THE IMPORTANCE OF SOLUBLE UROKINASE PLASMINOGEN ACTIVATOR RECEPTOR IN PATIENTS WITH ACUTE BRUCELLOSIS

Nazlım Aktuğ Demir[1], Hatice Türk Dağı[2], Duygu Fındık[2], Şua Sümer[1], Onur Ural[1], Servet Kölgelier[3]

[1] Selçuk University, Faculty of Medicine, Department of Infectious Disease and Clinical Microbiology, Konya Turkey

[2] Selçuk University, Faculty of Medicine, Department of Clinical Microbiology, Konya, Turkey

[3] Adıyaman University, Faculty of Medicine, Department of Infectious Disease and Clinical Microbiology, Adıyaman, Turkey

ABSTRACT

Objective: *Brucellosis is a common zoonotic infectious disease especially in Mediterranean countries. Inflammatory markers are elevated during the course of acute brucellosis. C-reactive protein (CRP) is the most commonly used biochemical marker in clinical practice. Soluble urokinase type plasminogen activator receptor (suPAR) is an interesting biomarker which has drawn attention recently. Purpose of this study is to examine correlation between suPAR and CRP levels as markers of infectious disease in patients diagnosed with acute brucellosis.*

Material and Method: *This study included 125 acute brucellosis patients and 50 healthy controls. Pretreatment blood samples were taken from the patients. suPAR levels were measured using ELISA and CRP levels were measured with nephelometry.*

Results: *There was a positive correlation between suPAR levels and CRP, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (p=0.045, 0.039, 0.040; respectively). When we compared patient and control groups, CRP and suPAR levels were significantly higher than controls (p=0.001, 0.001; respectively). Growth in blood culture was detected in 14 (11.2%) patients. There was not a significant difference between patients who have or did not have growth in blood cultures (p=0.117). In the ROC curve analysis performed for suPAR, area under the curve (AUC) was 93.6% (p=0.001). Sensitivity and specificity were calculated as 84.8% and 86.0%, respectively, when suPAR's cut-off value was taken as 3.85 ng/mL according to the ROC curve.*

Conclusion: *Results of this study suggest that suPAR, like CRP, is a promising biomarker in acute brucellosis.*

Key Words: *Brucellosis, C-reactive protein, soluble urokinase type plasminogen activator receptor*

SOLUBL ÜROKİNAZ PLAZMİNOJEN AKTİVATÖR RESEPTÖRÜ'NÜN AKUT BRUSELLOZLU HASTALARDAKİ ÖNEMİ

ÖZET

Amaç: Bruselloz özellikle Akdeniz ülkelerinde sık görülen zoonotik bir enfeksiyon hastalığıdır. Akut brusellozda inflamatuvar belirteçlerlerde artış izlenir. Klinik pratikte en sık kullanılan biyokimyasal belirteç C-reaktif proteindir (CRP). Son zamanlarda solubl ürokinaz tip plazminojen aktivatör reseptörü (suPAR) ilgi çekici bir biyomarker olarak ön plana çıkmaktadır. Bu çalışmanın amacı, akut brusellozis tanısı alan hastalarda enfeksiyon hastalığı göstergesi olarak suPAR düzeyleri ile CRP düzeyleri arasındaki korelasyonu değerlendirmektir.

Materyal ve Metot: Bu çalışma 125 akut brusellozis hastası ve 50 sağlıklı gönüllüde yapıldı. Hastalardan tedavi öncesi kan örnekleri alındı. suPAR düzeyleri ELISA kullanılarak ve CRP düzeyleri nefelometrik olarak ölçüldü.

Bulgular: suPAR düzeyleri ile CRP, alanin aminotransferaz (ALT) ve aspartat aminotransferaz (AST) arasında pozitif korelasyon vardı (sırasıyla, p=0,045, 0,039, 0,040). Hasta ve kontrol grubu karşılaştırıldığında CRP ve suPAR düzeyleri kontrol grubundan anlamlı düzeyde yüksekti (sırasıyla, p=0,001, 0,001). Kan kültüründe üreme 14 (%11,2) hastada saptandı. Kan kültüründe üremesi olan ve olmayan hastalar arasında anlamlı bir fark yoktu (p=0,117). suPAR için yapılan ROC eğrisi analizinde eğri altında kalan alan %93,6 idi (p=0,001). ROC eğrisine göre brusella için suPAR'ın sınır değeri 3,85 ng/mL olarak alındığında sensitivite %84,8 spesifite %86,0 olarak hesaplandı.

Sonuç: Bu çalışmanın sonuçları suPAR'ın CRP gibi akut brusellozda umut verici bir belirteç olduğunu düşündürmektedir.

Anahtar Kelimeler: Brusellozis, C-reaktif protein, solubl ürokinaz plazminojen aktivatör reseptörü

INTRODUCTION

Brucellosis is a common zoonotic infectious disease which is especially prevalent in Mediterranean countries, Middle East, India, Central and South America, and Arabic countries.[1-3] According to World Health Organization data, approximately 500,000 new cases are reported annually.[4] Main transmission routes are exposure to contaminated tissues and secretions of animals, consumption of infected milk and dairy products and inhalation.[2,3] Brucella is an intracellular microorganism which uses these cells as reservoirs.[3,5] Inflammatory markers are elevated during the course of acute brucellosis.[4]

Several biomarkers are used for diagnosis of infection and follow-up of the disease. The most commonly used biochemical marker in clinical practice is C-reactive protein (CRP).[6-10] Soluble urokinase type plasminogen activator receptor (suPAR) is an interesting biomarker which has drawn attention recently.[6-11] suPAR is the soluble form of urokinase type plasminogen activator receptor (Upar). It is released from neutrophils, lymphocytes, macrophages, endothelial cells and some types of malignant cells.[6,10,12] Although there are limited number of studies, results are promising.[10,13-19]

Purpose of this study is to examine the correlation between suPAR and CRP levels as markers of infectious disease in patients diagnosed with acute brucellosis.

MATERIAL and METHOD

This study included 125 acute brucellosis patients admitted to Infectious Diseases and Clinical Microbiology Clinics of Adıyaman University, Adıyaman Research and Education Hospital and Selcuk University, Faculty of Medicine and 50 healthy controls between March-May 2012.

Patients age, sex, profession, route of transmission, laboratory and culture results were recorded on follow up sheets. Acute brucellosis was diagnosed by a 1/160 or higher titer in the standard tube agglutination (STA) test, or a four-times increase in titer between two STA tests performed two weeks apart in the presence of clinical symptoms (a compatible clinical presentation such as arthralgia, fever, sweating, chills, headache, and malaise) within the previous eight weeks, and/or growth of Brucella spp. in appropriately prepared culture media. Patients who met criteria for acute brucellosis, who did not have previous treatment for or diagnosis of brucellosis, and who did not have accompanying immunosuppression were included. Control group involved 50 healthy controls. Control group consisted of healthy individuals without any disease, with negative results for brucella STA test, and with normal values for leukocyte count (WBC), erythrocyte sedimentation rate (ESR), and CRP.

A 5 cc blood sample was taken from each patient in the same day. These blood samples were centrifuged at 5000 cycles for 3 minutes to separate plasma. These samples were kept at -80C°. suPAR levels were measured using ELISA (suPARnostic, ViroGates A/S Denmark) and CRP levels were measured with nephelometry (Dade Behring bn ProSpec, USA) at microbiology laboratory of Selcuk University, Faculty of Medicine. Informed consent from the patients and ethical committee approval (2012/02-4.1) were obtained.

Data were recorded into SPSS 18.0 software. Chi-square test was used in the analysis of categorical variables between the control and case groups, such as gender and clinical findings. Continuous variables were analyzed by Kolmogrov-Smirnow test, and Mann Whitney-U test was used for the analysis of data like suPAR and CPR which did not demonstrate normal distribution after normality analysis. T-test was used in independent groups in the analysis of data with normal distribution. Correlation between suPAR, CRP, lymphocytes, age, WBC, platelets (PLT), STA, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and ESR was tested by using Pearson's correlation analysis. AUC for suPAR was calculated by ROC curve. A p value of <0.05 was accepted as statistically significant.

RESULTS

This study involved 125 acute brucellosis patients and 50 healthy controls. Fifty three of 125 acute brucellosis cases (42.5%) were males, 72 (57.6%) were females, and mean age of the group was 42.1±16.3. Control group consisted of 24 (48%) males, and 26 (52%) females. Mean age of the control group was 41.8±16.2. There was no statistically significant difference in suPAR levels of acute brucellosis patients related to sex (p=0.133). Three most common symptoms observed in patients were malaise, arthralgia and night sweating. No relation was detected between symptoms and suPAR levels (Table 1).

Laboratory parameters of the patients are given in Table 2.There was a positive correlation between suPAR levels and CRP, ALT and AST (p=0.045, 0.039, 0.040; respectively). When we compared patient and control groups; CRP and suPAR levels were significantly higher than controls (p=0.001, 0.001; respectively) (Table 3).

Growth in blood culture was detected in 14 (11.2%) patients. There was not a significant difference →

between patients who have or did not have growth in blood cultures (p=0.117).

In the ROC curve analysis performed for suPAR, area under the curve (AUC) was 93.6% (p=0.001) (Figure 1). Sensitivity and specificity were calculated as 84.8% and 86.0%, respectively, when suPAR's cut-off value was taken as 3.85 ng/mL according to the ROC curve. The suPAR level was found to be higher than 3.85 ng/mL in 106 of 125 (84.4%) patients and 7 of 50 (14%) control groups (p=0.01).

DISCUSSION

SuPAR has recently drawn attention as a potential biomarker in the diagnosis of infectious diseases.[1,6,20] There are studies that examine diagnostic value of suPAR in several infectious diseases such as malaria, HIV, tuberculosis, Crimean-Congo hemorrhagic fever (CCHF) and especially in sepsis and systemic inflammatory response syndrome.[10,13-19]

Brucellosis is a very common zoonosis with a high morbidity and low mortality.[21,22] Brucellosis affects both sexes at same rate.[22,23] In our study, from 125 subjects 53 were males, 72 were females and mean age was 42.1±16.3.

Brucellosis has a wide clinical spectrum because brucella can affect many organs of the host. It generally has a nonspecific course with fever, chills, night sweats, malaise, and arthralgia.[2] The three most common symptoms in patients evaluated in our study were malaise, arthralgia, and night sweats. Fever and low back pain were behind these symptoms. There was not a difference between patients, symptoms and suPAR levels. In our study, laboratory findings changed according to involved organs and systems.

Acute phase reactants may be elevated at various levels in acute brucellosis patients as a part of acute phase response.[4,5] CRP is the most commonly used acute phase reactant for this purpose and it is a valuable marker for diagnosis and follow up of infectious diseases.[6,12,23] In our study CRP levels were higher at various degrees. This elevation in CRP levels were statistically significant when compared with the control group. Assessment of the correlation between laboratory parameters and suPAR levels revealed a positive correlation between suPAR and CRP, ALT, and AST. Literature review did not reveal any correlation between suPAR and ALT and AST in brucellosis.

SuPAR is the soluble form of urokinase receptor that takes place in plasminogen activation during coagulation cascade and is an important biological marker which plays role in inflammatory processes.[24] suPAR generally takes roles in many processes including inflammation, cellular migration, differentiation, and plasminogen activation.[6,23] suPAR level is elevated in blood in cases of immune system activation.[10,13,14,17,18] Diseases which cause significant elevations in suPAR levels include sepsis, bacterial meningitis, pneumonia, tuberculosis, HIV, malaria, CCHF, and many similar diseases.[10,13,14,16-19] Among them, there is evidence that suPAR levels may be used especially in tuberculosis, sepsis, and HIV infections.[10,14,16,18]

Table 1. Relation between symptoms and suPAR levels

		Number (%)	suPAR (ng/mL)	p value
Sex	Male	53 (42.4%)	5.2 (3.0-25.8)	0.133
	Female	72 (57.6%)	6.5 (2.6-22.4)	
Malaise	Yes	93 (74.4%)	5.2 (3.0-25.8)	0.447
	No	32 (25.6%)	6.5 (2.6-22.4)	
Fever	Yes	78 (62.4%)	6.0 (2.6-18.2)	0.139
	No	47 (37.6%)	6.0 (3.2-25.8)	
Night Sweating	Yes	90 (72.0%)	6.4 (2.6-22.6)	0.136
	No	35 (28.0%)	5.6 (3.2-25.8)	
Low Back Pain	Yes	44 (35.2%)	6.4 (2.6-22.6)	0.640
	No	81 (64.8%)	5.0 (3.0-25.8)	
Arthralgia	Yes	91(72.8%)	6.2 (2.7-21.4)	0.143
	No	34 (27.2%)	6.1 (2.9-25.8)	

suPAR: Soluble urokinase type plasminogen activator receptor

Table 2. Laboratory parameters of the patients

	Patient group median (min.-max.)	Control group median (min.-max.)
WBC (K/ul)	6.8 x10^3	7.4 x10^3
	(1.2 x103 - 15x10^3)	(4.5x103 - 9.3 x10^3)
Platelet (K/ul)	232x10^3	265x10^3
	(6x103 - 4.6 x10^3)	(198x103 - 432x10^3)
STA	1/320	
	(1/160 - 1/5120)	Negative
ALT (U/L)	26 (9 - 540)	24 (9 - 34)
AST (U/L)	26 (10 - 231)	25 (10 - 39)
ESR (mm/h)	25 (2 - 67)	11 (2 - 67)
CRP (mg/L)	14 (1 - 101)	4 (1 – 8.6)
suPAR (ng/mL)	6 (2.6 - 25.8)	3.2 (0-4.8)

WBC:white blood cell, **STA**: standard tube agglutination,
ALT: alanine aminotransferase, **AST**: aspartate aminotransferase,
ESR: erythrocyte sedimentation rate, **CRP**: C-reactive protein,
suPAR: Soluble urokinase type plasminogen activator receptor

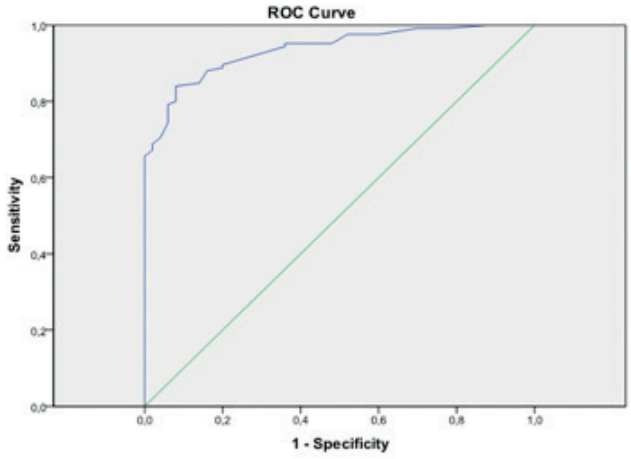

Figure 1. ROC Curve

Table 3. CRP and suPAR levels of patient and control groups

	Patient	Control	p value
CRP (mg/L)	23.0 ± 23.2	5.0 ± 2.3	0.001
suPAR (ng/mL)	7.2 ± 4.3	3.1 ± 0.8	0.001

CRP: C-reactive protein, **suPAR**: Soluble urokinase type plasminogen activator receptor

Advantages of suPAR use include stable circadian change in plasma concentration within defined limits, independence from fasting, and ability to be measured from plasma, CSF, and urine with ELISA.[6,17,25] Its main disadvantage is lack of a defined threshold value for infections.[6] Previous studies reported that suPAR production in peripheric blood mononuclear cells increase during endotoxemia.[6,26] suPAR levels significantly increase when endotoxins are increased and decrease when endotoxins are decreased in the environment.[27] Accordingly Koch et al. reported that suPAR is diagnostically and prognostically significant in critically ill patients.[27] The only study exploring the diagnostic value of suPAR in brucellosis is the study of Karsen et al.[29] In that study, Karsen et al. assessed the suPAR levels of 30 patients with acute brucellosis during diagnosis and follow-up treatment.[28] The change in the suPAR level during diagnosis and treatment was found to be statistically significant in patients with acute brucellosis. In our study suPAR levels of 125 acute brucellosis patients changed between 2.6-25.8 ng/mL. Comparison of patient and control groups revealed that CRP and suPAR levels were significantly elevated in patients. In addition, when the cut-off value of suPAR in the ROC analysis was taken as 3.85 ng/mL, sensitivity and specificity were detected as 84.8% and 86.0%, respectively.

In acute brucellosis, the reproduction levels in blood cultures differ depending on the culture method used and the subtypes of the parameter.[4] In our study, growth in blood culture was detected in 14 patients. When suPAR levels of patients with acute brucellosis who had reproduction in culture or not were compared, no statistically significant difference was found in between.

CONCLUSION

Results of this study suggest that suPAR, like CRP, is a promising biomarker in acute brucellosis. Further research is needed in order to support this observation and to determine threshold level of suPAR for infectious diseases.

* The authors declare that there are no conflicts of interest.

CORRESPONDING AUTHOR: Şua Sümer Selçuk Uni. Fac. of Med. Dep. of Infectious Disease and Clinical Microbiology Alaeddin Keykubat Campus 42250 Konya/Turkey **E-mail:** suasumer@gmail.com

DELIVERING DATE: 02 / 01 / 2014 • **ACCEPTED DATE:** 12 / 09 / 2014

REFERENCES

1. Young EJ. An overview of human brucellosis. Clin Infect Dis 1995; 21: 283-290.
2. Young EJ. Brucella Species. Mandell GL, Bennett JE, Dolin R. (eds). Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. Churchill Livingstone, Philadelphia 2010: 2921-2925.
3. Turgut M, Turgut AT, Koşar U. Spinal brucellosis: Turkish experience based on 452 cases published during the last century. Acta Neurochir (Wien) 2006; 148: 1033-1044.
4. Doganay M, Aygen B. Human brucellosis: an overview. Int J Infect Dis 2003; 7: 173-182.
5. Celli J. Surviving inside a macrophage: the many ways of Brucella. Res Microbiol 2006; 157: 93-98.
6. Donadello K, Scolletta S, Covajes C, Vincent JL. suPAR as a prognostic biomarker in sepsis. BMC Med 2012; 10: 2-9.
7. Dellinger RP, Levy MM, Carlet JM, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. Intensive Care Med 2008; 34: 17-60.
8. Marshall JC, Reinhart K. Biomarkers of sepsis. Crit Care Med 2009; 37: 2290-2298.
9. Pierrakos C, Vincent JL. Sepsis biomarkers: a review. Crit Care 2010; 4: 15.
10. Yilmaz G, Köksal I, Karahan SC, Mentese A. The diagnostic and prognostic significance of soluble urokinase plasminogen activator receptor in systemic inflammatory response syndrome. Clin Biochem 2011; 44: 1227-1230.
11. Huttunen R, Syrjänen J, Vuento R, et al. Plasma level of soluble urokinase-type plasminogen activator receptor as a predictor of disease severity and case fatality in patients with bacteraemia: a prospective cohort study. J Intern Med 2011; 270: 32-40.
12. Thuno M, Macho B, Eugen-Olsen J. suPAR: the molecular crystal ball. Dis Markers 2009; 27: 157-172.
13. Yilmaz G, Mentese A, Kaya S, et al. The diagnostic and prognostic significance of soluble urokinase plasminogen activator receptor in Crimean-Congo hemorrhagic fever. J Clin Virol 2011; 50: 209-211.

14. Andersen O, Eugen-Olsen J, Kofoed K, Iversen J, Haugaard SB. Soluble urokinase plasminogen activator receptor is a marker of dysmetabolism in HIV-infected patients receiving highly active antiretroviral therapy. J Med Virol 2008; 80: 209-216.

15. Mölkänen T, Ruotsalainen E, Thorball CW, Järvinen A. Elevated soluble urokinase plasminogen activator receptor (suPAR) predicts mortality in Staphylococcus aureus bacteremia. Eur J Clin Microbiol Infect Dis 2011; 30: 1417-1424.

16. Savva A, Raftogiannis M, Baziaka F, et al. Soluble urokinase plasminogen activator receptor (suPAR) for assessment of disease severity in ventilator-associated pneumonia and sepsis. J Infect 2011; 63: 344-350.

17. Wittenhagen P, Kronborg G, Weis N, et al. The plasma level of soluble urokinase receptor is elevated in patients with Streptococcus pneumoniae bacteraemia and predicts mortality. Clin Microbiol Infect 2004; 10: 409-415.

18. Eugen-Olsen J, Gustafson P, Sidenius N, et al. The serum level of soluble urokinase receptor is elevated in tuberculosis patients and predicts mortality during treatment: a community study from Guinea-Bissau. Int J Tuberc Lung Dis 2002; 6: 686-692.

19. Ostrowski SR, Ullum H, Goka BQ, et al. Plasma concentrations of soluble urokinase-type plasminogen activator receptor are increased in patients with malaria and are associated with a poor clinical or a fatal outcome. J Infect Dis 2005; 191: 1331-1341.

20. Kofoed K, Andersen O, Kronborg G, et al. Use of plasma C-reactive protein, procalcitonin, neutrophils, macrophage migration inhibitory factor, soluble urokinase-type plasminogen activator receptor, and soluble triggering receptor expressed on myeloid cells-1 in combination to diagnose infections: a prospective study. Crit Care 2007; 11: 38.

21. Bodur H, Erbay A, Akıncı E, et al. Neurobrucellosis in an endemic area of brucellosis. Scand J Infect Dis 2003; 35: 94-97.

22. Yingst S, Hoover DL. T cell immunity to Brucellosis. Crit Rev Microbiol 2003; 29: 313-331.

23. Ariza J, Bosilkovski M, Cascio A, et al. Perspectives for the treatment of brucellosis in the 21st century: the Ioannina recommendations. PLoS Med 2007; 4: 317.

24. Eugen-Olsen J. suPAR-a future risk marker in bacteremia. J Intern Med 2011; 270: 29-31.

25. Rabna P, Andersen A, Wejse C, et al. Urine suPAR levels compared with plasma suPAR levels as predictors of post-consultation mortality risk among individuals assumed to be TB-negative: a prospective cohort study. Inflammation 2010; 33: 374-380.

26. Ostrowski SR, Plomgaard P, Fischer CP, et al. Interleukin-6 infusion during human endotoxaemia inhibits in vitro release of the urokinase receptor from peripheral blood mononuclear cells. Scand J Immunol 2005; 61: 197-206.

27. Koch A, Voigt S, Kruschinski C, et al. Circulating soluble urokinase plasminogen activator receptor is stably elevated during the first week of treatment in the intensive care unit and predicts mortality in critically ill patients. Crit Care 2011; 15: 63.

28. Karsen H, Cesur S, Karaağaç L, et al. Can mannose-binding lectin and plasma level of soluble urokinase receptor be used in diagnosis and treatment monitorization of Brucellosis patients? Mikrobiyol Bul 2012; 46: 519-521.