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Original Article

# Evaluation of Hematological Parameters and Characteristics of Preterm Infants Born at ≥ 32 Weeks' Gestation with Retinopathy of Prematurity

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## ABSTRACT

***Background:*** To investigate the possible effect of hematological parameters and other risk factors on the development of retinopathy of prematurity (ROP) in preterm infants born at 32 weeks' gestational age (GA) and older.
***Methods:*** Infants having a birth weight (BW) above 1250 g and born at 32–35 weeks' GA with any stage of ROP disease were included in the study, while infants without ROP and born at 32–35 weeks' GA were randomly selected to form the control group (non-ROP group). Complete blood cell count (CBC) parameters obtained on the first day of preterms' life were analysed, and delivery type, cause of preterm birth, total number of births and smoking status during pregnancy were recorded. Infant-related factors, such as the GA at birth, BW) and total time spent in NICU were also recorded, and all parameters were compared between groups.
***Results:*** In total, 50 preterm infants were included in the study, 20 in the ROP group and 30 in the control group (non-ROP). No significant differences in hematological parameters were found between groups, though red blood cell (RBC) transfusion was found to be more common in the ROP group (p: 0.03).
***Conclusion:*** There were no statistically significant differences in hematological parameters measured on the infants' first day of life following preterm birth at 32–35 weeks' GA and a 1250 g BW. Therefore, RBC transfusion may be a risk factor for ROP development in late preterm infants.

***Keywords:*** Hematological parameters, Inflammatory markers, Late preterms, Retinopathy of prematurity, Risk factors

## Introduction

Retinopathy of prematurity (ROP) is a retinal vasoproliferative disorder among preterm infants (hereafter referred to as preterms) and is the leading cause of childhood blindness (1). In developed countries, ROP occurs primarily in extremely premature infants born at less than 28 weeks' gestational age (GA), but in low- to middle-income countries, older and heavier babies are also at risk of developing ROP (2). Therefore, screening guidelines can vary even within countries. According to the Turkish Neonatal and Turkish Ophthalmology societies' consensus guideline, in 2018, it was accepted to screen babies with a GA at birth of ≤32 weeks or a birth weight (BW) of ≤1500 g (3); while there are reports of heavier and older infants affected by ROP, it generally occurs in low-BW preterms (4-7). When evaluating the results of the TR-ROP study conducted Turkey, serious ROP cases were found in older and late preterm babies (4); as such, in the updated guidelines, the Turkish ROP screening criteria include babies having a BW of

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≤1700 g or GA at birth of <34 weeks. If neonatologists consider it necessary, an ophthalmologic examination can also be performed on older babies.

Recently, studies on potential risk factors for ROP development have focused on imbalances in angiogenic or anti-angiogenic cytokines and inflammatory or anti-inflammatory cytokines because of the aetiopathogenesis of ROP development. Hematological parameters were thus studied to determine an indicator risk factor for ROP development (8-11). Among these studies, none focused on preterms born at 32 weeks' GA and older. In response, this study aimed to investigate the possible effects of hematological parameters and other risk factors on ROP development in preterms born at 32 weeks' GA and older.

## Methods

This retrospective, cross-sectional study was performed at the Departments of Ophthalmology and Neonatology of Sakarya University, Education and Research Hospital, in Turkey, in adherence to the Declaration of Helsinki, with prior approval received from the Institutional Review Board (IRB number: 7152247 / 050.01.04 / 307) and written informed consent obtained from the parents of each subject.

The data of preterms born between January 2016 and January 2021 were retrospectively reviewed from 1852 medical records of infants who underwent ROP screening. Preterms with birth weights above 1250 g, born at 32–35 weeks' GA, and diagnosed with any stage of ROP were included in the study, while thirty infants without ROP, born at the same GA range, were randomly selected as the control group (non-ROP group). The sample size was determined to ensure reliable statistical analysis.

Preterms born before 32 weeks' GA, as well as those with pre-existing eye diseases such as congenital glaucoma, cataracts, or corneal or retinal diseases, were excluded, along with infants who did not undergo a blood test within the first 24 hours of life.

Fundus examination was performed under mydriasis, with one drop of 0.5% tropicamide eye drops combined with 1% phenylephrine eye drops instilled thrice in each eye, with a 10-min interval in between, before the examination. The same two ophthalmologists (ÖB, BK) performed all the examinations using a lid speculum and scleral depressor with a binocular indirect ophthalmoscope (Heine Video Omega, Germany) and 20 or 28 dioptre lenses. For each examination, the stage, zone and presence or absence of plus or preplus disease were recorded, though when a difference in ROP stage between the eyes of a single infant was indicated, the more advanced stage was recorded. All patients were examined at 4 weeks postpartum at the earliest and followed up with until the third zone of the retina was vascularised completely. After the ROP examination was complete, the patients were referred to the Department of Strabismus and Pediatric Ophthalmology of Sakarya University, Education and Research Hospital.

Complete blood cell counts (CBC) of all cases were routinely performed by neonatologists within the first 24 h of life, including an analysis of CBC parameters. The lymphocyte-to-monocyte ratio (LMR), neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) were calculated, and hemogram parameters, along with LMR, NLR and PLR values, were compared statistically between the ROP and non-ROP groups.

Delivery type, cause of premature birth (preeclampsia, premature membrane rupture, cervical insufficiency, etc.), total number of births and smoking status during pregnancy were recorded, as were infant-related factors, such as GA at birth, BW and length of stay in NICU. All recorded parameters were also compared between both groups.

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS Statistics, Version 24.0; IBM Corp., Armonk, NY, USA). The distribution of the variables was assessed using both visual (histograms, probability plots) and analytical methods (Kolmogorov-Smirnov and Shapiro-Wilk tests). Descriptive statistics were presented as means and standard deviations for variables with normal distribution. Since hematological parameters were normally distributed, comparisons between groups were made using the Student's t-test. The chi-square test was used to compare categorical variables. A p-value of <0.05 was considered statistically significant for all analyses.

### *Ethical approval*

Prior approval received from the Institutional Review Board (IRB number: 7152247 / 050.01.04 / 307) and written informed consent obtained from the parents of each subject.

## Results

In total, 50 preterms were included in the

**Table 1.** Demographics of the study groups

| | ROP group (n=20) | Non-Rop group (n=30) | p value |
|---|---|---|---|
| Gender (M:F) | 10:10 | 14:16 | 0.81 |
| Birth weight(g) | 1754.1±483 | 1909±413 | 0.39 |
| Range (g) | 1280-3190 | 1300-3500 | |
| GA (weeks) | 32.4±0.5 | 32.8±0.7 | 0.49 |
| Range (weeks) | 32-35 | 32-35 | |
| Time of initial examination (postpartum age) | 4.95±1.53 wk | 5.53 ±2.37wk | 0.07 |
| Range | 4-9 weeks | 4-13 weeks | |
| Time of staying in NICU (day) | 20.7±10.9 | 16.4±13.5 | 0,89 |

GA: Gestational age, BW:Birth weight, GA and BW are expressed as mean±standard deviation NICU: neonatal intensive care unit

study, with 20 in the ROP group and 30 in the control group (non-ROP). All infants were delivered by caesarean section, the basic characteristics of whom are shown in Table 1. There were no statistically significant differences between the two groups in terms of GA and BW (p: 0.49, p: 0.39, respectively), and the initial examination length did not differ statistically between the groups (p: 0.07). Further, no significant difference was found in the duration of NICU stay between the ROP and non-ROP groups (p: 0.89).

In the ROP group, Stage 1 ROP was detected in 25 eyes and Stage 2 ROP in 11 eyes, while only one infant had stage 3 ROP in both eyes. Five infants had different ROP stages in both eyes, but the more advanced ROP stage was recorded. While preplus disease was observed in two infants, plus disease was not observed in any of the infants, and in the follow-up examinations, complete retinal vascularisation was observed, meaning no treatment was required in the ROP group. Similarly, in the control group, complete retinal vascularisation was observed, and no signs of a regression of any ROP stage were identified.

The Student's t-test.was used for the comparison of hematological parameters between the groups. Further, no significant differences in hematological parameters or in the NLR, LMR and PLR values were found, with mean blood parameters; NLR, LMR and PLR values; standard deviations of the ROP and non-ROP groups; and p values shown in Table 2. Meanwhile, there were no significant differences between the groups in terms of the total number of pregnancies, maternal smoking history, consanguineous marriage history or mother's age and parity (Chi-square test). Three infants in the ROP group and none in the non-ROP group had a history of blood transfusion, this difference was statistically significant (p: 0.03; Table 3).

**Table 2.** Mean blood parameters, NLR, LMR, PLR and the standart deviations of ROP and non-ROP groups at the first 48 hours and the p values

| | ROP group | Non-ROP group | P value |
|---|---|---|---|
| WBC($x10^3$ /mcL) | 10±4.58 | 12.98±6.09 | 0.35 |
| RBC($x10^6$/mcL) | 4.57±0.60 | 4.78±0.60 | 0.86 |
| Hgb(g/dL) | 16.81±2.39 | 17.3±2.16 | 0.27 |
| HCT(%) | 50.95±6.82 | 52.03±6.75 | 0.67 |
| PLT($x10^3$ /mcL) | 244.11±70.16 | 240.68±90.64 | 0.38 |
| MCH(pg) | 36.76±1.8 | 35.22±5.85 | 0.14 |
| MCHC(g/dL) | 32.99±1.05 | 33.22±1.32 | 0.37 |
| RDW(ratio) | 17.7±1.26 | 17.6±1.47 | 0.65 |
| NEU($x10^3$ /mcL) | 3.38±2.88 | 4.54±3.09 | 0.64 |
| LYM($x10^3$/mcL) | 5.22±3.14 | 6.92±5.1 | 0.24 |
| MONO($x10^3$/mcL) | 0.72±0.34 | 0.73±0.48 | 0.26 |
| NLR | 0.74±0.61 | 0.86±0.64 | 0.41 |
| LMR | 9.6±10.31 | 13.4±11.07 | 0.21 |
| PLR | 59.5±39.31 | 46.34±28.84 | 0.95 |

ROP: Retinopathy of prematurity, WBC: White blood cell, RBC: Red blood cell, Hgb: Hemoglobin, HCT: Hematocrite, PLT: Patelets, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, RDW: Red cell distrubition width, NEU: Neutrophils, LYM: Lymphocytes, MONO :Monocytes, NLR: Neutrophil to lymphocyte ratio, LMR: Lymphocyte to monocyte ratio,PLR: Platelet to lymphocyte ratio

**Table 3.** Other characteristics of groups

| | ROP group (n=20) | Non-Rop group (n=30) | p value |
|---|---|---|---|
| Maternal age (Mean±STD) | 30.94±6.92 years | 29.29±4.40 years | 0.06 |
| Maternal smoking | 0 | 5 | 0.06 |
| Consanguineous marriage | 3 | 2 | 0.36 |
| RBC transfusion | 3 | 0 | 0.03 |
| Multiple pregnancy | 8 | 6 | 0.12 |

RBC: red blood cell, STD:standard deviation

## Discussion

A low BW and GA at birth are proven risk factors for premature retinopathy (8), as confirmed by most studies that screened infants born at <32 weeks' GA and at a BW of <1500 g (12). In the Multicenter Trial of Cryotherapy for Retinopathy of Prematurity (CRYO-ROP), 4099 infants weighing less than 1251 g were included, indicating its association with ROP (3), while studies from developing countries and from Turkey reported that severe ROP can develop in late preterms (born at >1500 g BW or 32–35 weeks' GA) (14-16). In the current study, infants having a BW of ≥ 1250 g and GA at birth of ≥32 weeks were included, as these are considered low risk factors for ROP. Hematological parameters and additional factors were also investigated to identify potential risk factors for ROP development.

Systemic inflammation may cause vascular anomalies in the retina by activating microglia in the retina.[17] For instance, such inflammatory factors as cytokines, growth factors, leukocytes, monocytes and macrophages are effective in retinal vascular development in ROP and may induce ROP independent of GA at birth (17, 18). Meanwhile, in a study conducted in China, Hu et al. showed that infants with ROP had lower NLR and higher LMR values than infants without ROP,[18] while Akdoğan et al. determined that in the first 24 h after birth, WBC, lymphocyte and monocyte counts were significantly lower in infants with ROP, with the same study showing a correlation between high LMR values and ROP development (17). In our study, there was no statistically significant difference between the groups in terms of mean NLR, PLR, LMR and WBC counts, which might suggest that in late preterms, systemic inflammation markers are unrelated to ROP development.

Platelets also play a critical role in ROP development because of the pro- and antiangiogenic factors they accumulate and carry, such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF), insulin like growth factor (IGF) and vascular endothelial growth factor (VEGF) (19, 20). Some studies even report a correlation between low platelet values and ROP in the early days of life (21), though platelet counts assessed in the first 24 h did not differ statistically between groups in our study.

Mean hemoglobin (Hb), RBC, hematocrit (HCT) and major histocompatibility complex (MHC) values were not found to be risk factors for ROP development in late preterms in this study, but in preterms with a lower BW and GA at birth, Niranjan et al. found that the absolute nucleated RBC count could be used as a screening tool in the early prediction of ROP (10). Further, Akyuz Ünsal et al. reported that mean HB, RBC and HCT were lower in preterms with ROP (9), while a recent study by Çömez et al. found that mean RBC distribution width (RDW) was higher in preterms with ROP than in those without (22), though these studies were focused on preterms with a lower BW and GA at birth. Overall, no study has focused on late preterms, in whom the characteristics of ROP development might differ.

In infants born with a predominancy of foetal Hb (HbF), within 1–2 years, HbF disappears, as it has a high affinity for oxygen when compared to adult Hb (HbA) and thus greater difficulty in unloading oxygen to tissues. With a blood transfusion, HbF is replaced with HbA, and the percentage of HbF decreases, delivering more oxygen to retina. However, non-physiological early reduction of HbF may lead to premature retinopathy,[23] and the increase in iron intake following a blood transfusion heightens the level of its oxidation product, and oxidative damage is found to be associated with ROP.[19] In the current study, there was a history of blood transfusion in three infants in total, all of whom were in the ROP group, meaning the absence of a transfusion history in the non-ROP group was considered statistically significant. (p: 0.03). Many studies consider that RBC transfusion is an independent risk factor for developing ROP, but there are no reports of its effects on ROP development in late preterms (19, 24, 25).

The hematological parameters were obtained on the first day of the preterms' lives, but obtaining these parameters in the first week or first month might have given us more information for a deeper evaluation. Because no preterms in the ROP group had a severe form of the disease, sample homogeneity might produce more accurate results. However, the evaluation of hematological parameters in late preterms with severe ROP was not possible. The main limitation of this study was the relatively small sample sizes of the groups. There is a need for more comprehensive studies with larger sample sizes.

## Conclusion

There were no statistically significant differences between preterms in the ROP and non-ROP groups in terms of hematological parameters measured on the first day of life of infants born at

a GA of 32–35 weeks and BW of at least 1250 g. RBC transfusion was found to be more common in the ROP group compared to non-ROP group, indicating its potential as a risk factor for ROP development in late preterms.

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## Conflicts of interest

The authors declare no conflict of interest.

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