Original article:

Significance of brucellosis in backache patients

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ABSTRACT

Introduction: India is an agricultural country. The dairy farming and animal husbandry are one of the major small-scale industries employing a large number of people. Evidence of zoonotic infections therefore, can be easily traced throughout the history in the community. Human brucellosis is not too uncommon disease in India. In our country, the disease has been found wherever it is looked for.

Materials and methods: A total of 250 patients of various ages attending orthopaedics outpatient department with complaints of backache were included in the study. 50 Medical students as healthy volunteers comprised the control group. Screening of these patients was carried out using rapid slide agglutination test. Then further tube agglutination test was put for patients positive with slide test for titer determination. Blood culture was sought in every patient who had agglutination in diagnostic titers and after confirmation of growth antibiotic susceptibility was done.

Results: 250 patients who complained of backache, 17 were positive on screening by slide agglutination test. All the patients positive by screening had tube agglutination positive in diagnostic titers. This was a significant finding of Standard tube agglutination test with backache (p = 0.041). Brucella melitensis could be recovered from two patients who were positive by tube agglutination test with a significant p value(0.003).

Conclusion: Brucella cannot be ruled out in patients of backache who hail from land of agriculture like ours. Differential diagnosis of brucellosis needs to be kept in mind while treating such patients as antibiotics play a role in treating such patients who are otherwise prescribed NSAIDs on long term. Morbidity can be reduced if accurate history, sample collection and processing are done.

Keywords: Brucellosis, NSAIDs

INTRODUCTION

Brucellosis is an important zoonotic disease. It is a global problem. As human brucellosis has a paucity of characteristic manifestations, it is difficult and often impossible for a physician to give clinical diagnosis with certainty. Important reasons to miss could be multiplicity of illness it mimics, lack of adequate facilities in most hospitals for establishing the diagnosis and lack of awareness of the existence of the disease. So it is not considered as probable diagnosis.1 Though it is disease of animals, it poses a public health hazard. Human affected by contact with infected animals or their products. Occupation plays a great role in the incidence of the disease. Brucellosis is characterized by its protean manifestations. It is often misdiagnosed as typhoid or pyrexia of unknown origin (P.U.O.). Backache is one of important complaints of disease.1

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complications which may develop during acute or chronic stages includes brucella spondylitis, arthritis, neurobrucellosis, brucella endocarditis etc. The most frequent suppurative complications of human brucellosis are osteoarticular. These complications include spondylitis, arthritis, osteomyelitis, tendinitis and bursitis. The lumbar spine is most frequently involved site although no part of spine is spared. So Brucellosis is often overlooked in the diagnosis of back pain. Clinical diagnosis is often impossible. A high index of suspicion and optimal use of laboratory is essential for accurate diagnosis. An absolutely certain diagnosis of brucellosis is made only when causative organism is isolated in culture. However, Brucella grow rather slowly in vitro. So the preliminary diagnosis often depends on the results of serological tests. The most widely used serological tests are agglutination tests. The present study has been carried out to screen the backache patients attending orthopaedic outpatient department, for antibrucella antibodies. 

MATERIAL AND METHODS

The present study has been carried out at Department of Microbiology, Government Medical College, Miraj after clearance from the ethical committee. Serum samples from the patients having complaints of backache attending orthopaedic out patient department were subjected to rapid slide agglutination test for detection of antibrucella antibodies. The samples positive by slide agglutination test were further tested by standard tube agglutination test to determine the titre. Efforts were made to collect blood from all the seropositive patients for blood culture. The samples in which growth suggestive of Brucella spp. was observed were subjected to further laboratory tests for confirmation. A total of 250 patients belonging to various age groups and either sexes were included in this study. 50 healthy individuals of different age and sex were also studied as controls.

STATISTICAL ANALYSIS

Cases and controls were compared to determine the significance of seropositivity in backache patients. Culture positivity in seropositive patients were further analysed statistically by using SPSS software for windows version 20.0 for windows (IBM software, Newyork,USA). Chi square test was applied for univariate analysis.

OBSERVATIONS AND RESULTS

Out of 250 cases of backache, 17 (6.8 %) showed presence of Brucella agglutinins, of which 17 (6.8 %) showed it in diagnostic titres. Out of 50 healthy individuals none showed the presence of Brucella agglutinins. Blood culture was done in each and every case. Brucella melitensis was recovered from 2 (13.3%) cases who showed seropositivity.

The age group of 31-40 years included maximum number of cases (65/250 i.e. 26 %). The study included 56% of males and 44% of females (table.1). A total of 17 cases were positive for presence of Brucella antibodies of which 14 were males and 3 were females. The age group of 41-50 years included highest number of cases (6/17 i.e. 26.6%). All the age groups seem to give uniform distribution of seropositivity.

The controls included medical students and voluntary blood donors, which were apparently healthy and completely asymptomatic (table.2). None of the controls gave history of any significant illness in the recent past, which required antibiotic therapy. The control group
included 80% males as compared to 20% females.

Titer wise distribution of seropositive cases of brucellosis in backache patients (table.3). The titres ranged from less than 320 IU to 2560 IU (Fig.1). The diagnostic titer suggested by IVRI is 80 IU. All cases (17/17) showed titres in the diagnostic range (Fig.2) and culture was positive in two samples (table.no 4). Growth of *Brucella spp* on Trypticase soy agar (Fig.3). Table No. 5 shows significance of brucellosis in backache patients with seropositivity (p=0.041) and distribution of culture positivity in seropositive cases of Brucellosis which was also significant (p=0.03). All seropositive cases were subjected to blood culture. Two samples were positive for culture and all of them yielded *Br. melitensis*. Out of these 2/17 (11.7%) one of them had displayed high titer of more than 2560IU.

**DISCUSSION**

Brucellosis is an endemic or epidemic disease. Brucellosis is a classical example of bacterial zoonosis. It is caused by gram-negative coccobacilli belonging to Genus Brucella. Human beings are susceptible hosts and acquire the infection by consuming raw infected milk and unpasteurized milk products. Direct contact with infected animals and their products is another common cause of acquiring the pathogen.6 Though majority of Brucella infections are acquired through direct or indirect contact with infected animals; it is not unusual to find cases without apparent history of contact with such animals.1 The human infection may be latent, acute or chronic, febrile type. It is often mistaken for typhoid, malaria or may remain undiagnosed as Pyrexia of Unknown Origin (PUO) for sometime. Besides this, the disease may as well present in a wide range of signs and symptoms, which often lead the disease to go undiagnosed or misdiagnosed.7-9 Osteoarticular complications are more common in Brucellosis. Backache is one of complaint of disease. Although backache is very common, the differential diagnosis may sometimes be very difficult. Both inflammation and infections of spinal or sacroiliac joints are examples of such causes.10 Low backache of brucella spondylitis closely simulates pain of prolapsed intervertebral disc.3 Brucellosis must be differentiated from tuberculosis as an important cause of backache especially in areas in which the disease is endemic.11-12

Isolation of Brucellae from blood is the gold standard of diagnosis of brucellosis. In the absence of positive cultures the most reliable indicators of Brucella infection are the agglutination tests. This is particularly true in cases of acute brucellosis in which a high serum agglutination titer will be found in most cases.13

**Screening of sera:**

In the present study 250 patients attending Orthopaedic out-patient department with complaint of backache were screened for antibrucella antibodies. (Table 1). Of 250 cases of backache 140 (56%) were males and 110 (44%) were females. Out of 250 cases 17 (6%) showed the presence of Brucella agglutinins by both rapid slide agglutination test and standard tube agglutination test. Out of 17 seropositive cases 14(82%) were males and 3(18%) were females. This male preponderance can be explained by the higher exposure of males to infected animals and their occupation. It has been seen that the disease is more common in adult age group. Maximum number of patients
in the present study belonged to the fifth decade of life followed by second and third decade. Youngest patient was a seventeen years male and the eldest patient was of 84 years age. In a study of Koshi and Myers (1967) 35% were males and 35% were females. In Mathur’s study 70% were males and 30% were females.\textsuperscript{1,4} 

Koshi and Myers (1967) have also reported that maximum number of cases belonged to the third decade of life. They have also reported the male preponderance for the disease.\textsuperscript{1} However Cooper (1991) has reported a higher incidence amongst women than men and a remarkable increase in brucellosis with increasing age. This was felt to have been due either to an increased exposure to infected livestock, or to an increased susceptibility to the disease in women and with increasing age.\textsuperscript{14} In our study the maximum number of cases where observed in fifth decade. There are several possible explanations for striking increase in the incidence of the brucellosis. First, the older individuals may be more likely to follow a traditional pastoral life style. Secondly, adult in general are more likely than children to be involved in the day to day care of livestock, and particularly to be involve in certain particularly high risk practices such as assisting in animal parturition.

**The control group:**

Fifty healthy individuals were also screened for antibrucella antibodies. Majority were between 11-20 years of age. (Table 2). The controls included medical students and voluntary blood donors, which were apparently healthy and asymptomatic and had no history of animal contact. None of them showed the presence of Brucella agglutinins. This indicates that the healthy individuals who are not exposed to animals and have a healthy life style are unlikely to catch Brucella infections. Sharma et al (1974)\textsuperscript{15} and Roy et al (1965)\textsuperscript{16} also found 0% incidence of Brucella agglutinins in healthy individual like our study.

**Evaluation of seropositive cases:**

Out of 17 seropositive cases of brucellosis in backache patients, all i.e., 17(6.2%) cases showed the presence of Brucella agglutinins in diagnostic titers\textsuperscript{(17-18)} The agglutination test if properly performed can be used as a very dependable laboratory procedure for rapid diagnosis of brucellosis. However, it has its limitations and false positive reactions may be seen after Cholera vaccination and in tularemia. But in Cholera vaccination titers are seen only in the first month and even then titers are usually insignificant. As a rule Brucella agglutinins are present in a suggestive titer by the end of second week and in frank cases of undulant fever, generally rise to high titers. Various workers have used various diagnostic titers. Usually the diagnostic titer varies with the manufacturer of antigen. Following are the diagnostic titers used by various workers and the antigens used -

<table>
<thead>
<tr>
<th>Study</th>
<th>Antigen</th>
<th>Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Koshi and Myers</td>
<td>IVRI</td>
<td>80 IU</td>
</tr>
<tr>
<td>Mathur</td>
<td>IVRI, Weybridge</td>
<td>80 IU</td>
</tr>
<tr>
<td>Mahakur and Panda</td>
<td>IVRI</td>
<td>160 IU</td>
</tr>
<tr>
<td>Present study</td>
<td>IVRI</td>
<td>80 IU</td>
</tr>
</tbody>
</table>
The FAO/WHO expert panel on brucellosis (1953) recommends the minimum diagnostic titer in brucellosis as 1/10 to 1/12 of the 50 percent agglutination obtained when the antigen employed is tested with freeze dried international standard anti *Brucella abortus* serum. 19

**Blood culture:**

In our study, cultures were done using trypticase soy broth and subcultured on trypticase soy agar and brain heart infusion agar. Various workers have used Castaneda’s biphasic method with Trypticase soy agar and broth, Tryptose agar and broth, or “Albimi” medium. Some prefer the method of subculturing at regular intervals as it allows earlier recovery and identification. Our experience with the Castaneda’s biphasic media has not been encouraging. Culture in trypticase soy broth incubated at 37°C in an aerobic atmosphere and in CO2 atmosphere with frequent subculturing onto trypticase soy agar and brain heart infusion agar plates was the method used by us.

One of the culture positive had titer of 2560 IU and other had 320 IU. 2 out of 17 cases (11.7%), from brucellosis in backache patients, cultured yielded the growth of *Br. melitensis*. The rest 15 (88.3%) cases were negative for Brucella.

Another important reason of failure to isolate Brucella from blood could be the menace of contamination. Contamination of blood cultures is a common problem, which has troubled the microbiologist time and again. 20 All seropositive cases were subjected to blood culture. Of these 17 cases two (11.7%) were positive by culture. The patients who were positive by culture had titers of 320 IU and 2560 IU. It is rare to have an insignificant agglutination titer when Brucella isolation has been made though there are some reports in which isolations have been made even when the titers were low and even when serology was negative. This has been explained by presence of blocking antibodies in blood. 1, 21

Moller et al (1951) have reported the isolation of *Br. melitensis* from 2 blood samples and 1 synovial fluid in patients with chronic brucellar spondylitis. 22 Samuel L. Turek (1989) in his book on Orthopedics described that blood cultures may rarely be successful. 23 All the two culture positive cases yielded the growth of *Br. melitensis*. The isolates were sent to IVRI, Izatnagar for confirmation and typing. Both the isolates were confirmed as *Br. melitensis*. All the two isolates had identical antibiotic sensitivity pattern. All the isolates were sensitive to tetracycline, doxycycline, streptomycin, amikacin, kanamycin, gentamicin, cotrimoxazole, ciprofloxacin and rifampicin. In a study by Bosch et al (1986) they had studied in vitro activity of ciprofloxacin, doxycycline, tetracycline, streptomycin, ceftriaxone, rifampicin and cotrimoxazole against 95 strains of *Br. melitensis*. All the strains were sensitive to all these antibiotics. 24

Human cases are also not uncommon. Many aspects of the disease are not adequately and substantially dealt with. A few workers who have done some work in limited geographical pockets cannot give full justice to the nationwide problem of brucellosis. Teamwork on a large scale is but a necessity. The morbidity and mortality leading to economic loss due to the disease should be given wide publicity to make the people aware of the condition. Due to the ignorance of the people, it is very difficult to get enough co-operation.
which is most necessary in the study of brucellosis. The disease cannot be eradicated from India unless and until it is studied comprehensively in India. To conclude, Brucellosis should be often overlooked in the differential diagnosis of back pain. The changing risk pattern for this disease requires a high index of suspicion, which can result in early diagnosis and predictably favorable results to treatment.

**Table No. 1: Age/Sex wise distribution of Brucellosis in backache patients.**

<table>
<thead>
<tr>
<th>Age</th>
<th>No. of Patients (Positive Cases)</th>
<th>Males (Positive Cases)</th>
<th>Females (Positive Cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-20</td>
<td>22(3)</td>
<td>15(3)</td>
<td>07(0)</td>
</tr>
<tr>
<td>21-30</td>
<td>58(3)</td>
<td>34(3)</td>
<td>24(0)</td>
</tr>
<tr>
<td>31-40</td>
<td>65(2)</td>
<td>30(1)</td>
<td>35(1)</td>
</tr>
<tr>
<td>41-50</td>
<td>46(6)</td>
<td>28(4)</td>
<td>18(2)</td>
</tr>
<tr>
<td>51-60</td>
<td>28(1)</td>
<td>15(1)</td>
<td>13(0)</td>
</tr>
<tr>
<td>61 and above</td>
<td>23(2)</td>
<td>16(2)</td>
<td>7(0)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>250(17)</strong></td>
<td><strong>140(14)</strong></td>
<td><strong>110(3)</strong></td>
</tr>
</tbody>
</table>

**Table No. 2: Age/Sex wise distribution of healthy controls.**

<table>
<thead>
<tr>
<th>Age</th>
<th>Total screened Persons</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-20</td>
<td>25</td>
<td>19</td>
<td>6</td>
</tr>
<tr>
<td>21-30</td>
<td>14</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>31-40</td>
<td>6</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>41-50</td>
<td>5</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>50</strong></td>
<td>40(80%)</td>
<td>10(20%)</td>
</tr>
</tbody>
</table>

**Table No. 3: Titer wise distribution of seropositive cases of Brucellosis in backache patients**

<table>
<thead>
<tr>
<th>Titer</th>
<th>No. of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>320IU</td>
<td>7</td>
</tr>
<tr>
<td>640IU</td>
<td>8</td>
</tr>
<tr>
<td>1280IU</td>
<td>1</td>
</tr>
<tr>
<td>2560IU</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>17</strong></td>
</tr>
</tbody>
</table>
Table No. 4: Distribution of culture positivity in seropositive cases of Brucellosis in backache patients

<table>
<thead>
<tr>
<th>Titer</th>
<th>Culture done</th>
<th>Culture Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>160 IU</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>320 IU</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>640 IU</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>1280 IU</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2560 IU</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5120 IU</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>2</td>
</tr>
</tbody>
</table>

Table No. 5: Cases studied and their results

<table>
<thead>
<tr>
<th>Source of Samples</th>
<th>Total No. of Samples tested</th>
<th>Total No. of seropositive cases (%)</th>
<th>p value</th>
<th>No. of cases Positive in diagnostic titres</th>
<th>No. of cultures done</th>
<th>No. of Culture positive cases (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brucellosis in backache patients</td>
<td>250</td>
<td>17 (6.8)</td>
<td>0.041</td>
<td>17</td>
<td>17</td>
<td>2 (11.7)</td>
<td>0.003</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>50</td>
<td>0</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1: Standard tube agglutination test
Figure 2 : Distribution of titers among brucella seropositive patients

![Pie chart showing distribution of titers among brucella seropositive patients.](image)

Figure 3 : Growth of *Brucella melitensis* on trypticase soy agar

![Image showing growth of Brucella melitensis on trypticase soy agar.](image)

REFERENCES:

17. Indian Veterinary research Institute. Protocol supplied with Rose Bengal Plate Test Antigen for Brucella.

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