International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

Original Research Article

Brucellosis! An Unusual Etiology in PUO!

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Received: 02/08//2012 *Revised: 24/08/2012* *Accepted: 25/08/2012*

ABSTRACT

Introduction: Brucellosis is one of the most common zoonotic disease in the world. Human brucellosis is known for its protean manifestations. However the most common presenting symptom is fever. The disease may be overlooked and misdiagnosed because of difficulty in diagnosis and lack of experience with laboratory testing. The present study therefore, was carried out, to see the prevalence of brucellosis in this area.
Methodology: Serum samples received in Microbiology department for Widal test were screened for antibrucella antibody by slide agglutination test. Positive serum samples, from these sera were confirmed for Brucellosis by Standard Tube Agglutination Test. An attempt was made for isolation of *Brucella* by blood culture.
Results: Of the 327 serum samples screened 6 were positive by slide agglutination. Four samples were positive by Standard Tube Agglutination Test.Brucellosis was confirmed in 1.22 % of the samples screened.
Conclusion: To conclude, though culture is gold standard, serology remains simple, cost effective, best diagnostic method for Brucellosis. Also physicians including pediatricians should have high degree of suspicion of brucellosis with history of animal contact, or simply of living in rural areas as three of four positive cases were from pediatric age group.
Key Words: Brucellosis, PUO, Rose Bengal Plate Test (RBPT), Standard Tube Agglutination Test (STAT).

INTRODUCTION

Brucellosis is one of the most common diseases in the world. [1] It is endemic in the Mediterranean region, the Middle East, Latin America and parts of Asia and Africa. [2] The reported incidence of human brucellosis worldwide in endemic areas varies widely, from < 0.01 to >200 per lakh population. [3] Human brucellosis is

known for its protean manifestations. However the most common presenting symptom is fever. The disease may be overlooked and misdiagnosed because of difficulty in diagnosis and lack of experience in laboratory testing. Clinical diagnosis is often impossible. A high index of suspicion and optimal use of laboratory is essential for accurate diagnosis of the disease. Absolutely certain diagnosis of brucellosis is made only when the causative organism is isolated in culture. [4] However brucella takes longer time to grow in vitro. Hence a diagnosis can be made by serological tests. [1, 3]

Considering the physicians lack of suspicion of brucellosis in any pyrexia of unknown origin (PUO) case, present study was carried out to find prevalence of the same in this area.

METHODOLOGY

Total 327 serum samples received in department of Microbiology, KIMS Karad for Widal test were included for study. Standard serological screening methods were used. Isolation of brucella was done from seropositive cases by standard methodology. [5] Serum samples from PUO cases received in serology laboratory for Widal test were first subjected for rapid slide agglutination test for detection of antibrucella antibodies. The samples positive by slide agglutination test further, were tested by standard tube agglutination test to determine the titres. An effort was made to collect blood from all the seropositive patients for blood culture with their consent.

Rapid slide agglutination test:- Rose Bengal Plate Test (RBPT) antigen is a pink coloured suspension of pure, smooth, killed cells of *Brucella abortus* strain 99, phenolised and stained with Rose Bengal dye. [5, 6] The antigen and sera stored at 4^{o}C were removed from refrigerator and left at room temperature for ½ hour. Just prior to use antigen bottles were shaken to ensure homogenous suspension. With the help of glass marking pencil the plate was divided into two halves. One drop (0.03ml) of antigen was placed in the square marked on each half of the plate. With a micropipette, 0.03ml of the serum was placed along with side of the antigen. With a spreader, the antigen and serum were mixed thoroughly and were spread to an area of about 2.5cms diameter. The plate was rotated for 4 minutes and the readings were immediately recorded. A known positive and negative serum was included in each day's tests. The test was read as positive on noting any degree of agglutination or negative when no agglutination was noted.

Standard Tube Agglutination Test (STAT):- Brucella standard agglutination test antigen is a suspension of a pure smooth culture of *Brucella abortus* strain 99 in phenol saline. Doubling dilution of test serum was done with 0.5 % phenol saline starting from 1:10. [5, 7] Equal amount of (0.5 ml) of *B.abortus* plain antigen was added to each tube. The tubes content were mixed and incubated at 37 ^{0} C for 20 hours. A set of 5 antigen control tubes with opacity corresponding to 0,25,50,70 and 100 percent in 0.5 % phenol saline were included for comparing the result of the test samples. A known positive and negative control sera were also simultaneously tested.

Interpretation of results: - The test tubes were compared with antigen control tubes for degree of opacity of the supernatant fluid. The maximum dilution that exhibited 50 % agglutination was considered as the end point of serum activity and was taken as titer of antibodies against brucella. Titer of 1:160 or above is considered diagnostically significant for brucellosis in humans. [5, 7]

Blood Culture:- Blood was collected under strict aseptic conditions from the patients

who showed the presence of antibrucella antibodies by standard tube agglutination test. About 10 ml of blood was collected from each patient whose STAT test was positive.5 ml of blood was added to each of the two bottles containing 50ml of Brain Heart Infusion Biphasic Media. [5] One bottle was incubated at 37°C and other was incubated in candle jar to provide the atmosphere of CO_2.

Subcultures were made onto BHI Agar plates in duplicate every 4th day of incubation. Plates were incubated at 37°C aerobically as well as in CO_2 jar. Plates were incubated at least for six weeks before being discarded as negative. [5]

RESULTS

Of the 327 serum samples screened 6 were positive by slide agglutination test (RBPT).

Table 1: Results of STAT test and Blood culture in six positive (RBPT) samples

Sr. No	Patient Age (Years)	Slide Agglutination Test (RBPT)	Standard Tube Agglutination Test (STAT) Titer	Culture
1	13	Positive	1: 2560	Negative
2	26	Positive	1:160	Negative
3	35	Positive	1:40	-
4	13	Positive	1: 1280	Negative
5	19	Positive	1:20	-
6	12	Positive	1: 1280	Negative

Four samples showed a diagnostic titer of equal to or more than 1:160 by STAT method.

DISCUSSION

Identifying causative agent for PUO poses a major health challenge in many countries including India. When it comes to unusual etiology like brucellosis, there is added difficulty because of lack of suspicion from physicians and limited laboratory expertise. [8]

Clinical findings, isolation from the blood and serological tests are valuable diagnostic methods for brucellosis. [5] Isolation of brucella though gold standard in practice is difficult due to early tissue localization and exacting culture requirements. [5, 8] Serological tests plays major role in diagnosis of the disease. Commonly used test include RBPT for screening and STAT for confirmation of brucellosis. [5, 8, 9]

In the study, 327 samples were screened by RBPT for brucella antibody, total six (1.83 %) were positive with slide agglutination method. Four of these samples showed STAT titre of more than or equal to 1:160 and confirmed brucellosis. [5] False positive reaction in RBPT may be due to cross reacting antibodies which are seen in *Francisella tularensis*, *E.coli* O :116 and O :157 , *Salmonella urbana* O:30, *Yersinia enterocolitica* O:9 ,*Pseudomonas matophilia* and *Vibrio cholera* infections. [10]

Seroprevalence by STAT method was 1.22 % in the present study. Similar findings were observed by Metri et al 1.6 % [9] while Kadri et al found it in 0.8 %. [11] In

other studies carried out by Handa et al, [12] and Sen et al [13] found it 3.3% and 6.8 % respectively in PUO cases. The prevalence rate in this study may be considered less as only Widal specimens suspected for enteric fever were screened for brucella. The prevalence rate might be more if general population (symptomatic / asymptomatic) is screened for brucella. Out of four positive cases in present study , three cases belong to paediatric age group. Nagmoti et al has reported three cases of childhood brucellosis. [14] Although human brucellosis affects all age groups, it is said to be rare in childhood. However in areas, where *B. melitensis* is endemic, pediatric cases are seen. [1] All four positive cases from our study gave history of contact with cattle with one giving history of consumption of raw milk. Blood culture of these patients was negative for brucella as though gold standard has its own limitations with isolation rate varying between 17-85 %. [15]

Many a times, a differential diagnosis of brucellosis in PUO cases is not considered by clinicians due to several reasons, such as physicians and surgeons failure to suspect the disease, misdiagnosis for enteric fever, failure to isolate organism on culture, absence of epidemiological approach, protean manifestations and atypical presentations. [9]

CONCLUSION

Serology plays important role in diagnosis of brucellosis. Also physicians should have high degree of suspicion of Brucellosis with history of animal contact or simply of living in rural areas, in PUO even in pediatric cases.

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