Serological Interpretation of Rheumatic Manifestations in Brucellosis

Nihan Cuzdan[1], Alev Cetin Duran[2] and Tugba Kula Atik[3]

[1]*Department of Rheumatology, Balikesir Ataturk City Hospital, Balikesir, Turkey*
[2]*Department of Microbiology and Basic Immunology, Balikesir Ataturk City Hospital, Balikesir, Turkey*
[3]*Department of Microbiology, Faculty of Medicine, Balikesir University, Cagis Kampusu, Balikesir, Turkey*

ABSTRACT

Objectives: To investigate the profile of autoimmune markers and to evaluate the status of vitamin D in the autoimmune process in brucellosis.

Study Design: Descriptive study.

Place and Duration of Study: Department of Rheumatology and Microbiology-Basic Immunology, Balikesir Ataturk City Hospital, Turkey, between June 2017 and December 2020.

Methodology: *Brucella* seropositive patients (mean age 46.58 ± 15.43 years, 43.7% females) were investigated retrospectively in terms of clinical manifestations, rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP), antinuclear antibody (ANA), and vitamin D (25OHD) levels. Patients were grouped as low *Brucella* (<1/160), high *Brucella* (≥1/160) titers, and the control group consisted of *Brucella* negative patients. Clinical manifestations, RF, anti-CCP, ANA, and 25 OHD levels were compared among the three groups. Correlation analysis was performed between autoimmune markers and 25OHD levels.

Results: The most common symptom among all *Brucella* seropositive patients was polyarthralgia (57.7%). RF positivity was found higher in two patient-groups than the control group ($p = 0.008$). Anti-CCP positivity was found higher in patient-groups than the control group ($p < 0.001$). ANA levels were similar among the three groups ($p = 0.077$). Median 25OHD levels were found significantly lower in patient-groups than the control group ($p < 0.001$). No correlation was found among vitamin D, RF, anti-CCP, and ANA levels ($p = 0.501$, $p = 0.613$ and $p = 0.616$, respectively).

Conclusions: Increased rates of RF, anti-CCP; and decreased 25OHD levels in *Brucella* seropositive patients. It is important to consider brucellosis in the differential diagnosis of patients with rheumatologic manifestations in the presence of autoimmune markers.

Key Words: *Anti-nuclear anti-body, Anti-cyclic citrullinated peptide, Brucella, Rheumatoid factor, Vitamin D.*

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INTRODUCTION

Brucellosis, also known as 'Malta fever' or 'Mediterranean fever,' is a zoonotic infectious disease caused by *Brucella spp*. The first cases of *Brucella* infection in Turkey were described in 1932.

The incidence was gradually increased and reported as 2.56/1000 in 2004.[1] The *Brucella* bacterium can invade multiple organs such as the liver, lymph nodes, spleen, joints, and nerves, resulting in a wide range of clinical presentations. The incubation period of brucellosis is usually 1-3 weeks, but it rarely takes a few months. Symptoms are mostly similar to other diseases presenting with fever; however, myalgia and hyperhidrosis are much more intense.

Correspondence to: Dr. Nihan Cuzdan, Department of Rheumatology, Balikesir Ataturk City Hospital, Balikesir, Turkey
E-mail: nihancuzdan@hotmail.com
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The duration of the disease varies from a few weeks to several months. The bacterial persistence may lead to varying pathological changes; that may cause granulomatous hepatitis, arthritis, spondylitis, anemia, leukopenia, thrombocytopenia, meningitis, uveitis, optic neuritis, and endocarditis.[2] Having a wide range of symptoms and clinical findings and showing clinical features similar to other rheumatologic diseases, it can be challenging for physicians at the diagnostic stage.

Brucellosis may also trigger some immunological reactions. As a result of these reactions, some autoantibodies that are important in diagnosing and managing rheumatological diseases may overlap. Autoinflammatory markers, such as rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP), antinuclear antibody (ANA), and extractable nuclear antigens (ENA) are important biomarkers in the diagnosis of inflammatory rheumatic diseases. There are data in the literature that these antibodies may become positive by the immunological reaction caused by *Brucella*.[3-5] Since brucellosis may present mimicking rheumatological disease both by means of clinical symptoms and serological markers, this false positivity may cause a challenge in the diagnostic and treatment phases.

However, there is little information in the literature regarding the autoantibody levels in brucellosis patients. Past studies showed 12.9-30.6% RF, 16.3-20.9% anti-CCP, and 8.2-25% ANA positivity in brucellosis patients.[3-6] However, the previous studies were limited due to their small sample size.

Vitamin D is known to have various immunomodulatory effects.[7] The level of vitamin D was shown to be decreased in both granulomatous infections and some autoimmune diseases; and it has been reported that vitamin D deficiency may be associated with autoantibody production.[8,9] Vitamin D levels were found significantly lower in brucellosis patients in a previous study.[10] The relation among vitamin D, brucellosis, and autoantibody levels has not yet been studied.

This study aimed to evaluate the clinical presentation and the profile of rheumatologic autoantibodies in brucellosis patients, as well as the vitamin D status and its relation with the autoimmune response in brucellosis patients.

METHODOLOGY

A retrospective analysis of 679 *Brucella* seropositive patient records was carried out from the Clinical Microbiology/Immunology Department between June 2017 and December 2020. The first measured *Brucella* titer, which was at the diagnostic stage, was noted. The patients, who had manifestations compatible with brucellosis and positive *Brucella* tests, included if the test was positive at least at two consecutive measures. As the control group, *Brucella* negative patients without any specific diagnosis were randomly selected, including one out of three patients, among whose RF, anti-CCP, and ANA serologies were available from the Internal Medicine and Rheumatology Departments. Patients < 18 years, patients with malignancy, rheumatologic diseases, active coexistent infections, active chronic liver, kidney, or gastrointestinal disease, and patients who received brucellosis treatment before the test, were excluded from the study.

All patients' demographic and clinical data were extracted from the hospital database. The clinical presentation and musculoskeletal involvement were classified and recorded as constitutional and systemic symptoms; and signs such as fever, hyperhidrosis, weight loss, weakness, abdominal pain, headache, hepato-splenomegaly, musculoskeletal symptoms and signs such as myalgia, arthralgia, arthritis, bursitis, sacroiliitis, and spondylitis were also noted.

Patients were divided into three groups. Group 1 was determined as the low *Brucella* titer positivity and included clinically compatible patients with positive *Brucella* titer <1/160 at least at two consecutive measures. Group 2 was determined as the high *Brucella* titer positivity and included clinically compatible patients with *Brucella* titer ≥1/160. Group 3 was determined as the control group whose *Brucella* agglutination test was negative.

Hemogram, biochemical parameters, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) were studied simultaneously with the *Brucella* agglutination test and were noted. RF, anti-CCP, and ANA levels were accepted, if studied within one month of the *Brucella* agglutination test. RF was studied by nephelometric method, and anti-CCP chemiluminescent microparticle immunoassay method on architect device (Abbott Diagnostics). A positive result for RF was above the cut-off value of 20 IU/ml; whereas, >5 IU/ml value was positive for anti-CCP. The IIF-ANA screening test was performed using HEp20-10/liver biochip (Monkey) (Euroimmun AG, Lübeck, Germany) conjugated with a specific anti-human IgG (Euroimmun AG). Sera were considered positive for ANAs, if IIF staining was observed at a serum dilution of 1:100. Patterns were evaluated semi-quantitatively 1+ to 4+, according to the intensity. 25(OH) vitamin D_3 measurement was done by using the chromatographic based method (HPLC-UV).

Brucellosis was diagnosed with *Brucella* Coombs gel test (BCGT), [ODAK *Brucella* Coombs Gel Test, Odak Diagnostics, Istanbul, Turkey], which is a new and rapid agglutination-based method. This test was performed in microtubes with a gel matrix containing Coombs antibodies (anti-human IgG). Serial dilutions (1/40-1/5120) were prepared. Microtubes were incubated for 20 minutes at 37°C and then centrifuged for 20 minutes at a speed recommended by the manufacturer. Results were evaluated visually. The precipitation of pink *Brucella* antigens to the bottom of the tube was considered negative, and the presence of the pink antigen and antibody complex on the gel was considered positive. Bursitis, spondylitis and sacroiliitis were diagnosed on magnetic resonance imaging.

The data obtained in the study were analysed with the SPSS version 22.0 (SPSS INC, Chicago, IL, USA) programme. Categorical variables were given as percentages. Quantitative variables were given as mean ± standard deviation (SD) and median (25th percentile-75th percentile). Kolmogorov-Smirnov test was used to show whether the groups showed normal distribution. Since none of the groups conform to the normal distribution, Kruskal-Wallis test was used to compare three independent groups in numerical data. Chi-square test or Fisher's Exact test was used to compare independent groups with categorical variables. The correlation between dilution rates and inflammatory markers was evaluated with the Spearman correlation coefficient. A p-value less than 0.05 was considered as statistically significant.

This study has been performed as per the ethical standards of the WMA Declaration of Helsinki-Ethical principles for medical research involving human subjects. Ethical approval for this study was obtained from the Ethics Committee of the local university (Date: 10.06.2020, No. 2020/88).

RESULTS

Of the 979 Brucella seropositive patients recorded in the database, 679 patients (mean age 46.58 ± 15.43 years, 43.7% females) were recruited in the analysis after the application of inclusion/exclusion criteria. The demographic and clinical characteristics of all the groups are shown in Table I.

Table I: Comparison of demographic data, clinical manifestations and laboratory measures of the three groups.

		Group 1 (Low Brucella Titer) n:145	Group 2 (High Brucella Titer) n:534	Group 3 (Brucella Negative Control) n:226	p-value
Age (years) Median IQR		49.00 (36.00-59.00)	47.00 (35.00-58.00)	46.00 (38.00-56.00)	0.501
Gender (female), n (%)		90 (62.1)	207 (38.8)	175 (77.4)	<0.001
Clinical Manifestations (n, %)	Fever	17 (11.7)	269 (50.4)	-	<0.001
	Hyperhidrosis	8 (5.5)	169 (31.6)	-	<0.001
	Weight loss	2 (1.4)	35 (6.6)	-	0.015
	Lumber pain	10 (6.9)	69 (12.9)	-	0.045
	Myalgia	40 (27.6)	172 (32.2)	-	0.287
	Polyarthralgia	68 (46.9)	324 (60.7)	-	0.003
	Weakness	22 (15.2)	243 (45.5)	-	<0.001
	Headache	3 (2.1)	63 (11.8)	-	<0.001
	Abdominal pain	8 (5.5)	41 (7.7)	-	0.361
	Hepato/Splenomegaly	3 (2.1)	24 (4.5)	-	0.185
	Peripheral Arthritis	8 (5.5)	46 (8.6)	-	0.292
	Bursitis	3 (2.1)	19 (3.6)	-	0.596
	Spondylitis	0 (0)	41 (7.7)	-	0.001
	Sacroiliitis	4 (2.8)	13 (2.4)	-	0.770
WBC Median IQR		6.80 (5.65-8.20)	6.60 (5.37-8.00)	6.50 (5.50-7.70)	0.204
Hemoglobin g/dL median IQR		13.10 (12.10-14.30)	13.40 (12.17-14.50)	12.90 (12.20-13.70)	0.052
Platelet 10^3/µL median IQR		252.00 (207.00-300.50)	238.00 (194.75-290-25)	283.50 (231.00-325.00)	<0.001
ALT U/L median IQR		17.00 (12.00-23.00)	19.00 (14.00-30.00)	15.00 (12.00-19.25)	<0.001
AST U/L median IQR		19.00 (16.00-22.50)	21.00 (16.00-28.00)	17.00 (14.00-21.00)	<0.001
ESR mm/hr median IQR		14.00 (10.00-23.50)	18.00 (11.00-35.25)	11.00 (8.00-14.00)	<0.001
CRP mg/dL median IQR		0.30 (0.20-0.80)	0.60 (0.20-2.00)	0.30 (0.10-0.40)	<0.001
RF n/n* (%)IU /mL		9/75(12.0)	24/273 (8.8)	7/226 (3.1)	0.008
Anti-CCP n/n* (%) U/mL		6/47(12.8)	14/120 (11.7)	3/226 (1.3)	<0.001
ANA n/n* (%)		7/32 (21.9)	9/60 (15.0)	21/226 (9.3)	0.077
Vitamin D ng/mL (n*) median IQR		14.90 (7.90-24.80)	13.90 (7.40-22.00)	22.50 (12.95-30.95)	<0.001

IQR: Interquartile range: (25th percentile-75th percentile), n: Number, n: Number of available patient results, WBC: White blood cell, ALT: Alanine aminotransferase, AST: Aspartate atransferase, ESR: Erythrocyte sedimentation rate, CRP: C reactive protein, RF: Rheumatoid factor, anti-CCP: Anti-cyclic citrullinated peptide, ANA: Antinuclear antibody.*

The most common symptom among all *Brucella* seropositive patients was polyarthralgia (57.7%), followed by fever (42.1%); and the most common musculoskeletal complication was peripheral arthritis (7.9%). Peripheral arthritis was mostly presented as mono-articular (87.0%) and was most common in the knee joint (59.3%). Spondylodiscitis (6.0%) was mostly seen in L4-5 vertebrae (26.8%); whereas, bursitis (3.2%) was most common in the shoulder region (45.5%). Sacroiliitis was found in 2.5% of *Brucella* seropositive patients (Table II). When the groups were compared in terms of clinical symptoms, all symptoms and signs were seen with higher frequency in brucellosis patients with high titers (Table I).

Table II: Frequency of symptoms and signs in brucellosis patients.

			n (%)	
Polyarthralgia			392 (57.7)	
Fever			286 (42.1)	
Weakness			265 (39.0)	
Myalgia			212 (31.2)	
Hyperhidrosis			177 (26.1)	
Headache			66 (9.7)	
Lumber pain			79 (11.6)	
Abdominal pain			49 (7.2)	
Peripheral arthritis			54 (7.9)	
	Monoarthritis		47 (87.0)	
	Oligoarthritis		3 (5.6)	
	Poliarthritis		4 (7.4)	
	Localisation	Knee	32 (59.3)	
		Hand	8 (14.8)	
		Ankle	6 (11.1)	
		Hip	5 (9.3)	
		Shoulder	2 (3.7)	
		Elbow	1 (1.8)	
Spondylitis			41 (6.0)	
	Localisation		T1-2	2 (4.9)
			T6-7	1 (2.4)
			T7-8	2 (4.9)
			T8-9	2 (4.9)
			T9-10	4 (9.7)
			T11-12	2 (4.9)
			T12-L1	7 (17.1)
			L3-4	2 (4.9)
			L4-5	11 (26.8)
			L5-S1	8 (19.5)
Weight loss			37 (5.4)	
Bursitis			22 (3.2)	
	Localisation		Shoulder	10 (45.5)
			Knee	6 (27.3)
			Wrist	5 (22.7)
			Hip	1 (4.5)
Hepato/splenomegaly			27 (4.0)	
Sacroiliitis			17 (2.5)	

n: Number, T: Thoracal, L: Lumber, S: Sacral.

The median values of white blood cell count, hemoglobin, platelet, alanine aminotransferase (ALT), aspartate aminotransferase (AST), ESR, and CRP levels of *Brucella*-positive patients were shown in Table I. ESR, CRP, AST, and ALT values were higher in patients with high *Brucella* titers ($p < 0.001$).

RF was available for 348 *Brucella* seropositive patients (75 with a low titer, and 273 with a high titer). RF was found positive in 9/75 (12.0%) patients with a low titer, 24/273 (8.8%) patients with a high titer, and 7/226 (3.1%) in the control group. RF positivity rates were similar between the two patient groups ($p = 0.401$), which was significantly higher than the control group ($p = 0.008$). Anti-CCP was available for 167 *Brucella* seropositive patients (47 with a low titer and 120 with a high titer). Anti-CCP positivity was found in 6/47 (12.8%) patients with low titer, 14/120 (11.7%) patients with high titer, and 3/226 (1.3%) patients in the control group. Anti-CCP levels did not differ between the two patient-groups ($p = 0.844$), which was significantly higher than the control group ($p < 0.001$).

ANA levels were available for 92 *Brucella* seropositive patients (32 with a low titer and 60 with a high titer). ANA was found positive in 7/32 (21.9%) patients with low titer, 9/60 (15.0%) patients with high titer, and 21/226 (9.3%) in the control group. ANA levels were similar in all the three groups ($p=0.077$).

Vitamin D was available for 239 *Brucella* seropositive patients (80 with low titer and 159 with high titer), and 111 patients in the control group. Median vitamin D levels were found significantly lower in patient-groups with low 14.90 (7.90-24.80) and high titers 13.90 (7.40-22.00) than the control group 22.50 (12.95-30.95) ($p < 0.001$). Vitamin D levels were significantly lower in *Brucella* patients with high titer compared to the low titer group ($p < 0.001$).

A low level correlation was found between *Brucella* dilution rate and WBC, platelet, ALT, and AST levels ($p = 0.002$ Rho = (-) 0.120, $p = 0.001$ Rho = (-) 0.132, $p = 0.001$ Rho = 0.260, $p < 0.001$ Rho = 0.223, respectively). There was no correlation between *Brucella* dilution rates and RF, anti-CCP, and ANA levels ($p = 0.180$, $p = 0.549$, and $p = 0.649$; respectively). No correlation was found between vitamin D and *Brucella* titers, RF, anti-CCP, ANA, levels ($p = 0.394$, $p = 0.501$, $p = 0.613$, $p = 0.616$, respectively) (Table III).

DISCUSSION

This study focused on the clinical manifestations, autoantibody profile of *Brucella* seropositive patients, and association of vitamin D with autoimmune markers. Brucellosis is an endemic disease in Turkey. The disease may present with a wide range of clinical symptoms. The most common complication of brucellosis is reported to be osteoarticular involvement.[11] It can mimic rheumatologic diseases that may cause misdiagnosis and lead to malpractice.

Table III: Correlation analysis of hemogram, biochemical tests, autoantibody levels and vitamin D levels.

	Vitamin D level	
	Rho	***p-value***
Brucella antibody titer	(-)0.056	0.394
WBC	(-)0.026	0.626
Hemoglobin	0.160	0.003
Platelet	(-)0.137	0.011
ALT	(-)0.008	0.888
AST	(-)0.053	0.322
ESR	(-)0.184	0.001
CRP	(-)0.124	0.020
RF	(-)0.044	0.501
Anti-CCP	(-)0.038	0.613
ANA	0.043	0.616

WBC: White blood cell, ALT: Alanine aminotransferase, AST: Aspartate transferase, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, RF: Rheumatoid factor, anti-CCP: Anti-cyclic citrullinated peptide, ANA: Antinuclear antibody.

According to this study, the most common symptom among all *Brucella* seropositive patients is polyarthralgia (57.7%). It was found that the most common osteoarticular complication of the disease is peripheral arthritis, presented as monoarticular. The most common site of arthritis was the knee joint. It was observed that higher *Brucella* titers are associated with higher frequency of symptomatic disease. Brucellosis symptoms and signs have been widely covered in previous studies.[12-15] In some studies, sacroiliitis was reported to be the most common presentation of osteoarticular involvement;[12,13] whereas others have reported it as peripheral arthritis.[15] The discrepancy may be due to the age groups of the study populations. Sacroilitis is seen more common in younger populations; whereas, peripheral arthritis is seen in older ages.[16]

A significant increase was found in ALT, AST, ESR, and CRP values in brucellosis patients with high titers in the present study. It has been reported that ESR and CRP values may be higher, especially in patients with osteoarticular involvement.[6] CRP value has been reported to be the most frequently increased inflammatory marker in osteoarticular involvement.[11] In the study by Ahmedinejad *et al.*, elevation in ESR and CRP levels was observed in the acute phase of the disease.[3] Moreover, the liver function tests are expected to be mildly elevated in brucellosis patients,[2] as was seen in this study. A weak correlation was found between WBC, platelet, ALT, AST levels, and *Brucella* antibody titer in this study.

Brucellosis is known to cause some immunological reactions. According to some previous studies, RF, anti-CCP, and ANA may become positive in the serum of brucellosis patients and may cause a diagnostic challenge for rheumatologists. RF is the most commonly studied auto-antibody in brucellosis; however, the sample size of the study groups is small. The study by Ahmedinejad *et al*. showed that out of 49 patients, 15 (30.6%) were RF positive, and it was higher than the healthy control group (2.3%).[3] Similarly, Al-Eissa *et al.* showed RF positivity in 5 (21%) of 24 patients in their study.[4] In Gökhan *et al*.'s study, RF was positive in 8 (12.9%) of 62 brucellosis patients with arthritis;[5] whereas, Bosilkovski *et al.* were unable to show RF positivity in any of their brucellosis patients.[6] In the present study, RF positivity was 12.0% in patients with low titer *Brucella* seropositivity; whereas, 8.8% in patients with high titer. The patient-groups in the above--mentioned studies differ widely. Ahmedinejad *et al*. investigated RF both in the acute and chronic phases of the disease, Al-Eissa *et al.* studied the pediatric population, and Gokhan *et al.* has only included patients with osteoarticular involvement.[3,4,6] RF positivity tends to appear in the acute phase of the disease.[3] This study has included all positive titers of the *Brucella* test, since those with inconclusive serology could also present with clinical symptoms and might be at the acute stage of the disease. No difference between the groups was found with low and high *Brucella* titers; whereas, RF positivity was higher in the *Brucella* seropositive patients than the control group. Interestingly, even not statistically significant, patients with low titer had also high RF frequency. Severe clinical presentation involving multiple organ systems in low agglutinating titers is reported in endemic areas.[17] It should be kept in mind that the low *Brucella* tittered group, whose RF was available, was relatively small in this study. Still, the high RF positivity rates in low titers of *Brucella* antibody support the hypothesis that the *Brucella* antigen may induce autoimmune markers, regardless of clinical status.

Although anti-CCP and ANA tests were examined in some previous studies, the results are contradictory. In Gökhan *et al.*'s study, the anti-CCP rate was 20.9% in *Brucella* patients with peripheral arthritis and 70% in rheumatoid arthritis patients. Patients, whose anti-CCP were positive were re-examined after *Brucella* treatment, and all anti-CCP values had converted to negative. In the same study, ANA positivity was found at a rate of 12.9%.[5] In the study by Ahmedinejad *et al*., anti-CCP was 16.3%; whereas, ANA was found to be 8.2% positive.[3] Al-Eissa *et al.* found a higher ANA positivity rate (25%) in the pediatric group.[4] In contrast with these results, Gotuzzo *et al.* reported ANA positivity at very low titers; whereas, Arıdogan *et al.* reported no anti-CCP positivity in any of their *Brucella* patients.[14,18] In this study, anti-CCP positivity was detected in both low and high titer *Brucella* positive patients, and it was found to be significantly higher in both groups compared to the control group. However, there was no difference in the three groups in terms of ANA positivity.

According to these results, there was no significant relationship between RF, CCP, ANA values, and the *Brucella* titers. RF and anti-CCP are not autoimmune markers used to measure RA patients' activity but are associated with more severe disease and worse clinical outcomes.[19] There is still no clear data available regarding the role of autoantibodies in the pathology and activity of brucellosis. It may be agreed that the polyclonal B cell activation may lead to the formation of RF, anti-CCP, and ANA in brucellosis.[5] RF is an antibody that develops against the Fc part of IgG, and it is thought to be pathogenic with the formation of immune-complex in rheumatoid arthritis. Anti-CCP autoantibodies are against citrulline residues of proteins; and the peptides and have high specificity for rheumatoid arthritis.[20] RF was reported to be positive (2.8 %) in the healthy population;[21] whereas, anti-CCP can be positive in low titers in 1-3%.[22] It has been reported that anti-CCP can also lead to immuno--complex formation similar to RF, causing secretion of pro-inflammatory cytokines such as TNF-alpha. RF is claimed to potentiate anti-CCP mediated secretion of pro-inflammatory cytokines. However, the fact that both autoantibodies have also been seen in various inflammatory conditions than rheumatoid arthritis, it suggests that they are mostly part of the general autoimmunity-related phenomenon.[23]

There are only a few studies that explore the vitamin D levels in brucellosis patients. Moreover, as far as the authors' knowledge, this is the first study that analysed the association of vitamin D with RF, anti-CCP, and ANA autoantibodies in *Brucella* seropositive patients. It was found that vitamin D levels were significantly lower in both the patient--groups than the control group. Vitamin D levels were at the lowest levels in patients with high titers of *Brucella* antibody. Hence, there was no correlation between RF, anti-CCP, ANA, and vitamin D levels. Two studies evaluated vitamin D status in *Brucella* patients with contradictory results in the literature. Kurtaran *et al.* found lower vitamin D and VDR levels in 86 brucellosis patients compared to the control group.[10] On the contrary, the prevalence of hypovitaminosis D did not differ between brucellosis patients and healthy controls in Keramat *et al*.'s study.[24] A previous study in another granulomatous disease, tuberculosis, has shown lower vitamin D levels in ANA positive patients as compared to ANA negative patients. Besides, they have found a significant correlation between the ANA titers and the levels of vitamin D.[8] In Wang *et al*.'s study, where they studied RA patients, serum Vitamin D level is found inversely associated with anti-CCP levels.[25] These results do not support the results of previous studies that claim hypovitaminosis D is associated with the presence of autoimmune markers.

The present study has some limitations. Due to its retrospective design, confounding factors could not be controlled completely. Although three subjects were included in the control group to prevent selection bias, gender remained different between the control and the patient-groups. The large sample size, the evaluation of clinical data, autoimmune markers, and their association with vitamin D levels in the same sample group are the strength of this study. In this study, unlike previous studies, all *Brucella* positive patients were evaluated, the low titer positivity could also cause seroconversion. Prospective controlled studies, which evaluate the clinical importance of RF, anti-CCP, ANA autoantibodies, and vitamin D's role in the autoimmune response of brucellosis patients, are needed.

CONCLUSIONS

According to these results, RF and anti-CCP positivity prevalence are higher in brucellosis patients than the control group. Vitamin D levels were negatively correlated with *Brucella* antibody titers; whereas, no correlation was observed between vitamin D levels and autoantibody presence. Physicians should become aware of and consider brucellosis in their differential diagnosis of febrile diseases with osteoarticular involvement, even in cases with positive rheumatologic autoimmune markers.

ETHICAL APPROVAL:
Ethical approval from Institutional Review Board (IRB) of Faculty of Medicine, Balikesir University was obtained prior to initiation of the research work.

PATIENTS' CONSENT:
Informed consents were not obtained due to the study's retrospective design and fully anonymised data.

CONFLICT OF INTEREST:
The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:
NC: Conception and design, acquisition, analysis, and interpretation of the data, drafting and final approval of the version to be published.
ACD, TKA: Drafting, or revising it critically for important intellectual content, designing, analysis and interpretation of data.

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