

# Inflammation and anemia in simple febrile seizures and complex febrile seizures

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## Abstract

**Aim:** This is a unique study that aimed to determine anemia and inflammatory status in simple febrile seizure vs complex febrile seizure patients. Neutrophil/lymphocyte ratio and platelet/lymphocyte ratio are positively correlated with inflammatory markers including TNF alpha and IL-6. They are practical, inexpensive, and valuable tools for evaluating inflammation.

**Materials and Methods:** Patients presenting with first febrile seizures were enrolled retrospectively. We investigated hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration and red blood cell distribution width values and white blood cell count, neutrophil, lymphocyte count, neutrophil/lymphocyte ratio, platelet/lymphocyte ratio, and mean platelet volume, C - reactive protein.

**Results:** Our study showed that higher neutrophil/lymphocyte ratio, platelet/lymphocyte ratio, and lower mean platelet volume values in complex febrile seizure cases than simple febrile seizure cases. We determined cut-off values for neutrophil/lymphocyte ratio, platelet/lymphocyte ratio, and mean platelet volume of 2.5, 10523.3, and 7.3 respectively.

**Conclusion:** High neutrophil/lymphocyte ratio, platelet/lymphocyte ratio, and low mean platelet volume values can help distinguish simple febrile seizure and complex febrile seizure patients and predict the clinic. The optimal cut-off values that we determined may guide clinicians.

**Keywords:** Anemia; inflammation; febrile seizure

## INTRODUCTION

Febrile seizures (FS) are the most common seizure disorder in children between 6 months to 6 years of age, affecting %2-5 of the pediatric population (1,2). According to the clinical presentation, FS are divided into two groups. Simple febrile seizures (SFS) have a short duration of fewer than 15 minutes. The seizures are generalized and they do not recur within the same febrile illness. Complex febrile seizures (CFS) have a longer duration, more than 15 minutes, may present with focal signs, and recur in the same febrile illness. (2,3).

The exact underlying mechanism of FS seems to be multifactorial. Infectious agents, genetic factors, vaccines, anemia, and inflammation were proposed as possible causes (3-5).

Iron deficiency is one of the most frequent micronutrient deficiencies. Iron is essential for neurogenesis and differentiation of brain cells. Iron deficiency causes abnormal brain structure and alters hippocampal development, metabolism of synaptic neurotransmitters

including norepinephrine, dopamine, serotonin, glutamine, and gamma-aminobutyric acid (6). The higher predominance of anemia in CFS was demonstrated in previous studies (5).

SFS was reported to be followed by epilepsy in about 2% of patients, while epilepsy development after CFS was reported from 4% to 12% of individuals (7). Prolonged FS directly damage the hippocampus and surrounding structures (8). Inflammation plays a role in the pathogenesis of FS and epilepsy after FS (9,10). As the fever is induced by cytokines like TNF- $\alpha$ , IL-1 $\beta$ , IL-6, these inflammatory cytokines lead to the development of FS (11,12). Endogenous pyrogenic cytokine; IL-1 $\beta$  is involved in the febrile response generation of FS (13). Cytokine gene polymorphisms, which affect the amount of cytokine produced, are thought to be related to the development of FS (9,14). However, there are conflicting results in the literature. Nur et al. showed the role of IL-6 single nucleotide polymorphisms in the pathogenesis of FS (14). Haspolat et al. reported that gene polymorphisms of IL-1 $\beta$ , IL-1 $\alpha$ , and IL-1Ra did not show a significant

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role in FS (15). But they are impractical and expensive to evaluate. Cytokines alter white blood cell, neutrophil and lymphocyte counts. Leukocytosis, neutrophilia, lymphopenia and high C- reactive protein (CRP) levels have been widely used in various febrile and inflammatory disorders (16-18). On the other hand, neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) are practical, inexpensive and valuable tools for evaluating inflammation (19). NLR and PLR positively correlate with inflammatory markers including TNF alpha and IL-6 (19,20). NLR, PLR and anemia presence were not studied together in children with FS before.

In the present study, we aimed to investigate the biomarkers that might be useful for detecting inflammation in SFS and CFS patients. We also evaluated anemia presence in patients with SFS and CFS.

## MATERIALS and METHODS

In our retrospective study, we enrolled 66 children with FS, who were consulted to Dokuz Eylul University Pediatric Neurology Clinics between the years of 2014 and 2015. Patients with a chronic or systemic disease and a history of epilepsy, prematurity, central nervous system (CNS) abnormalities, CNS infections, CNS hemorrhage, electrolyte disorders, toxic encephalopathies, static encephalopathy, stroke, mental retardation, hydrocephalus were excluded. This study was approved by the Local Ethics Committee of Dokuz Eylul University.

The patients were divided into two groups as SFS and CFS groups. A single FS with no focal features and lasting less than 15 minutes were defined as SFS. If the FS lasted more than 15 minutes, or had focal features or repeated in the first 24 hours, it was defined as CFS (2,3). Age, sex, birth weight, family history of epilepsy and FS were recorded. Complete blood counts and CRP levels were investigated at administration. Hemoglobin (Hgb), hematocrit (Htc), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), neutrophil, lymphocyte, platelet (PLT), mean platelet volume (MPV) and CRP results were recorded.

Based on the former studies Hgb lower than 10.5 g/dL for 6-24 months and lower than 11.5 g/dL for 25-72 months of age, Htc lower than 33 % for 6-24 months and lower than 34% for 25-72 months of age, MCV lower than 70 fL for 6-24 months and lower than 75 fL for 25-72 months of age, MCHC lower than 30 g/dL for 6-24 months and lower than 31 g/dL for 25-72 months of age were determined as anemia (1,9). Normal MPV ranged between 7.4-10.4 fL and the normal CRP level was under 8 mg/L. As red cell distribution width varies per laboratory, we used a cut-off value identified by each laboratory with the lowest acceptable value being %14.3 in our laboratory. NLR was defined as the absolute neutrophil count divided by the absolute lymphocyte count. PLR was defined as the platelet count divided by absolute lymphocyte count.

SPSS 22.0 for Windows was used to analyze the data. The distribution of the dependent variables was evaluated by

the Kolmogorov-Smirnov test. Results were expressed as mean and standart deviation. Chi-Square Test was used to compare categorical variables. Analysis for the association between the seizure types and laboratory findings was done by the Mann-Whitney-U test. p values less than 0.05 were considered to be statistically significant. Cohen's d test was used to indicate the standardized difference between the two mean values of our groups. ROC curve analysis was used for calculating the optimal cut-off values, sensitivity and specificity of NLR, PLR and MPV.

## RESULTS

### Patients and demographic characteristics

Sixty-six children (mean age  $25.6 \pm 2.0$  months) with FS were evaluated. Thirty-four (53.1%) patients were in the SFS group. Thirty (46.9%) patients were male. There were 5 (9.6%) patients with a family history of epilepsy and 28 (53.8%) patients with a family history of FS. Anemia was found in 12 (18.8%) children with FS. There was no statistically significant difference regarding age, gender, birth weight and family history of epilepsy and febrile seizure between the two groups (Table 1).

**Table 1. The demographic characteristics of Simple febrile seizure and Complex febrile seizure groups**

	SFS (n:34)	CFS (n:32)	P
Age (months)	25.5 ±18.3	25.8 ±14.2	0.942
Male (n/%)	19 (55.9%)	11 (36.7%)	0.141
Birth weight (grams)	3097.3 ±636.9	3228.6 ±606.6	0.530
Family history of epilepsy (n/%)	3 (13%)	2 (6.9%)	0.644
Family history of febrile seizure (n/%)	12 (52.2%)	16 (55.2%)	1.000
Anemia	5 (14.7%)	7 (23.3%)	0.523

n: number, SFS: simple febrile seizures, CFS: complex febrile seizures

### Laboratory Findings

There was no statistically significant difference regarding Hg, Htc, MCV, MCHC, RDW, PLT, CRP, and WBC values. Neutrophil count ( $56.7 \pm 16.5$  % vs.  $68.6 \pm 15.1$  %,  $p= 0.004$ ), NLR ( $2.6 \pm 2.6$  vs.  $4.5 \pm 4.1$ ,  $p= 0.035$ ), and PLR ( $10802 \pm 8209.2$  vs  $19209.3 \pm 18919.7$ ,  $p=0.022$ ) were found higher and lymphocyte count ( $31.8 \pm 15.3$  % vs.  $22.8 \pm 11.7$  % ,  $p= 0.012$ ), and MPV ( $7.5 \pm 0.7$  vs  $7.1 \pm 0.6$ ,  $p=0.027$ ) were found lower in CFS group than SFS group. Mean values and standart deviations of laboratory findings are listed in Table 2.

According to the ROC curve analysis, the optimal cut-off value for NLR, PLR and MPV were found to be 2.5, 10523.3 and 7.3 respectively (Figure 1-3) (Table 3).

Table 2. Mean values and standart deviations of laboratory findings				
	SFS (n:34)	CFS (n:32)	p	Cohen's d <sup>†</sup>
Hb (g/dL)	11.4 (±0.9)	11.3 (±0.9)	0.668	
Htc (%)	33.7 (±3)	33.5 (±2.6)	0.853	
MCV (fL)	76.2 (±5.8)	77.9 (±4.7)	0.197	
RDW (%)	14.4 (±1.8)	14.4 (±1.2)	0.950	
MCHC (g/dL)	33.7 (±2.2)	33.8 (±1.1)	0.878	
PLT (mm <sup>3</sup> /dL)	272421.3 (±97751.4)	302033.3 (±1008346)	0.239	
MPV (fL)	7.5 (±0.7)	7.1 (±0.6)	<b>0.027*</b>	<b>0.58</b>
CRP (mg/dL)	8.5 (±3.4)	8.5 (±3.6)	0.987	
WBC (mm <sup>3</sup> /dL)	18447 (±31219.5)	12730 (±5260.8)	0.326	
Neu (%)	56.7 (±16.5)	68.6 (±15.1)	<b>0.004*</b>	<b>0.75</b>
Lym (%)	31.8 (±15.3)	22.8 (±11.7)	<b>0.012*</b>	<b>0.65</b>
NLR	2.6 (±2.6)	4.5 (±4.1)	<b>0.035*</b>	<b>0.54</b>
PLR	10802 (±8209.2)	19209.3 (±18919.7)	<b>0.022*</b>	<b>0.57</b>

\* Difference is statistically significant; <sup>†</sup> Only results with a modarate effect size between groupswere added; n: number, SFS: simple febrile seizures, CFS: complex febrile seizures, dL: deciliter, fL: femtoliter, g: grams, L: liter, mg: miligrams, Hgb: hemoglobin, Htc: hematocrit, MCV: mean corpuscular volume, MCHC: mean corpuscular hemoglobin concentration, MPV: mean platelet volume, RDW: red cell distribution width , PLT: platelet, NLR: neutrophil to lymphocyte ratio, PLR: platelet to lymphocyte ratio

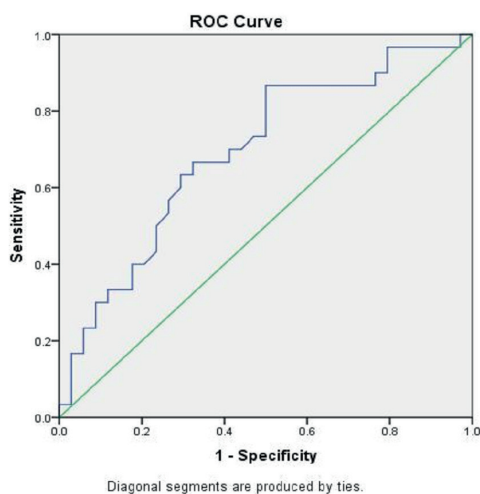


Figure 1. ROC curve analysis for Neutrophil/Lymphocyte ratio in differentiating simple febrile seizure and complex febrile seizure. AUC (95%CI): 0.694 (0.564-0.824)

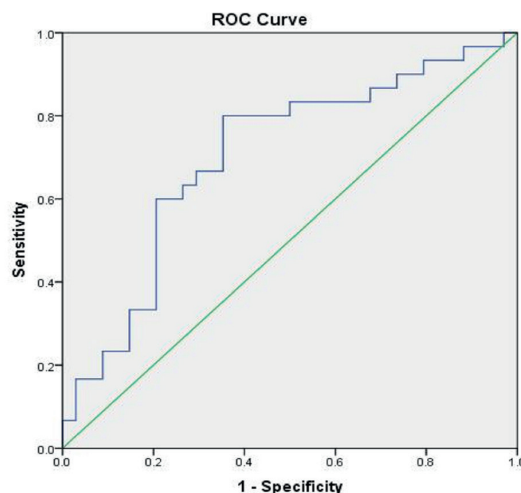


Figure 2. ROC curve analysis for Platelet/Lymphocyte ratio in differentiating simple febrile seizure and complex febrile seizure. AUC (95%CI): 0.704 (0.573-0.835)

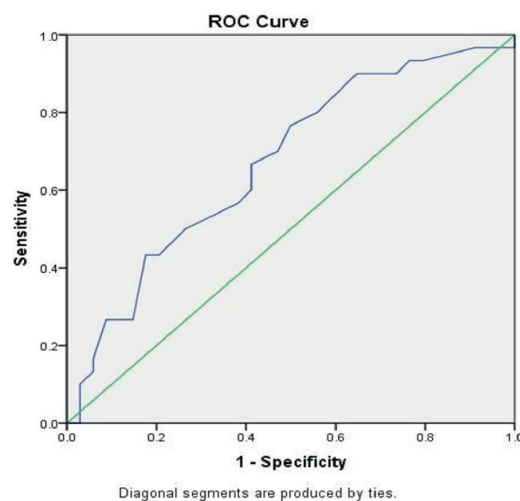


Figure 3. ROC curve analysis for Mean platelet volume in differentiating simple febrile seizure and complex febrile seizure. AUC (95%CI): 0.670 (0.537-0.802)

Table 3. ROC curve analysis results of NLR, PLR and MPV in simple febrile seizure and complex febrile seizure groups					
Risk Factor	AUC (95% CI)	Cut-off	p	Sensitivity (%)	Specificity (%)
NLR	0.694 (0.564-0.824)	2.5	<b>0.008</b>	66.7	67.6
PLR	0.704 (0.573-0.835)	10523.3	<b>0.005</b>	66.7	67.6
MPV	0.670 (0.537-0.802)	7.3	<b>0.020</b>	60	58.8

ROC: receiver operating characteristic, AUC: area under curve, CI: confidence interval, MPV: mean platelet volume, NLR: neutrophil to lymphocyte ratio, PLR: platelet to lymphocyte ratio

## DISCUSSION

This is a unique study that aimed to determine both anemia and inflammation in SFS vs CFS patients by using simple biomarkers including complete blood cell count, NLR and PLR which can be easily obtained. Our study showed that higher neutrophil count, NLR, PLR and lower lymphocyte count and MPV can be considered as independent biomarkers of inflammation in patients with CFS.

Iron deficiency anemia was found to be related to raising the threshold for the first febrile seizure. Fallah et al. demonstrated that iron deficiency and iron deficiency anemia was significantly higher in febrile patients with seizure. And it was found to be more common in patients with CFS (21). Daoud et al showed that anemia was significantly related to first febrile seizure (22). Ozaydin et al found that Hgb, Htc, MCV levels were lower and RDW levels were higher in children with CFS than those with SFS. These studies suggest that anemia may be a risk factor for CFS (17). In the present study microcytic anemia was found in %14.7% and %23.3% of the SFS and CFS patients respectively. Although we did not have a control group, the percentage of anemia was not different from previous studies which reported anemia between 15.5%-48% in Turkish children (23).

In animal models, hyperthermic seizures as a model of CFS were found to be related to alterations in brain structure. Inflammation in early life was found to be associated with increased brain excitability in adulthood (24). Pavlidou et al. reported that CFS increases the risk for epilepsy 3.6 times (25). In the previous studies, the relationship between FS and inflammation has been reported. It is therefore important that CFS is predictable. Higher NLR and PLR values were found in the CFS group. In the differentiation of SFS and CFS, the optimal cut-off values for NLR and PLR were 2.5 and 10523.3, respectively. In the previous FS studies, cut-off values for NLR were reported 1.98 and 2.31 (26,27). In our study both the cut-off value, sensitivity and specificity for NLR were slightly higher. Liu et al showed higher NLR and MPV in all FS patients than control (28). Similar to their study, we got the same cut-off value of NLR for differentiating CFS from SFS.

The MPV values reflect the size of platelets which was shown to be inversely correlated with inflammation (29). High-grade inflammatory conditions are associated with the circulation of predominantly small platelets with low levels of MPV (19). Low MPV values were reported in various inflammatory diseases including cardiovascular diseases, rheumatologic diseases, cancer and some infectious diseases (29-31). There are conflicting reports about MPV in patients with FS. Ozaydin et al. reported lower levels of MPV as an inflammatory biomarker in patients with CFS (17). In Chinese children, MPV was found to be higher in SFS group (28). Other 2 studies from Turkey revealed no difference in MPV values for SFS and CFS patients (26,27). In our study patients with CFS had lower MPV values, and the cut-off value of 7.3 may be useful for differentiating CFS.

## LIMITATIONS

Limitations in our study should be addressed, as it is a retrospective study, serum cytokines and serum iron were not measured and we could not compare our findings with healthy subjects. We could not evaluate the other parameters of anemia and inflammation. Also, we would like to underline that our study has a small sample size.

Our study has some strength. It is the first study that investigated both anemia and inflammation together in FS patients. Diagnostic testing is not needed in SFS. However, the physicians following patients with FS may not be sure how long follow-up time should be. The present study provided some suggestive parameters.

## CONCLUSION

High NLR, PLR and low MPV values compared to cut-off values can guide in terms of CFS. We determined cut-off values for NLR, PLR and MPV in our study. Cases presenting with the first FS and high NLR, PLR and low MPV values may be at risk for developing CFS and should be followed for a longer period.

*Competing Interests: The authors declare that they have no competing interest.*

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*Ethical Approval: Local Ethics Committee of Dokuz Eylul University approved the study. Approval date and number: 15.06.2017, 2017/16-03.*

## REFERENCES

1. Bidabadi E, Mashouf M. Association between iron deficiency anemia and first febrile convulsion: A case-control study. *Seizure* 2009;18:347-51.
2. Pavlidou E, Hagel C, Panteliadis C. Febrile seizures: recent developments and unanswered Questions. *Childs Nerv Syst* 2013;29:2011-17.
3. McClellanda S, Dubéa CM, Yanga J, Baram TZ. Epileptogenesis after prolonged febrile seizures: Mechanisms, biomarkers and therapeutic opportunities. *Neuroscience Letters* 2011;497:155-62.
4. Hesdorffer DC, Shinnar S, Lewis DV, et al. Consequences of Prolonged Febrile Seizures in Childhood (FEBSTAT) Study Team. Risk Factors for Febrile Status Epilepticus: A Case-Control Study. *J Pediatr* 2013;163(4):1147-51.
5. Sfaihi L, Maaloul I, Kmiha S, et al. Febrile seizures: an epidemiological and outcome study of 482 cases. *Childs Nerv Syst* 2012;28:1779-84.
6. Johnston MV. Iron Deficiency, Febrile Seizures and Brain Development. *Indian Pediatr* 2012;49:13-4.
7. Kuang YQ, Kong B, Yang T, Cheng L, Gu JW, Zhou HT, et al. Epileptiform Discharges and Frontal Paroxysmal EEG Abnormality Act as Predictive Marker for Subsequent Epilepsy in Children with Complex Febrile Seizures. *Clin EEG Neurosci* 2014;45:299-303.

8. Yoong M, Martinos MM, Chin RF, Clark CA, Scott RC. Hippocampal volume loss following childhood convulsive status epilepticus is not limited to prolonged febrile seizures. *Epilepsia* 2013;54:2108-15.
9. Chou IC, Lin WD, Wang CH, et al. Interleukin (IL)-1b, IL-1 Receptor Antagonist, IL-6, IL-8, IL-10, and Tumor Necrosis Factor a Gene Polymorphisms in Patients With Febrile Seizures. *J Clin Lab Anal* 2010;24:154-9.
10. Vezzani A, Maroso M, Balosso S, et al. IL-1 receptor/Toll-like receptor signaling in infection, inflammation, stress and neurodegeneration couples hyperexcitability and seizures. *Brain Behav Immun* 2011;25:1281-9.
11. Atici Y, Alehan F, Sezer T, T et al. Serum S100B levels in children with simple febrile seizures. *Seizure* 2012;21:175-7.
12. Teran CG, Medows M, Wong SH, et al. Current Role of the Laboratory Investigation and Source of the Fever in the Diagnostic Approach. *Pediatr Emerg Care* 2012;28:493-7.
13. Dubé CM, Ravizza T, Hamamura M, Zha Q, Keebaugh A, Fok K, et al. Epileptogenesis provoked by prolonged experimental febrile seizures: mechanisms and biomarkers. *J Neurosci* 2010;30:7484-94.
14. Nur BG, Kahramaner Z, Duman O, Dundar NO, Sallakci N, Yavuzer U, et al. Interleukin-6 Gene Polymorphism in Febrile Seizures. *Pediatric Neurology* 2012;46:36-8.
15. Haspolat S, Baysal Y, Duman O, et al. Interleukin-1a, Interleukin-1b, and Interleukin-1Ra Polymorphisms in Febrile Seizures. *J Child Neurol* 2005;20:565-8.
16. Feng JF, Huang Y, Liu JS. Combination of neutrophil lymphocyte ratio and platelet lymphocyte ratio is a useful predictor of postoperative survival in patients with esophageal squamous cell carcinoma. *Oncol Targets Ther* 2013;6:1605-12.
17. Ozaydin E, Arhan E, Cetinkaya B, et al. Differences in iron deficiency anemia and mean platelet volume between children with simple and complex febrile seizures. *Seizure* 2012;21:211-4.
18. Tosun A, Koturoglu G, Serdaroglu G, et al. Ratios of Nine Risk Factors in Children With Recurrent Febrile Seizures. *Pediatr Neurol* 2010;43:177-82.
19. Bucak A, Ulu S, Oruc S, et al. Neutrophil-to-Lymphocyte Ratio as a Novel-Potential Marker for Predicting Prognosis of Bell Palsy. *Laryngoscope* 2014;124:1678-81.
20. Farah R, Khamisy-Farah R. Association of Neutrophil to Lymphocyte Ratio With Presence and Severity of Gastritis Due to Helicobacter pylori Infection. *J Clin Lab Anal* 2014;28:219-23.
21. Fallah R, Tirandazi B, Akhavan Karbasi S, Golestan M. Iron Deficiency and Iron Deficiency Anemia in Children with Febrile Seizure. *Iran J Ped Hematol Oncol* 2013;3:200-3.
22. Daoud AS, Batieha A, Abu-Ekteish F, et al. Iron Status: A Possible Risk Factor for the First Febrile Seizure. *Epilepsia* 2002;43:740-3.
23. Akin F, Solak ES, Kilicaslan C, Boke SB, Arslan S. Iron Deficiency Anemia among Hospitalized Children in Konya, Turkey. *Anemia* 2013;2013:514801.
24. Reid AY, Riazi K, Campbell Teskey G, Pittman QJ. Increased excitability and molecular changes in adult rats after a febrile seizure. *Epilepsia* 2013;54:45-8.
25. Pavlidou E, Panteliadis C. Prognostic factors for subsequent epilepsy in children with febrile seizures. *Epilepsia* 2013;54:2101-7.
26. Yigit Y, Yilmaz S, Akdogan A, et al. The role of neutrophil-lymphocyte ratio and red blood cell distribution width in the classification of febrile seizures. *Eur Rev Med Pharmacol Sci* 2017;21:554-9.
27. Goksugur SB, Kabakus N, Bekdas M, Demircioglu F. Neutrophil-to-lymphocyte ratio and red blood cell distribution width is a practical predictor for differentiation of febrile seizure types. *Eur Rev Med Pharmacol Sci* 2014;18:3380-5.
28. Liu Z, Li X, Zhang M, et al. The role of Mean Platelet Volume/platelet count Ratio and Neutrophil to Lymphocyte Ratio on the risk of Febrile Seizure. *Sci Rep* 2018;8:15123.
29. Gunluoglu G, Yazar EE, Veske NS, et al. Mean platelet volume as an inflammation marker in active pulmonary tuberculosis. *Multidiscip Respir Med* 2014;9:11.
30. Oncel MY, Ozdemir R, Yurttutan S, et al. Mean platelet volume in neonatal sepsis. *J Clin Lab Anal* 2012;26:493-6.
31. Kisacik B, Tufan A, Kalyoncu U, et al. Mean platelet volume (MPV) as an inflammatory marker in ankylosing spondylitis and rheumatoid arthritis. *Joint Bone Spine* 2008;75:291-4.