ORIGINAL ARTICLE

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Diagnostic role of mean platelet volume and neutrophil to lymphocyte ratio in childhood brucellosis

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Background/Aims: Brucellosis patients present various non-specific clinical symptoms, such as fever, fatigue, sweating, joint pain, arthritis, myalgia, and headache. Based on the nonspecifity of its clinical signs and symtoms, we decided to evaluate whether mean platelet volume (MPV) , neutrophil to lymphocyte ratio (NLR), and platelet to lymphocyte ratio (PLR) will contribute to the diagnosis.

Methods: In this retrospective study, we reviewed hospital-records of 60 children with a confirmed diagnosis of brucellosis in Kayseri between January 2013 and January 2016, and compared the hematological parameters; white blood cell (WBC) count, hemoglobin (Hb), neutrophil count, lymphocyte count, platelet count, MPV, NLR, and PLR with 55 healthy age and gender matched children. Also, the well known inflammation markers; erytrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were compared between the patient and control group.

Results: We found significant difference among the Hb, platelet count, MPV and NLR values between the patient and control group ($p < 0.05$). There was no difference between WBC, neutrophil count, lymphocyte count and PLR between the patient and control group ($p > 0.05$). When the patients were divided into groups as arthritis positive and arthritis negative and compared to the control group; we found that the NLR is more significant in between the arthritis positive and control group ($p = 0.013$). Also, we found significant difference among the ESR and CRP values between the patient and control group ($p < 0.001$).

Conclusions: The results of this study indicates that MPV and NLR values can be used as markers of inflammation in childhood brucellosis. Also, NLR is more valuable in children with brucella arthritis.

Keywords: Brucellosis; Child; Mean platelet volume; Neutrophil to lymphocyte ratio; Diagnosis

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INTRODUCTION

Brucellosis is a bacterial zoonotic disease transmitted directly or indirectly by contact with infected animals or contaminated dairy products. It is a common disease in middle, east, and southeast region of Turkey, and affects the children as well as adults [1,2]. Different from the adults, almost all of the pediatric cases are due to unpasteurized milk or milk products consumption [3-6]. Following the initial infection, the bacteria proliferates in

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the regional lymph nodes, passes into blood and causes systemic infection involving tissues and several organs, primarily the reticuloendothelial system. Brucellosis patients present non-specific clinical symptoms; such as fever, fatigue, sweating, joint pain, arthritis, myalgia, and headache [1,5-8]. Although the gold standart test for the diagnosis of brucellosis is culture method, standart tube agglutination test with a titre of 1/160 is used most commonly [9]. Laboratory findings such as leukocytosis/leukopenia, relative lymphocytopenia, anemia, thrombocytopenia, high C-reactive protein (CRP), and high erytrocyte sedimentation rate (ESR) can be found in patients, but does not help directly to the diagnosis [10]. While searching for other markers contributing to the diagnosis of childhood brucellosis, we decided to evaluate the mean platelet volume (MPV), neutrophil to lymphocyte ratio (NLR), and platelet to lymphocyte ratio (PLR) in our patient group. There are many studies investigating the hematological parameters MPV, NLR, and PLR as markers of inflammation in several rheumatologic, cancer, and/or infectious diseases [11-15]. In this study, we compared the MPV, NLR, and PLR values of our brucellosis patients with healthy children to determine their usability in the diagnosis of childhood brucellosis on admission.

METHODS

This retrospective study was performed in the Pediatric Clinic of Kayseri Training and Research Hospital. The medical records of patients who were diagnosed and treated for brucellosis in the pediatric clinic between January 2013 and January 2016 were evaluated. A total of 60 children with brucellosis and 55 age- and gender-matched healthy controls were enrolled in the study. Healthy children were selected through children who applied to hospital for routine check-up, or vaccination status screening or for preoperative evaluation of minor elective surgery (for example, hernia repair). Children with any sign of infection or systemic illness were excluded from the control group. The diagnosis of brucellosis was established according to the presence of isolation of *Brucella* species from blood culture and/or, positive brucella serology test equal or higher than the titre of 1:160, using standard agglutination test (SAT) for patients presenting symptoms suggestive of brucellosis. Other demographic, clinical and laboratory data, and treatment modalities and outcomes were obtained from patients' follow-up folders and hospital records.

Patients with an underlying pathology (like rheumatic disease, autoimmune disease), or evidence of other bacterial infection (culture positivity for another bacteria), or who were over 18 years old and whose file records were unreachable were excluded from the study.

Hematological parameters including white blood cell (WBC) count, hemoglobin (Hb), neutrophil count, lymphocyte count, platelet count, and MPV were recorded for both groups. NLR and PLR were calculated as the ratio of neutrophils to lymphocytes and platelets to lymphocytes, respectively. CRP of all patients and controls and ESR of patients and controls whose existing were recorded. Comparison between the patients and the control subjects was performed with regards to WBC, neutrophil count, lymphocyte count, platelet count, MPV, NLR, and PLR. White blood cell, Hb, neutrophil count, lymphocyte count, platelet count, and MPV, NLR and PLR values, CRP, and ESR were compared between the patients and control groups. Also, patients were divided into two groups as arthritis positive and artritis negative groups and WBC, neutrophil count, lymphocyte count, platelet count, MPV, NLR, and PLR values compared to the controls one by one. All kinds of blood cell counts were made in Sysmex XN-350 (Sysmex, Kobe, Japan) and CRP measures were held on BN Prospec (Dade Behring, Siemens, Deerfield, IL, USA) Nephelometer.

The Non-Interventional Clinical Ethics Committee of Erciyes University Medical Faculty approved the study protocol.

Statistical analysis

The normality of data distribution was determined using the Kolmogorov-Smirnov test. Normally distributed numerical variables were expressed in mean ± standard deviation. Normally distributed numerical variables were compared using the Student *t* test or One-way analysis of variance test. Tukey test was used for *post hoc* tests. Data corresponding to an abnormal distribution were expressed in median (minimum–maximum). Abnormally distrubuted numerical data were compared using the non-parametric Mann-Whitney *U* test or Kruskal-Wallis test. The chi-square test was used to compare cat-

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Table 1. Demographic and clinical characteristics of the 60 patients

Characteristic	Value
Age, mon	30 (1–204)
Male sex	49 (81.7)
Symptoms and clinical signs	
Fever	60 (100)
Arthralgia	56 (93.3)
Weakness	54 (90.0)
Myalgia	50 (83.3)
Anorexia	34 (56.7)
Sweating	27 (45.0)
Arthritis	17 (28.3)
Peripheral lymphadenopathy	10 (16.7)
Abdominal pain	8 (13.3)
Hepatosplenomegaly	7 (11.7)
Splenomegaly	1 (1.7)
Serum agglutination test results	
1/20[a]	1 (1.7)
1/160	30 (50.0)
1/320	14 (23.4)
1/640	8 (13.3)
1/1,280	7 (11.7)
Erythrocyte sedimentation rate, mm/hr	9.5 (2–56)
C-reactive protein, mg/dL	8.9 (3.17–252)
Treatment	
Rifampicin + doxycycline	22 (36.7)
Rifampicin + doxycycline + gentamicin	15 (25.0)
Rifampicin + TMP-SMT	13 (21.7)
Rifampicin + TMP-SMT + gentamicin	8 (13.3)
Rifampicin + TMP-SMT + cefotaxime/ceftriaxone[b]	2 (3.3)

Values are presented as median (range) or number (%).
TMP-SXT, trimethoprim-sulfamethoxazole.
[a]Case with congenital infection.
[b]One case with relapsing disease and one case with congenital infection.

egorical variables between the groups. *p* values of less than 0.05 were considered statistically significant. The data were analyzed using SPSS version 22.0 (IBM Co., Armonk, NY, USA).

RESULTS

The median age of the patients was 130 months (range, 1 to 204) and 81.7% (n = 49) of the patients were male. The median age of the control group was 104 months (range, 1 to 204) and 70.9% (n = 39) were male. There were no significant differences in the median ages (p = 0.249) and gender distribution (p = 0.174) between the patient and the control group. The most common symptoms at admission were fever (100%), arthralgia (93.3%), and weakness (90%). Seven patients had hepatosplenomegaly (11.6%), one patient had only splenomegaly (1.7%). The frequency of arthritis was 28.3% (17/60) and the most commonly affected joint was knee (47%, 8/17). The presence of brucella arthritis was not related to serum aglutination titer (p = 0.507) and blood culture positivity (p = 0.646). The most frequent SAT was 1/160 in 50% of all patients. One case of congenital infection had a SAT of 1/20. The culture positivity rate was 31.2%. Most of the patients (36.7%, 22/60) were treated with a combination of rifampicin plus doxycycline. Frequency of SAT and treatment modalities are listed in Table 1. The ESR was studied in 46 patients (76.70%) and in 18 controls (32.7%), and the median values were 9.5 mm/hr (range, 2 to 56) and 2.5 mm/hr (range, 2 to 10), respectively. There was a significant difference between the patient and control group (p < 0.001). The median CRP value was 8.93 mg/L (range, 3.17 to 252.0) in patient group, 3.28 mg/L (range, 3.17 to 5.04) in control group and there was a significant difference between the patient and control group (p < 0.001) (Table 1). Median WBC was 6,870/mm^3 (range, 1,220 to 18,370), Hb was 12.6 ± 2.0 mg/dL, platelet count was 272,366 ± 91,913/mm^3, MPV value was 8.9 ± 1.14 fL, NLR was 0.99 (range, 0.31 to 10.2) and PLR was 95.2 (range, 22.2 to 370) in the patient group. Median WBC was 6,500/mm^3 (range, 3,590 to 10,770), Hb was 13.4 ± 1.4 mg/dL, platelet count was 309,963 ± 73,000 mm^3, MPV value was 9.3 ± 0.76 fL, NLR was 0.89 (range, 0.17 to 1.39) and PLR was 93.05 (range, 1.4 to 172) in the control group. There was statistically significant difference in Hb, platelet count, MPV, and NLR values between the two groups (p < 0.05). There was no statistically significant difference between the WBC, neutrophil count, lymphocyte count and PLR values between the two groups (p > 0.05) (Table 2). When the patient group was divided into two groups

Table 2. Comparison of the laboratory findings of patient and control groups

Variable	Patient (n = 60)	Control (n = 55)	*p* value
WBC, /mm³	6,870 (1,220–18,370)	6,500 (3,590–10,770)	0.186
Neutrophil, /mm³	2,820 (620–15,690)	2,790 (890–4,850)	0.103
Lymphocyte, /mm³	3,020 (270–6,930)	3,100 (1,520–5,840)	0.223
NLR	0.99 (0.31–10.2)	0.89 (0.17–1.39)	0.032
PLR	95.2 (22.2–370)	93.1 (1.4–172)	0.853
Hemoglobin, g/dL	12.6 ± 2.0	13.4 ± 1.4	0.017
Platelet, /mm³	272,366 ± 91,913	309,963 ± 73,000	0.017
MPV, fL	8.9 ± 1.14	9.3 ± 0.76	0.049
ESR, mm/hr	9.5 (2–56)	2.5 (2–10)	< 0.001
CRP, mg/dL	8.93 (3.17–252)	3.28 (3.17–5.04)	< 0.001

Values are presented as median (range) or mean ± SD.
WBC, white blood cell; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; MPV, mean platelet volume; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein.

Table 3. Comparison of the laboratory findings of artritis positive, arthritis negative patients, and control group

Variable	Arthritis (+)	Arthritis (–)	Control	*p* value
WBC, /mm³	6,850 (3,590–13,710)	6,890 (1,220–18,370)	6,500 (3,590–10,770)	0.310
Neutrophil, /mm³	3,540 (950–10,570)[a]	2,780 (620–15,690)[b]	2,790 (890–4,850)[b]	0.007
Lymphocyte, /mm³	2,710 (1,450–5,650)	3,050 (270–6,930)	3,100 (1,520–5,840)	0.405
NLR	1.38 (0.37–5.9)[a]	0.95 (0.31–10.2)[b]	0.90 (0.17–1.3)[b]	0.002
PLR	92.4 (22.2–214)	99.1 (46.5–370)	93.0 (1.4–172)	0.944
Hemoglobin, g/dL	12.8 ± 1.7	12.6 ± 2.1	13.4 ± 1.43	0.059
Platelet, /mm³	251,823 ± 72,550[a]	280,488 ± 98,102[b]	309,963 ± 73,003[b]	0.035
MPV, fL	9.3 ± 1.08[b]	8.8 ± 1.14[a]	9.3 ± 0.76[b]	0.034

Values are presented as median (range) or mean ± SD.
WBC, white blood cell; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; MPV, mean platelet volume.
[a]Indicates the difference.
[b]Indicates the similarity.

as arthritis positive and negative group, the comparison of NLR resulted more significant between the arthritis positive and control group ($p = 0.002$). Also, the difference of MPV between arthritis negative and control group was found more significant ($p = 0.034$) (Table 3).

DISCUSSION

Changes in hematological parameters are commonly seen in brucellosis patients, but they do not have diagnostic value and turn to normal values rapidly with the treatment of brucellosis. Aypak et al. [16] reported that the most frequent hematological abnormality in a total of 69 children with brucellosis was thrombocytopenia with 15.9% (11/69). The other abnormalities were thrombocytosis with 7.3%, leukopenia with 5.8%, anemia 4.3%, and bicytopenia 4.3%. Tanir et al. [17] reported the hematological abnormalities in a total of 90 children with brucellosis as anemia with 26.7%, leukopenia 10%, thrombocytosis 5.6%, thrombocytopenia 3.3%, and leukocytosis 1.1%. Karakukcu et al. [18] reported the frequency of pancytopenia as 14.8% among a total of 54 patients. In our study, hematological abnormalities were anemia 10%, bicytopenia 5%, and pancytopenia 3.3%, respectively. These results show that the hematological parameters in brucellosis patients vary from patient to patient. However, the most frequent ab-

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normalities are announced as mild anemia, leukocytosis/leukopenia, and thrombocytopenia in literature [3,16,19].

The physiological immune responses of circulating leukocytes to various stressful events are characterized by an increased neutrophil count and decreased lymphocyte count. An increase in total leukocyte and neutrophils is an inflammatory reaction, particularly when caused by a bacterial infection. Brucella lipoproteins possess proinflammatory properties that could contribute to the localize tissue injury and inflammation by direct activation of neutrophils [20,21]. Lymphocytopenia has also been described as a diagnostic marker of bacterial infection [22,23]. In the lightning of these data, NLR is becoming a more valuable inflammation marker than neutrophilia or lymphocytopenia alone for predicting bacterial infection. As a result of changes caused by the inflammation in neutrophils, platelets, and lymphocytes, NLR and PLR have turned into inflammatory markers. Also, NLR and PLR can easily be calculated from a routine total blood count with no additional cost. Recently, NLR and PLR are used to predict the disease activity, prognosis, and survival rates in some systemic inflammatory diseases such as juvenile idiopatic artrit, Crohn disease; in cancer patients with hepatocellular, breast and other cancers; and in bacterial infections like tuberculosis and bloodstream infections.

In literature search, we found only two studies investigating NLR and PLR in brucellosis. In the study by Olt et al. [10], Hb and NLR were found to be significantly associated with brucellosis in adult patients. In the study by Aktar et al. [24], NLR and PLR were found as indirect markers of inflammation in children with brucella arthritis. There was no other study investigating the NLR and PLR values in children with brucellosis. In our study, we found that Hb and NLR are significantly different in children with brucellosis from healthy control children ($p < 0.05$). Also, we evaluated the, NLR and PLR in our patients by dividing the group as arthritis positive and arthritis negative. Similar to the study of Aktar et al. [24], we found that NLR is significantly different in arthritis positive group compared to the control group ($p = 0.002$). However, different from the previous studies, we found no significant difference in PLR either between patients and control group, or arthritis positive/arthritis negative and control group ($p > 0.05$).

As another hematological parameter, MPV was investigated in many systemic inflammatory diseases, also in brucellosis. Previous studies reported that interferon-γ, tumor necrosis factor α, interleukin 1 (IL-1), and IL-12 secreted in brucellosis. Over releasing of these proinflammatory cytokines might influence the maturation of platelets and result in a reduction in size of platelets during brucellosis infection [25,26]. In literature search, we found four studies investigating MPV in brucellosis patients. In the study by Okan et al. [27] there was statistically significant difference in MPV ($p < 0.001$) and platelet count ($p < 0.05$) between a total of 96 adult brucellosis patient and control group. In the study by Kucukbayrak et al. [28] platelet count and MPV were found significantly meaningfull markers of inflammation in a total of 40 brucellosis patients. However, in the study by Togan et al. [29] MPV was found to be insignificant ($p = 0.897$) in a total of 250 patients with brucellosis. In our study, we found the MPV significantly different in children with brucellosis from healthy control children ($p = 0.049$). However, different from NLR value, the difference for MPV was more significant between the arthritis negative and control group ($p = 0.034$).

The limitations of our study are its resrospective nature and the relatively small sample size of patients; especially with brucella arthritis.

In conclusion, MPV and NLR can be used as inflammatory markers of brucellosis, and NLR can be used especially in patients with arthritis on admission. Further prospective studies are needed to compare our results and make decision.

KEY MESSAGE

1. Brucellosis can cause anemia, thrombocytopenia, and neutrophilia in children.
2. Changes of mean platelet volume and neutrophill to lymphocyte ratio (NLR) can be used as inflammatory markers in childhood brucellosis.
3. NLR is a sensitive marker of inflammation in children with brucella arthritis.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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