



Article

Serum Cytokine Profiles and Inflammatory Markers in Brucellosis-Associated Arthritis—A Cross Sectional Study

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Simple Summary

Brucella is an intracellular bacterium 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 higher in brucellosis-positive patients compared to brucellosis-negative arthritis patients. Inflammatory markers such as ESR and CRP were also elevated in brucellosis-positive cases. These findings describe variations in cytokine and inflammatory marker levels among study groups. 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 bacterium, 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 numerically higher in brucellosis-positive arthritis patients compared to controls; however, these differences were not statistically significant ($p > 0.05$). Similarly, no statistically significant differences were observed for IL-6 and IFN- γ between the groups. Inflammatory markers such as ESR and CRP were elevated in brucellosis-positive patients, although these differences did not reach statistical significance. These findings indicate variability in cytokine and inflammatory marker levels between groups. The observed associations with raw dairy consumption, contact with



Academic Editor: Stephen K. Wikel

Received: 17 January 2026

Revised: 21 April 2026

Accepted: 27 April 2026

Published: 6 May 2026

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unvaccinated livestock, and direct animal exposure highlight the need for early diagnosis, effective livestock immunisation programs, and strengthened One Health-based public health interventions to reduce disease burden.

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

1. Introduction

Brucella is an intracellular bacterium 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 [1]. Humans contract it via infected animals or by consuming unpasteurized dairy products. Till now, ten species have been identified within the genus *Brucella*: *Brucella abortus*, *Brucella canis*, *Brucella ceti*, *Brucella inopinata*, *Brucella melitensis*, *Brucella microti*, *Brucella neotomae*, *Brucella ovis*, *Brucella pinnipedialis*, and *Brucella suis*. Among these species, *B. abortus* primarily infects cattle, *B. suis* infects pigs, and *B. melitensis* infects sheep and goats. *B. canis* is linked to dogs, *B. ceti* and *B. pinnipedialis* are associated with marine mammals, *B. ovis* infects sheep, *B. neotomae* infects rodents, *B. microti* has been isolated from voles, *B. papionis* has been isolated from baboons, and *B. vulpis* has been identified in foxes [2]. Among all species, *B. abortus* and *B. melitensis* are the main causes of human brucellosis worldwide [3].

In humans, brucellosis can present with a variety of symptoms, including fever and musculoskeletal issues such as arthritis [4]. 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 [5].

Although diagnosing human brucellosis remains clinically challenging, particularly in complicated cases presenting with osteoarticular involvement, such as arthritis, Brucellosis continues to be a major public health burden with notable economic effects despite advancements in control techniques [6]. The illness is frequently characterised by nonspecific clinical symptoms that overlap with a variety of inflammatory and viral illnesses, making early identification more difficult and perhaps resulting in misdiagnosis [7,8]. Bacterial culture and serological assays have been vital methods of laboratory diagnosis. Culture is still the gold standard for a brucellosis diagnosis, but it is time-consuming, requires biosafety infrastructure, and has reduced sensitivity in chronic or acute cases.

Although serological assays, including SAT, ELISA, and RBPT, are frequently employed, their diagnostic precision may be limited by cross-reactivity and the inability to differentiate between acute and chronic infections [9].

Molecular diagnostic techniques, especially those based on the polymerase chain reaction (PCR), have emerged as valuable tools. PCR directly identifies *Brucella* DNA in clinical samples, providing increased sensitivity and specificity as well as fast turnaround times. This improves early and precise diagnosis, particularly in complex cases such as *Brucella*-associated arthritis [10].

Mainly, human brucellosis is treated with doxycycline, rifampin, trimethoprim, aminoglycosides (gentamicin or streptomycin), and quinolones (ciprofloxacin or ofloxacin) [11]. Currently, ceftriaxone is a safe drug and has potential as a combined treatment for certain *Brucella*-associated complicated cases [12].

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, because cytokines play a vital role in the pathogenesis of arthritis, where an

imbalance between pro- and anti-inflammatory cytokines promotes autoimmunity, chronic inflammation, and joint damage [13]. Most of the cytokines play important roles in brucellosis, but because the study focused on a small number of reliable cytokines essential to immune regulation and disease development, IL-2, IL-6, IL-10, and IFN- γ were chosen to analyse the balanced representation of protective and regulatory immune mechanisms relevant to *Brucella*-associated arthritis.

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, whereas anti-inflammatory cytokines such as IL-10 regulate the immune response 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 [14]. Interleukin-10 (IL-10) is 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 [15]. 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 [16]. 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 from arthritis and to identify a potential biomarker to assess 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, of whom 43 patients belonged to the acute group, and 29 patients belonged to the chronic group. The study also included a control group consisting of 39 patients presenting with arthritis or polyarthralgia who tested negative for brucellosis based on serological (SAT, RBPT, ELISA) and molecular methods (PCR).

A total of 5 mL of blood was collected in plain (serum) vials and in EDTA (plasma) vials at room temperature. Samples were centrifuged at 3000 rpm for 4 min, and the separated serum was aliquoted and stored at -20°C until further analysis. Repeated freeze–thaw cycles were avoided. Blood samples were collected from the patients with clinical symptoms before the treatment of brucellosis.

Additionally, a pre-designed questionnaire was used to gather demographic data and risk factors associated with the disease.

Inclusion criteria:

- Patients who provided informed consent.
- Patients aged ≥ 15 years with clinical features of *Brucella*-associated arthritis.
- Suspected brucellosis symptoms including animal contact and a history of consumption of unpasteurized products.

- Evidence of musculoskeletal involvement consistent with *Brucella* infection.
- Exclusion criteria:
- Pregnancy.
 - Non-symptomatic patients.

2.3. Laboratory Diagnosis

2.3.1. Definition of Brucellosis

A case of brucellosis-associated arthritis was defined based on a combination of clinical and laboratory criteria. Patients presenting with arthritis or polyarthralgia along with suggestive clinical features (such as fever, malaise, history of animal contact, or consumption of unpasteurized dairy products) were evaluated using serological, molecular, and culture-based methods.

2.3.2. Classification of Disease Duration

Patients were categorised based on duration of symptoms into acute and chronic brucellosis for descriptive and clinical interpretation. Acute brucellosis was defined as a symptom duration of ≤ 3 months, while chronic brucellosis was defined as duration of > 3 months, based on established clinical criteria.

2.3.3. Laboratory Confirmation Was Defined as the Presence of at Least One of the Following

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, India. 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 h at 37 °C with one drop of *Brucella* antigen. After 24 h, agglutination was observed. The highest dilution showing agglutination was recorded as a titre of 1:160, considered positive [17].

RBPT (Rose Bengal Plate Test): RBPT, a rapid slide agglutination test, was done using antigen obtained from IVRI, Izatnagar, Bareilly, India. Equal volumes (30 μ L) of serum and antigen were mixed for 4 min, and visible agglutination indicated a positive result [18].

ELISA (Enzyme-linked Immunosorbent Assay): ELISA is 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 and IgM antibodies, we used coated microplates, reagents, positive and negative controls, a calibrator, and, most importantly, patient sera, and then processed the samples according to the manufacturer's instructions [ELISA IgG Calbiotech Inc. (El Cajon, CA, USA) & (ELISA IgM Calbiotech Inc. (El Cajon, CA, USA))]. After performing the test, we quantified specific antibodies generated in response to human brucellosis at optical densities of 450 and 655 nm, and then the results were interpreted accordingly. We successfully identified antibodies in the patient's serum after performing the 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 the reaction was carried out in a BIO-RAD device. A positive control was obtained from ICAR, Mathura. After completion of RT-PCR cycles, a graph was created.

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 of master mix was prepared in a master mix tube, and then the 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: Blood cultures were performed using the BacT/ALERT system (bioMérieux, Inc., Durham, NC, USA) according to the manufacturer's instructions. We collected 5 mL blood samples from OPD/IPD patients with suspected symptoms of brucellosis and injected them into FA Plus bottles for incubation for up to 4 weeks. Positive samples were subcultured on *Brucella* agar and incubated at 37 °C under 10% CO₂. Bacterial identification was based on Gram staining and standard biochemical tests (urease, oxidase, and catalase).

In cases with discordant results, classification was based on clinical correlation along with serological findings, considering the known limitations in sensitivity of culture and molecular methods in brucellosis.

Cytokine analysis: In this study, we selected only four cytokines because of their importance in the brucellosis-associated arthritis. The concentration levels of various cytokines (IL-6, IL-10, IL-2, and IFN- γ) were determined using commercially available ELISA kits (Diaclone, Besançon, 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 h 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 of stop solution. The optical density was measured at 450 nm and at dual wavelengths (450 nm & 655 nm). 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 accordingly. All cytokine levels (IL-6, IL-10, IL-2, and IFN- γ) are expressed in pg/mL. All samples, standards, and controls were analysed in duplicate to ensure accuracy and reproducibility of the assay.

2.4. Statistical Analysis

Data were entered and analysed using IBM SPSS Statistics for Windows, Version 11.0 (IBM Corp., Armonk, NY, USA). Continuous variables were assessed for normality using the Shapiro–Wilk test. As several variables, particularly cytokine levels, showed a non-normal distribution, non-parametric tests were applied.

Continuous variables (e.g., cytokine levels, ESR, CRP) are presented as mean $\pm$ standard deviation or median (interquartile range), as appropriate, and were compared between groups using the Mann–Whitney U test. Categorical variables (e.g., demographic characteristics and risk factors) were analysed using the Chi-square (χ^2) test. No multivariable modelling was performed due to the limited sample size and the exploratory study design. No formal correlation analysis between cytokine levels and inflammatory markers was performed; therefore, no correlation coefficients are reported. Missing data were handled using a complete case analysis approach, and the number of observations for each variable is reported in the respective tables. A *p*-value < 0.05 was considered statistically significant.

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, 37 (51.3%) 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.

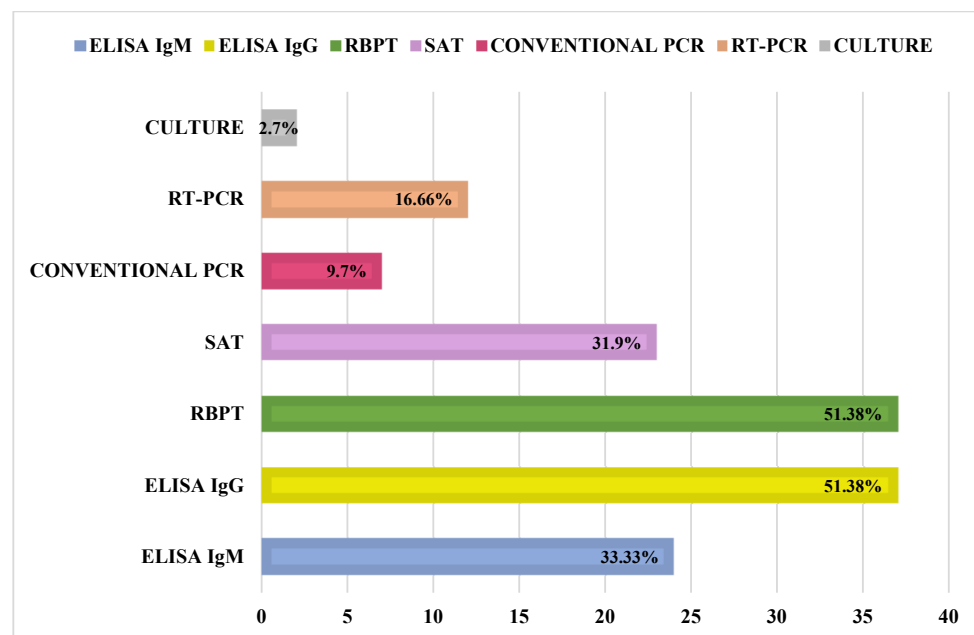


Figure 1. Positive cases by different diagnostic methods in brucellosis-positive arthritis patients ($n = 72$): Among *Brucella*-positive arthritis patients, most (51.3%) were positive for ELISA IgG and RBPT, followed by ELISA IgM, SAT, RT-PCR, conventional PCR and culture.

Patients were categorised into acute and chronic brucellosis based on disease duration. The main demographic and clinical characteristics of the 72 *Brucella*-positive cases are summarised 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 ($n = 72$): Demographic and clinical variables summarised using descriptive statistics and presented as percentages and frequencies.

Clinical Indicators	<i>Brucella</i> -Positive Arthritis Patients ($n = 72$)
Sex	
Male	21 (29.1%)
Female	51 (70.8%)
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%)

Table 1. *Cont.*

Clinical Indicators	<i>Brucella</i> -Positive Arthritis Patients (<i>n</i> = 72)
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%)
Epididymo-orchitis	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 and other biochemical parameters between *Brucella*-positive arthritis patients and controls: As demonstrated in Table 2, the brucellosis-positive patient group had considerably higher mean levels of IL-10 (1.0 ± 0.5) and IL-2 (4.5 ± 6.6) than the control group, whereas 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 numerically higher ESR (21.8 ± 11.4) and CRP (4.5 ± 6.7), although there were no significant haematological differences between the groups, as indicated by comparable haemoglobin (Hb) and ALP levels. There were no statistically significant differences in any of the variables ($p > 0.05$); however, ESR had the lowest p -value (0.093), which was still not significant.

Table 2. Comparison of cytokine levels and other biochemical parameters between *Brucella*-positive arthritis patients and controls ($n = 111$): Table shows the group statistics (means $\pm$ SD) for variables of *Brucella*-positive and *Brucella*-negative arthritis patients.

Variable	<i>Brucella</i> – (<i>n</i>)	Mean (SD)	<i>Brucella</i> + (<i>n</i>)	Mean (SD)	<i>p</i> Value
Haemoglobin (g/dL)	39	10.33 (2.27)	71	10.49 (2.03)	0.697
TLC (/mm ³)	39	4133 (4445)	71	4724 (11,697)	0.763
ESR (mm/h)	38	18.21 (9.18)	71	21.86 (11.46)	0.093
ALP (IU/L)	37	102.6 (54.9)	68	104.9 (40.9)	0.813
CRP (mg/dL)	38	3.84 (5.38)	71	4.57 (6.71)	0.561
RA factor (IU/mL)	37	13.55 (51.01)	70	13.07 (38.82)	0.957
IL-6 (pg/mL)	39	2.45 (8.99)	72	1.47 (2.54)	0.386
IL-10 (pg/mL)	39	0.96 (0.47)	70	1.02 (0.55)	0.582
IL-2 (pg/mL)	39	3.14 (4.42)	72	4.52 (6.66)	0.250
IFN- γ (pg/mL)	38	1.83 (3.30)	72	1.57 (2.74)	0.652

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. The sex distribution value ($\chi^2 = 1.308$, $p = 0.253$) demonstrated that *Brucella* exposure risk is not limited to a particular gender. None of the associations reached conventional statistical significance (all $p > 0.05$). The largest χ^2 value observed was for vaccination status ($\chi^2(3) = 6.94$, $p = 0.074$), which approached but did not reach statistical significance. Other variables with non-significant χ^2 included contact with cows ($\chi^2(1) = 2.35$, $p = 0.126$), which is consistent with known *B. abortus* transmission patterns, while other livestock exposures indicated no significant relationships socio-economic status ($\chi^2(1) = 2.48$, $p = 0.115$) and shared water source ($\chi^2(3) = 5.72$, $p = 0.126$). Symptoms such as fever, malaise, joint pain, and weakness were similarly distributed, showing diagnostic overlap between the control group and *Brucella*-positive arthritis patients.

Table 3. Association between *Brucella* seropositivity, various risk factors and clinical symptoms ($n = 72$): A series of chi-square tests were performed to examine associations between *Brucella* seropositivity, various risk factors and clinical symptoms.

Variable (Row)	χ^2 (Pearson)	df	p (2-Tailed)
Sex	1.308	1	0.253
Cow (contact)	2.347	1	0.126
Buffalo (contact)	0.910	1	0.340
Goats (contact)	0.234	1	0.629
Duration (exposure)	1.898	1	0.168
Native place	0.460	1	0.498
Socio-economy	2.483	1	0.115
Smoker	2.862	3	0.413
Drinker	2.629	2	0.269
Recent travel	2.695	3	0.441
Raw meat consumption	3.199	3	0.362
Raw dairy consumption	3.422	3	0.331
Fever (symptom)	3.173	3	0.366
Malaise	3.318	3	0.345
Joint/back pain	2.501	3	0.475
Weakness in limbs	3.985	3	0.263
Bodyache	3.824	3	0.281
Rashes	3.051	3	0.384
Neck stiffness	2.957	3	0.398
Vaccinated	6.943	3	0.074
Animals near sleeping area	5.365	3	0.147
Share water source	5.720	3	0.126

As shown in Figure 2, a graphical comparison of cytokine levels was performed to assess the distribution and variability of cytokine levels between *Brucella*-positive and *Brucella*-negative arthritis patients. *Brucella*-positive arthritis patients exhibited elevated levels of IL-10 and IL-2, but no statistically significant differences were observed in the levels of IL-2, IL-6, IL-10, and IFN- γ between both groups.

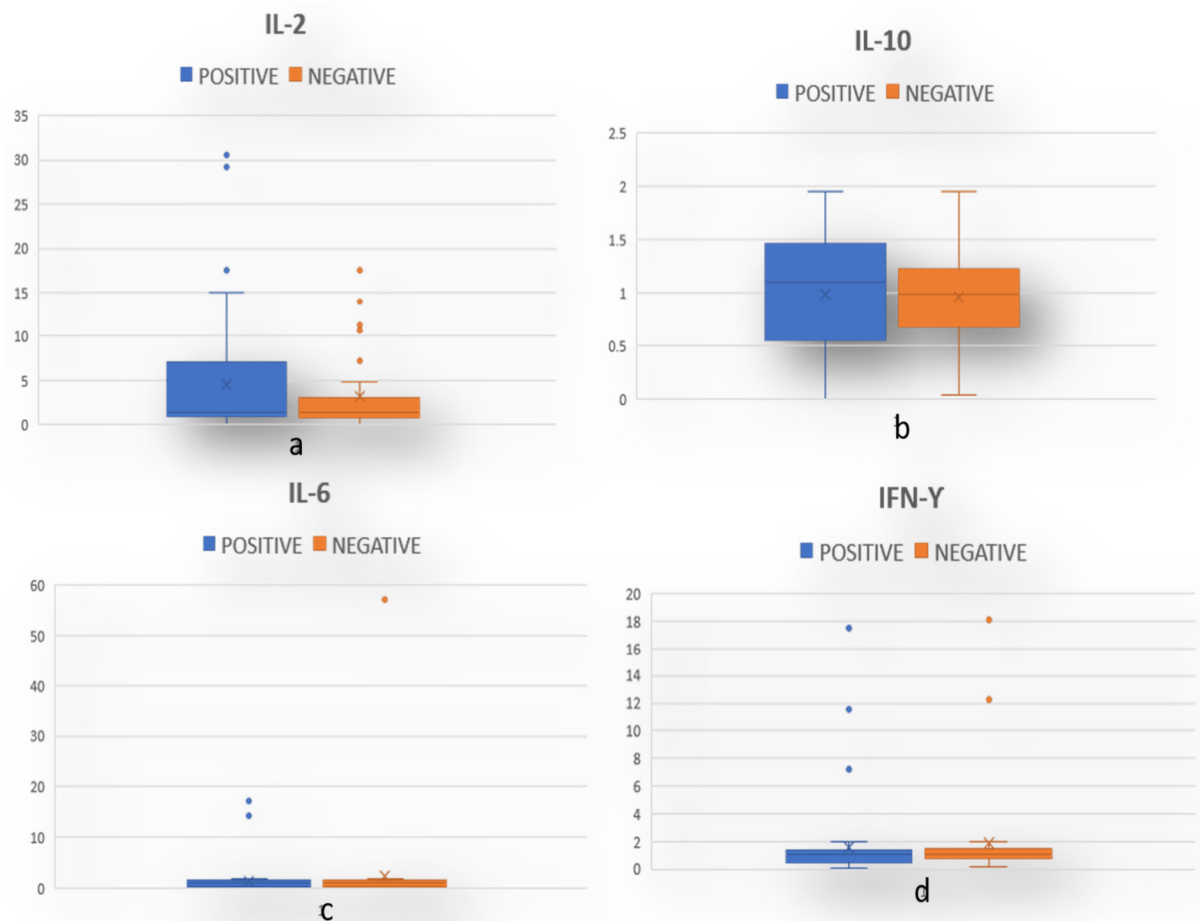


Figure 2. Box plots illustrate the distribution of cytokine levels (a) IL6, (b) IL10, (c) IL6, and (d) IFN γ among *Brucella*-positive and *Brucella*-negative arthritis patients. The median is represented by the central line, and the whiskers indicate the range of values. The 'x' symbol denotes the mean. The Y-axis represents the concentrations of cytokines, while the X-axis denotes the study groups.

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 [19]. 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 [20,21].

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 [22]. 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 [23]. To determine the relationship between *Brucella* and arthritis, diagnosis confirmation is essential, as the disease presents with non-specific clinical symptoms that often resemble those of other rheumatologic conditions [24]. 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% [25]. Another study by Ebrahimpour et al. (2017) demonstrated that 10.8% of the brucellosis-positive pa-

tients with focal problems had polyarthritis [26]. Another study by Colmenero et al. (1996) showed 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 from osteoarticular complications [27]. Ariza et al. (1993) also emphasised the high prevalence of musculoskeletal symptoms in brucellosis [28]. 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 finding was consistent with previous research conducted by Gotuzzo et al. (1987) [29]. 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) [30], 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 plays an important role in the transmission of the disease, as also demonstrated by Gwida et al. (2010) [31] and Corbel (2006) [32]. 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) [33], who 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) [34] and Mantur & Amarnath (2008) [35], emphasising the importance of close human–animal contact in brucellosis transmission. No statistically significant association was observed between cow contact and brucellosis status ($p = 0.126$). Similarly, environmental factors such as animals near sleeping areas and shared water sources did not show statistically significant associations with disease status ($p > 0.05$). These findings should be interpreted as exploratory and descriptive, without evidence of a statistically supported association. However, these exposures are consistent with known epidemiological risk factors for brucellosis reported in previous studies (Gwida et al., 2010) [31].

Inflammatory markers such as ESR and CRP showed numerical differences between groups; however, no statistically significant between-group differences were observed ($p > 0.05$) [36]. 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 finding was consistent with previous research conducted by S Almohrij et al. (2025) [37], 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) [38], 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) [27] 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 it helps *Brucella* to survive inside the host cells. Similar to our findings, research conducted by Saadati et al. (2019) [4] 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 shows similarity with the research conducted by Tang et al. (2021) [39], which demonstrated that a higher level of IL-2 cytokine is mostly present in patients with acute brucellosis. These findings should be interpreted as exploratory, as no statistically significant differences were observed between groups.

On the other hand, opposite cytokine effects were observed, in which we detected lower levels of IFN- γ and IL-6 cytokines in *Brucella*-positive arthritis patients. Our findings are corroborated by the study of Murphy et al. (2001) [40], 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) [41] showed that decreased IFN- γ responses were observed 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 in macrophage activity, which favours persistent infection over efficient inflammatory clearance. Our study is supported by previous research in which, in *Brucella*-negative inflammatory arthritis, especially rheumatoid arthritis, increased levels of IL-6 and IFN- γ are frequently observed, indicating ongoing joint inflammation [42]. 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 between groups, demonstrating a variable host response pattern and explaining the complexity of brucellosis-related immunological alterations. However, the non-normal distribution of cytokine data required non-parametric analysis, which may limit comparability with studies using parametric methods.

5. Conclusions

This study explored cytokine profiles in brucellosis-associated arthritis and observed numerical differences between groups; however, no statistically significant differences were identified. Similarly, no robust associations were demonstrated between cytokine levels and clinical or epidemiological variables. These findings suggest variability in immune response but do not provide statistically conclusive evidence of cytokine-based differentiation or predictive utility. Therefore, the results should be interpreted as exploratory and hypothesis-generating.

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. Further studies with larger sample sizes and more homogeneous populations are required to better understand the role of cytokines in brucellosis-associated arthritis.

Author Contributions: K.N.: Formal analysis, Investigation, Methodology, Writing—original draft. H.S.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing—review & editing, Writing—original draft.

P.A.K.: Formal analysis, Investigation, Methodology, Writing—original draft. A.B.S.: Supervision, Writing—review & editing. L.Z.J.: Writing—Supervision, review & editing. H.A.: Investigation, Methodology, Z.M.: Data curation, Formal analysis, N.F.: Supervision, Writing—review & editing. H.M.K.: Supervision, Validation, Writing—review & editing. A.R.: Supervision, Writing—review & editing. All authors have read and agreed to the published version of the manuscript.

Funding: This study is part of a project funded by the Indian Council of Medical Research, New Delhi. (Project ID: ZON/65/2022/ECD-II).

Institutional Review Board Statement: This study was approved by the Institutional Ethics Committee, Faculty of Medicine, AMU, Aligarh (IEC JNMC/270 Dated 1 March 2021).

Informed Consent Statement: Informed consent was obtained from all the subjects, including participants involved in this study.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Acknowledgments: We thank our institution's Viral Research and Diagnostic Laboratory, Department of Microbiology, for providing study infrastructure. We acknowledge Syed Ghazanfar Ali, Sivan, Shibli Javed, and Sanaullah for their assistance with various technical aspects of the study. We are extremely thankful to M. Suman Kumar, Division of Veterinary Public Health, ICAR-IVRI, Izatnagar, India and K. Gururaj, Animal Health Division, ICAR-CIRG, Makhdoom, Farah, Mathura, Uttar Pradesh for providing necessary support to standardize molecular testing in this study.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Qureshi, K.A.; Parvez, A.; Fahmy, N.A.; Abdel Hady, B.H.; Kumar, S.; Ganguly, A.; Atiya, A.; Elhassan, G.O.; Alfadly, S.O.; Parkkila, S.; et al. Brucellosis: Epidemiology, pathogenesis, diagnosis and treatment—A comprehensive review. *Ann. Med.* **2023**, *55*, 2295398. [\[CrossRef\]](#) [\[PubMed\]](#) [\[PubMed Central\]](#)
2. O'Callaghan, D.; Whatmore, A.M. *Brucella* genomics as we enter the multi-genome era. *Brief. Funct. Genom.* **2011**, *10*, 334–341. [\[CrossRef\]](#)
3. Conrad, P.A.; Meek, L.A.; Dumit, J. Operationalizing a One Health approach to global health challenges. *Comp. Immunol. Microbiol. Infect. Dis.* **2013**, *36*, 211–216. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Saadati, N.; Khodashahi, M.; Naghibzadeh, B.; Khodashahi, R. Relation between cytokines and *Brucella* arthritis, spondylitis, and sacroiliitis. *Rheumatol. Res.* **2019**, *4*, 57–61. [\[CrossRef\]](#)
5. Spervanasilis, N.; Karantanas, A.; Markaki, I.; Konsoula, A.; Ntontis, Z.; Koutserimpas, C.; Alpantaki, K. *Brucella* spondylitis: Current knowledge and recent advances. *J. Clin. Med.* **2024**, *13*, 595. [\[CrossRef\]](#)
6. Singh, B.B.; Khatkar, M.S.; Aulakh, R.S.; Gill, J.P.S.; Dhand, N.K. Estimation of the health and economic burden of human brucellosis in India. *Prev. Vet. Med.* **2018**, *154*, 148–155. [\[CrossRef\]](#)
7. Rasheduzzaman, M.; Hossain, M.A.; Paul, S.K.; Nasreen, S.A.; Haque, N.; Akter, A.; Muhammadullah, S.; Kabir, H.; Sonia, S.J.; Ahmed, S. Serological and molecular Epidemiology of human brucellosis in Mymensingh region Bangladesh. *Bangladesh J. Med. Microbiol.* **2020**, *14*, 19–24. [\[CrossRef\]](#)
8. Asaad, A.M.; Alqahtani, J.M. Serological and molecular diagnosis of human brucellosis in Najran, Southwestern Saudi Arabia. *J. Infect. Public Health* **2012**, *5*, 189–194. [\[CrossRef\]](#) [\[PubMed\]](#)
9. Pathak, A.D.; Dubal, Z.B.; Doijad, S.; Raorane, A.; Rodrigues, S.; Naik, R.; Naik-Gaonkar, S.; Kalorey, D.R.; Kurkure, N.V.; Naik, R.; et al. Human brucellosis among pyrexia of unknown origin cases and occupationally exposed individuals in Goa Region, India. *Emerg. Health Threats J.* **2014**, *7*, 23846. [\[CrossRef\]](#) [\[PubMed\]](#)
10. Halling, S.M.; Tatum, F.M.; Bricker, B.J. Sequence and characterization of an insertion sequence, IS711, from *Brucella ovis*. *Gene* **1993**, *133*, 123–127. [\[CrossRef\]](#)
11. Ariza, J.; Bosilkovski, M.; Cascio, A.; Colmenero, J.D.; Corbel, M.J.; Falagas, M.E.; Memish, Z.A.; Roushan, M.R.H.; Rubinstein, E.; Sipsas, N.V.; et al. Perspectives for the treatment of brucellosis in the 21st century: The ioannina recommendations. *PLoS Med.* **2007**, *4*, e317. [\[CrossRef\]](#)
12. Fatani, D.F.; Alsanoosi, W.A.; Badawi, M.A.; Thabit, A.K. Ceftriaxone use in brucellosis: A case series. *IDCases* **2019**, *18*, e00633. [\[CrossRef\]](#)
13. McInnes, I.; Schett, G. Cytokines in the pathogenesis of rheumatoid arthritis. *Nat. Rev. Immunol.* **2007**, *7*, 429–442. [\[CrossRef\]](#)

14. Franzoni, G.; Signorelli, F.; Donniacuo, A.; Schiavo, L.; Napoletano, M.; De Matteis, G.; Grandoni, F.; Zinellu, S.; Bove, V.; Giudici, S.D.; et al. Exploring potential cytokine profiles as diagnostic biomarkers for brucellosis in Mediterranean buffaloes. *Front. Vet. Sci.* **2025**, *12*, 1583858. [\[CrossRef\]](#)
15. Zafari, P.; Zarifian, A.; Alizadeh-Navaei, R.; Taghadosi, M.; Rafiei, A. Association between polymorphisms of cytokine genes and brucellosis: A comprehensive systematic review and meta-analysis. *Cytokine* **2020**, *127*, 154949. [\[CrossRef\]](#)
16. Skyberg, J.A.; Thornburg, T.; Kochetkova, I.; Layton, W.; Callis, G.; Rollins, M.F.; Riccardi, C.; Becker, T.; Golden, S.; Pascual, D.W. IFN- γ -deficient mice develop IL-1-dependent cutaneous and musculoskeletal inflammation during experimental brucellosis. *J. Leukoc. Biol.* **2012**, *92*, 375–387. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Copur, B.; Pasa, O. The role of the serum tube agglutination test in the monitoring of human brucellosis: Evaluation of post-treatment SAT titers. *Rev. Assoc. Med. Bras.* **2022**, *68*, 1234–1239. [\[CrossRef\]](#)
18. Patil, D.P.; Ajantha, G.S.; Shubhada, C.; Jain, P.A.; Kalabhavi, A.; Shetty, P.C.; Hosamani, M.; Appannanavar, S.; Kulkarni, R.D. Trend of human brucellosis over a decade at tertiary care centre in North Karnataka. *Indian J. Med. Microbiol.* **2016**, *34*, 427–432. [\[PubMed\]](#)
19. Laine, C.G.; Johnson, V.E.; Scott, H.M.; Arenas-Gamboa, A.M. Global estimate of human brucellosis incidence. *Emerg. Infect. Dis.* **2023**, *29*, 1789–1797. [\[CrossRef\]](#) [\[PubMed\]](#)
20. Dagnaw, G.G.; Mamuye, Y.; Dejene, H. Human and animal brucellosis and risk factors for human infection in Ethiopia: A systematic review and meta-analysis (2015–2024). *BMC Public Health* **2024**, *24*, 3495. [\[CrossRef\]](#)
21. Suresh, K.P.; Patil, S.S.; Nayak, A.; Dhanze, H.; Rajamani, S.; Shivamallu, C.; Cull, C.A.; Amachawadi, R.G. Prevalence of brucellosis in livestock of African and Asian continents: A systematic review and meta-analysis. *Front. Vet. Sci.* **2022**, *9*, 923657. [\[CrossRef\]](#)
22. del Pozo, J.S.G.; Solera, J. Systematic review and meta-analysis of randomized clinical trials in the treatment of human brucellosis. *PLoS ONE* **2012**, *7*, e32090. [\[CrossRef\]](#)
23. Buzgan, T.; Karahocagil, M.K.; Irmak, H.; Baran, A.I.; Karsen, H.; Evirgen, O.; Akdeniz, H. Clinical manifestations and complications in 1028 cases of brucellosis: A retrospective evaluation and review of the literature. *Int. J. Infect. Dis.* **2010**, *14*, e469–e478. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Pappas, G.; Akritidis, N.; Bosilkovski, M.; Tsianos, E. Brucellosis. *N. Engl. J. Med.* **2005**, *352*, 2325–2336. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Turan, H.; Serefhanoglu, K.; Karadeli, E.; Togan, T.; Arslan, H. Osteoarticular involvement among 202 brucellosis cases identified in Central Anatolia region of Turkey. *Intern. Med.* **2011**, *50*, 421–428. [\[CrossRef\]](#)
26. Ebrahimpour, S.; Bayani, M.; Moulana, Z.; Hasanjani Roushan, M.R. Skeletal complications of brucellosis: A study of 464 cases in Babol, Iran. *Casp. J. Intern. Med.* **2017**, *8*, 44–48.
27. Colmenero, J.D.; Reguera, J.M.; Martos, F.; Sánchez-de-Mora, D.; Delgado, M.; Causse, M.; Martín-Farfán, A.; Juarez, C. Complications associated with *Brucella melitensis* infection: A study of 530 cases. *Medicine* **1996**, *75*, 195. [\[CrossRef\]](#)
28. Ariza, J.; Pujol, M.; Valverde, J.; Nolla, J.M.; Rufí, G.; Viladrich, P.F.; Corredoira, J.M.; Gudiol, F. Brucellar sacroiliitis: Findings in 63 episodes and current relevance. *Clin. Infect. Dis.* **1993**, *16*, 761–765. [\[CrossRef\]](#)
29. Gotuzzo, E.; Seas, C.; Guerra, J.G.; Carrillo, C.; Bocanegra, T.S.; Calvo, A.; Castañeda, O.; Alarcón, G. Brucellar arthritis: A study of 39 Peruvian families. *Ann. Rheum. Dis.* **1987**, *46*, 506–509. [\[CrossRef\]](#)
30. Kumari, R.; Kalyan, R.K.; Jahan, A.; Jain, A.; Kumar, P.; Gupta, K.K.; Manoj, A. Human brucellosis: An observational study from a tertiary care centre in North India. *Cureus* **2023**, *15*, e42980. [\[CrossRef\]](#)
31. Gwida, M.; Al Dahouk, S.; Melzer, F.; Rösler, U.; Neubauer, H.; Tomaso, H. Brucellosis—Regionally emerging zoonotic disease? *Croat. Med. J.* **2010**, *51*, 289–295.
32. Corbel, M.J. *Brucellosis in Humans and Animals*; World Health Organization: Geneva, Switzerland, 2006.
33. Faddane, K.; Moumni, H.; Cherkaoui, I.; Lakranbi, M.; Hamdi, S.; Ezzikouri, S.; Saile, R.; El Azhari, M. Seroprevalence of human brucellosis in Morocco and associated risk factors. *Vet. World* **2022**, *15*, 2224–2233. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Zhang, N.; Zhou, H.; Huang, D.S.; Guan, P. Brucellosis awareness and knowledge in communities worldwide: A systematic review and meta-analysis of 79 observational studies. *PLoS Neglected Trop. Dis.* **2019**, *13*, e0007366. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Mantur, B.G.; Amarnath, S.K. Brucellosis in India—A review. *J. Biosci.* **2008**, *33*, 539–547. [\[CrossRef\]](#)
36. Xu, N.; Dong, X.; Yao, Y.; Guan, Y.; Chen, F.; Zheng, F.; Wang, G. Improved early detection of focal brucellosis complications with anti-*Brucella* IgG. *J. Clin. Microbiol.* **2020**, *58*, e00903-20. [\[CrossRef\]](#)
37. Almohrij, S.; Bahabri, I.; Alghamdi, R.M.; Bosaeed, M. *Brucella* septic arthritis: A case series and review of the literature. *J. Infect. Public Health* **2025**, *18*, 102993. [\[CrossRef\]](#)
38. Bosilkovski, M.; Krteva, L.; Caparoska, S.; Dimzova, M. Osteoarticular involvement in brucellosis: Study of 196 cases in the Republic of Macedonia. *Croat. Med. J.* **2004**, *45*, 727–733.
39. Tang, Y.; Ma, C.; Sun, H.; Yang, S.; Yu, F.; Li, X.; Wang, L. Serum levels of seven general cytokines in acute brucellosis before and after treatment. *Infect. Drug Resist.* **2021**, *14*, 5501–5510. [\[CrossRef\]](#)

40. Murphy, E.A.; Sathiyaseelan, J.; Parent, M.A.; Zou, B.; Baldwin, C.L. Interferon-gamma is crucial for surviving a *Brucella abortus* infection in both resistant C57BL/6 and susceptible BALB/c mice. *Immunology* **2001**, *103*, 511–518. [[CrossRef](#)]
41. Ghaznavi Rad, E.; Khosravi, K.; Zarinfar, N.; Mosayebi, G. Reduced IFN- γ production in chronic brucellosis patients. *Iran. J. Immunol.* **2017**, *14*, 215–222. [[PubMed](#)]
42. Lama, M.; Sarkar, R.; Ghosh, B. Serum Cytokine Profiles in Patients with Rheumatoid Arthritis Before and After Treatment with Methotrexate. *J. Interferon Cytokine Res.* **2023**, *43*, 344–350. [[CrossRef](#)]

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