Original Article

Serological Detection of Human Brucellosis among the Fever of Unknown Origin (FUO) Patients in Risk Group at Mymensingh, Bangladesh

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Abstract

Objectives: *FUO is one of the unresolved challenges in medicine despite the presence of modern diagnostic facilities and therapeutic advancement. The prevalence of FUO in hospitalized patient is reported approximately 3% and has a higher impact on health care system. Among the infective causes of FUO, more than 3% cases are due to Brucellosis. Brucellosis is a zoonotic disease and occurred mainly in occupationally exposed individuals such as farmers, veterinarians, slaughter house workers, cattle handlers, and meat inspectors (risk group). As Bangladesh is a developing country many livestock based industries are developed within the last 10 years. As a result the chance of human Brucellosis also increased. The true incidence of Human Brucellosis in our country is not known. The purpose of this study is to find out the prevalence of Brucellosis among the occupationally exposed FUO patients.*

This cross sectional descriptive type of study was carried out in the Department of Microbiology, Mymensingh medical college, Mymensingh, Bangladesh, from 01/01/2017 to 31/12/2017. Total 300 (three hundreds) patients of either sex were included in this study who met the inclusion criteria of FUO from occupationally exposed cases. 05 ml Whole blood was collected aseptically from each patient, serum were separated and used for Brucella specific latex agglutination test and ICT.

Among the study population 64.67 %(194/300) were male & 35.33%(106/300) were female. Majority of the population were from age group 20-40 yrs. The prevalence of brucellosis were found 13.33%(40/300) by brucella specific latex agglutination test and 5%(15/300) by immune chromatographic test (ICT). In brucella specific latex agglutination test and ICT 50%(20/40) and 46.67%(7/15) were found positive in dairy farm/ animal farm workers respectively. followed by cattle handlers 32.5%(13/40) and 33.33%(5/15), then slaughter house workers 15%(6/40) and 20%(3/15) .

The present study showed that a considerable number of FUO cases due to brucellosis is present in risk group of population.Consequently human brucellosis should be included as a differential diagnosis in FUO cases especially in FUO with history of occupational exposure.

Key wards: *Brucellosis, FUO, ICT.*

Introduction

FUO is one of the unresolved challenges in medicine despite the presence of modern diagnostic facilities and therapeutic advancement. Fever of unknown origin (FUO) is a major cause of debilitating illness worldwide. It was first described in 1961 by Petersdorf and Beeson[1] as fever with a body temperature ≥ 38℃ for at least 3 weeks duration with a failure to reach a diagnosis after 1 week of inpatient investigation or 3 outpatients' visits.[2]

The prevalence of FUO in hospitalized patient is reported approximately 3% and has a higher impact on health care system.[3] In 1930, 70% of FUO remain undiagnosed which has become 5-10% in 2004.[4] FUO classified under the following headings i) classical ii) nosocomial, iii) immune deficient or neutropenic, iv) HIV associated (Modified Durack and Street classification of FUO).[5] The classic FUO again subdivided into infectious, neoplastic, connective tissue diseases and miscellaneous conditions (1). Among the infective causes of FUO, more than 3% cases are due to Brucellosis.[6] Brucellosis is a zoonotic disease and occurred mainly in occupationally exposed individuals such as farmers, veterinarians, slaughter house workers, cattle handlers, and meat inspectors (Risk group).[7] As Bangladesh is a developing country many livestock based industries are developed within the last 10 years. As a result the chance of human Brucellosis also increased. The true incidence of Human Brucellosis in our country is not known. In A study done by Ahmed and Suman P singh[8,9] showed the prevallance of Human Brucellosis is 13% and 14.8% in risk group population respectively. The purpose of this study is to find out the prevalence of Brucellosis among the occupationally exposed FUO patients.

Materials and methods

This cross sectional descriptive type of study was carried out in the Department of Microbiology, Mymensingh medical college, Mymensingh, Bangladesh, on patients suffering from fever of unknown origin who were attending at outpatient and inpatient department from 01/01/2017 to 31/12/2017. Sample size was calculated by using Guilford and Frucher's formula, n= (z2xpq)/d2. Here prevalence of human Brucellosis in risk group population in Mymensingh region was 13%8. The calculated sample size was 172, for better study Total 300 (three hundreds) patients of either sex were included in this study who met the inclusion criteria of FUO Among high risk

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group. High risk group includes patients with history of occupational exposure such as cattle farmers, veterinarians slaughter house workers, animal handlers etc. Patients suffering from immunodeficiency due to any cause or had known malignancies or whom diagnosis were established during the study period were excluded from our study. Axillary and oral temperatures were used for temperature recording. 05 ml Whole blood was collected aseptically from each patient, serum were separated and used for Brucella specific latex agglutination test and ICT.

Laboratory procedure

At first screening test (Brucella specific latex agglutination test) were done with all collected samples and Titer ≥ 1:160 were consider as screening positive.[10] The Brucella specific latex agglutination test (Spinreact SA/SAU. ctra Santa calona. 7E.17176 SA NT ESTEVE 1E BAS (G1) Spain) was performed on each sample according to the manufacturer's instructions.

Immuno chromatographic (ICT) test

Brucella IgM/IgG LFA is an immune chromatographic lateral flow assay (one diagnostics, 1090HA, Amsterdam, The Netherlands). The assay was intended to be used as an aid in the sero diagnosis of Brucellosis. The Brucella IgM/IgG LFA consists of two devices, one for the detection of specific IgM antibodies and one for the detection of specific IgG antibodies.

Methods

The assay was based on the binding of specific antibodies to a broadly reactive antigen immobilized at the test line, located in the assay window of the device. The assay utilizes a dried detection reagent deposited within the device. The detection reagent consists of a red particles coated with antibodies. The brucella IgM assay uses antibodies specific for IgM and the brucella IgG assay uses antibodies specific for IgG. To perform the assay 5µl serum was placed in the sample port of 2 assay device one for detection of Brucella IgM and another for Brucella IgG and then 4 drops of assay fluid was added in each device to start the assay. Specific antibodies present in the patient sample migrate through the porous membrane by capillary force and attach to the antigen at the test line. The detection reagent in turn was bind to these antibody complexes that are attached to the membrane, resulting in the appearance of a red line. Test results were taken after 15 minutes under adequate light. The presence of specific IgM antibodies contained in the patient sample was revealed by the appearance of a red line in the test zone of the brucella IgM assay device. While the presence of specific IgG antibodies was revealed by the appearance of a red line in the test zone of the brucella IgG assay device. If the sample not containing any brucella specific IgM or IgG antibodies, the sample and detection reagent were pass over the test zone and no line appear in the test zone. With any sample, a red line should appear in the control zone. If a red line not appearing in the control zone, the test was considered as invalid.

Interpretation of the test

1) A positive result with the brucella IgM device and a negative result with the brucella IgG device demonstrate the presence of specific IgM antibodies and are indicative of an acute Brucellosis infection.
2) A positive result with the brucella IgM device and a positive result with the brucella IgG device demonstrate the presence of specific IgM and IgG antibodies and are indicative of a sub acute Brucellosis infection.
3) A negative result with the brucella IgM device and a positive result with the brucella IgG device demonstrate the presence of specific IgG antibodies and were indicative of a chronic form of Brucellosis.

Result

Brucellosis is one of the major cause of Fever of unknown origin (FUO) among the suspected risk group population. With this back ground the study was done in Mymensingh Medical college mymensingh, Bangladesh to detect the prevalance of Brucellosis in the suspected risk group population. This study was conducted in 300 patients suffering from Fever of unknown origin who had a history of occupational exposure. At first Brucellaspecific latex agglutination tests and then ICT tests were done from all samples to detect the true incidence of Human Brucellosis.

Table -1:Age and sex distribution study population(n=300)

Age distribution (yrs)	Male	Female	Total
< 20	49	24	73(24.33%)
20-40	90	60	150(50%)
41-60	40	13	53(17.67%)
>60	15	09	24(8%)
Total	194(64.67%)	106(35.33%)	300(100%)

Table 1 : showing the total distribution of study population regarding age and sex. Among the study population 64.67%(194/300) were male and 35.33%(106/300) female respectively. Majority of the study population 50%(150/300) were in age group 20-40 yrs and minimum number 8%(24/300) were from age group >60 yrs.

Table-2:Result of Brucella specific latex agglutination test according to titer in study population(n=300)

Titer	No of cases	Percentage	Commulative percentage
1:20	115	38.33%	
1:40	80	26.67%	
1:80	65	21.67%	
1:160	38	12.66%	13.33%
1:320	02	0.67%	
1:640	0	0%	
Total	300	100%	

Table 2 : showing the result of Brucella specefic latex agglutination test in study population. Among them 12.66%(38/300) and 0.67% (2/300) having a titer of 1:160 and 1:320 respectively and the comulative percentage were 13.33%.

Table-3: Prevalance of Brucellosis detected by Brucella specific latex agglutination test and ICT test(n=300).

Test name	Positive	Negative
Brucella specific latex agglutination test	40(13.33%)	260(86.67%)
ICT	15(5%)	285(95%)

Table 3: shows 13.33% (40/300) study population were sero positive by Brucella specific latex agglutination test and 5% (15/300) were positive by Immunochromatographic test (ICT).

Table-4: Distribution of brucellosis among different occupations by Brucella specific latex agglutination test (n=40) (titer1:≥160 considered as positive cases by Brucella specific latex agglutination test)10.

Occupatins	Positive
Live stock fermers/cattle handelars	13(32.5%)
Veterinarians	01(2.5%)
Slaughter house workers	06(15%)
Dairy farm/animal farm workers	20(50%)
Total (n=40)	40(100%)

Table 4: shows the sero prevallence of brucellosis among different occupations in study population. Total 13.33% (40/300) were Brucella specific latex agglutination test positive. Among them highest 50%(20/40) cases were found in dairy farm /animal farm workers, followed by 32.5%(13/40) in live stock farmers/cattle handelars, then 15%(06/40) in slaughter house worker and 2.5%(1/40) cases were found in vaterinarians.

Table-5: Distribution of brucellosis among different occupations by ICT test(n=15)

Occupatins	Positive
Live stock fermers/cattle handelars	05(33.33%)
Veterinarians	00 (00%)
Slaughter house workers	03(20%)
Dairy firm/animal farm workers	07((46.67%)
Total (n=15)	15(100%)

Table 5: shows the sero prevallence of brucellosis among different occupations in study population. Total 5% (15/300) were ICT positive. Among them highest 46.67%(7/15) cases were found in dairy farm /animal farm workers, followed by 33.33% (05/15) in live stock farmers/cattle handelars, then 20% (03/15) in slaughter house worker and we were not found any positive cases in vaterinarians by ICT method.

Table-6: Distribution of ICT positive cases from Brucella specific latex agglutination test positive and Brucella specific latex agglutination test negative cases (n=300). (titer1:≥160 considered as positive by brucella specific latex agglutination test).

	ICT positive	ICT negative
Brucella specific latex agglutination test positive (n=40)	13(32.5%)	27(67.5%)
Brucella specific latex agglutination test negative (n=260)	02(0.77%)	258(99.23%)
Total (n=300)	15(5%)	285(95%)

Table 6: shows the distribution of ICT positive cases from Brucella specific latex agglutination test positive and from negative cases repectively. Total 5%(15/300) cases were ICT positive. Among the positive cases 32.5% (13/40) were found from Brucella specific latex agglutination test positive cases and 0.77%(2/260) were found from Brucella specific latex agglutination test negative cases.

Discussion

Human Brucellosis is a major zoonotic and occupation related disease which is caused by a bacteria belonging to genus Brucella.They are small, gram negative, non spore forming, non capsulated coccobacili. Globally more than 500000 new cases are reoorted every year with the annual incidence of varying widely from <2 to >500 per 1000000 population among different regions of world including Latin America,The Middle East, Africa, Asia and the mediterranean basin.[11]

There are several methods for diagnosis of human Brucellosis among them Blood culture and isolation of the organism in labratories is gold standerd, but this process is laboreous and time consuming.[12] therefore serological based test methods are considered as the most practical method for screening and for diagnosis of the disease. Keeping this view in mind we used brucella specific latex agglutination test and immunochromatographic test (ICT) to detect the prevallance of Brucellosis among FUO patients.

In the present study the majority of the cases 50% (150/300) were in the age group between 20-40 yrs and total 64.67% (194/300) were male and 35.33%(106/300) were female (table-1). The age group between 20-40 yrs and male predominance was due to people in this age group are more active and main earning members of the family in our society which causes them more commonly infected. The seroprevallance of Brucellosis by brucella specific latex agglutination test was 13.33% (40/300) Table-3. The prevallance of Brucellosis by using IgM & IgG kit were 5%(15/300).Table-3. The sero prevallance study of Brucellosis in Human in risk group was conducted by Nahar & Ahmed in 2009 and reported it was 6%.[13] Which was inaccordance with our current study. Several study reported that among high risk population 4.4%- 12.8% were sero positive in some selected areas in Bangladesh.[14,15,16] This was due to high risk people were in close contact with animals and their products.

In this study we found total 15 (5%.15/300) ICT positive cases. Among them dairy farm / animal farm workers were found highest sero positivity for brucella infection and it was 46.67% (7/15). Another study by Rahman et al reported that milker

(18.2%. n=55), showed maximum positivity.[16] which was inaccordance with our current study. It is due to their occupational exposure with animals during milking and other farm activities. In this study other occupational group such as live stock farmers/cattle handallers and slaughter house workers found 33.33%(5/15) and 20%(3/15) brucella seropositive respectively. In a recent study in Sylhet by Akhtar J et al showed 31.3% slaughter house workers were seropositive.[17] another study coducted in Pakistan by Mukhter showed 21.7% seropositivity among slaughter house workers.[18] cattle handelars are directly contacted with their domestic animals, they are responsible for feeding, taking care of their animal during sickness, assisting animal during parturation as well as handling of still birth. So they have high chance of brucella infection. It is also well known that, the slaughter house workers were exposed to organs of infected animals and most of them were work with bare hands. Hence the risk of brucella seropositivity to them increase through cuts or injuries and splashing of infected fluid.

Conclusion

The present study showed that a considerable number of human brucellosis is present in risk group of population in mymensingh district. more sensitive and specific tests such as PCR and Rt- PCR is required for diagnosis of brucellosis. Vaccine against animal brucellosis is less effective and absence of vaccine against human brucellosis will increase the chance of human brucella infection. Hance human brucellosis should be included as a differential diagnosis in FUO patients espicially with history of occupational exposure.

Limitations

The study was done in a limited period of time so the sample size was small which causes the findings sparse. Further study on the subject from different region of Bangladesh should be carried out in long scale to find out the actual prevalence of the disease.

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