Süleyman Demirel Üniversitesi
Sağlık Bilimleri Dergisi
Cilt 13, Sayı 3, 410 - 418, 2022

Suleyman Demirel University
Journal of Health Sciences
Volume 13, Issue 3, 410 - 418, 2022

Serum Metalloproteinase-2 and Tissue İnhibitor Metalloproteinase-1 Levels As a Biomarker in Patients With Osteoarthritis Due To Brucella İnfection

Brucella Enfeksiyonuna Bağlı Osteoartritli Hastalarda Biyobelirteç Olarak Serum Metalloproteinaz-2 ve Doku İnhibitörü Metalloproteinaz-1 Seviyeleri

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ABSTRACT

Objectives: It was aimed to investigate the usability of MMP-2 and TIMP-1 levels as biomarkers in the oosteoarticular complications of brucellosis.

Methods: The subjects were categorized into three groups as the healthy control group, brucella group, and brucella patients with osteoarticular involvement groups. Before medical treatment, serum samples from patients and control groups were stored at -80ºC until the day of study. MMP-2 and TIMP-1 serum levels were quantified by the ELISA method.

Results: Serum level of MMP-2 (mean ± SD) in healthy control group was 1.71 +/- 0.10 ng / mL. Brucella patient group and Osteoarticular complıcation group were 14.3 +/- 2.52 ng / ml 20.65 +/- 2.33 ng / ml respectively (p=0.001). The mean TIMP-1 level in the control group was 3578.96 +/- 67.2 ng / mL, while in the Brucella group, this rate was 998.27 +/- 66.7 ng / mL and in the bone involvement group, 1656. 17 +/- 17.3 ng / ml. The difference between the control group and the brucella patients and the complicated group was statistically significant (p= 0.001).

Conclusions: We think that the significant change in serum levels of MMP-2 and TIMP-1 when evaluated together with the radiological method, can be used as a biochemical indicator of the development of osteoarticular complications.

K eywords: : Brucellosis, MMP-2, TIMP-1 ,osteoarticular complication, biomarker

Alınış / Received: 01.07.2022 Kabul / Accepted: 14.09.2022 Online Yayınlanma / Published Online: 20.12.2022

DOI: 10.22312/sdusbed.1138497
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ÖZET

Amaç: Brusellozun oosteoartiküler komplikasyonlarında MMP-2 ve TIMP-1 düzeylerinin biyobelirteç olarak kullanılabilirliğinin araştırılması amaçlandı.

Metot: Olgular sağlıklı kontrol grubu, brusella grubu ve osteoartiküler tutulumu olan brusella hastaları olarak üç gruba ayrıldı. Medikal tedavi öncesi hasta ve kontrol gruplarından alınan serum örnekleri çalışma gününe kadar -80ºC'de saklandı. MMP-2 ve TIMP-1 serum seviyeleri, ELISA yöntemiyle ölçüldü.

Bulgular: Sağlıklı kontrol grubunda MMP-2 (ortalama ± SD) serum düzeyi 1,71 +/- 0,10 ng / mL idi. Brusella hasta grubu ve Osteoartiküler komplikasyon grubunda sırasıyla 14,3 +/- 2,52 ng / ml 20,65 +/- 2,33 ng / ml idi (p=0,001). Kontrol grubunda ortalama TIMP-1 düzeyi 3578,96 +/- 67,2 ng/ml iken, Brucella grubunda bu oran 998,27 +/- 66,7 ng/ml, kemik tutulumu grubunda 1656, 17 +/- 17,3 ng / ml idi . Kontrol grubu ile brusella hastaları ve komplike grup arasındaki fark istatistiksel olarak anlamlıydı (p= 0,001).

Sonuç: Radyolojik yöntemle birlikte değerlendirildiğinde, MMP-2 ve TIMP-1 serum seviyelerindeki önemli değişiklik, brusellozun osteoartiküler komplikasyonlarının gelişiminin biyokimyasal bir göstergesi olarak kullanılabileceğini düşünüyoruz.

Anahtar kelimeler: Brusellozis, MMP-2, TIMP-1 ,osteoartiküler komplikasyon, byiobelirteç

1. Introduction

Brucellosis is a zoonotic bacterial infection that affects many organs and systems. It is transmitted by direct or indirect contact with infected animals or their products. Although brucella infection can be transmitted to humans in various forms, the most common way of spreading is by consuming unpasteurized milk and dairy products from an infected animal [1,2]. Besides, it can also be transmitted directly through the damaged skin, conjunctival inoculation, and inhalation of infectious aerosols. There is no specific clinical finding as it can involve all organs and tissues. Although the liver, bone marrow, spleen, and lymph nodes are the most frequently involved organs, they can also include organs and tissues such as the heart, genitourinary system organs, central nervous system, and joints [1,2,3]. Bone and joint involvement, with a rate of about 40%, are the most common complications of brucellosis [2,4,5]. The most commonly affected areas are sacroiliac joints, peripheral joints and spinal regions. Various clinical conditions have been reported, including peripheral arthritis, osteomyelitis, sacroiliitis, bursitis, spondylitis, and tenosynovitis [5,6,7].

Although the clinical and radiological features of osteoarthritic brucellosis are well known, the mechanisms of bone involvement are still poorly understood. Under different conditions, matrix metalloproteinases (MMPs) are released in the inflammatory environment. MMPs are produced not only by macrophages and neutrophils but also by several osteoblasts, including MMP-2. Type I collagen in Bone and MMP-2 degrades type II collagen in cartilage [8].TIMPs, which are specific tissue inhibitors, play a crucial role in the control of MMP activity. Tetracyclines, α 2-macroglobulin, heparin, and synthetic inhibitors are among the active MMP inhibitors [9]. TIMPs are proteins necessary for regulating connective tissue metabolism. They are found in many tissues and body fluids. It activates the latent enzyme form by binding irreversibly and non-covalently to MMPs and preventing catalytic activity maintenance [10,11].

We aimed to investigate the levels of MMP-2 and TIMP-1, which play a role in the regulation of connective tissue metabolism in patients with brucellosis who have osteoarticular involvement.

2. Material and Method

Ethical Approval

The study was conducted by Research Ethics Committee of the Suleyman Demirel University (no:116137).

Study Population

Laboratory and radiological data of outpatient and inpatient brucellosis patients admitted to xxx İnfection diseases clinic were evaluated between January 2018 and January 2020. People aged 25 to 65 years without another inflammatory, autoimmune and malignant diseases were included in the study. The subjects were categorized into three groups, the healthy control group (n=30), the brucella group (n=30), and the brucella patients with osteoarticular complıcation group (n=30).

Group-A (Healthy control group)

A control group was created with individuals without acute or chronic disease, symptoms, or pathological physical examination. It was similar in age and gender in both groups.

Group-B (Brucella group)

Patient serums for the Rose Bengal test positive were examined by immunocapture-agglutination technique to eliminate the factors that caused false negativity/positivity. A brucella patient group (n = 30) was created by patients without complications.

Group-C (Osteoarticular complıcation group)

Brucella patients with osteoarticular involvement were evaluated as a separate group. Traditional radiological methods were used to assess morphological changes in the osteoarticular system. Patients with osteoarthritic participation were identified with cases of sacroiliitis, peripheral arthritis, spondylitis, and osteomyelitis.

Diagnosis of Brucellosis

Brucellosis was diagnosed based on clinical, bacteriological, and serological findings. Patient serums were first screened by the Rose Bengal slide agglutination test (Seromed, Istanbul, Turkey). Then the Brucellacapt test (Vircell SL, Granada, Spain) was performed according to the manufacturer's instructions. Antibody titers of 1/160 and above were considered positive for brucellosis, whereas those lower than 1/160 were considered negative. Blood cultures were performed by using BacT/ALERT 3D (bioMérieux, France) automated blood culture system. The isolated bacterial strains were identified using conventional methods and Phoenix 100 (Becton Dickinson, USA) automated system. Osteoarticular brucellosis is diagnosed with clinical inflammatory signs of the affected joints, with positive serological tests and positive cultures. Radiological evaluations such as joint sonography, direct radiography, computed tomography, and magnetic resonance imaging were performed to diagnose osteoarticular brucellosis.

ELISA detection of MMP-2 and TIMP-1

Blood samples were centrifuged, and serum was stored at -80°C until testing. Stored serum was analyzed for MMP-2 (Human MMP-2 ELISA Kit, Elabscience, USA) and their specific inhibitors TIMP-1(Human TIMP-1 ELISA Kit, Elabscience, USA) with commercially available sandwich ELISA according to the manufacturer guidelines. ELISA testing was performed at the Suleyman Demirel University research laboratory using the ELİSA plate washer (Medispec ESW 300, Palmcity 72, USA) and ELISA plate reader (Biotek FLX50, Absorbance Microplate Reader, ABD).

Statistical analysis

SPSS 16.0 (for Windows) version was used for statistical evaluation. For the assessment of the results, standard statistical methods were used. Average, standard deviation, minimum and maximum values of the data were determined. The student's t-test was used to compare independent quantitative data with normal distribution. Mann Whitney U-test was used to compare independent quantitative data without normal distribution. Comparing the categorical and continuous variables between the groups was performed using the chi-square test and ANOVA. Correlation between the investigated variable was found using Pearson's coefficient linear correlation. The data were evaluated in the 95% confidence interval, and p <0.05 were considered significant. The ROC curves were generated to assess the sensitivity and specificity for the prediction of osteoarticular complıcation.

3. Results

The mean ratios (mean ± SD) of MMP-2 in the control group, Brucella patient group and osteoarticular complıcation group were 1.71 +/- 0.10 ng / mL,14.3 +/- 2.52 ng / ml 20.65 +/- 2.33 ng / ml respectively. The difference between the control group, the brucella patients, and the complicated group was statistically significant (p = 0.001). Compared with the control group, the MMP-2 ratios increased about sevenfold in the brucella group and about tenfold in the bone involvement group (Figure-1).

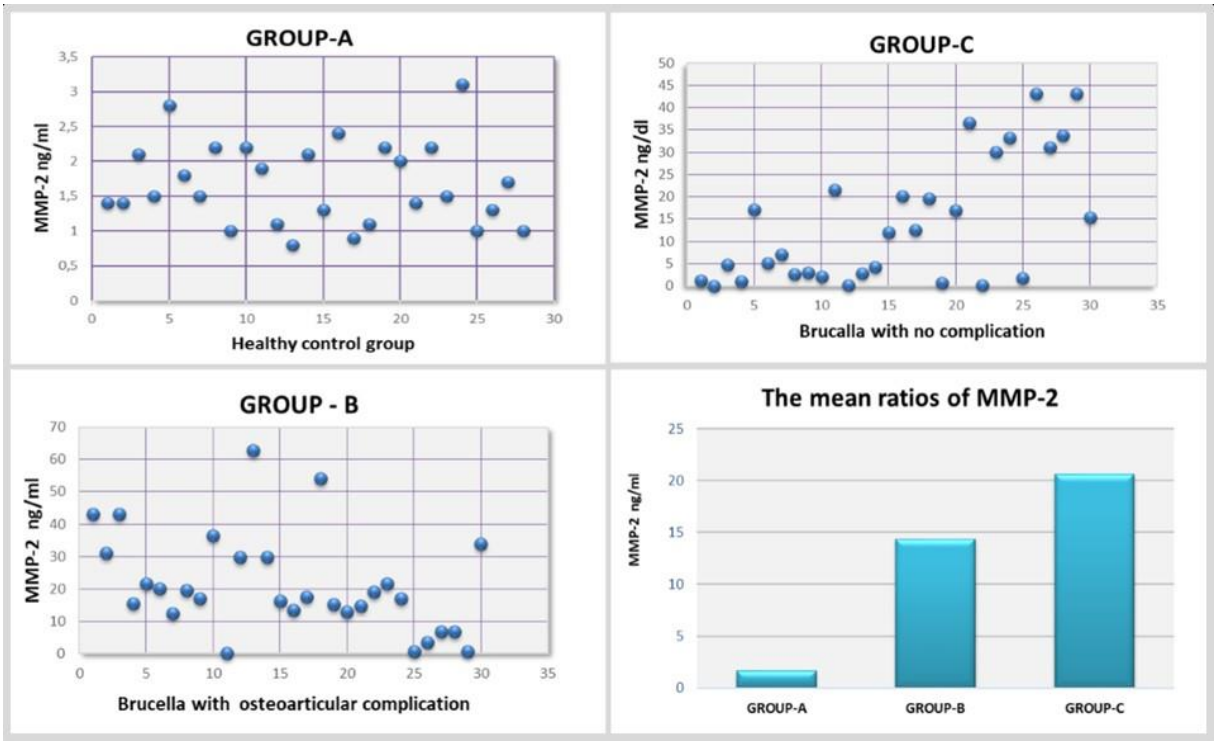

Figure 1: Distribution of MMP-2 serum levels and mean rates by study groups

The mean TIMP-1 level in the control group (n = 30) was 3578.96 +/- 67.2 ng / mL, while in the Brucella group this rate was 998.27 +/- 66.7 ng / mL and in the bone involvement group, 1656, 17 +/- 17.3 ng / ml. The difference between the control group, the brucella patients, and the complicated group was statistically significant (p: 0.001). Compared with the control group, TIMP-1 ratios decreased about four times in the brucella group and about twice in the bone involvement group (Figure-2).

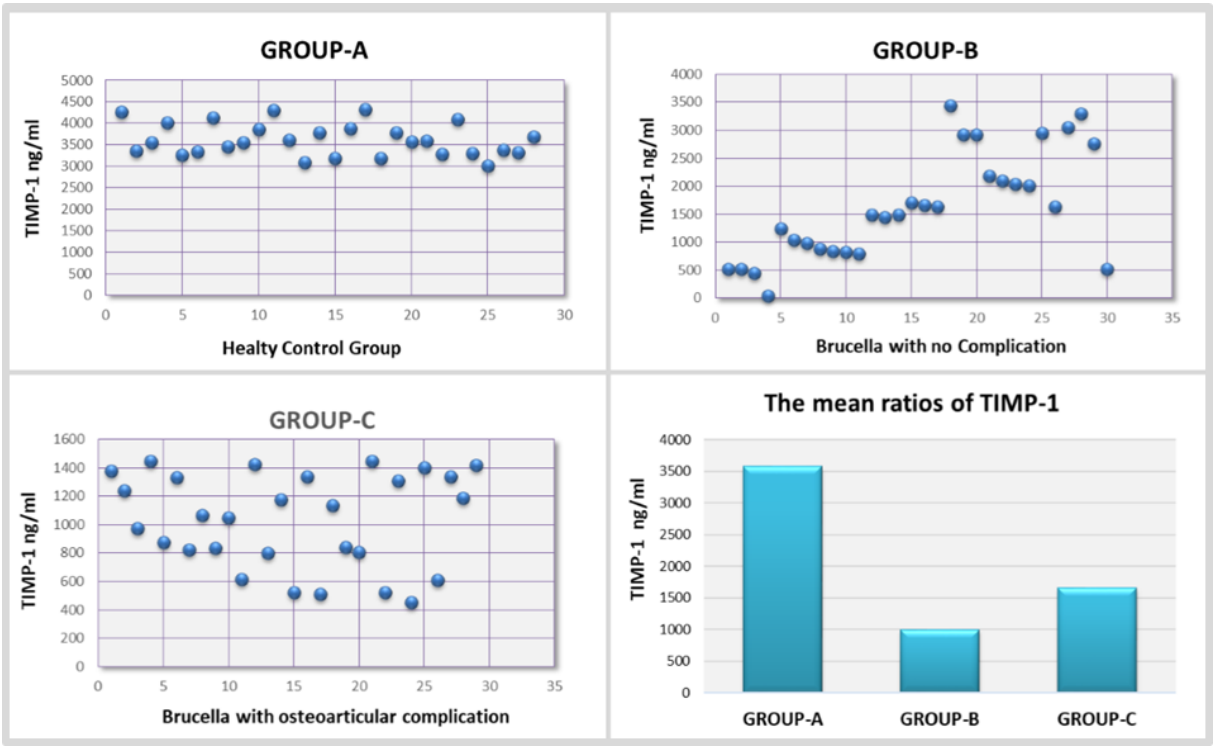

Figure-2: Distribution of TIMP-1 serum levels and mean rates by study groups

This difference was statistically significant (p: 0.001). When all groups were evaluated, a negative correlation was found between serum levels of MMP-2 and TIMP-1 ($p < 0.05$; $p = 0.001$). When we compared the MMP-2 / TMP-1 ratios, this ratio was 0.045 +/- 0.029 µg / ml in the control group and 1.438 +/- 0.588 µg / ml in the Brucella patient group and 1.246 +/- 0.456 µg / ml in the bone involvement group (Figure-3)

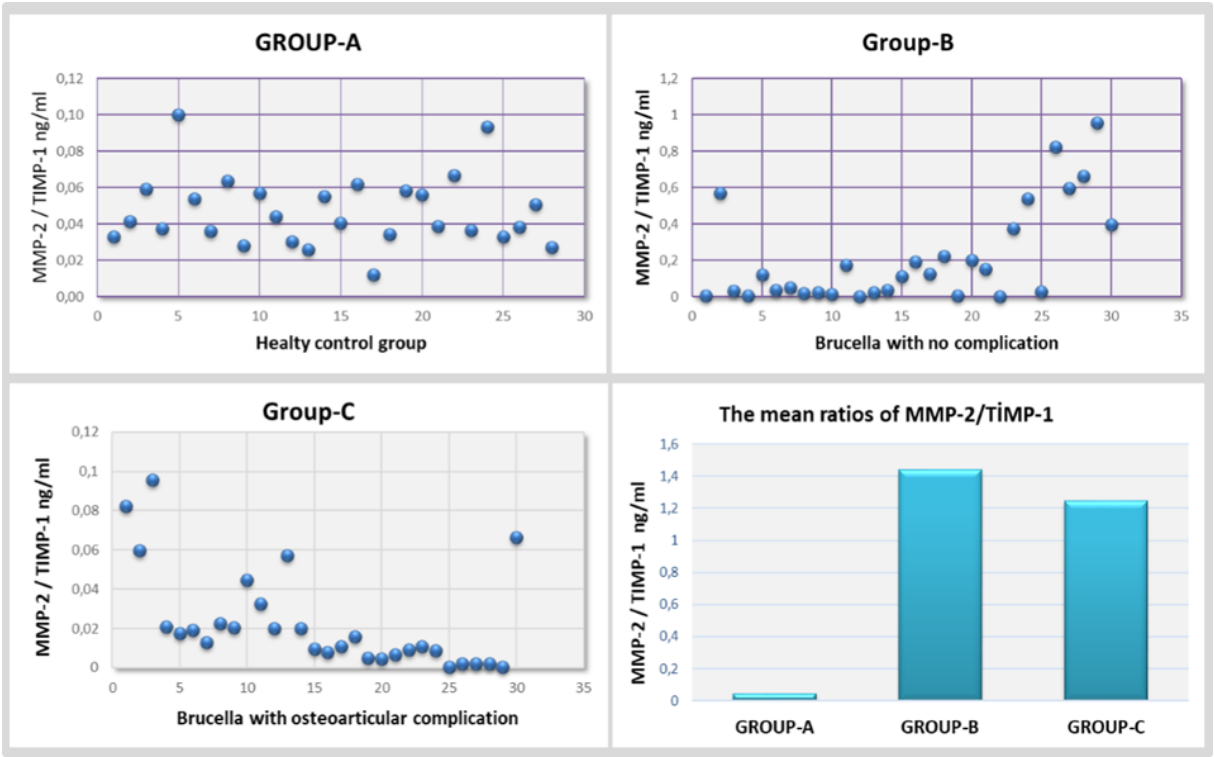

Figure-3: Distribution of MMP-2/TIMP-1 serum levels and mean rates by study groups

The difference between the control group, the brucella patients, and the complicated group was statistically significant (p: 0.001). ROC analysis was performed to evaluate the osteoarticular complıcation of brucella, which demonstrated that areas under the curve (AUC) of MMP-2, TIMP-1 ROC analysis also showed a cut-off value of the sensitivity, specificity, positive predictive value, and negative predictive value. (Table 1, Figure 4). MMP2 and TMP1 measurement results had a significant ROC curve, whereas MMP2 / TMP1 ratios did not have a significant curve between brucella patients and bone involvement groups.

Table 1: The area under the curve (AUC) values for MMP-2 and TIMP-1 serum levels

Variables	AUC (95% CI)	Sensitivity	Specificity	PPV	NPV	P-Value	Accuracy
MMP-2	0.84 (0.79-0.89)	53%	83%	0.76	0,64	0,054	68%
TIMP-1	0.78 (0.65-0.87)	100%	56%	0.69	1	0,005	78,3%

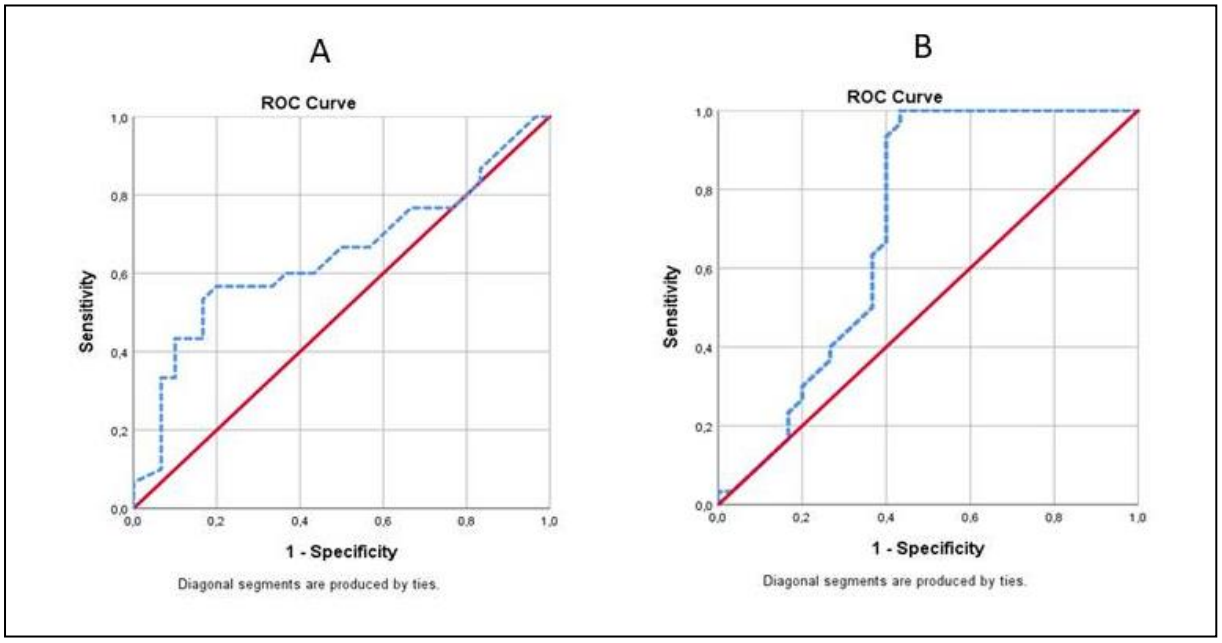

Figure-4: A; ROC curves of MMP-2 serum level for discriminating osteoarticular involvement B; ROC curves of TIMP-1 serum level for determining osteoarticular involvement

4. Discussion and Conclusion

Brucellosis is a common disease worldwide and causes a considerable disease burden in endemic countries such as Turkey. Brucellosis may involve many organs and systems. The clinical manifestations of brucellosis are related to inflammatory processes in acute and chronic periods [3,4]. Brucellosis may become chronic, resulting in osteoarticular complications leading to bone and joint damage. In most cases of osteoarticular involvement, bone and joint damage are caused by the inflammatory reaction, including increased MMP activity, provoked by the infection [11]. While clinical and radiological evaluations are revealed in brucellosis, immunopathological mechanisms have not been fully elucidated yet. In recent studies, the role of matrix metalloproteinases in the immunopathogenesis of human brucellosis has been tried to be explained. It is assumed that MMPs have essential in developing brucella complications [12,13].

Osteoblasts have been shown to produce several MMPs, including MMP-2. MMP-2 is extremely important because it degrades the type-II collagen in the bone and the cartilage. The potential contribution of MMPs to tissue damage in osteoarticular brucellosis has not been evaluated [8]. We aimed to investigate the levels of MMP-2 and TIMP-1 in patients with brucellosis who have osteoarticular involvement. The current study showed an increased serum MMP-2 level in the brucella group and the bone involvement group compared with the control group (Figure-1). This study showed increased serum matrix metalloproteinase levels in all brucellosis patients (p = 0001). Serum MMP-2 levels were higher in all patients with osteoarticular involvement. This increase was statistically significant between the group with osteoarticular involvement and other groups. ($p < 0.05$; $p = 0.001$). The results of the presented study suggested that MMP-2 has a vital role in the development of osteoarticular complications.

Studies on several cell lines have shown that MMP production can be induced by GM-CSF (Granulocyte macrophage-colony Stimulating Factor) [14,15]. This factor, produced by Brucella-infected osteoblasts, may stimulate MMP-2 in the same cells [12,13]. Different studies have shown that GM-CSF is an essential mediator of MMP-2 production by brucella-infected osteoblasts. Cytokines such as TNF- and IL-1 induce MMP-2 secretion by osteoblasts [15,16]. But Brucella-infected osteoblasts do not produce detectable levels of TNF- or IL-1. Therefore, the role of GM-CSF secreted by Brucella-infected osteoblasts is essential in increasing MMP-2 production [8,17]. Šiširak et al. demonstrated that the detection of matrix metalloproteinases in the serum is necessary for assessing the disease activity and predicting the development of complications of brucellosis [18]. MMPs are capable of breaking down the components of the extracellular matrix. However, they also break down some proteinases, chemotactic molecules, adhesion molecules, and cell surface receptors. The activated forms are all inhibited by TIMPs that bind tightly to each activated enzyme and block its action. However, the localization and clearance of MMPs are also tightly controlled [11,8,19]. In vivo, the activity of MMPs is counterbalanced by the activity of TIMPs. This balance between MMPs and their inhibitors TIMPs is of great importance in maintaining physiological events in organisms, such as remodeling of the ciliate, wound healing, angiogenesis, inflammation, apoptosis, and development of the immune response [20,21,22]. Our study showed that TIMP-1 was expressed in serum samples of all brucellosis patients. Compared with the control group, the serum level of TIMP-1 decreased about two times in the bone involvement group (figure-2). This difference was statistically significant (p: 0.001). The area under the curve of TMP1 was found to be more important. In osteoarticular infections or inflammatory conditions, TIMPs generally do not increase to the same extent as MMPs. Therefore, increasing the MMP / TIMP ratio is a biomarker that supports cartilage and joint damage [23,24,25].

When all groups were evaluated, a negative correlation was found between MMP-2 and TIMP-1. Our study showed that serum levels of MMP-2 / TMP-1 ratios were higher in the brucella and bone involvement group compared to the control group. Compared with the Brucella patient group, the serum level of the MMP-2 / TMP-1 ratios was lower in the bone involvement group. The presented study showed no statistically significant correlation between serum levels of MMP-2 / TMP-1 ratios and the development of osteoarticular complications (p 0.05). In other words, our results show that MMP-2 / TIMP-1 ratios cannot be used as a biomarker to show osteoarticular complications. Serum levels of MMP-2 dominated in all patients with osteoarticular complications. The equilibrium MMP activity bias shift leads to uncontrolled destruction of the matrix and, ultimately, pathophysiological events. Osteoarthritic joints contain increased MMP and, less frequently, increased TIMP [8,24]. It was demonstrated that osteoarticular complications in human brucellosis were manifested as cartilage degradation and bone loss [12,13,25].

Measurement of metalloproteinase concentration in serum is non-invasive, easy to administer, and relatively fast. It could be a promising procedure for determining osteoarticular involvement of brucella. Although the significant change in serum levels of MMP-2 and TIMP-1 does not provide a definitive

clinical diagnosis, they can be used as a biochemical indicator of the development of osteoarticular complications of brucellosis when evaluated together with the radiological method.

Declaration of Ethical Code

In this study, we undertake that all the rules required to be followed within the scope of the "Higher Education Institutions Scientific Research and Publication Ethics Directive" are complied with, and that none of the actions stated under the heading "Actions Against Scientific Research and Publication Ethics" are not carried out.

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