

Sero-Diagnosis of Human Brucellosis Among TB Suspected Patients

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Abstract

In order to verify the frequency of human brucellosis among a TB suspected population, a study was conducted on the basis of sero- diagnosis of a total of 229 blood samples from TB suspected patients. Serological tests carried out were slide and semi- quantitative agglutination tests. Of the tested samples, 44(19.21%) were SAT positive and of these, 28(12.23%) were positive for the semiquantitative agglutination test. Twelve (42.86%) had a brucella titer of 1:80; 14 (50%) had a brucella titer of 1:160 and 2 (7.14%) had a titer of 1:320. The majority of patients positive for brucellosis, exhibiting sero-agglutination titers ranging between 1:40 and 1:320, were of rural background. However, 35.72% persons positive for brucella antibody with titer of 1:160 were from urban localities. Fever (84.48%), headache/dizziness (71.43%) and weakness/fatigue (46.43%) were the most common presenting symptoms among the brucellosis positive patients. The frequency of brucellosis among these TB suspected patients was found to be higher in women (73.91%), as compared to men (52.38%)(JPMA 47:243, 1997).

Introduction

Brucellosis is a bacterial disease which affects about 500,000 individuals worldwide each year. It is also a disease of animals and causes significant losses among livestock, therefore, is one of the most distressing and economically ravaging zoonotic disease of immense public health importance. Its incidence in humans and livestock has been reported in many countries¹. The primary hosts of *Brucella* species are cattle, goats, sheep, swine and dogs. During infection in animals, the blood, spleen, liver and kidneys carry the pathogen. *Brucella* is also localized in the pregnant uterus, causing abortion and in the mammary glands where the organisms are shed in the milk². The established mode of transmission of *Brucella* spp. to humans occurs usually through direct contact with infected animals or their carcasses, or by ingestion of unpasteurized milk or dairy products from infected animals³. Airborne transmission is thought to occur by contaminated aerosols in meat packing plants and laboratories⁴. Brucellosis is a zoonosis with protean multisystem manifestations, classically referred to as a disease of farmers, meat handlers and persons who drink raw milk from infected animals and consume infected carcasses. Brucellosis presents a set of approximately 150 symptoms with the major manifestations like, undulating fever, sweating, chills, cough, lassitude, malaise, gastritis, weight loss/anorexia, dizziness, arthritis and backache⁵.

Brucellosis is gradually being eliminated in industrialized countries, but is on the rise in developing countries, may be due to an attempt to an increase in the supply of milk and meat through the expansion of the domestic animal industries. A few studies are already on record in Pakistan reporting brucella infection among PUO patients⁷. Animal brucellosis in Australia is on the decline since 1986, as a result of mandatory pasteurization of domestic milk supplies and control of bovine infections. This was achieved through a National Brucella and Tuberculosis Eradication Campaign⁸. However, human brucella infection has been reported among abattoir workers in Australia through the processing of infected cattle meat. The present study therefore, goes inline with the Australian Brucella and Tuberculosis Eradication Campaign i.e., both infections are likely to be of animal origin, therefore,

when there are chances of tuberculosis infection, a possibility of brucellosis is always there. In view of a variety of symptoms being presented by TB suspected patients, which are similar to those explained for brucellosis, the present study was undertaken to conduct serological studies for brucella infection among TB suspected patients. Although a variety of tests, including ELISA are available for serodiagnosis of brucellosis, yet many of the workers still advocate the efficacy of slide agglutination and semiquantitative agglutination tests to be reliable methods for the detection of brucella infections⁶. In the present study, these two tests were employed to detect sero-positivity for recording brucellosis among TB suspected individuals.

Materials and Methods

A total of 229 samples were collected for the serological study to ascertain the frequency of human brucellosis among a TB suspected population. A proforma was filled out at the time of blood collection, with the help of the concerned physician, detailing the patient's history and ailment. Three to five cubic centimetre of blood was collected using a sterile syringe and left overnight to allow for clotting. Later, the serum was extracted by centrifugation at 2500 rpm for 5 minutes, dispensed into sterile eppendorf vials and immediately processed. If serological tests were not carried out immediately, the serum was stored at -20°C till subsequent analysis. Collected sera were tested for the presence or absence of brucella antibodies using slide test antigen produced by SLITEST W-W Cod. R 1710. The test consists of observing the reaction of direct agglutination between bacterial suspension and patient serum which may contain the specific antibodies. The test procedure prescribed by the manufacturer was followed. The results were interpreted as suggested in the literature provided with the kits.

Results

Of the total 229 patients tested, 44 (19%) were found to be SAT positive and of these 28 (12%) showed positivity to the semiquantitative agglutination test. Among patients that were positive to the semiquantitative agglutination test, showing varied titer distribution, 16 had titre 1:40, 12 (43%) 1:80, 14 (50%) had a titer of 1:160 and 2 (7%) showed a titer of 1:320. As for the locality of the patients tested for brucellosis, 77 % of those with a 1:40 titer had a rural background having frequent contact with farm animals whereas, 23% with the same titer had a urban background. All those patients with a titer of 1:80 lived in villages as was the case with the 2 patients with a titer of 1:320. Of those patients with a titer count of 1:160 (64%) had their homes in villages and 36% lived in the city (Table 1).

Table I. Titer-wise locality percentage of sero-positive TB suspected patients.

Titer	No. of sero-positive patients	Locality percentage	
		Rural	Urban
1:40	44	77	23
1:80	12	100	0.00
1:160	14	64	36
1:320	2	100	0.00

Among the TB suspected patients who were sero-positive for brucellosis it was recorded that the most common presenting symptom was fever (84%), followed by headache/dizziness, weakness/fatigue, cough, arthritis/ rheumatic pains, chills, backache, naus. a/anorexia, chest pain and vomiting (Table II).

Table II. Presenting symptoms (%) among semiquantitative sero- positive TB suspected patients.

Presenting symptoms/ Physical signs	Percentage (n=28)
Fever	84.48
Headache/Dizziness	71.43
Weakness/fatigue	46.43
Cough	26.43
Arthritis/Rheumatic pain	25.00
Chills	17.86
Backache	14.29
Nausea/Anorexia	10.71
Chest pain	7.14
Vomiting	3.57

Among those TB suspected patients who were positive for the semiquantitative test for brucellosis, the incidence of brucella infection recorded was higher in females (74%) as compared to males (52%). Age wise distribution of TB suspected patients who were positive for the semiquantitative test for brucellosis is shown in Table III.

Table III. Age-wise incidence of brucellosis among TB suspected patients.

Age range (years)	SAT Positive	Semiquantitative agglutination test positive
0-9	-	-
10-19	4	4
20-29	11	7
30-39	11	7
40-49	4	2
50-59	7	3
Above 60	7	5

Majority of the cases were in the age group 20-40 years.

Discussion

Two hundred and twenty-nine TB suspected patients were included in the present study. Of these, 19% were found to be SAT positive for brucellosis. Out of these, 12% were confirmed to be positive for brucellosis based on the semiquantitative agglutination test. Varied antibody titers are claimed to declare positive individuals to be brucella infected⁹. The present study considered the titer of 1:80 to be the lowest antibody titer to claim a person brucella positive. On the higher side, we could record a titer of 1:320 among these patients. Twelve (42%) of the confirmed brucella positive TB suspected patients, had a titer of 1:80, 14 (50%) had a titer of 1:160 and 2 (7%) showed a titer of 1:320. A majority of the persons confirmed to be brucella positive were from rural areas. These results clearly imply that brucellosis is more concentrated in the rural areas, where consumption of raw milk, contact with animal carcasses, secretions and uterine discharges is often encountered, as compared to the urban areas. These findings are similar to those of other scientists who suggested that contact with infected animals is more dangerous than consumption of contaminated food stuffs¹⁰. However, other scientists were of the opinion that infection may be communicated from infected animals by the ingestion of raw milk, or milk products¹¹ and yet others claimed that the brucella organism may enter the body via the alimentary tract, conjunctival mucosa, respiratory tract, or skin¹². Some documented reports claim among the various risk factors, drinking of raw milk, consumption of home-made dairy products and close contact with infected animals were the most likely to cause active brucellosis^{13,14}. Since a wide range of symptoms occur with this infection, therefore, an attempt was made to record the most frequently exhibited symptoms among the TB suspected patients who were brucella positive. In the present study under discussion, low grade fever was recorded to be the most common symptom. Similar to this, a TB patient also presents a low grade fever as a symptom. Therefore, both diseases may be misdiagnosed. This necessitates that in case of prolonged fevers, may be among, TB suspected persons, or otherwise, an effort should be made to look for brucellosis. Earlier reports confirm these observations¹⁴⁻¹⁶. The other set of symptoms prevalent among brucella positive patients were headache, weakness, cough, rheumatic pains, chills and backache. Our results endorse the importance of brucellosis as a cause of backache among brucella sero-positive patients with complaints of backaches and among spinal brucellosis, diagnosed by positive serology¹⁷⁻¹⁸. Arthritis and rheumatic pain were the third set of symptoms abundantly reported by brucella positive TB suspected patients. This syndrome, too, has been recorded by various workers^{16,19}. Some of the less salient symptoms presented were nausea, anorexia, chest pain and vomiting (Table II). Most of these symptoms except for arthritis and rheumatic pain are also some of the presenting symptoms of tuberculosis. Therefore, possibilities of misdiagnosing brucellosis is there if serological studies are not carried out. Most of the clinics in Pakistan diagnose tuberculosis on the basis of radiology and symptomatology. Therefore, a large number of brucella positive cases receive treatment for tuberculosis. The present study stresses upon invariably testing the TB suspected persons for brucellosis. The positivity rate was considered for the frequency of brucellosis on the basis of sex as well. The rate of brucella infection was predominant in females²⁰, as compared to males. This was probably due to the fact that in rural Pakistan the caring of livestock i.e., milking, cleaning of animal stalls and taking the animals to pasture are the responsibilities of the women. This increases their chances for close contact with animals, as compared to the male population. On the other hand, males are more connected with the heavier tasks mostly dealing with the male farm animals impounding the fields and other draft purposes. An interesting point to be mentioned here is that, all the teenagers found to be sero-positive for brucellosis with a titer \geq 1:80 were girls with a rural background. This further establishes the role of rural females in the rearing of farm animals. Considering the age-wise distribution of the seropositive

brucella infected persons, the highest exposure rate was recorded in the age group of 20-40 years. These findings are similar to those of other researchers^{13,16}. In addition, our study also detected a sharp rise in brucella infection in persons above 60 years old. This is also in agreement with other reports¹³. Although an antibody titer of 1:80 is a confirmatory titer to declare a patient to be suffering from this infection, yet in many studies higher titers are on record. In case of our studies only two patients showed a titer of 1:320. The probable reason for this is that in ascending infections, the antibody titer is high, while in case of chronic brucellosis and in a descending phase the antibody titer drops. Therefore, only two patients with the titer of 1:320 are believed to be recently infected, while the remaining are suspected to be chronic patients of brucellosis. Similar views are expressed by previous workers²¹. During this study, a few TB positive patients were also investigated for brucella infection, as the signs and symptoms of both these ailments are similar. The findings of our study showed that only one case of TB positive individual was also found to be positive for brucellosis. This young male had a titer of 1:320 and had been initially diagnosed as TB positive, then subsequently cleared of the disease after treatment, but afterwards found to be once again TB positive. It was then that the patient was also found to be brucella positive as well. Since the antibiotic treatment for both tuberculosis and brucellosis is almost similar, only differing in duration, it may be possible that this individual did not take the prescribed medication for the correct duration, resulting in a relapse of the disease, which may originally have been brucellosis. Brucellosis is a treatable disease that requires the antibiotic therapy based on a combination of drugs, such as tetracycline/doxycycline associated with either, streptomycin or rifampicin. A higher relapse rate in case of the doxycycline - rifampicin combination as compared to the doxycycline - streptomycin antibiotic combination has been reported²². Hence, the latter regimen is probably more effective. The TB infected and suspected patients reporting to these two TB centers were receiving antibiotic therapy as explained above. Therefore, the chances of treating brucella infections in TB centers as TB suspected persons is confirmed. This necessitates that all TB suspected patients should be invariably examined for brucellosis and upon confirmation of the brucella infection they can be cured by short term antibiotic therapy and could also be saved of economic losses and mental anguish. Tuberculosis patients are often regarded as a source of infection for the rest of the society. Therefore, in diagnosing these patients as brucella and not TB, shall be of social service and provide mental relief to their families in particular and to the society in general.

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