiral antibodies were detected in the age group of 41- 60(100%).

Table 1: Age wise distribution of patients

Age group (in years)	No. of patients	Percentage (%)	IgM Brucella Positive	IgG Brucella positive	IgM leptospira positive
<20	11	9.91	0	1*	0
21-40	75	67.57	2	2(1*/2)	0
41-60	14	12.61	0	0	2
>60	11	9.91	0	1*	0
Total	111	-	2	4	2

*=positive by SAT for Brucella

Majority of patients were males (51.35%).

Table 2: Sex wise distribution of febrile patients

	Study group	Percentage (%)	IgM Brucella Positive	IgG Brucella positive	IgM Leptospira positive
Males	57	51.35	1	3*	1
Females	54	48.65	1	1	1
Total	111	-	2	4(3*/4)	2

*=positive by SAT for Brucella

All the patients included in the study were shown the 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%
Veterinary Doctor	01	0.90%

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

Table 4: Result of serological tests with reported relevant history

	IgM for Brucella	IgG for Brucella	SAT for Brucella	Animal contact H/O	Occupational H/O	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 vender	Fever with arthralgia

DISCUSSION

In the present study, antibodies against brucella and leptospira were found in 8 patients of PUO out of 111 patients (7.02%). Out of these 8 patients, 6 were seropositive for brucella. In the region of rural western Maharashtra, Goel *et al* reported prevalence of brucellosis in PUO by serology is 2%.[4] Basavarajappa *et al* showed that prevalence of brucellosis in Davangere is 2.4% by serological test. Thakur and Thapliyal *et al* reported a seroprevalenceof 17.39% in field veterinarians and abattoir workers.[6]In Kashmir study done by Kedari *et al* found 28 of 3532 (0.8%) patients of pyrexia of unknown.[7] In the present study, out of 111 patients, 2 were seropositive for leptospira (1.8%). Both the patients positive for Leptospira IgM ELISA had travelled to areas of flood and presented with fever, muscle pain and headache. Modified Faine's total score for both the patients resulted in confirmed diagnosis of leptospirosis. Modified Faine's criterion has two parts, first includes clinical features and epidemiological features and second includes result of ELISA and/or MAT. We need to add results of serological test to the first part of the criteria to come to be final diagnosis. In the present study, when first part was considered in case of both patients the diagnosis was presumptive. This is because the symptoms presented by leptospirosis were not specific enough to diagnose the infection. So, on clinical basis the diagnosis was not possible. This underlines the importance of serological support to confirm the clinical suspicion of leptospirosis. Shiv *et al* concluded that Modified Faine's criterion is valuable in diagnosis of leptospirosis when serological test is available.[8] Uy-Lumandas *et al* however reported Modified Faine's criteria had poor sensitivity and low PPV and cannot be recommended as a screening test for the early diagnosis.[9] Study done by Sharma *et al* revealed sensitivity of 3.03% and specificity of 80% and PPV 66%.[10] In the present study, using modified Faine's criteria we could come to the definitive diagnosis of leptospirosis in 2 patients. Utility of modified Faine's criteria in any set up needs an extensive study using large patient base. 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%, 4.96% samples detected by SAT and IgG ELISA.[11] Mantur *et al* studied 92 provisionally diagnosed Brucella patients in which IgM and IgG together were positive in 56(60.9%) patients.[12] 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 presented study, SAT was performed on all samples and 3 (2.7%) were found to be positive. Assad *et al* reported that out of 54 confirmed brucellosis cases, SAT gave positive in 50 (92.6%).[13] Agasthya *et al* reported 2.26% samples positive by SAT among high risk population.[14]Nawihi *et al* tested 304 serum samples to detect Brucella antibodies of which 87 (28.6%) were positive by SAT.[15] 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. 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 to comment on specific symptoms for acute and chronic brucellosis we need to extend the study to large population to comment and confirm clinical symptoms in acute and chronic brucellosis. Diagnosis of zoonotic infection is important when history of patient is indicating animal contact, occupation in which animal contact is obvious and /or travel to endemic area of infection especially with respect to leptospirosis.

CONCLUSION

As clinical symptoms of zoonotic infections such as brucellosis and leptospirosis are not specific and are similar to PUO, it is beneficial to include diagnostic test for brucellosis and leptospirosis in the battery of test for PUO. This will help in early diagnosis and treatment of these infections resulting in decreased morbidity and mortality. Also, SAT being cumbersome, time consuming and shows false negativity due to blocking antibodies, IgG ELISA and IgM ELISA can be used for diagnosis of brucellosis.

REFERENCES

1. Doganay M, Bilgehan A. Brucellosis: an Overview. Int J Infect Dis. 2003; 7: 173–82.
2. Himani D, Suman MK, Mane BG. Epidemiology of leptospirosis: an Indian perspective. J foodborne zoonotic Dis [Internet]. 2013; 1(1):6–13.
3. Agarwal SK, Rajani AR, Hussain K, Dande MM. Brucella endocarditis: an occupational hazard. BMJ Case Rep. 2013; 2013: 10–3.
4. Goel S, Goyal P, Singh A, Kumar A, Gupta A, Surana A, *et al*. Incidence and sero epidemiology of brucellosis from a tertiary care centre of rural Maharashtra. 2015;2(8):71–7.
5. Basavaraj Metri C, Jyothi P, Baragundi Mahesh C, Lava R, Basavarajappa, Hanumanthappa AR, *et al*.

Seroprevalence of brucellosis in Davangere, Karnataka. J Clin Diagnostic Res. 2011; 5(1):41–4.
6. Thakur SD, Thapliyal DC. Seroprevalence of brucellosis in man. J Commun Dis [Internet]. 2002; 34(2):106–9.
7. Kadri SM, Rukhsana A, Laharwal MA, Tanvir M. Seroprevalence of brucellosis in Kashmir (India) among patients with pyrexia of unknown origin. J Indian Med Assoc. India; 2000 Apr; 98(4):170–1.
8. Shivakumar S, Shareek PS. Diagnosis of leptospirosis utilizing modified Faine's criteria. J Assoc Physicians India. 2004 Aug; 52:678-9.
9. Uy-Lumandas M, Ong-Lim AL, Gonzales ML. Validation of modified Faine's criteria in the diagnosis of leptospirosis in children using microscopic agglutination test as the gold standard. Pediatr Infect Dis Soc Philipp J. 2013; 14(1):42–8.
10. Sharma N, Sethi S, Bhalla A. Evaluation of the Modified WHO Faine's Criteria for Diagnosing Human Leptospirosis in a Tertiary Care Hospital of North India. 2016; 1(December):0–1.
11. Pathak AD, Dubal ZB, Doijad S, Raorane A, Rodrigues S, Naik R, *et al*. Human brucellosis among pyrexia of unknown origin cases and occupationally exposed individuals in Goa Region, India. Emerg Health Threats J. 2014; 7(1):1–5.
12. Mantur B, Parande A, Amarnath S, Patil G, Walvekar R, Desai A, *et al*. ELISA versus conventional methods of diagnosing endemic brucellosis. Am J Trop Med Hyg. 2010; 83(2):314–8.
13. Asaad AM, Alqahtani JM. Serological and molecular diagnosis of human brucellosis in Najran, Southwestern Saudi Arabia. J Infect Public Heal. 2012; 5(2):189–94.
14. Agasthya A, S I, Prabhudas K. Brucellosis in high risk group individuals as. Indian J Med Microbiol. 2007; 25: 28–31.
15. Nowihi M, Abdullah QY, Alkhyat SH, Almahbashi AA, AlThobahni A, Al Bana MN, *et al*. Comparison of Standard Agglutination Test and Enzyme- Linked Immunosorbent Assay to Detect Brucella Infection in Yemeni Pregnant Women. J Microbiol Exp [Internet]. 2017; 5(5):10–2.

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