Determination of serum YKL-40 levels in patients with brucellosis

Abstract

**Aim:** To determine serum YKL-40 levels in patients diagnosed with brucellosis.

**Material and Methods:** This study included 40 Brucellosis patients with positive serum agglutination tests and 41 healthy control subjects. The two groups were compared with regard to their serum YKL-40, hematological parameters, and C-reactive protein (CRP) values.

**Results:** The brucellosis patient group presented YKL-40 values of 15.0 ± 8.8 ng/mL, CRP values of 0.2 ± 0.1 mg/dL, a Neutrophil/Lymphocyte ratio (NLR) of 1.5 ± 1.0, and a Platelet/Lymphocyte ratio (PLR) of 109.3 ± 46.4. The control group presented YKL-40 values of 12.7 ± 6.1 ng/mL, CRP values of 1.48 ± 2.1 mg/dL, a Neutrophil/Lymphocyte ratio (NLR) of 2.1 ± 0.7, and a Platelet/Lymphocyte ratio (PLR) of 147.8 ± 59.0.

**Conclusion:** No significant differences were determined between the YKL-40 levels of the patient and control groups. The patient group manifested significantly higher levels of CRP compared to the control group, and YKL-40 was determined to be positively correlated with CRP, PLT, PLR, NLR and age. However, further studies are needed for the role of YKL-40 in infectious diseases to be understood more clearly.

**Keywords:** brucellosis; YKL-40; diagnostic value

Determination of serum YKL-40 düzeylerinin belirlenmesi

Öz

**Amaç:** Bruselloz tanılı hastalarında serum YKL-40 düzeylerini belirlemek.

**Gereç ve Yöntemler:** Bu çalışma serümlü aglutinasyon testi pozitif 40 Bruselloz hastası ve 41 sağlıklı kontrol grubu dahil edilmiştir. İki grup arasında serum YKL-40, hematolojik parametreler ve C reaktif protein (CRP) değerleri karşılaştırılmıştır.

**Bulgular:** Bruselloz hastalarında, YKL-40 değeri 15.0 ± 8.8 ng/mL, CRP 0.2 ± 0.1 mg/dL, Nötrofil/Lenfosit oranı (NLR) 1.5 ± 1.0 ve Platelet/Lenfosit oranı (PLR) 109.3 ± 46.4 idi. Kontrol grubunda, YKL-40 değeri 12.7 ± 6.1 ng/mL, CRP 1.48 ± 2.1 mg/dL, Nötrofil/Lenfosit oranı (NLR) 2.1 ± 0.7 ve Platelet/Lenfosit oranı (PLR) 147.8 ± 59.0 idi.

**Sonuç:** YKL-40 düzeylerinde hasta ve kontrol grubu arasında anlamlı bir fark bulunmamıştır. Hasta grubunda kontrol grubuna göre CRP anlamlı olarak yüksek, bunun yanında YKL-40 ile CRP, PLT, PLR, NLR ve yaş arasında pozitif korelasyon saptanmıştır. Ancak YKL-40’in enfeksiyon hastalıklarında rolünü daha iyi anlaşılmasını için ileri çalışmalara ihtiyaç vardır.

**Anahtar kelimeler:** bruselloz; YKL-40; tanışal değeri
**Introduction**

Brucellosis is an endemic zoonosis that is commonly encountered in humans and animals in developing countries. It is transmitted by the intake of food contaminated with the gram-negative coccobacilli *Brusella spp.*, through breaks in the skin and mucosa during direct contact with infected animals or by the inhalation of infective material. The disease may manifest as a septisemic inflammatory disease or could affect bones, tissues, and other organs in a localized region [1-3].

Brucellosis may present various clinical symptoms including fever, chills, sweats, fatigue, arthralgia, back pain and headache [4]. The clinical diagnosis of brucellosis is made complicated by the variability of the symptoms, lack of differentiating physical symptoms, and the manifestation of subclinical and atypical forms of the disease during both acute and chronic stages. The gold standard test for the diagnosis of brucellosis is bacterial growth in culture [5]. However, serological methods such as the Rose Bengal test, standard tube agglutination (STA) test, STA with Coombs, and enzyme-linked immunosorbent assay (ELISA) are used more frequently as Brucella cultures are difficult, risky, and associated with low bacterial isolation rates [6, 7]. The patients may present laboratory findings such as leukocytosis/leukopenia, relative lymphocytopenia, anemia, trombositopenia, high C-reactive protein (CRP), and high erythrocyte sedimentation rate (ESR); however, these do not make a direct contribution to the diagnosis [8].

**YKL-40** is a 40 kDA chitinase-like protein. It can be secreted by numerous cells including macrophages, fibroblasts, epithelial cells, vascular endothelial and smooth muscle cells [9, 10]. Its functions in physiological and pathological phenomena are not yet clear; however, it is believed to be involved in certain processes such as inflammation, cell proliferation, apoptosis prevention, extracellular tissue remodelling, and stimulation of angiogenesis [9]. It was also reported to be active in inflammatory situations such as malign diseases, autoimmune disorders, and infections [9, 11, 12].

Brucellosis is a chronic inflammatory disease, and for this reason, it is important that the molecules involved in the inflammatory and immunological cascades are investigated and understood better. We thought that the YKL-40 molecule could contribute to the diagnosis of brucellosis patients due to its role in the inflammatory process. To our knowledge, no studies exist that have investigated the relationship between Brucellosis and YKL-40. The aim of this study is to compare the serum YKL-40 levels of Brucellosis patients to those of control subjects of similar age and gender.

**Material and Methods**

**Study Population**

A total of 40 patients with brucellosis aged between 18-70 years and 41 healthy gender-matched controls were enrolled in the study. The diagnosis of brucellosis was established according to a serology test result equal to or higher than a titre of 1:160, using the standard tube agglutination test (STA). The STA test was performed to give a final dilution of 1/40-1/5120. The tubes that did not present agglutination in the end of a 24-hr incubation period were assessed with the Coombs test.

Patients with inflammatory diseases, malignancies, diabetes mellitus, cardiovascular diseases, acute-chronic kidney or liver diseases were excluded from the study. The common clinical finding across patients was knee arthritis. The study was performed in accordance with the Declaration of Helsinki's Good Clinical Practice guidelines and approved by the local ethical committee (2017-18/212). All subjects provided written informed consent before participation in the study.

**Biochemical measurements**

Blood samples were obtained following overnight fasting. Hematologic parameters were obtained using a Sysmex XN-1000 (Sysmex Corporation ™, Hyogo, Japan) analyser. NLR and PLR were calculated as the ratio of neutrophils to lymphocytes and platelets to lymphocytes, respectively. For CRP and YKL-40 parameters, collected blood samples were centrifuged at 1500 rpm for 10 minutes to separate the serum. CRP was measured by the immunoturbidimetric method with a commercially available kit using an (Roche Diagnostic Corp., Mannheim, Germany) autoanalyzer. Serum was stored at -80°C until the analysis of YKL-40 ELISA test.

We used an enzyme-linked immunosorbent assay kit (Boster Biological Technology, Catalog #:EK0974, USA) in accordance with the manufacturers’ instructions to measure serum YKL-40 levels. The measuring range of the YKL-40 ELISA kit was 62.5-4000 pg/mL. Serum samples were diluted 10-fold with a dilution buffer and two wells were used per sample. We read the absorbance at 450 nm with the SPECTRO star Nano microplate reader (BMG Labtech). The data were processed...
with the MARS software (BMG Labtech). Standard curves were generated using a four-parameter curve fitting equation, and YKL-40 levels were calculated according to this curve, with values given as ng/ml.

**Statistical analysis**

Analyses were performed using the SPSS software (version 16.0, SPSS, Chicago, IL). The Kolmogorov–Smirnov test was used to evaluate the normality of the distributions of variables. The independent samples t-test was used for normally distributed parameters and the Mann-Whitney U test was used for parameters of non-normal distribution. Parametric data were expressed as mean ± standard deviation (SD). Correlations between two variables were assessed by using the Pearson correlation coefficient. P values of less than 0.05 were considered statistically significant.

**Results**

The mean ages of the patients (23 male, 57.5%) and control subjects (26 male, 63.4%) were 44.8 ± 13.2 years and 46.9 ± 15.5 years, respectively. There were no significant differences between the patient and the control group in terms of their mean ages (p = 0.467) and gender distributions (p = 0.753).

In the brucellosis group, YKL-40 levels were 15.0 ± 8.8 ng/mL, CRP was 1.48 ± 2.1 mg/dL, WBC was 7.2±2.3 × 10^9/L, NEU was 3.7 ± 2.1 × 10^9/L, LYM was 2.7 ± 0.8 × 10^9/L, PLT was 274.4 ± 80.2 × 10^9/L, MPV was 9.8 ± 1.1 fL, NLR was 1.5 ± 1.0, and PLR was 109.3 ± 46.4. Also, patients were divided into four groups according to standard tube agglutination test titres levels and all demographic and biochemical parameters were compared across these groups. However, no statistically significant differences were found (p > 0.05).

In the control group, YKL-40 levels were 12.7 ± 6.1 ng/mL, CRP was 0.2 ± 0.1 mg/dL, WBC was 6.7±1.5 × 10^9/L, NEU was 4.0 ± 1.1 × 10^9/L, LYM was 1.9 ± 0.6 × 10^9/L, PLT was 272.6 ± 84.7 × 10^9/L, MPV was 9.4 ± 1.0 fL, NLR was 2.1 ± 0.7, and PLR was 147.8 ± 59.0. CRP levels (P < 0.001) were significantly higher, and NEU (P = 0.023) and PLR (P < 0.001) were significantly lower in patients with brucellosis compared to the controls. There were no statistically significant differences between the two groups with regard to the other parameters (p > 0.05) (Table I). The demographic characteristics and the biochemical parameters of the patients have been shown in Table I in detail.

**Table I.** Demographic features and laboratory findings of the study population.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patient (n = 40)</th>
<th>Control (n = 41)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>44.8 ± 13.2</td>
<td>46.9 ± 15.5</td>
<td>0.467</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>23/17</td>
<td>26/15</td>
<td>0.753</td>
</tr>
<tr>
<td>YKL-40 (ng/mL)</td>
<td>15.0 ± 8.8</td>
<td>12.7 ± 6.1</td>
<td>0.106</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>1.48 ± 2.1</td>
<td>0.2 ± 0.1</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>14.2 ± 1.5</td>
<td>13.8 ± 1.8</td>
<td>0.072</td>
</tr>
<tr>
<td>RDW</td>
<td>13.7 ± 1.5</td>
<td>14.5 ± 2.1</td>
<td>0.334</td>
</tr>
<tr>
<td>WBC (x10^9/L)</td>
<td>7.2±2.3</td>
<td>6.7±1.5</td>
<td>0.186</td>
</tr>
<tr>
<td>Neutrophil (x10^9/L)</td>
<td>3.7 ± 2.1</td>
<td>4.0 ± 1.1</td>
<td>0.023*</td>
</tr>
<tr>
<td>Lymphocyte (x10^9/L)</td>
<td>2.7 ± 0.8</td>
<td>1.9 ± 0.6</td>
<td>0.085</td>
</tr>
<tr>
<td>Platelet (x10^9/L)</td>
<td>274.4 ± 80.2</td>
<td>272.6 ± 84.7</td>
<td>0.891</td>
</tr>
<tr>
<td>MPV, fL</td>
<td>9.8 ± 1.1</td>
<td>9.4 ± 1.0</td>
<td>0.597</td>
</tr>
<tr>
<td>NLR</td>
<td>1.5 ± 1.0</td>
<td>2.1 ± 0.7</td>
<td>0.306</td>
</tr>
<tr>
<td>PLR</td>
<td>109.3 ± 46.4</td>
<td>147.8 ± 59.0</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Pearson correlation analyses showed a positive correlation between YKL-40 and CRP (r = 0.291, P = 0.008), PLT (r = 0.312, P = 0.005), PLR (r = 0.288, P = 0.009), NLR (r = 0.225, P = 0.043), and age (r = 0.296, P = 0.007) (Figure 1 and 2). There was also a positive correlation between CRP and WBC (r = 0.255, P = 0.021) and NEU (r = 0.232, P = 0.037).

**Figure I.** The correlation analysis of NLR and YKL-40.
Figure II. The correlation analysis of PLR and YKL-40.

Discussion

Clinical symptoms and laboratory tests are not specific in brucellosis. A review of the literature reveals that YKL-40 levels are increased in cases of inflammatory disease [13-16]. Numerous studies have investigated YKL-40. However, fewer studies exist in association to infectious diseases. And as far as we now, it has never been studied in relation to brucellosis. Thus, we aimed to investigate YKL-40 levels in patients with brucellosis.

According to the results of our study, no significant differences exist between the patient and control groups in terms of their YKL-40 levels. The patient group manifested significantly higher CRP, and significantly lower PLR and Neu compared to the control group. In addition, YKL-40 was shown to be positively correlated with CRT, PLT, PLR, NLR, and age.

In the literature, Kronborg et al. have determined higher serum YKL-40 levels in patients with pneumococcus bacteremia [17]. Hattori et al. reported significantly higher levels of serum YKL-40 in patients diagnosed with sepsis. They also stated that it could be a biomarker of sepsis based on the proteomic analysis they had conducted but further studies were needed to clarify its clinical benefits [18]. Ostergaard et al. determined high YKL-40 levels in patients with purulent meningitis, suggesting that YKL-40 is produced by activated macrophages in the central nervous system [19].

It is important to consider that a significant aspect of brucella pathogenesis comprises the interaction with macrophages. Indeed, studies that investigate the Brucella/macrophage relationship in vitro are important to understand how Brucella survives inside the cell [20]. The permanence of Brucella while it creates chronic infection depends on macrophages as the primary target cells and its interaction with the host immune system. In humans, Brucella spp. is characterized by high proinflammatory cytokine levels. Interestingly, the antiinflammatory cytokine IL-10 is induced and its levels are increased in Brucella infections [21, 22]. YKL-40 is secreted in vitro by human macrophages at the late stages of differentiation, however, is not expressed in monocytes. YKL-40 was shown to be secreted only by activated macrophages and at a specific stage [23, 24]. Based on the results of our study, we think that chronic brucella patients do not manifest high YKL-40 levels because of the inhibition of macrophage activation due to the disease becoming chronic and the increase in IL-10.

YKL-40 is also synthesized by joint chondrocytes and synovial cells in patients with rheumatoid arthritis (RA) [25, 26]. In a study that investigated the relationship of YKL-40 with joint pathologies affecting the knee joints of patients with RA, YKL-40 was determined to be higher in RA patients with moderate/severe or none/mild synovitis of the knee joint compared to osteoarthritis patients with moderate/severe or none/mild synovitis of the knee joint. Moreover, RA patients demonstrated higher levels of YKL-40 in the synovial fluid than in the serum [13]. We did not obtain synovial fluid from the affected knee joints of patients with Brucella and we report this as a limitation of our study.

Earlier studies have investigated the neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) in Brucella patients. Bozdemir et al. detected a significant difference in the NLR values of pediatric patients diagnosed with Brucella compared to arthritis-positive patients, whereas no significant differences were found in PLR values [27]. In another study conducted by Olt et al. on Brucella patients, the NLR was significantly correlated with Brucella, with no significant difference in the PLR [8]. In our study, we did not detect a significant difference in NLR values of Brucella patients, however, the PLR value was determined to be significantly lower. In addition, YKL-40 was found to be positively correlated with NLR and PLR values.

The primary limitations of our study include the small number of patients with brucella arthritis and use of single blood samples. Another limitation is that synovial samples were not tested for YKL-40.

Conclusion

The physiological function of YKL-40 is not known, however, its secretion during normal and disease periods suggest that it could be involved in inflammation. We believe that more studies must be conducted at this time to investigate whether or not YKL-40 has a biological function in infectious diseases and reveal the pathophysiological importance of the YKL-40 protein.
Declaration of conflict of interest
The authors received no financial support for the research and/or authorship of this article. There is no conflict of interest.

References