

**BRUCELLOSIS PRESENTING AS CHRONIC ARTHRITIS: A STUDY IN EASTERN
INDIA WITH A REVIEW OF BRUCELLAR ARTHRITIS**

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ABSTRACT

Although brucellosis commonly presents as fever of unknown origin, it has polymorphic features that affect different organ systems. Involvement of osteoarticular system is also not uncommon in brucellosis with arthritis being the main feature. In this paper we attempt to find out brucellosis as a cause in patients with chronic arthritis. This study was carried out over a 1 year period from January 2015 to December 2015. Blood samples were collected from cases of chronic arthritis which were subjected to three serological and one genus specific PCR test for the detection of *Brucella* infection. Out of 170 cases of chronic arthritis, 86 (50.59%) patients showed serological positivity for all the three tests and 24 (14.11%) patients cases showed positivity for genus specific PCR. This finding clearly indicates that a significant number of brucellosis cases are there presenting as chronic arthritis. Thus every case of chronic arthritis not responding to conventional treatment should be investigated for brucellosis, especially if there is a history of contact with animals and animal products.

KEYWORDS: Brucellosis, Arthritis, ELISA, PCR.

INTRODUCTION

Brucellosis is a re-emerging infectious disease with varied clinical manifestations depending on the site of involvement. Complications can be diverse with bone and joint involvement being one of the most frequent, presenting with arthritis, sacroilitis, spondylitis and osteomyelitis.^[1,2,3] There have been several reports of skeletal involvement of brucellosis from different parts of the world, however, that from India being very few. The purpose of the present study is to reveal the scenarios of brucellar arthritis in this part of India.

MATERIALS AND METHODS

The study was carried out over a 1- year period from January 2015 to December 2015, in a tertiary care teaching hospital in Eastern India. After getting permission from Institutional Ethical Committee, 170 patients of chronic arthritis were included in this study after obtaining informed consent from them. Arthritis was diagnosed by the presence of signs and symptoms of pain or tenderness or both, restriction of movement and swelling.^[4] Blood samples were collected from them for serological and polymerase chain reaction (PCR) tests. Serum samples were kept in separate aliquots and stored at -20°C before further processing. Each sample was subjected to a panel of *Brucella* specific serological tests- Rose Bengal Plate Agglutination Test (RBPT;

IAHVB, Bengaluru), Standard Tube Agglutination Test (SAT; Tulip Diagnostics Pvt. Ltd.), ELISA (Immunolab GmbH, Germany) for the detection of IgM and IgG antibodies and genus specific PCR (Prime). For PCR tests, the serum samples were first subjected to DNA extraction using QIAmp DNA Mini Kit (Qiagen). The extracted DNAs were then subjected to PCR. The PCR was carried out in 50µL reaction mixture in each PCR tube that contained 5µL PCR buffer, 1µL dNTP, 0.2 µL Taq Polymerase, 5µL template DNA and 1 µL each of forward (F) and reverse (R) primers and 36.8 µL nuclease free water. The primer sequences used were as follows^[5] – BCSP- B4 (F) TGG CTC GGT TGC CAA TAT CAA ;BCSP- B5 (R) CGC GCT TGC CTT TCA GGT CTG; amplicon size was 223bp. The steps in PCR were followed as described by Baily *et al.*^[5] The amplified products were then subjected to agarose gel electrophoresis using a 100 bp DNA ladder and a positive and a negative control and the bands formed were seen in a gel doc (Biorad). Other investigations including C reactive protein (CRP), rheumatoid factor (RA), antistreptolysin O titre (ASO), uric acid (UA) were performed with each sample. Radiological examination was done with each affected joint.

RESULTS

All patients under study gave positive history of animal contact. Number of female patients was 50 and number of male patients 120 (1:2.4). Of the 170 patients studied, *Brucella* specific serological test positivity has been depicted in Fig.1. Among these 170 patients, both serological and *Brucella* specific PCR tests are positive in 24(14.11%) patients. Regional distribution of the joints involved in the 86 patients showing positivity in all serological tests is shown in Table 1. Age wise distribution of these 86 positive patients is given in Fig. 2. In these 86 patients, occupationally farmers constitute the majority (N=48), followed by housewives (N=24), labourers (N=14). Distribution of involved joints in all the *Brucella* genus specific PCR positive patients (N=24) is shown in Table 2. Age wise distribution of the 24 PCR positive patients is given in Fig. 3. In these 24 patients, occupationally, farmers constitute the majority (N=17), followed by housewives (N=6), labourers (N=1). Gel pictures of the PCR positive cases are shown in Fig. 4-6.

Among the 170 patients studied, C reactive protein was positive in 77 patients, rheumatoid factor was positive in 14 patients; serum uric acid was high in 11 patients, antistreptolysin O titre positive in 1 patient. Radiography of the affected joints showed synovitis and few destructive changes. Gender wise distributions of the different parameters are shown in Table 3. Both male and female were found to be almost equally likely to have brucellosis, i.e., there was no gender specificity in this study. This was also applicable for both RA and CRP positivity. All the RA factor positive patients (except one female) were *Brucella* negative and their CRP levels was normal. So there was no such association. Brucellosis positive patients had significantly higher propensity of having CRP positivity (p value=0.0033) in our study. There was also no such significant difference between antibiotic users and non users in brucellosis. Hence it seems that antibiotics used by the patients were not specific.

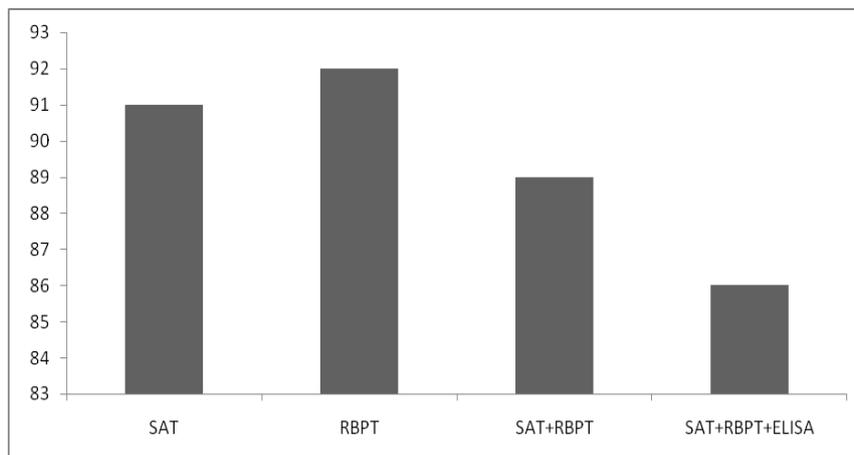


Fig.1: Showing SAT positivity in 91(53.52%) patients, RBPT positivity in 92(54.1%) patients, SAT and RBPT both positive in 89(52.35%) patients, SAT, RBPT and ELISA all three positivity in 86(50.58%) patients.

Table 1: Distribution of involved joints

Joints involved	Number of patients
Bilateral knee joints	23
Bilateral hip joints	38
Bilateral knee and hip joints	12
Bilateral ankle joints	5
Bilateral shoulder joints	3
Left knee joint	1
Right knee joint	3
Right hip joint	1

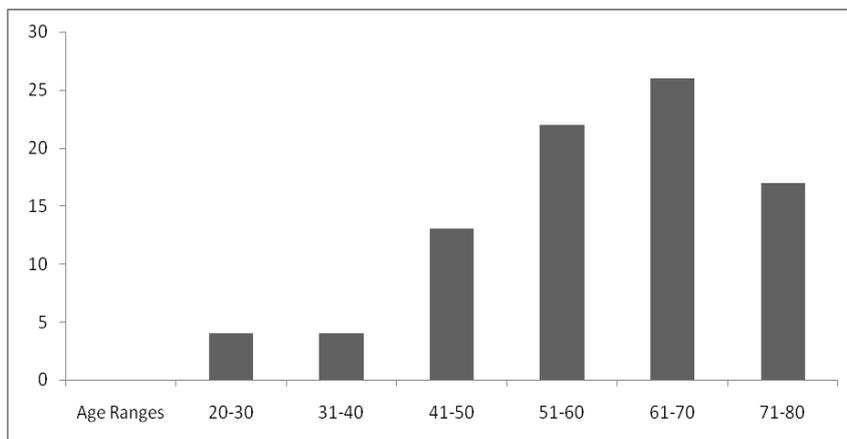


Fig. 2: Number of patients in 20-30 years was 4, between 31-40 years was 4, 41-50years was 13, 51-60 years was 22, 61-70 years was 26 and 71-80 years was 17.

Table 2: Distribution of involved joints in PCR positive cases

Joints involved	Number of patients
Bilateral knee joints	13
Bilateral hip joints	6
Bilateral knee and hip joints	4
Right hip joint	1

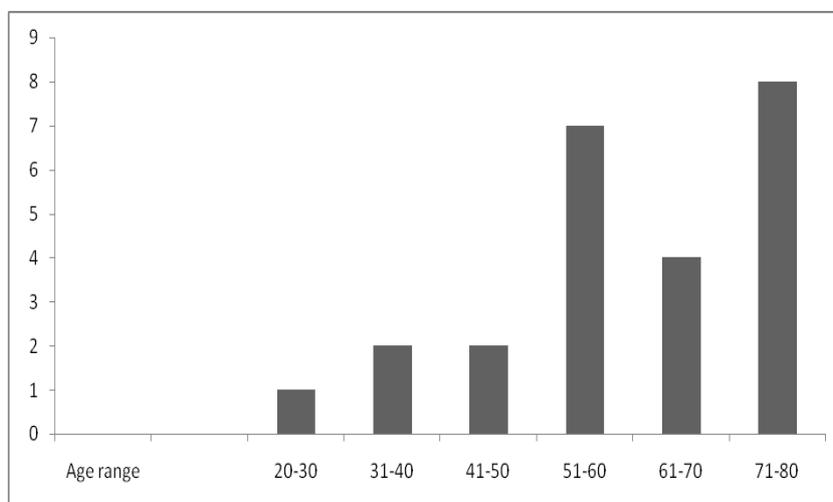


Fig. 3: Number of PCR positive patients in 20-30 years was 1, between 31-40 years was 2, 41-50 years was 2, 51-60 years was 7, 61-70 years was 4, 71-80 years was 8.

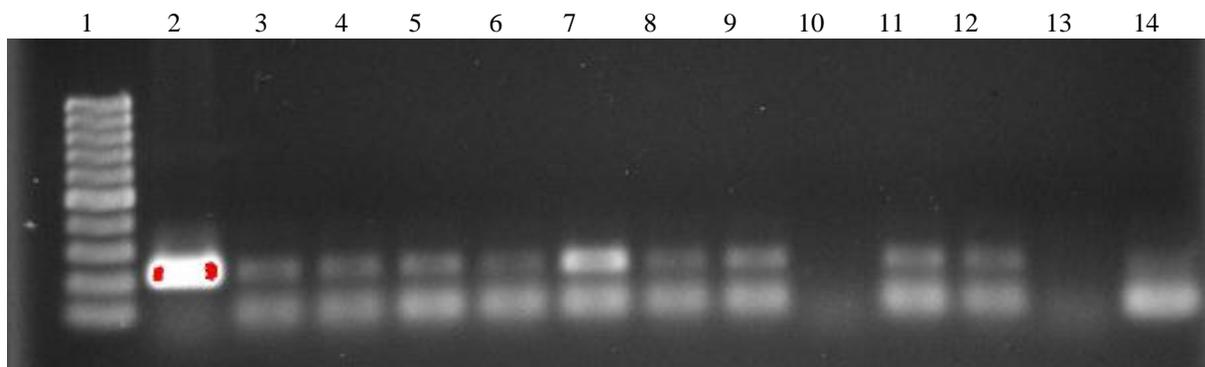


Fig 4: Lane 1- DNA ladder, Lane 2- positive control, Lanes 3,4,5,6,7,8,9- positive cases, Lane10-negative case, Lanes 11,12- positive cases, Lane 13- negative control, Lane 14- positive case.

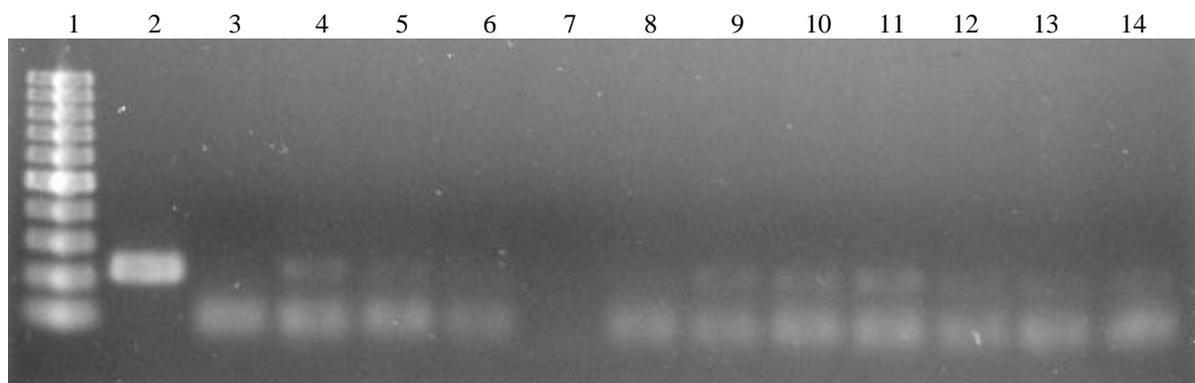


Fig 5: Lane 1- DNA ladder, Lane 2- positive control, Lane 3- negative control, Lanes 4,5- positive cases, Lanes 6,7- negative cases, Lanes 8,9,10,11,12,13,14- positive cases.

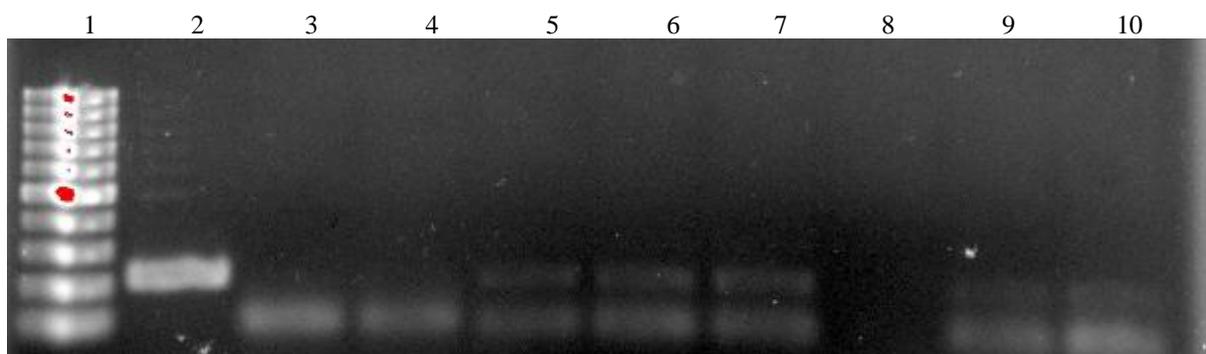


Fig 6: Lane 1- DNA ladder, Lane 2- positive control, Lane 3- negative control, Lane 4- negative case, Lanes 5,6,7- positive cases, Lane 8- negative case, Lanes 9, 10- positive cases

Table 3: Gender wise distribution of positive results of different parameters.

Gender	SAT \geq 1:80	RBPT	IgM	IgG	PCR	RA(+)	CRP (high)	UA (high)	ASO titre (high)
F=50	30 (60%)	29 (58%)	27 (54%)	27 (54%)	8 (16%)	7 (14%)	24 (48%)	11 (22%)	0
M=120	61 (50.83%)	63 (52.5%)	59 (49.16%)	59 (49.16%)	16 (13.33%)	7 (5%)	53 (44.16)	0	1
Total =170	91	92	86	86	24	14	77	11	1

DISCUSSION

Kennedy made the first report of the skeletal involvement of brucellosis in 1904, about 20 years after the discovery of the Malta fever bacterium by Sir David Bruce.^[6] A study published in 1982^[7] was on the articular involvement of human brucellosis. A retrospective study was made in this paper and 304 patients of human brucellosis caused by *B. melitensis* were studied over a period of 12 years in Lima hospital in Peru. Four syndromes were recognized in a total of 33.8% patients of brucellosis. The most frequent pattern was sacroiliitis (46.6%) usually non-destructive and which were either uni- or bilateral. The second most frequent articular syndrome was peripheral arthritis (38.8%), manifested either as a single large lower extremity joint or as an asymmetric pauciarthritis. Rarely patient presented with a rheumatoid like arthritis. Mixed arthritis was a combination of the first two (7.8%). Spondylitis (6.8%) was the least common form of arthritis. The arthritis resolved with a combined

treatment of tetracycline and streptomycin for 21 days without sequelae.

In another study published in 1990^[8], in Kuwait, out of 400 studied patients of brucellosis, 104 patients had arthritis, of which 96 patients could be followed up. The systemic disease in these 96 patients were acute in 54 patients, subacute in 24 and chronic in 18 patients. The main symptoms were joint pain, fever, sweating and easy fatigability. The joints mainly affected were sacroiliac (26%), knee (25%); followed by hip (18%) and spine (8%). Joint effusion occurred in 30% of the cases predominantly in the acute group. Culture of synovial fluid was negative in all cases. Treatment with a combination of streptomycin and tetracycline or rifampicin resulted in an excellent cure rate and resolution of arthritis without sequelae.

In a study published in 1991^[9], 263 patients with a diagnosis of brucellosis between January 1984 and

December 1987 were studied prospectively. 25% of these patients developed osteoarticular complications. Spondylitis in 58% and sacroilitis in 45% were the most prevalent findings. At diagnosis 72% patients showed radiological abnormalities commonly at axial site but rarely at peripheral sites. Fifty seven of these patients received medical treatment alone and 51 of them got cured with a single course of treatment. Treatment failed or there was relapse in 11% patients of whom 5 patients had spondylitis; 12% patients all of whom had spondylitis and par vertebral or epidural abscess required surgical treatment.

In another study published in 1993^[10], 530 patients with brucellosis were studied prospectively between 1974 and 1989. The finding for 62 patients with 63 episodes in which sacroiliac joint was involved, were analysed, 10 of the older patients had concomitant spondylitis. Systemic symptoms of pain and findings of sacroilitis were observed in 75% of them. The most frequent radiographic findings were blurring of articular margins and widening of sacroiliac space. No radiographic abnormality was detected in 13 cases. Results of 99mTc and gallium67 bone scan were abnormal in 90% of the cases.

In a separate study published in 1993^[11], 1729 cases of brucellosis were diagnosed and treated over a period of 21 years. 7 patients were identified as having arthritis of sternoclavicular joint. 4 patients had involvement of another joint. All patients recovered without sequelae.

In another study published in 1999^[12], 35 patients in the age group of 14-74 yrs, who had *Brucella* spondylitis were treated between January 1991 and December 1997. Back and neck pain, fever and constitutional symptoms were the most common findings. Cultures of blood specimens were positive in 26 patients for *B.melitensis*. 1 of the 35 patients underwent surgical treatment for spinal epidural abscess. Therapy failed in 9 patients and 5 patients had relapse. There was no death or severe sequelae.

In a study published in 2001^[13], a single case of a 47 years old man, with a known history of rheumatoid arthritis for 5 years was found to have para vertebral epidural abscess, vertebral fracture and nerve root compression due to brucellosis. The person underwent surgery for L3-L4 vertebral stabilization and medical treatment for knee arthritis.

Another work published in 2011^[14] was on a retrospective study conducted in a referral children centre in Tehran from 1997 to 2005. Of 96 patients diagnosed with brucellosis, 24 had *Brucella* arthritis. Most common features were fever and fatigue. Monoarthritis was recorded in 15 patients with knee in 8, hip in 5 and ankle in 2 patients, while 9 had polyarthritis. None of the patients had axial joint involvement. Recurrence was not seen in any of the 24 patients who

received a combination of cotrimoxazole for 6 weeks and Gentamicin for 5 days.

In a review article published in 2013^[15], an observational study was made on skeletal involvement in children due to *B.melitensis*. Frequency of skeletal involvement in children has been found as between 6.4 and 73.5%. There has been controversies regarding the most common site involvement; sacroiliac verses peripheral joints. It has been found that in a vast majority of cases, peripheral joint involvement in paediatric brucellosis has a monoarticular pattern although there has been no agreement about the most common peripheral joint involved.

In a work published in 2014^[16], a 36 months study was conducted from December 2008 to October 2011 to evaluate epidemiology, clinical presentation and treatment outcome of brucellosis among children who were referred to Infectious Disease Clinic in Boo-Ali hospital in Zahedan city in Iran. Among 32 patients with brucellosis, 83% had chronic bone pain. Fever was reported in 39% and the least frequent complication was osteomyelitis in 3% patients. Patients were treated medically and all of them survived.

A study published in 2015^[17], identified a rare case of osteoarticular disease in Gonabad city of Iran where a 71 yrs old woman, who presented with severe pain in limbs specially left pelvis was diagnosed as serologically positive for brucellosis and after 10 days treatment with rifampicin, doxycyclin and streptomycin patient presented with good health without pelvic pain.

In another study in 2015^[18], a case of osteomyeloarthritis due to brucellosis was identified in a 54 yrs old male in B M Patil Medical College in Vijayapura. Patient had a one and half year's history of pain in right hip. Xray and MRI findings of the patient revealed destruction and erosion of right femoral head and acetabulum with abnormal heterogeneous enhancement of marrow, synovial and joint collection, and superior subluxation of right hip joint with severe joint space narrowing and lumbar spondylosis. Patient was detected to be positive for RBPT test, positive SAT and 2ME tests with a titre of 1280 IU each and showed positive result for the presence of *Brucella* antibody. The patient underwent Girdle stone osteotomy.

In a separate study in 2015^[19], isolation of *B.melitensis* was done from synovial fluid of 5th metatarsophalangeal joint of a 39 yrs old lady having unusually chronic, asymmetric, additive, peripheral polyarthritis. The isolation was confirmed by PCR. RBPT and SAT tests were positive for the patient. The patient had a history of contact with an aborted goat. The patient was treated with rifampicin and doxycycline and recovered fully.

In the present study, 170 patients of chronic arthritis were assessed for the presence of *Brucella* infection in

them. Out of them serological positivity by all the three *Brucella* specific serological tests (SAT, RBPT, ELISA) were found in 86 patients and 24 patients showed *Brucella* genus specific PCR positivity in addition to serological positivity. A correlation between CRP positivity along with other symptoms may be considered as the diagnostic criteria for brucellosis among arthritis patients. All patients with serological and PCR positivity were treated with combination of rifampicin and doxycycline and they responded well.

CONCLUSION

It is important to be aware of the brucellosis as a cause of chronic arthritis especially in occupationally exposed community so that prompt treatment can be initiated to relieve patients of any locomotor disability and cure the disease permanently.

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