Original Article

DOI: 10.7860/JCDR/2019/42205.13242

Seroprevalence of Anti-Brucella Antibodies IgG and IgM in Acute Polyarthritis in a Tertiary Care Center in Southern India

Microbiology Section

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ABSTRACT

Introduction: Acute polyarthritis is a clinical manifestation of diverse aetiologies. Infectious diseases like Brucellosis, Lyme disease, Viral infections like Chikungunya are some of the causes for Acute polyarthritis. Brucellosis can present with fever, malaise and arthralgia. Osteoarticular complications leading to polyarthritis is not uncommon with Brucellosis.

Aim: To find out the seroprevalence of Brucellosis in acute polyarthritis in a Tertiary Care Center.

Materials and Methods: A prospective study was done in a Tertiary Care Center, Tamil Nadu for a period of six months from January 2017 to June 2017. Blood samples were collected from 60 patients with acute polyarthritis and also from 20 age and sex matched healthy controls. Sera were tested for the presence of anti IgM and anti IgG *Brucella* antibodies by Enzyme Linked Immuno Sorbent Assay (ELISA). Also, the acute phase reactant C-Reactive Protein (CRP) levels were measured by latex agglutination test. Statistical analysis was done by Chi-square test.

Results: In patients with acute polyarthritis serum IgM was found significant (p=0.000347) compared to IgG. The mean CRP in the diseased group was 24.12±12.10 mg/dL.

Conclusion: Even though there was no statistical significance between *Brucella* antibodies and acute polyarthritis 16.66% were *Brucella* antibody positive. Hence screening for brucella antibodies on acute polyarthritis has a definite role while evaluating a case of acute polyarthritis.

Keywords: Brucellosis, C-reactive protein, Enzyme linked immunosorbent assay

INTRODUCTION

Human Brucellosis is a zoonotic disease which can culminate in serious morbidities and mortality. The clinical presentation is multiorgan disease with wide array of symptoms due to systemic infection [1]. Brucellosis is transmitted to man through infected animals and their products. The human disease is named as Mediterranean fever, Malta fever and Undulant fever. Clinical presentation can be latent infection, acute or sub-acute brucellosis or chronic brucellosis. The clinical manifestations of acute brucellosis are irregular fever, asthmatic attacks, nocturnal drenching sweats, exhaustion, anorexia, chills, nervous irritability, muscular and articular pains. The symptoms of chronic brucellosis are low grade fever, sweating, lassitude and joint pains. There is no gender predisposition and individuals between 15 and 45 years. of age are commonly affected. Involvement of osteoarticular system presenting as arthritis is common in Brucellosis [2].

Though arthritis occurs as a common complication of Brucellosis it is under -reported and under diagnosed in India since isolation of organisms from affected joints is often unyielding and hence isolation is seldom attempted [3]. *Brucella* arthritis usually involves the spine in adults, whereas in children and adolescents joints like hip, knee and ankle are more commonly affected. There are reports showing septic bursitis due to *Brucella* in prepatellar bursa [4] and olecranon bursa [5].

Polyarthritis is defined as pain of synovial or articular origin, with or without inflammation, in four or more joints and poses a diagnostic challenge to the clinicians. A good knowledge of the various causes of polyarthritis facilitates an accurate diagnosis and proper management. Eliciting a history with regards to onset of the disease, gives clues in the diagnosis of arthritis. When the onset is abrupt occurring within hours or days one should think about infection, gout or injury as the cause for polyarthritis. If the symptoms are present for months or years the cause may be Rheumatoid Arthritis (RA), Psoriatic Arthritis (PsA), Osteoarthritis (OA) or due to chronic infections like syphilis, hepatitis, Human Immunodeficiency Virus (HIV). Bacteria which are associated with polyarthritis are Staphylococci, Streptococci, Enterococci, *Neisseria gonorrhoeae*, *Borrelia* and Gram-Negative bacilli [6]. As *Brucella* species are intracellular organisms; they grow intracellularly causing variable bacteremia phases followed by localisation of infections in tissues of the genital tract, mammary glands and reticulo-endothelial system [7].

On the basis of the failure to grow micro-organism from joint fluid and the poor response to antibiotic therapy, Brucella arthritis is thought to be reactive [8]. The role of bacterial endotoxins, exotoxins, bacterial peptidoglycans and circulating immune complexes in the pathophysiology of arthritis in Brucellosis remains controversial [9]. The genus Brucella consists of Gram negative nonmotile aerobic coccobacilli which grow on special media. This organism was named after David Bruce, the organism was isolated and established it as a causative agent of brucellosis in 1887 by a British army doctor. There are about 10 species in the Genus *Brucella*. *Brucella abortus*, *Brucella suis*, *Brucella canis* and *Brucella ovis* were isolated from cattle, pig, canines and sheep respectively in later period. *Brucella melitensis, B. abortus, B.suis, and B.canis* species cause infections in human.

Several diagnostic tests are available, isolation of *Brucella* by blood cultures is confirmatory of brucellosis; however, in practice it is difficult because of early tissue localisation of the bacteria and the exacting culture requirements. As blood cultures are positive only in 10%-30% of brucellosis case, a greater number of cases are diagnosed serologically [10,11]. The sensitivity of blood culture is variable and is between 58% and 90%. Repeated subcultures on periodic basis upto a period of 30 days is necessary to maximise yield. As the automated systems like BACTEC 9000 series systems detects 95% of cultures within 7 days, subcultures are not necessary [12]. The sensitivity of bone marrow biopsy and culture is around 90% [13].

Serum Agglutination Test (SAT) which measures both IgG and IgM is the gold standard diagnostic test in Brucellosis. IgM remains

elevated longer than IgG, though IgM is the first isotype which rises after infection with brucellosis. In active brucellosis, the SAT titer is ≥1:160. Other diagnostic tests like micro agglutination, direct fluorescent antibody, and Rose Bengal agglutination test have no advantage over the SAT.

An ELISA test to detect IgM and IgG antibody isotypes is more sensitive than the SAT. Antibodies to *Brucella* appear within 1-2 weeks after infection [14]. Very rarely antibodies appear before the onset of symptoms [15]. In the acute stage there is an initial production of IgM antibodies followed by IgG which decline after treatment. The highly sensitive and specific PCR assay appears promising [16]. Competitive enzyme immunoassay (cELISA) has high specificity and sensitivity (99.7% and 98.3), and is useful for evaluating the response to treatment and to assess the prognosis [17].

In the treatment of Brucellosis, monotherapy is not recommended. Dual therapy with drugs like doxycycline for 30 days and aminoglycosides for 2-3 weeks have low relapse rate [18]. The above dual therapy is more effective in the management of *Brucella* spondylitis [19].

The World Health Organisation (WHO) recommended dual therapy with Doxycycline and Rifampicin in 1986. Clinical trials comparing the efficacy of doxycycline with either rifampicin or streptomycin proved to be equivalent [20].

The arthritis can become destructive unless treated early. It may lead to spinal stenosis, a worst complication of *Brucella* arthritis. Data on the sero-prevalence of human brucellosis in developing countries is very limited. Prior studies carried out in the Mediterranean region have reported sero-prevalence ranging from 8% in Jordan [21] to 15% in Saudi Arabia [22]. In sub-Saharan Africa sero-prevalence of 5.3% in Nigeria [23] and 10%-13.3% in Isreal and Uganda [24,25] have been reported.

As Brucellosis is one of the causes of polyarthritis either acute or chronic, a study was carried out at a Tertiary Care Center to find out the prevalence of IgM and IgG antibodies by ELISA test in patients with acute polyarthritis. The study also focused on the response of acute phase reactant CRP in seropositive *Brucella* arthritis.

MATERIALS AND METHODS

A prospective study was done at a Tertiary Care Center for a period of six months from January 2017 to June 2017. The study was approved by institutional ethical committee (No. 1274/2016) and informed consent was taken from participants. Study population included 60 patients with acute polyarthritis and 20 age and sex matched healthy controls. Assuming the prevalence rate as 8% sample size of 80 individuals was estimated using a standard formula for prospective studies.

Inclusion criteria: Patients with acute polyarthritis.

Exclusion criteria: Patients with autoimmune rheumatic diseases like Rheumatoid Arthritis, Spondyloarthritis, and Psoriatic arthritis. Antistreptolysin O (ASO), Rheumatoid Factor (RF), VDRL, Hepatitis B surface antigen positive.

Sample: Under aseptic precautions blood sample was collected from the study group and serum was separated. Sera were stored in -20°C deep freezer. Samples were subjected to IgM and IgG ELISA test. The test kits used were procured from Euroimmun (Germany).

Procedure: The test method was an Indirect ELISA in which the microtiter plate wells were coated with *Brucella* antigen. On addition of diluted samples, controls, the antibody present bound to the antigen. On subsequent addition of enzyme linked conjugate, substrate and stop solution colour development occurs. The intensity of the colour is directly proportional to the concentration of antibodies in serum. Similarly, the test was done using IgG conjugate.

Test results were interpreted as per the kit protocol. Antibody index of <0.8 was reported negative, ratio between 0.8 <1.1 borderline positive and ratio of ≥1.1 was positive.

The samples which were positive for *Brucella* antibodies were further tested for the acute phase reactant CRP levels. Latex agglutination test was done to detect the CRP level. The qualitative and semi quantitative tests were performed. The lower limit of detection of the test was 6 mgm/L. Dilution factor multiplied with 6 was the CRP level in the sample. A CRP level of >1.1 mgm/L in acute infections had a sensitivity, specificity, positive predictive value and negative predictive value of 57.14%, 86.44%, 50.00% and 89.47%, respectively in a study done at Kilimanjaro, Tanzania [26].

STATISTICAL ANALYSIS

Statistical analysis was done using SPSS 20.0 version. Chi-square test was used to analyse the significance of Brucella antibodies in patients with Polyarthritis. The significance of isotype IgM and IgG *Brucella* antibodies in acute polyarthritis was deduced by Chi-square test. The p-value <0.05 was taken as significant.

RESULTS

Fever was the commonest symptom observed in patients with acute polyarthritis. Of the 60 patients with polyarthritis, 49 (81.66%) presented with either high grade or low-grade fever. Fatigue and weakness were the clinical manifestations in 46 (76.66%) patients out of the 60 diseased group. The joints involved were knee, hip, ankle, elbow and hands. Few patients presented with sacroilitis also. This is depicted in [Table/Fig-1].

Joints involved	No. of patients	%
Knee, Hip, Elbow, Wrist	41	68.33
Wrist, Ankle, Hip, Knee	13	21.66
Knee, Hip, Ankle, Elbow, Wrist	6	10

[Table/Fig-1]: Distribution of joint involvment in acute polyarthritis (n=60).

Since the aim of this study was to detect the seroprevalence of *Brucella* antibodies in patients with polyarthritis, all polyarthritis patients were screened for Antistreptolysin O, VDRL, Widal, HbsAg and Rheumatoid factor to rule out the causes of acute polyarthritis other than from Brucella infection.

Study group	Antibody positive	Antibody negative
Diseased (n=60)	10	50
Controls (n=20)	1	19

[Table/Fig-2]: *Brucella* antibodies positivity in diseased Vs controls (n=80). Chi-square 1.7261; p-value 0.1894; Not significant

Seroprevalence of *Brucella* antibodies in the study group was detected by ELISA test and the results are tabulated in [Table/Fig-2].

In the diseased group 16.66% patients with polyarthritis were *Brucella* antibody positive. The seropositivity in healthy controls was (5%).

The *Brucella* antibody isotypes which appear in the sera of infected patients are IgM, IgG and IgA. During the initial period, IgM is the only immunoglobulin appearing. In acute and sub-acute brucellosis IgM,IgG and IgA can consistently rise. With the progression of disease, IgM recedes quantitatively and IgG becomes predominant. In patients with chronic brucellosis IgG and IgA raise often and elevation ofIgM is seldom seen.

Among the 10 patients with *Brucella* antibody positivity the isotypes detected by ELISA test are shown in [Table/Fig-3]. Studies have shown that IgM positivity is 100% in acute brucellosis in contrast to 33% in chronic brucellosis.

CRP is an acute phase reactant and is a predictor of disease activity. CRP level was measured in all the 10 *Brucella* antibody positive polyarthritis cases by latex agglutination test. Results of the CRP test are tabulated in [Table/Fig-4].

Isotype	Positive	Negative
IgM	9	1
IgG	1	9

[Table/Fig-3]: Brucella antibodies isotypes in diseased (n=10).
Chi-square 12.8; p-value 0.000347; significant

CRP	No	%
Normal	4	40%
Elevated	6	60%

[Table/Fig-4]: CRP levels in brucella antibody positive patients (n=10).

In patients with elevated CRP levels, the semi quantitative test was deployed and the mean CRP levels detected were 24.12±12.10 mg/dL.

DISCUSSION

Acute polyarthritis remains a challenge to the practicing physicians. It needs to be evaluated in a broadspectrum considering the diverse aetiologies of acute polyarthritis. Brucellosis is one among the infectious causes of polyarthritis. People who are in contact with infected animals and consume unpasteurized milk and milk products are the vulnerable group [27,28]. From the time of the Roman era, organisms similar to Brucellae have been found in carbonised cheese. This disease was fully elucidated by Sir David Bruce, Hughes, and Zammit working in Malta [29].

Most of the diseased group in our study was urban and semi urban population where consumption of unpasteurized dairy products like butter, cheese, curd etc. are prevalent. As the study population was small, significance of unpasteurized dairy products could not be attached in our study.

The symptoms observed in the diseased group were fever, fatigue, sweating, loss of appetite and arthralgias. This was in concordance with the earlier studies [30,31]. In the study by Aygen B et al, most frequent symptoms found were fever (63.2%), sweating (62.7%), arthralgia (59.1%) and back pain (58.5%) [32]. Clinical presentation was polyarthritis involving hip, knee, ankle, elbow and wrist. In our study, 68.3% presented with arthritis of knee, wrist, hip joints. Prevalence of focal involvement has been reported to range from 20% to 40% in many studies [33]. Sacroilitis and polyarthritis were frequent complications as showed by Geyik MF et al., [27]. Arthralgias either arthralgias has a predilection for bone ends and the sacroiliac joints. However, sacroilitis was found in very few patients in our study. Any patient with the above clinical features and history of contact with animals, consumption of unpasteurized dairy products should be evaluated for brucellosis.

Diagnosis of *Brucella* poses a real challenge for the Microbiologists. The various diagnostic methods are culture and serological tests as mentioned in the introduction. In our study, IgM and IgG ELISA tests were carried out to find out the seroprevalence of *Brucella* in polyarthritis. As certain studies have descried ELISA test as a sensitive test and not affected by the presence of rheumatoid factor in the diagnosis of *Brucella* [34], we adopted ELISA test. ELISA test is also popular as it can detect specific IgM and IgG *Brucella* antibodies [35].

The primary objective of our study was to find out whether there was any significant association between acute polyarthritis and brucellosis. Though statistical significance was not found with regards to acute polyarthritis and brucellosis 16.66% of the diseased population were seropositive.

The seroprevalence of *Brucella* was 5.4% in a Malaysian study [36]. Seroprevalence of *Brucella* in high risk population of Pakistan and Brazil were 21.7% and 4%, respectively [37,38]. Some of the publications say that human brucellosis is a fairly common disease in India [39]. Seroprevalence of *Brucella* in different types of population were 8.5% among dairy personnel, 4.2% in aborted women and 8.5% in human cases in Gujarat [40]. When compared to the prevalence of anti-brucella antibodies in humans of different regions across the world, the prevalence in our South Indian Population is similar to some of the above-mentioned studies.

In brucellosis, both IgM and IgG antibodies appear in 7-10 days after the onset of clinical infection. As the disease progresses, IgM antibodies level falls but the IgG antibodies persist or increase in titer [41]. In patients with chronic brucellosis IgM disappears and only IgG can be detected. Agglutination test detects IgM more than the IgG. ELISA can detect IgM and IgG separately and it is more sensitive and specific.

In the present study anti-brucella IgM antibody was positive in 90% of patients with acute polyarthritis and IgG was detected in 10% of the diseased group. In patients with brucellosis the percentage of IgM, IgA, IgG detected were 100%, 98%, and 97%, respectively. Our study is comparable to the study done by Christopher S et al., [42]. As the resources were limited, Anti-brucella IgA ELISA was not carried out.

The acute phase reactant CRP was raised in 60% of patients who were *Brucella* antibody positive. The epidemiological and clinical studies by Kooraki S et al., has shown elevated CRP in 62% of Brucella arthritis [43].

LIMITATION

The sample size was small as the resources for testing a greater number of samples were limited. Similarly, the presence of IgA anti-brucella antibodies was not tested because of the limited resources.

CONCLUSION

Eventhough statistical significance was not found between polyarthritis and brucellosis, the study revealed 16.66% were *Brucella* antibody positive. Also, the most common isotype identified was IgM in acute polyarthritis. As ELISA test is simple, sensitive, specific and cost-effective, anti-brucella antibody ELISA is recommended in clinical practice. In conclusion, all patients with constitutional symptoms and polyarthritis, brucellosis has to be ruled out so as to prevent the osteoarticular complications of Brucellosis.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jul 17, 2019
- Manual Googling: Aug 28, 2019
- iThenticate Software: Sep 26, 2019 (14%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: No
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Jul 17, 2019**
Date of Peer Review: **Aug 12, 2019**
Date of Acceptance: **Sep 08, 2019**
Date of Publishing: **Oct 01, 2019**