

Original Article

Are the Neutrophil-to-lymphocyte, Platelet-to-lymphocyte and Monocyte-to-lymphocyte Ratios Predictive Factors for the Retinopathy of Prematurity?

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Abstract

Introduction: In this study, we aimed to evaluate the possible relationship between the neutrophil-to-lymphocyte, monocyte-to-lymphocyte and platelet-to-lymphocyte ratio and retinopathy of prematurity (ROP). **Material and Method:** The data of 348 patients who applied to Erzincan University Ophthalmology Unit for screening the retinopathy of prematurity were analysed retrospectively. One hundred sixty seven patients who were collected CBC samples within the first 72 hours after delivery and who met the inclusion criteria were included in the study. Infants with a gestational age of ≤ 35 week were screened for ROP. Patients were divided into two groups. Group 1 was involved the patients with the diagnosis of ROP and group 2 was involved the normal patients (no ROP). The levels of NLR, PLR and MLR were determined in two groups. **Results:** Fifty-nine patients who were detected ROP are in group 1 and 108 patients who were not seen ROP are in group 2 in the study. The mean gestational age at birth of the patients was 30.4 ± 2.1 [26-34] weeks in group 1 and 33.7 ± 1.7 [27-35] weeks in group 2 ($P=0.004$). The mean gestational weight at birth was 1927.2 ± 158.4 [1690-2300] gram in group 1 and 2169.1 ± 283.1 [1750-3100] gram in group 2. ($P<0.001$) With the result of logistic regression analysis, gestational age [Odds Ratio(OR): 0.531, 95%CI: 0.388-0.726, $P<0.001$], NLR [OR:34.849, 95%CI: 2.091-580.779, $P=0.013$] and PLR [OR: 1.067, 95%CI: 1.034-1.110, $P<0.001$] were detected the independent risk factors for ROP. **Conclusion:** Our study revealed that higher NLR and PLR which are evaluated with in first 72h after birth is an independent predictor of ROP.

Key words

Lymphocyte; Monocyte; Neutrophil; Platelet; Retinopathy of prematurity

Introduction

Retinopathy of prematurity (ROP) is a vasoproliferative retinal disease characterised by an interception of normal retinal vascular development and subsequent disorganised vascular growth. ROP is a primary cause of preventable

vision loss worldwide in children and affects thousands of infants.^{1,2} The progression of ROP has been connected to different factors such as angiogenic factors, cytokines, and neuroprotective and oxidative growth factors.³ In recent years, inflammatory factors have also been stated to be associated with ROP.³⁻⁶

Complete blood count (CBC) is an easy, economical, and commonly used principal haematological test comprising the quantitative determination of white blood cells (WBC), red blood cells (RBC), and platelets. WBC contain neutrophils, lymphocytes, monocytes, eosinophils and basophils. WBC are found in blood, lymphatic fluid and all tissues of the body. Leukocyte count in the blood is an important indicator especially in infectious diseases.^{7,8} In healthy humans, the most plenty white blood cells are neutrophils, which play important roles during acute and chronic inflammatory reactions. The neutrophil-

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to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and monocyte-to-lymphocyte ratio (MLR) are the respective proportions of absolute neutrophil to lymphocyte, platelet to lymphocyte, and monocyte to lymphocyte counts obtained from the evaluation of the CBC test. As markers of inflammation, many studies have shown the relationship between NLR, PLR and MLR and many diseases, such as inflammatory and cardiovascular diseases, and cancer.⁷⁻¹⁰ The ratios of WBC have been recommended as indicators of general inflammatory reactions.¹¹ In this retrospective study, we purposed to evaluate the correlation between NLR, MLR and PLR and ROP.

Material and Method

The data of 348 infants that were referred to the Ophthalmology Unit of Erzincan University, Turkey between June 2018 and July 2019 for the screening of ROP were retrospectively analysed. A total of 167 patients, from whom CBC samples were collected within the first 72 hours after birth and who met the inclusion criteria were included in the study. Fifty-nine patients were found to have ROP on the first examination, but none had the severe form of the disease. This study was created in accordance with the Declaration of Helsinki, and verbal informed consent was taken from the parents of each patient before the study. All infants were participants who applied for ROP screening and had blood tests available. Performing the study no extra blood sample were needed and all of the parents accept including in the study of the babies. All the infants had CBC samples which were collected in intensive care unit for newborns. The approval of the Institutional Ethics Committee of Erzincan University was also obtained.

Clinical Evaluation

Infants with a smaller gestational age than 35 weeks were examined for ROP after administering tropicamide 1% (Tropamid[®], Bilimilaç[®], Gebze, Turkey) eye drops and phenylephrine hydrochloride 0.5% (Mydfrin[®], Alcon[®], Fort Worth, TX) eye drops for three times for pupillary dilatation. The ROP examination was performed by the same experienced eye professional using a binocular indirect ophthalmoscope combined with a sclera depressor after administering proparacaine hydrochloride 0.5% (Alcaine[®], Alcon[®], Fort Worth, TX) eye drops as the topical anesthetic. The ROP status was graded according to the international

classification of ROP in all infants. The ROP status was noted including the stage, zone and extent of the disease, and the presence or absence of plus disease for each infant.¹² Each infant was also graded according to the maximum stage of ROP examined in either eye.

All blood samples were gathered and investigated within the first 72 h after birth. Peripheral venous blood samples (1 mL) was gathered in tubes containing dipotassium ethylenediamine tetraacetate (EDTA-2K). CBC were investigated by an automated haematology analyser. (Sysmex Corporation, Kobe 651-0073, Japan). The NLR values were figured out by dividing the neutrophil count to the lymphocyte count, the PLR values by dividing the platelet count to the lymphocyte count, and the MLR values by dividing the monocyte count to the lymphocyte count.

The patients were separated into two groups: Group 1 comprised those with the diagnosis of ROP and Group 2 consisted of patients without ROP. All patients accepted in the study were evaluated by fundoscopic examination at four weeks after birth. The patients were called for a control visit at an appropriate time according to the stage and zone of ROP (after one, two, three or four weeks. Retinal vascularisation reached the ora serrata in the follow-up of all the patients. No subjects were taken ROP treatment (intravitreal anti-VEGF/laser photocoagulation) in the study.

Exclusion Criteria

Infants born with haematologic disorders, received postnatal steroid therapy or a blood product transfusion before the ROP examination were excluded from the study. And also patients with respiratory distress syndrome (RDS), asphyxia neonatorum, neonatal pneumonia, premature rupture of membranes (PROM), necrotising enterocolitis, hypoxic-ischemic encephalopathy (HIE) and blood culture-proven sepsis were excluded from the study. Infants who did not attend follow-up regularly and who had any other accompanying ocular or systemic diseases were excluded from the study. Also the infants with maternal risk factors such as antenatal steroid use, fever/sepsis and chorioamnionitis etc. were excluded the study.

Statistical Analysis

The SPSS (Statistical Package for the Social Sciences) version 22.0 statistical software package was used for statistical analyses. The Kolmogorov-Smirnov test was applied to observe the distribution of the parameters in the study groups. Continuous variables were expressed as mean

± standard deviation (SD), and categorical variables as frequencies and percentages. The chi-square test was used to analyse the categorical variables. Inter-group comparisons of the continuous variables were performed using Student's t-test in addition to the analysis of variation (ANOVA) for homogenous data and the Mann-Whitney U test for non-homogenous data. The Friedman variance analysis test was performed to compare the variables in repeated measures, Wilcoxon's signed rank test for the pairwise comparisons of these variables, and the Pearson and Spearman correlation tests for the assessment of the correlations between the variables. Logistic regression analysis was performed to predict the significant independent risk factors associated with the existence of ROP. Exact *P* values of <0.05 were considered statistically significant. The adjusted odds ratio (OR) and the 95% confidence interval (CI) for each possible risk factor were calculated. A receiver operating characteristic (ROC) curve analysis was applied to define the cut-off values of NLR and PLR in predicting the stage of ROP. All of the results were appraised at a confidence interval of 95% based on a significance level of *P*<0.05.

Results

In group 1, 34 patients of the 59 patients were male and 25 were female. In group 2, 68 of the 108 patients were male and 40 were female. There wasn't any statistically significant difference between the two groups in terms of gender distribution (*P*=0.745). The mean gestational age

at birth was 30.4±2.1 [26-34] weeks in group 1 and 33.7±1.7 [27-35] weeks in group 2, and there was a statistically significant difference between the two groups (*P*=0.004). The mean gestational weight at birth was 1927.2±158.4 [1690-2300] and 2169.1±283.1 [1750-3100] gram in group 1 and 2, respectively (*P*<0.001).

The CBC test results of the patients are shown in Table 1.

When the CBC test results of the patients were evaluated, the mean neutrophil count was 3.74±1.85 (10⁹/L) in group 1 and 1.91±0.87 (10⁹/L) in group 2. The difference in the neutrophil count between the two groups was statistically significant (*P*<0.001). The average lymphocyte count was 4.86±1.56 (10⁹/L) in group 1 and 5.22±1.58 (10⁹/L) in group 2. There wasn't any statistically significant difference between the two groups in terms of the lymphocyte count (*P*=0.394). The mean monocyte count was 1.43±0.49 (10⁹/L) in group 1 and 0.86±0.46 (10⁹/L) in group 2, with no significant difference between the two (*P*=0.088). The average platelet count was 453.36±121.96 (10⁹/L) and 304.33±117.58 (10⁹/L) in group 1 and 2, respectively, and the difference was not statistically significant (*P*=0.744).

The NLR value of the patients was 0.87±0.59 in group 1 and 0.39±0.18 in group 2. NLR was significantly higher in infants with ROP (*P*<0.001). When the MLR of the patients was examined, this ratio was calculated as 0.32±0.15 for group 1 and 0.18±0.11 for group 2, and there was a significant difference between the two groups (*P*=0.009). The PLR value of the patients was 98.94±30.97 in group 1 and 59.41±18.97 in group 2. PLR was significantly higher in group 1 than in group 2 (*P*<0.001).

The logistic regression analysis was performed to

Table 1 Clinical findings and laboratory measurements of the patients

	Group 1	Group 2	Total	<i>P</i> value
Number of patients	59 (35.3%)	108 (64.7%)	167 (100%)	–
Gender (male/female)	34 (57.6%) / 25 (42.4%)	65 (60.2%) / 43 (39.8%)	99 (59.3%) / 68 (40.7%)	0.745
GA	30.4±2.1	33.7±1.7	32.2±2.3	0.004
GW	1927.2±158.4	2169.1±283.1	2083.6±270.9	<0.001
Neutrophil	3.74±1.85 (10 ⁹ /L)	1.91±0.87 (10 ⁹ /L)	2.55±1.56 (10 ⁹ /L)	<0.001
Lymphocyte	4.86±1.56 (10 ⁹ /L)	5.22±1.58 (10 ⁹ /L)	5.09±1.57 (10 ⁹ /L)	0.394
Monocyte	1.43±0.49 (10 ⁹ /L)	0.86±0.46 (10 ⁹ /L)	1.06±0.54 (10 ⁹ /L)	0.088
Platelet	453.36±121.96 (10 ⁹ /L)	304.33±117.58 (10 ⁹ /L)	356.98±138.2 (10 ⁹ /L)	0.744
NLR	0.87±0.59	0.39±0.18	0.56±0.44	<0.001
MLR	0.32±0.15	0.18±0.11	0.23±0.14	0.009
PLR	98.94±30.97	59.41±18.97	73.38±30.34	<0.001

GW: gestational weight; GA: gestational age; NLR: neutrophil to lymphocyte ratio; PLR: platelet to lymphocyte ratio; MLR: monocyte to lymphocyte ratio

determine the independent risk factors for ROP. According to the results of this analysis, gestational age (OR: 0.531, 95% CI: 0.388-0.726, $P < 0.001$), NLR (OR: 34.849, 95% CI: 2.091-580.779, $P = 0.013$), and PLR (OR: 1.067, 95% CI: 1.034-1.110, $P < 0.001$) were detected as the independent risk factors for ROP (Table 2).

The ROC curve analysis was used to determine whether the cut-off values of NLR and PLR could be used to predict ROP. An NLR over 0.53 indicated higher risk of ROP with 81.4% sensitivity and 83.3% specificity while a PLR over 81.48 indicated higher risk of ROP with 71% sensitivity and 90% specificity (Figures 1 and 2).

Table 2 Logistic regression analysis results for independent predictors of ROP

Variable	OR	95% CI	P value
GW	0.998	0.996-1.001	0.281
GA	0.531	0.388-0.726	<0.001
Sex	0.654	0.213-2.008	0.458
Neutrophil	0.327	0.022-4.901	0.419
Lymphocyte	0.309	0.043-2.235	0.244
Monocyte	42.341	0.275-6524.413	0.145
Platelet	1.022	0.990-1.056	0.181
NLR	34.849	2.091-580.779	0.013
PLR	1.067	1.034-1.110	<0.001
MLR	0.641	0.005-81.611	0.857

GW: gestational weight; GA: gestational age; OR: odds ratio ; 95% CI: 95% confidence interval; NLR: neutrophil to lymphocyte ratio; PLR: platelet to lymphocyte ratio; MLR: monocyte to lymphocyte ratio

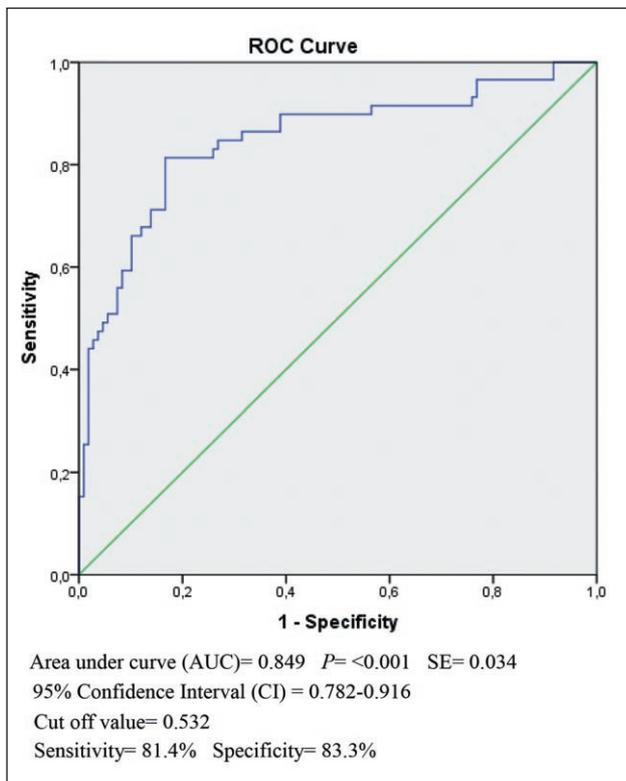


Figure 1 ROC curve analysis for NLR.

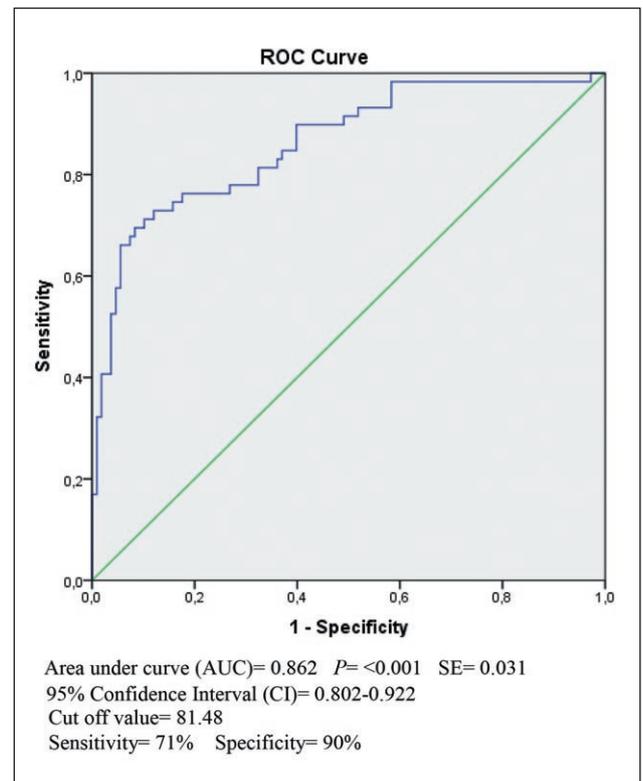


Figure 2 ROC curve analysis for PLR.

Discussion

It is certain that systemic inflammatory responses of maternal immune system and vigorous neonatal inflammatory reactions play a significant role in the development of ROP.¹²⁻¹⁵ Systemic inflammatory responses may affect the creation of abnormal retinal vascularisation and promote the risk of ROP, independent of gestational age and weight or intensity of early systemic disease.¹⁶ Woo et al also informed that umbilical cord blood cytokine levels and perinatal factors were significantly associated with the pathogenesis of ROP.¹⁷ NLR, PLR or MLR have been used in the prognostic diagnosis of cancer in many different branches of medicine and clinically significant results have been obtained.¹⁸⁻²¹

Previous studies indicated that increased neutrophil count and decreased lymphocyte count were associated with a systemic inflammatory response and physiological stress, respectively.²²⁻²⁵ In our study, the neutrophil count was meaningfully higher in the ROP group, but the logistic regression analysis indicated that the neutrophil count was not an independent risk factor for ROP. No significant difference was observed between the lymphocyte values of the groups with and without ROP. In the logistic regression analysis, the lymphocyte count was not associated with ROP, as in the neutrophil count. Similarly, the number of monocytes was not associated with ROP.

Guida et al found a relationship between sepsis and thrombocytopenia in babies with a very low birth weight, and they suggested that the pathogens that caused sepsis might affect the platelet kinetics.²⁶ In our study, no relationship was found between the presence of ROP and platelet count, and the exclusion of the infants with systemic problems, such as sepsis may have been effective in the emergence of this condition.

Several studies have shown that the NLR, PLR and MLR values, which are related to systemic inflammation, may be prognostic factors for ocular diseases, such as degenerative myopia, keratoconus and age-related macular degeneration.²⁷⁻²⁹

Kurtul et al examined the relationship between ROP and NLR and found no independent risk relationship.³ The authors only observed a relationship between the lymphocyte count and ROP. In the current study, the logistic regression analysis showed that PLR and NLR were independent risk factors for ROP. In addition, in the ROC analysis, the cut-off value was found to be 0.53 for NLR and 81.48 for PLR; however, no relationship was found between the lymphocyte count and ROP, unlike the results

reported by Kurtul et al.

The major limitation of our study is that severe ROP cases were not included in the sample. Some postnatal conditions, such as oxygen support, intraventricular haemorrhage, anaemia, apnoea, and sepsis have been shown to be associated with ROP progression.³⁰ These accompanying pathologies are likely to affect the NLR, PLR and MLR values. Accordingly, the patients with severe ROP were excluded from the study group. In addition, the relatively low number of participants and the absence of an evaluation of other inflammatory markers can be considered as limitations. Studies involving large-scale patient groups and infants with severe ROP and systemic diseases may be important in evaluating the relationship of ROP with NLR and PLR.

Conclusions

Our study revealed that increased NLR and PLR evaluated within the first 72 h after birth are independent predictors of ROP. In premature babies, the detection of NLR and PLR can be helpful to predict the possible development of ROP. Further investigations are needed including severe ROP cases for detecting the effects of CBC results on ROP progression.

Declarations

Ethics Approval and Consent to Participate

Informed consent was taken from the parents of each patient before the study. The approval of the Institutional Ethics Committee of Erzincan University was obtained (33216249-604.01.02-E.24306). Verbal informed consent was taken from the parents of each patient before the study.

Consent for Publication

Not applicable.

Availability of Data and Materials

All generated or analysed data during this study are included in the supporting file.

Competing Interests

In accordance with our ethical obligation as a researcher, all authors are reporting that we have not any financial or

non-financial conflicts of interest in this research. (Authors: Adem Uğurlu MD, Erel Icel MD).

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Authors' Contributions

AU and Eİ conceived and supervised this research. AU collected and analysed the data in the study. AU wrote the initial manuscript, revised by Eİ. All authors critically reviewed the manuscript for significant intellectual content and approved the final version.

References

- Kim SJ, Port AD, Swan R, Campbell JP, Chan RVP, Chiang MF. Retinopathy of prematurity: a review of risk factors and their clinical significance. *Surv Ophthalmol* 2018;63:618-37.
- Early Treatment For Retinopathy Of Prematurity Cooperative Group. Revised indications for the treatment of retinopathy of prematurity: results of the early treatment for retinopathy of prematurity randomized trial. *Arch Ophthalmol* 2003;121:1684-94.
- Kurtul BE, Kabatas EU, Zenciroglu A, et al. Serum neutrophil-to-lymphocyte ratio in retinopathy of prematurity. *JAAPOS* 2015; 19:327-31.
- Hu YX, Xu XX, Shao Y, et al. The prognostic value of lymphocyte-to-monocyte ratio in retinopathy of prematurity. *Int J Ophthalmol* 2017;10:1716-21.
- Lee J, Dammann O. Perinatal infection, inflammation, and retinopathy of prematurity. *Semin Fetal Neonatal Med* 2012;17: 26-9.
- Alan S, Tuna S, Türkoğlu EB. The relation of neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, and mean platelet volume with the presence and severity of Behçet's syndrome. *Kaohsiung J Med Sci* 2015;31:626-31.
- Wang X, Zhang G, Jiang X, Zhu H, Lu Z, Xu L. Neutrophil to lymphocyte ratio in relation to risk of all-cause mortality and cardiovascular events among patients undergoing angiography or cardiac revascularization: a meta-analysis of observational studies. *Atherosclerosis* 2014;234:206-13.
- Bowen RC, Little NAB, Harmer JR, et al. Neutrophil-to-lymphocyte ratio as prognostic indicator in gastrointestinal cancers: a systematic review and meta-analysis. *Oncotarget* 2017;8:32171-89.
- Bonaventura A, Liberale L, Carbone F, et al. Baseline neutrophil-to-lymphocyte ratio is associated with long-term T2D remission after metabolic surgery. *Acta Diabetol* 2019;56:741-8.
- Horne BD, Anderson JL, John JM, et al. Which white blood cell subtypes predict increased cardiovascular risk? *J Am Coll Cardiol* 2005;45:1638-43.
- International Committee for the Classification of Retinopathy of Prematurity. The International Classification of Retinopathy of Prematurity revisited. *Arch Ophthalmol* 2005;123:991-9.
- Hellström A, Smith LE, Dammann O. Retinopathy of prematurity. *Lancet* 2013;382:1445-57.
- Courtright P, Hutchinson AK, Lewallen S. Visual impairment in children in middle- and lower-income countries. *Arch Dis Child* 2011;96:1129-34.
- Cavallaro G, Filippi L, Bagnoli P, et al. The pathophysiology of retinopathy of prematurity: an update of previous and recent knowledge. *Acta Ophthalmol* 2014;92:2-20.
- Chen J, Stahl A, Hellstrom A, Smith LE. Current update on retinopathy of prematurity: screening and treatment. *Curr Opin Pediatr* 2011;23:173-8.
- Tremblay S, Miloudi K, Chaychi S, et al. Systemic inflammation perturbs developmental retinal angiogenesis and neuroretinal function. *Invest Ophthalmol Vis Sci* 2013;54:8125-39.
- Woo SJ, Park KH, Lee SY, et al. The relationship between cord blood cytokine levels and perinatal factors and retinopathy of prematurity: a gestational age-matched case-control study. *Invest Ophthalmol Vis Sci* 2013;54:3434-9.
- Ying HQ, Deng QW, He BS, et al. The prognostic value of preoperative NLR, d-NLR, PLR and LMR for predicting clinical outcome in surgical colorectal cancer patients. *Med Oncol* 2014; 31:305.
- Song X, Zhang GM, Ma XC, et al. Comparison of preoperative neutrophil-lymphocyte, lymphocytomonocyte, and platelet-lymphocyte ratios in patients with upper urinary tract urothelial carcinoma undergoing radical nephroureterectomy. *Oncol Targets Ther* 2016;9:1399-407.
- Yamagishi T, Fujimoto N, Nishi H, et al. Prognostic significance of the lymphocyte-to-monocyte ratio in patients with malignant pleural mesothelioma. *Lung Cancer* 2015;90:111-7.
- Seng D, Fang Q, Li P, Liu F, Liu S. Prognostic Value of the Pretreatment Neutrophil-to-Lymphocyte Ratio in Pediatric Parotid Cancer. *Front Pediatr* 2019;24:7:207.
- Alan S, Tuna S, Türkoğlu EB. The relation of neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, and mean platelet volume with the presence and severity of Behçet's syndrome. *Kaohsiung J Med Sci* 2015;31:626-31.
- Azab B, Camacho-Rivera M, Taioli E. Average values and racial differences of neutrophil lymphocyte ratio among a nationally representative sample of United States subjects. *PLoS One* 2014; 9:e112361.
- Fu H, Qin B, Hu Z, et al. Neutrophil- and platelet-to lymphocyte ratios are correlated with disease activity in rheumatoid arthritis. *Clin Lab* 2015;61:269-73.
- Qin B, Ma N, Tang Q, et al. Neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) were useful markers in assessment of inflammatory response and disease activity in SLE patients. *Mod Rheumatol* 2016;26:372-6.
- Guida JD, Kunig AM, Leef KH, McKenzie SE, Paul DA. Platelet count and sepsis in very low birth weight neonates: is there an organism-specific response? *Pediatrics* 2003;111:1411-5.
- Icel E, Ucak T, Karakurt Y, Yilmaz H, Tasli NG, Turk A. The Relation of Neutrophil to Lymphocyte Ratio and Platelet to Lymphocyte Ratio with High Axial Myopia. *Ocul Immunol Inflamm* 2019;17:1-6.
- Karaca EE, Ozmen MC, Ekici F, Yuksel E, Turkoglu Z. Neutrophil-to-lymphocyte ratio may predict progression in patients with keratoconus. *Cornea* 2014;33:1168-73.
- Ilhan N, Daglioglu MC, Ilhan O, et al. Assessment of neutrophil/lymphocyte ratio in patients with age-related macular degeneration. *Ocul Immunol Inflamm* 2015;23:287-90.
- Dogra MR, Katoch D, Dogra M. An Update on Retinopathy of Prematurity (ROP). *Indian J Pediatr* 2017;84:930-6.