Mean platelet volume in brucellosis: correlation between brucella standard serum agglutination test results, platelet count, and C-reactive protein.

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Abstract

Background: Brucellosis, a zoonotic infection, was most widely diagnosed by the Brucella standard serum agglutination test (SAT). No previous publication has demonstrated a correlation between the degree of Brucella SAT agglutination positivity and the severity of brucellosis infection.

Objective: To contribute to the clarification of the relationship between patelets and brucellosis. It is also aimed at evaluating the usefulness of the SAT titer as a measure of brucellosis severity.

Material and Methods: We compared the control (n=60) and patients (n=96) groups in terms of mean platelet volume (MPV), C-reactive protein (CRP) and platelet values. Patients were grouped according to their degree of agglutination positivity titers and compared by means of CRP, MPV and platelet values. We also investigated the relationship among logarithmic values of MPV, platelet and CRP parameters for each group.

Results: Although statistically meaningful difference was observed between control and patients group in terms of MPV and platelet value, there were no statistically significant differences observed among patients groups. The physiological negative correlation between MPV and platelet count was not encountered in group 2 and 3. Logarithmic values of CRP were not correlated with logarithmic values of MPV and platelet counts.

Conclusion: The MPV could be a new parameter to evaluate hematologic abnormalities in patients with brucellosis. The SAT titer was not a useful measure for evaluation of the severity of brucellosis.

Keywords: Brucella, CRP, platelet count, mean platelet volume, agglutination

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Introduction

Brucellosis is a zoonotic infection caused by Gram-negative coccobacilli[1]. The Brucella standard serum agglutination test (SAT) is the most widely used serologic test for the diagnosis of brucellosis as mentioned in the quoted reference[2]. The titer of agglutinating antibodies indicating active Brucella infection varies from country to country and depends on the endemicity of brucellosis[3]. The widely accepted SAT cut-off point was ≥ 1/160 as many textbooks advised and different studies performed in Turkey [4–7].

Mean platelet volume (MPV) generated by clinical hematology analyzer reflects platelet activation.

Previous studies have reported that there is a correlation between MPV and inflammatory disease [8-12]. Aydemir et al. indicated that different type of microorganisms might be cause specific MPV and platelet response[10].

Several mediators such as interleukin (IL)-1, tumor necrosis factor (TNF)-alpha and IL-6 influence the maturation of thrombopoetic cells [13,14]. Brucella-infected cells are activated to express IL-1 beta and IL-6 at both the mRNA and protein levels[15].

Several markers of inflammation have been used in diagnosis of bacterial infections. One of these markers is C-reactive protein (CRP) [16]. It has been reported in different studies that the elevated CRP might be associated with Brucellosis and might be used to determine the activity of acute Brucellosis [17-19].

There had been only two studies about the change of MPV value in Brucellosis [20,21] and no previous publication has demonstrated a correlation between the degree of Brucella SAT agglutination positivity and the severity of brucellosis infection. The importance of platelets in brucellosis has not been proven yet and our study aimed to contribute to the clarification of

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this relationship. The study also aimed to assess the usefulness of the SAT titer as a measure of brucellosis severity.

Material and methods

Study Population

In the current study, we retrospectively reviewed the records of 1336 brucellosis suspected patients from December 2010 to December 2011. The study population composed of 96 patients and 60 controls were examined. We evaluated MPV, CRP and platelet values of patients at first admission to our hospital. The control group included 60 healthy individuals (mean age: 45 ± 14, male / female ratio: 22/38) without known inflammatory disease and the use of drugs. Additionally the control groups had no anemia, no bleeding diathesis and had normal platelet counts. We obtained the records of all patients and controls from Ankara Numune Education and Training Hospital's laboratory information system. Samples were sent by physicians from various medical inpatient and outpatient clinics. We sought information regarding Brucella SAT results, CRP, MPV, platelet count, gender and age. The protocol was approved by local ethical committee.

Methods

The Brucella SAT test was performed in the microbiology laboratory of our hospital using Standard commercial Brucella abortus antigen (Refik Saydam Laboratories, Ankara, Turkey). It was performed according to the manufacturer's instructions. CRP levels were determined by the immunoturbidimetric method (Beckman Coulter Unicel DXC 800, USA). The normal CRP values in our laboratory are <5 mg/L. Platelet count was measured using an automatic blood counter (Beckman Coulter LH 780, USA). 4 ml blood samples were collected from each brucellosis suspected patients in ethylenediaminetetraacetic acid (EDTA) tubes. The mean platelet volumes were calculated using the same hematological analyzer. The expected MPV and platelet values in our laboratory ranged between 7-12 femtolitres (fl) and 150,000-450,000 per microlitre, respectively.

Statistical Analyses

Shapiro-Wilk's and Levene's tests were used to check the normality and the equal variances assumptions of the data. A logarithmic transformation was applied to CRP variable because of its highly skew distribution. Values were expressed as mean±standard deviation or geometric mean and 95% confidence intervals. The differences between groups were tested using independent-samples t test or one-way analysis of variance. Brown-Forsythe test was also performed when the assumption of equal variances does not hold.

Games-Howell post-hoc test was used for pair wise comparisons. Pearson correlation coefficients were calculated to see the relationships between MPV, PLT and CRP values for each group and a scatter-plot matrix was given for the visual representation. All analyses were performed using R 2.14.0 software considering $p<0.05$ statistically significant.

Results

Ninety six patients who had a titer of ≥ 1:160 enrolled in this study as a patient group. We grouped all patients according to their degree of agglutination positivity. The distribution of patients, according to their sexes and mean ages are given in Table I.

Table I The distribution of patients, according to their sexes and mean ages

Total # of patients (n=96)	Group 1(n=31)	Group 2(n=31)	Group 3(n=19)	Group 4(n=15)
***Brucella* SAT result**	1/160	1/320	1/640	≥1/1280
Male/Female	16/15	20/11	15/4	9/6
Mean Age, (*years*±SD)	42 ±14	45 ±14	41 ±16	42 ±15

SAT: Standard serum agglutination test

The mean values of MPV and platelet in control group were 11.01±0.6 and 268.16±58.21, respectively. However those of in patients were 8.66±1.44 and 254.94±82.40, respectively. According to Table II, statistically meaningful difference between patient and control groups was observed in terms of MPV and platelet values ($p<0.001$ and $p<0.05$).

Table II Comparison of MPV, platelet and CRP values between patients and controls

Variable	Brusellosis Patients (n=60)	Control (n=96)	p
MPV	11.01±0.6	8.66±1.44	<0.001
PLT	268.16±58.21	245.94±82.40	<0.05

MPV: Mean platelet volume, PLT: Platelet count, CRP: C-reactive protein. Values are expressed as mean±SD and %95 confidence intervals

No statistically meaningful difference was observed in terms of MPV, platelet and CRP values among patients groups (Table III).

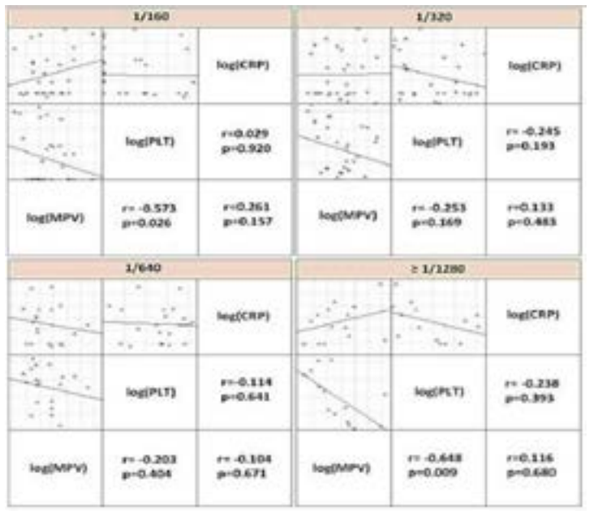
Table III Comparison of MPV, platelet and CRP values among patients groups

	Group 1	Group 2	Group 3	Group 4	*p*
MPV (fl)	8.44(7.98-8.92)	8.75(8.26-9.28)	8.40(7.84-9.00)	8.53(7.62-9.55)	0.503
PLT (10^3 µl)	265.03(227.92-308.18)	209.26(181.46-241.33)	254.40(222.52-290.85)	213.71(168.08-271.73)	0.112
CRP (mg/L)	1.41(0.91-2.18)	1.64(1.01-2.68)	1.50(0.96-2.34)	1.30(0.65-2.63)	0.600

MPV: Mean platelet volume, PLT: Platelet count, CRP: C-reactive protein. Values are expressed as geometric means and %95 confidence intervals

Physiological negative correlation between logarithmic values of MPV and platelet was not observed in group 2 (r= -0.253, p= 0.169) and in group 3 (r= -0.203, p= 0.404). Logarithmic values of CRP were not correlated with logarithmic values of MPV and platelet (Figure 1).

Figure 1 A scatterplot matrix with pearson correlation coefficients to display the relationships between logarithmic values of MPV, platelet and CRP parameters for each group.



Discussion

Hematologic abnormalities are observed in brucellosis. One of these abnormalities is thrombocytopenia. Brucella induced thrombocytopenia was reported in the range of 2.4- 33%[22]. It was encountered to statistical difference with regard to the platelet counts in patient and control groups. Also MPV values were found to be lower in patient group than in the control group (Table II). Over releasing of proinflammatory cytokines and acute-phase reactants can suppress the size of platelets [23]. Previous studies reported that interferon gamma (IFN-γ), TNF-α, IL-1 and IL-12 secreted in brucellosis[15]. These cytokines might influence the maturation of platelets and the release of platelets into the circulation [13]. Becchi et al. also found a negative MPV value trend in sepsis[24]. Ozturk et al reported that MPV values were lower in patients with brucellosis than control groups[20]. These results are in agreement with the result of our study. Considering the above findings, it could be contemplated that investigating MPV values in combination with platelet counts might be useful in patients with brucellosis. MPV is an indicator of platelet activation and production[9, 25]. In this context therefore, it could be inferred that in addition to platelet counts, the activation of platelets could be affected in patients with brucellosis. Accordingly, the determination of MPV values could be useful in evaluation of the progression of the disease.

MPV, platelet and CRP values changed with brucellosis were used for evaluating whether there was a correlation between the severity of brucellosis and a higher titer in Brucella SAT test. We could not find any significant difference among patients groups in terms of MPV, CRP and platelet value (Table III). It was reported that the role of CRP in the inflammatory processes may involve binding to platelet activating factor. [26]. Therefore, we also investigated the correlation between logarithmic values of MPV, CRP and platelet values by using Scatter plot matrix with Pearson correlation to evaluate the relationship between the severity of the disease and SAT titer. However, there was no correlation between logarithmic values of MPV, CRP and platelet values in patient groups. Yuksel et al., Ulaslı et al., as well reported that no correlation was observed between CRP and MPV in patients with ulcerative colitis and chronic obstructive pulmonary disease, respectively [8,27]. The findings of our study were in line with the results of these previous studies. The physiological inverse relationship between MPV and platelet count seen by Kim et al[28], was not observed in group 2 and 3 (Figure 1) and we also found that both MPV and platelet values decreased in patients with brucellosis. It was observed that brucellosis might affect the physiological inverse correlation seen between MPV and platelet count. However, the physiological inverse relationship did not disappear in all groups. All these findings support the suggestion that there is no correlation between the severity of brucellosis and a higher titer in the SAT test.

Conclusion

MPV value was thought to be used in evaluation of platelet function in patients with brucellosis. Additionally, it was thought that the SAT titer was not useful to evaluate the severity of the disease.

References

1. Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos EV. *The new global map of human brucellosis. Lancet Infect Dis* 2006;6:91-9.
2. Memish ZA, Almuneef M, Mah MW, Qassem LA, and Osaba AO. Comparison of the Brucella standard agglutination test with the ELISA IgG and IgM in patients with Brucella bacteremia. *Diagn Micr Infec Dis* 2002;44:129-32.
3. Memish Z, Mah MW, Mahmoud SA, Shaalan MM, Khan Y. Brucella bactraemia: clinical and laboratory observations in 160 patients. *Journal of Infection* 2000;40:59–63.
4. Young EJ. Brucella species. In: Mandell GL, Bennett JE, Dolin R, editors. *Principles and Practice of Infectious Diseases*, 5th ed. Churchill Livingstone, Philadelphia; 2000. p. 2386.
5. Young EJ. Brucella spp. In: Gillespie SH, Hawkey PM, editors. *Principles and Practice of Clinical Bacteriology*, 2nd ed. John Wiley & Sons Ltd, West Sussex, England; 2006. p. 265-72.
6. Tasova Y, Saltoğlu N, Sahin G, Aksu HSZ. Osteoarthricular Involvement of Brucellosis in Turkey. *Clin Rheumatol* 1999;18:214–19.
7. Akdeniz H, Irmak H, Anlar Ö, Demiröz AP. Central nervous system brucellosis: Presentation, diagnosis and treatment. *Journal of Infection*1998;36:297-301.
8. Yuksel O, Helvaci K, Basar O, Koklu S, Caner S, Helvaci N, Abaylı E, Altiparmak E. An overlooked indicator of disease activity in ulcerative colitis: mean platelet volume. *Platelets* 2009;20:277-81.
9. Sert A, Aypar E, Odabas D. Mean platelet volume in acute rheumatic fever. *Platelets*. 2012 [Epub ahead of print]
10. Aydemir H, Piskin N, Akduman D, Kokturk F, Aktas E. Platelet and mean platelet volume kinetics in adult patients with sepsis. *Platelets* 2012 [Epub ahead of print].
11. Kisacik B, Tufan A, Kalyoncu U, Akdogan A, Ozturk MA, Karadag O, Kiraz S, Ertenli I, and Calguneri M. Mean platelet volume (MPV) as an inflammatory marker in ankylosing spondylitis and rheumatoid arthritis. *Joint Bone Spine* 2008;75:291-94.
12. Yazici S, Yazici M, Erer B, Erer B, Calik Y, Ozhan H, Ataoglu S. The platelet indices in patients with rheumatoid arthritis: mean platelet volume reflects disease activity. *Platelets*. 2010;21(2):122-5.
13. Gasparyan AY, Ayvazyan Lilit, Mikhailidis DP, Kitas GD. Mean Platelet Volume: Alink Between Thrombosis and Inflammation? *Current Pharmaceutical Design* 2011; 17: 47-58.
14. Gasparyan AY, Sandoo A, Stavropoulos-Kalinoglou A, Kitas GD. Mean platelet volume in patients with rheumatoid arthritis: the effect of anti-TNF-α therapy. *Rheumatol Int*. 2010 Jun;30(8):1125-9.
15. Caron A, Cross A, Liautard JP, Dornand J. Brucella species release a specific, protease-sensitive, inhibitor of TNF-alpha expression, active on human macrophage-like cells. *The Journal of Immunology* 1996; 156: 2885-93.
16. Simon L, Gauvin F, Amre DK, Saint-Louis P, Lacroix J. Serum procalcitonin and Creactive protein levels as markers of bacterial infection: Asystematic rewiev and meta-analysis. *Clin Ðnfec Dis* 2004;39:206-17.
17. Dabdoob WA, Abdulla ZA. A panel of eight tests in the serodiagnosis and immunological evaluation of acute brucellosis. *East Mediterr Health J*2000;6:304-12.
18. Al-Kassab AS, Nur MA, Malik JM. Evaluation of serum C-reactive protein in the diagnosis of arthritic and non-arthritic brucellosis *J Trop Med Hyg* 1991;94:92-6.
19. Navarro JM, Mendoza J, Leiva J, Rodriguez-Contreras R, de la Rosa M. C- reactive protein as a prognostic indicator in acute brucellosis. *Diagn Microbiol Infect Dis* 1990;13:269-70.
20. Oztürk ZA, Sayiner H, Kuyumcu ME, Yesil Y, Savaş E, Sayıner ZA, Kısacık B, Kepekçi Y. Mean Platelet Volume in Assessment of Brucellosis Disease. *Biomed Res-India* 2012;23:541-46
21. Küçükbayrak A, Taş T, Tosun M, Aktaş G, Alçelik A, Necati Hakyemez I, Koçoğlu E, Akdeniz H. Could thrombocyte parameters be an inflammatory marker in the brucellosis? *Med Glas (Zenica)*. 2013 Feb;10(1):35-9.
22. Franco MP, Mulder M, Gilman R, Smits LH. Human brucellosis *Lancet infect Dis* 2007;7:775-86
23. Bath PM, Butterworth RJ. Platelet size: measurement, physiology and vascular disease. *Blood Coagul Fibrinolysis* 1996; 7:157-61.
24. Becchi C, Al Malyan M, Fabbri LP, Marsili M, Boddi V, Boncinelli S. Mean platelet volume trend in sepsis: is it a useful parameter? *Minerva Anestesiol* 2006;72:749-56.
25. Park Y, Schoene N, Harris W. Mean platelet volume as an indicator of platelet activation; methodological issues. *Platelets* 2002;13:301-6
26. Ballou SP, Kushner I. C reactive protein and the acute phase response. *AdvIntern Med* 1992;183:133-45.
27. Ulasli SS, Ozyurek BA, Yilmaz EB, Ulubay Gaye. Mean platelet volume as an inflammatory marker in acute exacerbation of chronic obstructive pulmonary disease. *Polskze Archwum Medycyny Wewnetrznej* 2012;122:284-90.
28. Kim JH, Kang SB, Kang JI, Kim JW, Kim SY, Bae HY. The relationship between mean platelet volume and fasting plasma glucose differs with glucose tolerance status in a Korean general population: Gender differences. *Platelets* 2012 [Epub ahead of print].