Turkish Journal of Clinics and Laboratory

To cite this article: Sezgin FM, Nar R. Determination of serum YKL-40 levels in patients with brucellosis. Turk J Clin Lab 2019; 10: 335-339.

Original Article

Determination of serum YKL-40 levels in patients with brucellosis

Bruselloz hastalarında serum YKL-40 düzeylerinin belirlenmesi

Fikriye Milletli SEZGIN*[1], Rukiye NAR[2]

[1]Ahi Evran University School of Medicine, Department of Medical Microbiology, Kirsehir/TURKEY
[2]Pamukkale University School of Medicine, Department of Biochemistry, Denizli/TURKEY

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 ± 6.1ng/mL, CRP values of 1.48 ± 2.1mg/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

Öz

Amaç: Bruselloz tanılı hastalarda serum YKL-40 düzeylerini belirlemek.

Gereç ve Yöntemler: Bu çalışmaya serum aglütinasyon 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 hasta grubunda, 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 ± 6.1ng/mL, CRP 1.48 ± 2.1mg/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'ın enfeksiyon hastalıklarında rolünün daha iyi anlaşılabilmesi için ileri çalışmalara ihtiyaç vardır.

Anahtar kelimeler: bruselloz; YKL-40; tanısal değeri

Corresponding author*: Fikriye MİLLETLİ SEZGİN, Ahi Evran University School of Medicine, Department of Medical Microbiology, Kirsehir/TURKEY
E-posta: fikriyemilletli@hotmail.com
ORCID: 0000-0002-8317-2312
Recevied: 20.10.2018 accepted : 19.11.2018
Doi: 10.18663/tjcl.472868

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

Volume 10 Number 3 p: 335-339

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 ± 2.1mg/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 aaccording to standart 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 ± 6.1ng/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.

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

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).

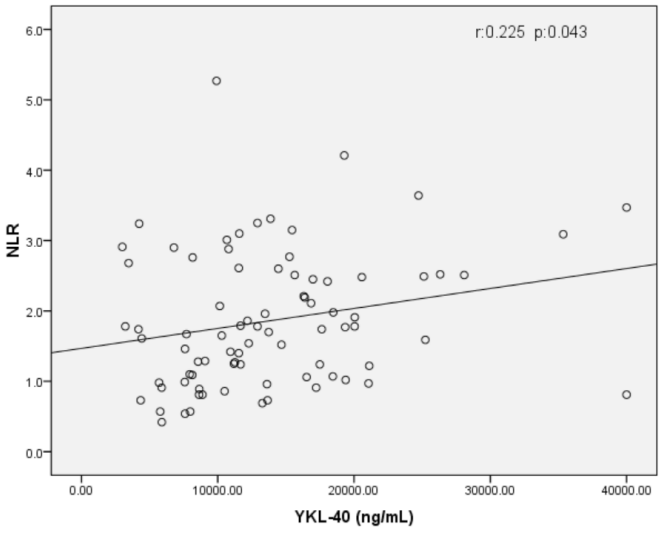

Figure I. The correlation analysis of NLR and YKL-40.

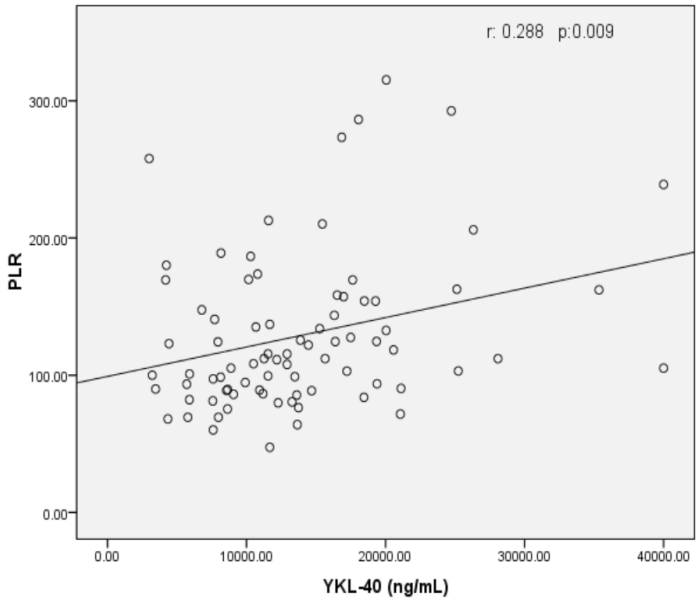

Figure II. The correlation analysis of PLR and YKL-40.

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 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

1. EJ Y. Brucella species. In: Mandell GL, Bennet JE, Dolin R, (eds). Principles and Practice of Infectious Diseases. 6th ed. New York: Churchill Livingstone, 2005: 2669-72.
2. Hussein MZ, Abou-Elnoeman SA, Al-Fikky AA, El-Samadouny EI, Shaaban AAM. Evaluation of Rose Bengal Test, Standard Tube Agglutination Test and Nested PCR for the diagnosis of Human Brucellosis. Egypt J Med Microbiol 2006; 15: 249-56.
3. Seleem MN, Boyle SM, Sriranganathan N. Brucellosis: a re-emerging zoonosis. Vet Microbiol 2010; 140: 392-8.
4. Galinska EM, Zagorski J. Brucellosis in humans-etiology, diagnostics, clinical forms, Ann Agric Environ Med 2013; 20: 233–38.
5. Hekmatimoghaddam S, Sadeh M, Khalili MB, Mollaabedin M, Sazmand A. Comparison of PCR, Wright agglutination test and blood culture for diagnosis of brucellosis in suspected patients. Pak J Biol Sci 2013; 16: 1589–92.
6. Gomez MC, Nieto JA, Rosa C, Geijo P et al. Evaluation of seven tests for diagnosis of human brucellosis in an area where the disease is endemic. Clin Vaccine Immunol 2008; 15: 1031-33.
7. Asaad AM, Alqahtani JM. Serological and molecular diagnosis of human brucellosis in Najran, Southwestern Saudi Arabia. J Infect Public Health 2012; 5: 189-94.
8. Olt S, Ergenc H, Acikgoz SB. Predictive contribution of neutrophil/lymphocyte ratio in diagnosis of brucellosis. Biomed Res Int 2015; 2015: 210502.
9. Łata E, Gisterek I, Matkowski R, Szelachowska J, Kornafel J. The importance of determining the prognostic marker YKL-40 in serum and tissues. Pol Merkur Lekarski 2010; 28: 505-8.
10. Roslind A, Johansen JS. YKL-40: a novel marker shared by chronic inflammation and oncogenic transformation. Methods Mol Biol 2009; 511: 159-84.
11. Libreros S, Iragavarapu-Charyulu V. YKL-40/CHI3L1 drives inflammation on the road of tumor progression. J Leukoc Biol 2015; 98: 931-36.
12. Faibish M, Francescone R, Bentley B, Yan W, Shao R. A YKL-40- neutralizing antibody blocks tumor angiogenesis and progression: a potential therapeutic agent in cancers. Mol Cancer Ther 2011; 10: 742-51.
13. Volck B, Johansen JS, Stoltenberg M et al. Studies on YKL-40 in knee joints of patients with rheumatoid arthritis and osteoarthritis. Involvement of YKL-40 in the joint pathology. Osteoarthritis Cartilage 2001; 9: 203–14.
14. Matsumoto T, Tsurumoto T. Serum YKL-40 levels in rheumatoid arthritis: correlations between clinical and laborarory parameters. Clin Exp Rheumatol 2001: 19; 655–60.
15. Vind I, Johansen, JS, Price PA, Munkholm P. Serum YKL-40, a potential new marker of disease activity in patients with inflammatory bowel disease. Scand J Gastroenterol 2003; 38: 599–605.
16. Koutroubakis IE, Petinaki E, Dimoulios P, Vardas E, Roussomoustakaki M, Maniatis AN, et al. Increased serum levels of YKL-40 in patients with inflammatory bowel disease. Int J Colorectal Dis 2003; 18: 254–59.
17. Kronborg G, Ostergaard C, Weis N et al. Serum level of YKL-40 is elevated in patients with Streptococcus pneumoniae bacteremia and is associated with the outcome of the disease. Scand J Infect Dis 2002; 34: 323–26.
18. Hattori N, Oda S, Sadahiro T et al. YKL-40 identified by proteomic analysis as a biomarker of sepsis. Shock 2009; 32: 393–400.
19. Ostergaard C, Johansen JS, Benfield T, Pric PA, Lundgren JD. YKL-40 is elevated in cerebrospinal fluid from patients with purulent meningitis. Clin Diagn Lab Immunol 2002; 9: 598–604.
20. Gorvel JP, Moreno E. Brucella intracellular life: from invasion to intracellular replication. Vet Microbiol 2002; 90: 281–97.
21. Fernandes DM, Baldwin CL. Interleukin-10 downregulates protective immunity to Brucella abortus. Infect Immun 1995; 63: 1130–33.
22. Xavier MN, Winter MG, Spees AM et al. CD4+ T cell-derived IL-10 promotes Brucella abortus persistence via modulation of macrophage function. PLOS Pathog 2013; 9: 1003454.
23. Volck B, Price PA, Johansen JS et al. YKL-40, a mammalian member of the chitinase family, is a matrix protein of specific granules in human neutrophils. Proc Assoc Am Physicians 1998; 110: 351–60.
24. Johansen JS, Møller S, Price PA et al. Plasma YKL-40: a new potential marker of fibrosis in patients with alcoholic cirrhosis? Scand J Gastroenterol 1997; 32: 582–90.
25. Hakala BE, White C, Recklies AD. Human cartilage gp-39, a major secretory product of articular chondrocytes and synovial cells, is a mammalian member of a chitinase protein family. J Biol Chem 1993; 268: 25803–10.
26. Kirkpatrick RB, Emery JG, Connor JR, Dodds R, Lysko PG, Rosenberg M. Induction and expression of human cartilage glycoprotein 39 in rheumatoid inflammatory and peripheral blood monocyte-derived macrophages. Exp Cell Res 1997; 237: 46–54.
27. Bozdemir ŞE, Altıntop YA, Uytun S, Aslaner H, Torun YA. Diagnostic role of mean platelet volume and neutrophil to lymphocyte ratio in childhood brucellosis. Korean J Intern Med 2017; 32: 1075-81.