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Article

Serum Cytokine Profiles and Inflammatory Markers in Brucellosis-Associated Arthritis

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Simple Summary

Brucella is an intracellular-bacteria that causes brucellosis, a zoonotic infection common worldwide. Due to the non-specific symptoms of brucellosis, which can overlap with rheumatologic complications, accurate diagnosis is essential; for this, haematological parameters and inflammatory markers play a crucial role. In this study we included 111 patients, comprising 72 brucellosis-positive arthritis patients and 39 brucellosis-negative arthritis patients to investigate the variation in levels of four cytokines (IL-2, IL-6, IL-10 and IFN- γ) among brucellosis-positive and brucellosis-negative arthritis patients and their relationship with clinical parameters. The mean serum levels of IL-2 and IL-10 were markedly higher in brucellosis-positive patients than in brucellosis-negative arthritis patients, and a significant correlation was observed between elevated levels of IL-2 and IL-10 and inflammatory markers, specifically ESR and CRP. Epidemiological data like cow contact and vaccination status showed positive trends toward association with brucellosis. Our results align with the One Health concept for controlling brucellosis-related arthritis.

Abstract

Brucellosis is a common zoonotic infection in India, caused by a facultative intracellular, Gram-negative coccobacillus and frequently presents with nonspecific symptoms. This study aimed to assess serum cytokine levels (IL-6, IL-10, IFN- γ , and IL-2) by ELISA and to correlate them with inflammatory markers (ESR, and CRP) in patients with suspected brucellosis presenting with rheumatoid arthritis-like manifestations and polyarthralgia. This study included 111 patients, comprising 72 brucellosis-positive arthritis patients and 39 brucellosis-negative arthritis patients as controls. In this study, we investigated the variation in levels of four cytokines (IL-2, IL-6, IL-10 and IFN- γ) among brucellosis-positive and brucellosis-negative arthritis patients and their relationship with clinical parameters. The mean serum levels of IL-2 and IL-10 were markedly higher in brucellosis-positive arthritis patients. As brucellosis is an important cause of inflammatory arthritis, characterized by elevated serum levels of IL-10 and IL-2 and increased inflammatory markers, including ESR and CRP, along with relatively lower levels of IFN- γ and IL-6, which are linked to the inflammatory rheumatoid arthritis. The observed associations with raw dairy consumption, contact with unvaccinated livestock, and direct animal exposure highlight the need for early diagnosis, effective livestock immunization programs, and strengthened One Health-based public health interventions to reduce disease burden.

Keywords: Brucellosis; arthritis; ELISA; cytokines; ESR; CRP

1. Introduction

Brucella is an intracellular-bacteria that causes brucellosis, a zoonotic infection common worldwide. It is a facultative, Gram-negative intracellular pathogen that can evade the host's immune system, leading to chronic infections in both humans and animals. In humans, brucellosis can present with a variety of symptoms, including fever and musculoskeletal issues such as arthritis [1]. Due to the non-specific symptoms of brucellosis, which can overlap with rheumatologic complications, accurate diagnosis is essential; for this, haematological parameters and inflammatory markers play a crucial role [2].

To understand the host immune responses and to differentiate infectious from non-infectious inflammatory diseases, the study of cytokines has emerged as a promising approach. Although both IFN- γ and IL-6 have been thoroughly studied in autoimmune arthritis, their function in *Brucella*-associated arthritis is still unidentified. Pro-inflammatory cytokines such as IL-6, IL-2, and IFN- γ play key roles in acute infection, while anti-inflammatory cytokines such as IL-10 show immunological regulation during chronic infection. In the case of brucellosis, IL-2 is essential for the growth of T cell populations, especially Th1 cells, which are necessary for efficient immune responses against intracellular infections [3]. Interleukin-10 (IL-10), a cytokine that reduces inflammation and controls immune responses to limit excessive tissue damage. IL-10 has been demonstrated to reduce the bactericidal activity of macrophages and prevent the synthesis of pro-inflammatory cytokines in brucellosis, which promotes the persistence of bacteria [4]. IL-6 has been shown to facilitate the development of CD8⁺ T cells and increase the bactericidal activity of macrophages, thereby aiding in the removal of *Brucella abortus* in the context of brucellosis, while Th1 cells release the cytokine interferon-gamma (IFN- γ), which stimulates macrophages to eliminate intracellular infections. IFN- γ is essential for regulating *Brucella* infection in brucellosis because it increases macrophage bactericidal activity and encourages naive T cells to differentiate into Th1 effector cells [5]. In this study, we aimed to investigate the levels of various cytokines (IL-6, IL-10, IFN- γ , and IL-2) in brucellosis patients suffering with arthritis, and to identify the perfect biomarker to enquire about the severity of the illness.

2. Materials and Methods

2.1. Patients

This study is a cross-sectional investigation conducted at JNMCH, AMU, Aligarh, from April 2023 to October 2025. A total of 111 patients presenting with arthritis and polyarthralgia were included in this study after obtaining appropriate informed consent.

2.2. Sample Collection

This study included 111 patients, comprising 72 brucellosis-positive arthritis patients and 39 brucellosis-negative arthritis patients. 5 ml of blood was taken from each individual, and the serum was extracted. Additionally, a pre-designed questionnaire was used to gather demographic data and risk factors associated with the disease.

2.3. Laboratory Diagnosis

SAT (Serum Agglutination Test): SAT was performed to detect anti-*Brucella* antibodies by serially diluting serum samples and adding *Brucella* antigen. The SAT antigen was purchased from Tulip Diagnostics, Goa. For the test, the serum samples were diluted from one tube to another with 0.25% phenol saline, from 1:5 to 1:320. Each sample was then incubated for 24 hours at 37°C with one drop of Brucel antigen. After 24 hours, agglutination was observed. The highest dilution showing agglutination was recorded as the 1:160 titre, considered positive [6].

RBPT (Rose Bengal Plate Test): RBPT, a rapid slide agglutination test, was done using antigen received from IVRI, Izatnagar, Bareilly. Equal volumes (30 μ L) of serum and antigen were mixed for 4 minutes, and visible agglutination indicated a positive result [7].

ELISA (Enzyme-linked Immunosorbent Assay): ELISA was designed as an indirect method of examining the patient's immune system to find antibodies that confirm prior and present exposure to the disease. To perform ELISA for IgG & IgM antibodies, we used coated microplates, reagents, positive and negative controls, a calibrator, and, most importantly, patient sera, then processed according to the manufacturer's instructions (ELISA IgG CALBIOTECH & ELISA IgM CALBIOTECH) After performing the test, we had to quantify specific antibodies generated in response to human brucellosis at an optical density of 450 and 655 nm, and then the result was interpreted accordingly. We have successfully identified antibodies in the patient's serum after performing assay.

RT-PCR: In our study, we employed real-time PCR to amplify a 731-base-pair segment of the omp31 gene from Brucella. We performed RT-PCR tests in a total volume of 20 μ L. Primers and fluorescently labelled probes were used, and reaction was carried out in a BIO-RAD device. Positive control was obtained from ICAR, Mathura. After completion of RT-PCR cycles, a graph was seen.

Conventional PCR: In our study, primers targeting the IS711 gene of B. abortus were used for conventional PCR. A total of 12.5 μ L volume Master mix was prepared in a Master Mix tube, then reaction was conducted in a thermal cycler. Afterwards a 1.5% agarose gel stained with ethidium bromide was employed for gel electrophoresis to determine positive PCR results.

Blood Culture: We have taken 5 ml blood samples from OPD/IPD patients with suspected symptoms of brucellosis and injected them into FA Plus bottles for incubation. Blood cultures were performed using the BacT/ALERT system (bioMérieux, Inc.) according to the manufacturer's instructions.

Cytokine analysis: The concentration levels of various cytokines (IL-6, IL-10, IL-2, and IFN- γ) were determined using the commercially available ELISA kits (Diacclone, France). The tests were performed according to the manufacturer's instructions. Briefly, 100 μ L of standards, controls, and patient samples were added to pre-coated wells along with 50 μ L of biotinylated antibody and incubated for 1 hours at room temperature. After washing three times, 100 μ L of streptavidin-HRP was added and incubated for 30 min at room temperature. Following another wash, 100 μ L of TMB substrate was added and incubated for 12–15 min in the dark. The reaction was stopped by adding 100 μ L stop solution. The optical density was measured at 450 nm as well as dual (450 nm & 655nm). The concentration of each cytokine was calculated by comparing the O.D. of each sample with the standards provided in the kit. A standard curve was constructed with the Human cytokine concentration on the y-axis and absorbance on the x-axis. Cytokine levels were calculated from the same.

2.4. Statistical Analysis

Chi-square tests, multiple logistic regression and independent t-tests were used to compare epidemiological variables, clinical symptoms, haematological, biochemical parameters and cytokine profiles.

3. Results

Subsections

Out of 111 patients presenting with joint pain and polyarthralgia, 72 (64.9%) were serologically positive for brucellosis. As shown in Figure 1, among the brucellosis-positive arthritis patients, 37 (51.3%) were positive for ELISA IgG, 24 (33.3%) for ELISA IgM, 27 (37.5%) for Rose Bengal Plate Test (RBPT), 23 (31.9%) for Standard Agglutination Test (SAT), 12 (16.6%) for RT-PCR, 7 (9.7%) for conventional PCR, and 2 (2.7%) for culture, respectively. In addition, 39 brucellosis-negative arthritis patients were also included as controls.

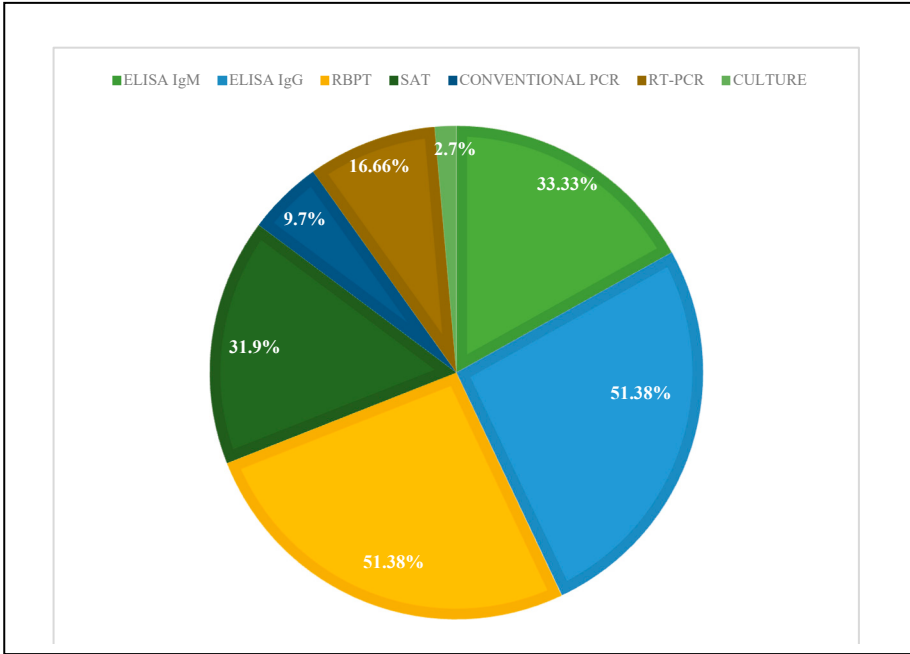


Figure 1. Positive cases by different diagnostic methods in Brucellosis-positive arthritis patients (n=72).

Patients were categorized into acute and chronic brucellosis based on disease duration. The main demographic and clinical characteristics of the 72 Brucella-positive cases are summarized in Table 1. The majority of patients were female, with a male-to-female ratio of 7:17. The age of the patients ranged from 15 to 80 years, with the highest prevalence (44.4%) observed in the 20–40-year age group. The most common risk factor among Brucella-positive arthritis patients was a history of close contact with animals (69.5%), and most patients belonged to rural areas. Arthralgia was the most frequent symptom, reported in 100% of cases, followed by body ache (70.8%), joint pain (69.4%), muscle pain (50%), fever (40.2%), and fatigue (26.3%).

Table 1. Demographic and clinical features of Brucellosis-positive Arthritis patients (n=72).

Clinical indicators	Brucella-positive arthritis patients (n=72)
Sex	21 (29.1%)
Male	51 (70.8%)
Female	
Age (Years)	
<20	4 (5.5%)
20-40	32 (44.4%)
40-60	30 (41.6%)
60-80	6 (8.3%)
Locality	
Rural	39 (54.2%)
Urban	33 (45.8%)
Animal contact	
Yes	50 (69.5%)
No	22 (30.5%)

Symptoms	
Fever	29 (40.2%)
Malaise	6 (8.3%)
Sweating	14 (19.4%)
Anorexia	9 (12.5%)
Muscle pain	36 (50%)
Yellow skin	7 (9.7%)
Arthralgia	72 (100%)
Joint pain	50 (69.4%)
Epididymalorcharditis	5 (6.9%)
Endocarditis	2 (2.7%)
Headache	18 (25%)
Fatigue	19 (26.3%)
Weakness	24 (33.3%)
Body ache	51 (70.8%)
Breathlessness	6 (8.3%)
Testicular swelling	3 (4.1%)
Confusion	4 (5.5%)

Comparison of cytokine levels & other biochemical parameters between Brucella-positive arthritis patients and controls: As demonstrated in Table 2, the brucellosis-positive patients group had considerably higher mean levels of IL-10 (1.0±0.5) and IL-2 (4.5±6.6) than the control group, and the mean levels of IL-6 (2.4±8.9) and IFN-γ (1.8±3.3) were higher in the control group than in the Brucellosis-positive arthritis individuals. Brucella-positive arthritis patients showed slightly elevated ESR (21.8±11.4) and CRP (4.5±6.7), suggesting a moderate but consistent inflammatory response, although there were no significant haematological differences between the groups, as indicated by comparable haemoglobin (Hb) and ALP levels. There were no statistically significant changes in any of the variables ($p > 0.05$); however, ESR had the lowest p-value (.093), but still not significant.

Table 2. Comparison of cytokines levels & other biochemical parameters between Brucella-positive arthritis patients and controls (n=111).

Variable	Brucella- (n)	Mean (SD)	Brucella+ (n)	Mean (SD)	P value
Hemoglobin (g/dL)	39	10.33 (2.27)	71	10.49 (2.03)	.697
TLC (/mm ³)	39	4,133 (4,445)	71	4,724 (11,697)	.763
ESR (mm/hr)	38	18.21 (9.18)	71	21.86 (11.46)	.093
ALP (IU/L)	37	102.6 (54.9)	68	104.9 (40.9)	.813
CRP (mg/dL)	38	3.84 (5.38)	71	4.57 (6.71)	.561
RA factor (IU/mL)	37	13.55 (51.01)	70	13.07 (38.82)	.957
IL-6 (pg/mL)	39	2.45 (8.99)	72	1.47 (2.54)	.386
IL-10 (pg/mL)	39	0.96 (0.47)	70	1.02 (0.55)	.582
IL-2 (pg/mL)	39	3.14 (4.42)	72	4.52 (6.66)	.250
IFN-γ (pg/mL)	38	1.83 (3.30)	72	1.57 (2.74)	.652

As shown in Table 3, a series of chi-square tests were performed to examine associations between Brucella seropositivity, various risk factors and clinical symptoms. Sex distribution value ($\chi^2 = 1.308$, $p = .253$), demonstrating that Brucella exposure risk is not limited to a particular gender. None of the associations reached conventional statistical significance (all $p > .05$). The largest χ^2 value observed was for vaccination status ($\chi^2(3) = 6.94$, $p = .074$), which approached but did not reach statistical significance. Other variables with non-significant χ^2 included cow contact ($\chi^2(1) = 2.35$, $p = .126$) which is consistent with known *B. abortus* transmission patterns, but other livestock exposures indicated no significant relationships, socio-economic status ($\chi^2(1) = 2.48$, $p = .115$), and shared water source ($\chi^2(3) = 5.72$, $p = .126$). Symptoms such as fever, malaise, joint pain, and weakness were similarly distributed, showing diagnostic overlap between the healthy group and Brucella-positive arthritis patients.

Table 3. Association between Brucella seropositivity, various risk factors and clinical symptoms (n=72).

Variable (row)	χ^2 (Pearson)	df	p (2-tailed)
Sex	1.308	1	.253
Cow (contact)	2.347	1	.126
Buffalo (contact)	0.910	1	.340
Goats (contact)	0.234	1	.629
Duration (exposure)	1.898	1	.168
Native place	0.460	1	.498
Socio-economy	2.483	1	.115
Smoker	2.862	3	.413
Drinker	2.629	2	.269
Recent travel	2.695	3	.441
Raw meat consumption	3.199	3	.362
Raw dairy consumption	3.422	3	.331
Fever (symptom)	3.173	3	.366
Malaise	3.318	3	.345
Joint/back pain	2.501	3	.475
Weakness in limbs	3.985	3	.263
Bodyache	3.824	3	.281
Rashes	3.051	3	.384
Neck stiffness	2.957	3	.398
Vaccinated	6.943	3	.074
Animals near sleeping area	5.365	3	.147
Share water source	5.720	3	.126

As shown in Table 4, a binary logistic regression was performed to examine whether IL-6, IL-10, IL-2, and IFN- γ predict seropositivity for Brucella. The full model did not significantly predict sero status, $\chi^2(4) = 3.53$, $p = .473$, and none of the cytokine predictors were statistically significant (all $p > .05$). The initial classification suggested poor specificity (0%) despite perfect sensitivity (100%), with overall accuracy 51.9%. These results indicate no evidence in the present sample that the tested cytokines are associated with Brucella seropositivity; however, the analysis is limited by sample size and model performance.

Table 4. Logistic Regression analysis to identify the cytokine levels, and seropositivity in Brucella-positive arthritis patients (n=72).

Predictor	B (β)	SE	Wald	df	p	Exp(B)	Score	Score p
Constant	0.074	0.193	0.148	1	.700	1.08	—	—
Cytokine	—	—	—	—	—	—	0.962	.327
IL-6								
Cytokine	—	—	—	—	—	—	0.031	.861
IL-10								
Cytokine	—	—	—	—	—	—	0.682	.409
IL-2								
Cytokine	—	—	—	—	—	—	2.071	.150
IFN- γ								
Model	χ^2	= df = 4	—	—	p = .473	—	—	—
(Omnibus)	3.529							

4. Discussion

Human brucellosis is still a neglected but significant zoonotic disease. Recent data indicate that there are about 2.1 million new cases in humans each year, with substantial regional variation in incidence [8]. Regional, livestock reservoir, monitoring intensity, and diagnostic approach each possess a significant impact on prevalence estimates. Close human-animal contact, the consumption of unpasteurized dairy products, and inadequate implementation of animal surveillance are among the causes of the highest burdens reported in endemic areas, especially in the Middle East, South Asia, the Mediterranean basin, and sub-Saharan Africa [9,10].

Brucellosis can cause major complications, if not diagnosed properly, as it affects several organ systems, including the central nervous system, cardiovascular system, gastrointestinal system, genitourinary system, hepatic system, and musculoskeletal system [11]. Peripheral arthritis, sacroiliitis, spondylitis, and osteomyelitis are examples of musculoskeletal disorders that can result in severe issues, in which osteoarticular involvement is the most frequent primary complication [12]. To determine the relationship between Brucella and arthritis, diagnosis confirmation is essential, as the disease presents with nonspecific clinical symptoms that often resemble those of other rheumatologic conditions [13]. According to a study by Turan et al. (2011), osteoarticular complications occurred in most of the brucellosis-positive patients, with a high percentage of 47%[14]. In another study by Ebrahimpour et al. (2017), demonstrated that 10.8% of the brucellosis-positive patients with focal problems had polyarthritis [15]. Another study by Colmenero et al. (1996) shows that bacterial localisation in the synovial and osseous tissues causes brucella arthritis and spondylitis, leading to persistent inflammation and joint deterioration, as they demonstrated that 66% of the patients suffered with Osteoarticular complications [16]. Ariza et al. (1993) also emphasized the high prevalence of musculoskeletal symptoms in brucellosis [17]. The present study found that a significant proportion (45.5%) of brucellosis-positive patients presented with arthralgia and arthritis.

In our study, we found that females showed a higher positivity rate (70.8%) among the *Brucella*-positive arthritis patients than males. This data showed similarity with previous research conducted by Gotuzzo et al. (1987)[18]. According to our study, most *Brucella*-positive arthritis patients had a history of close contact with animals (69.5%), and they mainly came from rural areas (54.2%), highlighting the occupational and environmental exposure linked to brucellosis. These findings are similar to those of Kumari et al. (2023)[19], who reported higher brucellosis prevalence among rural populations with a history of animal contact. A major risk factor identified in our study was the presence of unvaccinated animals (79.1%), which play an important role in the transmission of the disease, as also demonstrated by Gwida et al. (2010)[20] and Corbel (2006)[21]. In our study, consumption of raw dairy products was reported by 56.9% of *Brucella*-positive arthritis patients, supporting findings by Faddane et al. (2022)[22] demonstrated that unpasteurised milk is a key route of transmission of brucellosis. Additionally, exposures such as keeping animals near sleeping areas (37.5%), contact with cows (29.1%), and sharing water sources with animals (20.8%) were common, similar to studies reported by Zhang et al. (2019)[23] and Mantur & Amarnath (2008)[24], emphasising the importance of close human–animal contact in brucellosis transmission. We found a strong directional trend that indicated a connection with cow contact ($p = .126$). This pattern aligns with the known epidemiology of *B. abortus*, which is mainly associated with cattle, although this association is not statistically significant. Environmental factors, such as animals near sleeping areas and shared water sources, showed positive trends linked to disease transmission (Gwida et al., 2010)[20].

Inflammatory markers show a clinically significant trend towards elevated values in *Brucella*-positive patients, especially ESR and CRP [25]. In our study, *Brucella*-positive arthritis patients had higher levels of inflammatory markers such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), which indicate active systemic inflammation. This data showed similarity with previous research conducted by S Almohrij et al. (2025)[26], in which they demonstrated that elevated ESR and CRP in *Brucella*-positive arthritis patients served as more useful indicators of infection. According to another study, by Bosilkovski et al. (2004)[27], both acute and chronic brucellosis are frequently associated with elevated CRP and ESR, which are especially linked to osteoarticular involvement. These indicators show persistent inflammatory activity. However, another study by Colmenero et al. (1996)[16], demonstrated that ESR and CRP elevation are associated with tissue involvement and the severity of the disease, particularly in patients with sacroiliitis, spondylitis, and arthritis.

In our study, cytokine analysis showed higher IL-10 and IL-2 levels in *Brucella*-positive arthritis patients, suggesting that *Brucella* infection influences immune-modulating and T-cell-related responses. Conversely, IL-6 and IFN- γ levels were somewhat lower, indicating a reduced Th1 and pro-inflammatory response. Overall, the immunopathology of *Brucella* infection aligns with these data.

According to our analysis, brucellosis-positive arthritis patients had elevated levels of IL-10 and IL-2. IL-10, a crucial anti-inflammatory cytokine, can reduce pro-inflammatory cytokine production and suppress macrophage activation, and helps *Brucella* to survive inside the host cells. Similar to our findings, research conducted by Saadati et al. (2019)[1], demonstrated that a higher level of IL-10 cytokine played an important role in the brucellosis-positive arthritis patients. Another elevated cytokine in brucellosis-positive arthritis patients was IL-2, which increased T-cell activation, T-cell proliferation and immunological modulation. Our study is showing similarity with the research conducted by Tang et al. (2021)[28], in which they demonstrated that a higher level of IL-2 cytokine is mostly present in patients with acute brucellosis.

On the other hand, opposite cytokine effects were observed, in which we detected a lower levels of IFN- γ and IL-6 cytokines in the *Brucella*-positive arthritis patients. Our findings are corroborated by the study of Murphy et al. (2001)[29], which demonstrated that reduced IFN- γ production may be partly due to elevated IL-10, as IL-10 can affect the production of IFN- γ through altering the antigen-presenting capacity of macrophages. Another study by GhaznaviRad et al. (2017)[30] showed that

decreased IFN- γ responses were presented in individuals with chronic brucellosis, suggesting that insufficient Th1 responses may play a role in joint localisation and infection persistence. Chronic and relapsing brucellosis has also been associated with decreased IFN- γ production. Another cytokine with a lower level was IL-6. While IL-6 is frequently increased in acute infections, it also contributes to the elimination of bacteria and the control of inflammatory reactions. Our Brucella-positive arthritis patients with lower IL-6 levels might be the result of IL-10 mediated reduction of macrophage activity, which favours persistent infection over efficient inflammatory clearance. Our study is supported by the previous research in which Brucella-negative inflammatory arthritis, especially rheumatoid arthritis, increased levels of IL-6 and IFN- γ are frequently observed, indicating ongoing joint inflammation [31]. On the other hand, our patients with Brucella-positive arthritis had higher levels of IL-2 and IL-10 and lower levels of IFN- γ and IL-6. Overall, the data reveal that inflammatory and cytokine responses were largely comparable amongst groups, demonstrating a variable host response pattern and explaining the complexity of brucellosis-related immunological alterations.

5. Conclusions

This study identifies brucellosis as an important cause of inflammatory arthritis. Brucella-positive arthritis patients exhibited elevated levels of IL-10 and IL-2, along with increased ESR and CRP, reflecting ongoing inflammatory activity. In contrast, relatively lower levels of pro-inflammatory cytokines IFN- γ and IL-6 may indicate chronic immune adaptation in affected individuals. These results demonstrate that Brucella-positive arthritis has a different cytokine profile than Brucella-negative inflammatory arthritis, demonstrating the biological significance and importance of our study. Key risk factors included close contact with animals, exposure to unvaccinated livestock, and consumption of raw dairy products. Collectively, these findings underscore the importance of animal vaccination programs, early diagnosis, and strengthened public health control measures, particularly within a One Health framework, to reduce the burden of brucellosis-associated arthritis.

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Institutional Review Board Statement: The study was approved by the Institutional Ethics Committee, Faculty of Medicine, AMU, Aligarh (IEC JNMC/270 Dated 01/03/21).

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Conflicts of Interest: The authors declare that they have no known competing interest.

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