Evaluation of Blood Inflammatory Parameters of Infants with Premature Retinopathy Treated with Intravitreal Anti Vascular Endothelial Growth Factor

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ABSTRACT

Purpose: This study compared the blood inflammatory parameters between early preterm infants with retinopathy of prematurity (ROP) who were treated with intravitreal anti-vascular endothelial growth factor (VEGF)) and those who did not need treatment.

Materials and Methods: One hundred and six infants were included in the study. Early preterm infants with a gestational age (GA) ≤ 32 weeks and birth weight (BW) ≤ 1500 gm were included. These infants were divided into three groups: infants without ROP (group 1), infants with ROP who did not require treatment (group 2) and infants with ROP who were treated with intravitreal anti-VEGF (group 3). In the first week, neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR) and platelet-to-lymphocyte ratio (PLR) of the groups were calculated and compared.

Results: Of 106 infants, 38 (35.8%) were in group 1, 30 (28.3%) were in group 2 and 38 (35.8%) were in group 3. GA was 24.89 ± 1.48 weeks, and BW was significantly lower in group 3, 775.21 ± 175.11 gm. ($p < 0.001$). There was no significant difference between the groups in terms of NLR, MLR and PLR ($p = 0.833$, $p = 0.918$ and $p = 0.082$, respectively). Logistic regression analysis was performed; BW was a statistically significant independent risk factor (OR: 0,999 %CI: 0,992-0,999 $p = 0.022$) associated with ROP that required treatment.

Conclusion: It was shown that systemic inflammation parameters, including NLR, MLR and PLR, are not a reliable marker in the diagnosis and prognosis of ROP.

Keywords: Retinopathy of prematurity, Neutrophil-to-lymphocyte ratio, monocyte-to-lymphocyte ratio and platelet-to-lymphocyte ratio.

INTRODUCTION

Retinopathy of prematurity (ROP) is a physiopathological condition caused by abnormal vascular proliferation of the retina seen in low-birthweight and preterm infants. Today, with the development of standards in neonatal care units, the chances of survival of low-birthweight and preterm babies have increased. ROP also emerges as a more frequent problem and ranks first among the preventable causes of blindness in childhood[1]. It has also been reported that neuromotor growth retardation is common in children with a history of ROP. Therefore, ROP is an important global problem[2]. In 2010, 20,100 people worldwide experienced blindness or severe vision loss due to ROP[3].

The pathogenesis of ROP is not fully known, but it is thought to develop in two phases. While phase 1 occurs at 22-30 weeks of postmenstrual age, phase 2 occurs at 31-45 weeks of postmenstrual age[4]. In phase 1, the event develops as follows. The infant, who is normally in a hypoxic environment in the mother's womb, is exposed to high oxygen in the atmosphere (hyperoxia) at birth. Hyperoxia causes suppression of erythropoietin and vascular endothelial growth factor (VEGF). As a result of the absence of insulin-like growth factor 1 (IGF-1) and insufficient postnatal growth, the vascular development of the retina is inhibited (4-6 days after birth). The retina continues to develop, but since vascularisation is impaired, it cannot meet the oxygen need and switches to the hypoxic phase (phase 2). With the effect of hypoxia, the level of mediators such as VEGF, erythropoietin, and IGF-1 increases in the retina and neovascularisation begins. This neovascularisation is seen on the border of vascular and

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avascular retina. The newly formed vessels may cluster in the retina and rapidly thicken and form ridge tissue. If this neovascularisation is not treated in time, a process leading to retinal detachment begins[5].

In the pathogenesis of ROP, systemic inflammatory response in premature infants directly or indirectly affects retinal neovascularisation and ROP development[6]. Neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR) and platelet-to-lymphocyte ratio (PLR) can be used as a biomarker of systemic inflammation[7,8].

Treatments of severe ROP include laser therapy, cryotherapy and intravitreal drug injections that block the effects of endogenous VEGF. Successful ROP therapy has been reported with several types of anti-VEGF drugs[9-11]. Of these, bevacizumab is the most widely used because it is readily available and inexpensive[10].

Our study compared the blood inflammatory parameters between early preterm infants with ROP who were treated with intravitreal bevacizumab and those who have not developed ROP. The comparison was used to reveal whether these parameters can be used as a biomarker in the diagnosis and prognosis of ROP.

MATERIALS AND METHODS

One hundred and six infants hospitalised in the Neonatal Intensive Care Clinic at Dicle University between 2017 and 2020 were included in this retrospective study. Written informed constent was taken from infants' parents. Approval was obtained for our study from the local ethics committee of Dicle University (Date: 10.12.2020, Number: 387)

The Neonatal Study Group in Turkey suggests screening for ROP in infants with a gestational age (GA) ≤ 32 weeks or a birth weight (BW) ≤ 1500 gm [12]. Therefore, infants born in this range were included in the study based on this reference. The files of the infants were retrospectively examined, and their GA, BW and blood values and other diseases they had were recorded. Infants with haematological disorders, necrotising enterocolitis, sepsis, blood transfusion, postnatal steroid therapy and GA > 32 weeks or BW> 1500 gm were excluded from the study.

The first ROP examination was performed at 31 weeks of the postmenstrual age (GA + chronological age) in infants with GA < 27 weeks and after four weeks for those with GA ≥ 27 weeks. Before the infants were examined, 2.5% phenylephrine (Mydfrin, Alcon, USA) and 0.5% tropicamide (Tropamide, Bilim İlaç, Turkey) were administered three times at 10-minute intervals and the pupil was dilated. For topical anaesthesia, 0.5% proparacaine HCl (Alcaine, Alcon, USA) was administered 3-5 sec before the examination. Infants, examined with indirect ophthalmoscope and scleral depressor, were classified according to the standard of the International Committee for the Classification of ROP (ICROP) [13]. The treatment was planned according to the criteria determined by the Early Treatment for Retinopathy of Prematurity study group. According to their follow-up, the following infants received bevacizumab at a dose of 0.675 mg/0.025 ml intravitreal: (a) stage 2 or 3 in zone 2 in addition to plus disease, (b) stage 1 or 2 in zone 1 in addition to plus disease, (c) stage 3 in zone 1 and (d) aggressive posterior ROP [14].

The infants included in the study were divided into three groups. Group 1 included infants with no ROP (no stage), group 2 included infants with ROP but not requiring treatment, and group 3 included infants with ROP who were administered intravitreal anti-VEGF.

Peripheral venous blood (1 ml) taken from infants was placed in dipotassium ethylene diamine tetraacetate (EDTA) tubes. Complete blood count was evaluated by an automated haematological analyser (Sysmex XN 1000, Kobe, Japan). Blood parameters of the first week including white blood cell (WBC), neutrophil (N), lymphocyte (L), platelet (P) and monocyte (M) counts were included in the study. Using these parameters, NLR, MLR and PLR were calculated by simple division and recorded.

STATISTICS

All data were analysed using SPSS 26.0 (SPSS, Inc. Chicago, IL, USA). Continuous variables are presented as mean ± SD, and categorical variables are presented as numbers and percentages. The results were compared using the one-way ANOVA test and post-hoc Bonferroni analysis and were evaluated with a statistical significance level of $p < 0.05$. Logistic regression analysis was performed to predict the significant independent risk factors associated with the presence of ROP that required treatment. P values < 0.05 were considered statistically significant. The adjusted odds ratio (OR) and 95% confidence interval for each possible risk factor were calculated.

RESULTS

In our study, of the 106 infants, 38 (35.8%) were in group 1, 30 (28.3%) were in group 2 and 38 (35.8%) were in group 3.

According to mean GA, group 1 included infants with GA of 26.97 ± 1.82 (24-31) weeks, group 2, 26.60 ± 1.69 (24-30) weeks and group 3 24.89 ± 1.48 (22-28) weeks. There was a statistically significant difference in GA between the

groups (Table 1). The mean age of group 3 was significantly lower than groups 1 and 2 ($p < 0.001$). Post-hoc analysis for multiple comparisons is given in Table 2.

According to mean BW distribution, group 1 included infants with BW of 1006.97 ± 204.59 (630-1470) gm, group 2, 983.00 ± 176.86 (670-1435) gm and group 3, 775.21 ± 175.11 (430-1230) gm. There was a statistically significant difference between the groups in terms of BW (Table 1). BW was significantly lower in group 3 than groups 1 and 2 ($p < 0.001$). Post-hoc analysis for multiple comparisons is given in Table 2.

The mean WBC, N, L, M and P values and NLR, MLR and PLR of the groups are shown in Table 1.

In multiple comparisons with post-hoc Bonferroni test, WBC and N counts were significantly higher in group 2 than groups 1 and 3 (Table 2).

NLR was 1.19 ± 1.83 (0.10-9.31) in group 1, 1.13 ± 1.03 (0.07-4.58) in group 2 and 1.00 ± 1.09 (0.05-4.77) in group 3. There was no significant difference between the groups in terms of NLR ($p = 0.833$).

MLR was 0.31 ± 0.23 (0.03-0.98) in group 1, 0.29 ± 0.19 (0.02-0.67) in group 2 and 0.29 ± 0.25 (0.01-1.18) in group 3. There was no significant difference between the groups in terms of MLR ($p = 0.918$).

PLR was 89.39 ± 51.66 (15.40-261.21) in group 1, 64.81 ± 44.89 (6.06-160.00) in group 2 and 73.85 ± 39.27 (3.89-167.77) in group 3 was. There was no significant difference between the groups in terms of PLR ($p = 0.082$).

Logistic regression analysis was performed to determine the independent risk factors associated with ROP that required treatment. Although GA was not statistically significant, p value was borderline ($p = 0.058$), and BW was a statistically significant independent risk factor ($p = 0.022$). However, WBC and neutrophil counts were not independent risk factors ($p = 0.155$; $p = 0.756$) (Table 3)

Table 2: *Multipl comparison between three groups with post hoc bonferroni test.*

	P value
Gestational age	
Group 3<Group 1	**<0,001**
Group 3<Group 2	**<0,001**
Birth weight	
Group 3<Group 1	**<0,001**
Group 3<Group 2	**<0,001**
WBC	
Group 2>Group 1	**<0,001**
Group 2>Group 3	**<0,001**
Neutrophil	
Group 2>Group 1	0,063
Group 2>Group 3	0,045
P<0,05 was statistically significant	

Table 3: *Independent risk factors for treated ROP in the study population.*

	Odds ratio (%95 CI)	*p value
Gestational age	0,687 (0,466-1,013)	0,058
Birth weight	0,999 (0,992-0,999)	***0,022***
WBC count	0,889 (0,755-1,046)	0,155
Neutrophil count	1,041 (0,808-1,341)	0,756
Logistic regression analysis was used *p<0,05 was statistically significant		

Table 1: *Demographic characteristics of premature babies and distribution of blood parameters between groups.*

	Group-1 N=38 (%30,2)	**Group-2 N=30 (%23,8)**	**Group-3 N=38 (%30,2)**	**P value**
GA (week)	26,97 ± 1,82	26,60 ± 1,69	24,89 ± 1,48	**p<0,001**
BW (g)	1006,97 ±204,59	983,00 ± 176,86	775,21±175,11	**p<0,001**
Sex (Female/Male)	23/15	20/10	19/20	P=0,256
WBC ($\times 10^9$/L)	10,76 ± 4,42	16,04 ± 7,13	10,11 ± 3,94	**p<0,001**
Neutrophil ($\times 10^9$/L)	3,65 ± 3,54	5,63 ± 4,31	3,54 ± 2,46	0,027
Lymphocyte ($\times 10^9$/L)	4,84 ± 2,94	7,68 ± 6,42	5,73 ± 8,17	P=0,172
Monocyte ($\times 10^9$/L	1,12 ± 0,55	1,39 ± 0,74	1,07 ± 0,60	P=0,085
Platelet ($\times 10^9$/L)	334,95 ± 117,17	317,70 ± 131,81	300,90 ±151,72	P=0,548
NLR	1,19± 1,83	1,13 ± 1,03	1,00 ± 1,09	p=0,833
MLR	0,31 ± 0,23	0,29 ± 0,19	0,29 ± 0,25	p=0,918
PLR	89,39 ± 51,66	64,81 ± 44,89	73,85 ± 39,27	p=0,082
One way ANOVA test was used, P<0,05 was statistically significant				

DISCUSSION

In our study, there was no significant difference between the groups in terms of NLR, MLR and PLR, while GA and BW were significantly lower in group 3 compared with groups 1 and 2.

Both low GA and BW are major risk factors for developing ROP. There is a linear relationship between these two factors and neuronal and vascular development of the retina[15]. In the study where the Cryotherapy (CRYO-) ROP study group for ROP followed 4,099 babies born under 1251 gm, it was reported that every 100-gram increase in BW decreased ROP development by 27%, and every one-week increase in GA decreased ROP development by 19% [16]. In our study, lower GA and BW in infants in group 3 who received intravitreal treatment were found statistically significant ($p < 0.001$). Our study demonstrated by regression analysis that low BW and GA are significant risk factors associated with ROP that required treatment (OR = 0.999, p = 0.022 and OR = 0.687, p = 0.058).

Prenatal, perinatal and postnatal systemic inflammation (intrauterine infection, preeclampsia and sepsis, etc.) in preterm infants is one of the factors affecting the development of ROP[6]. Systemic inflammation affects ROP pathogenesis directly and indirectly. While the indirect effect of inflammation on ROP is disrupting retinal perfusion, its direct effect is retinal angiogenesis[6]. It has been shown in animal models that systemic inflammation in neonates impairs retinal vascular development and induces the pathological features of ROP[17]. In inflammation, nitric oxideis released from the tissue as a result of the infiltration of macrophages, monocytes and leukocytes. This released NO turns into peroxynitrite, and therefore, it increases the angiogenic factors (VEGF, b-VEGF and HIF1 alpha) in the molecule[18].

The body secretes cytokines, chemokines, nitric oxide, growth factors and hormones from immune cells (leukocytes, monocytes and macrophages) as a response to systemic inflammation in the blood. These factors have an important role in the formation of ROP[17]. Many studies have reported that NLR in inflammation is a better indicator than total leukocyte count[19,20].

Ünsal et al. reported that lymphocyte, neutrophil, monocyte and eosinophil counts tended to be higher in the ROP group and WBC count was significantly higher in their analysis although there were no signs of infection. This is likely due to bronchopulmonary dysplasia, ROP and so on. They suggested that such premature-related pathologies were caused by ongoing inflammation[21].

In our study, WBC and neutrophil were found to be significantly different in patients with ROP that did not require treatment and those who received treatment but were not an independent risk factor in logistic regression analysis (OR = 0.889, p = 0.155 and OR = 1.041, p = 0.756).

In case of systemic inflammation, the number of leukocytes in the blood increases. Moreover, the number of leukocytes in the blood increases, and the level of cortisol and catecholamines increases, but the number of lymphocytes decreases[22,23]. As an indicator of inflammation, increased neutrophil and low lymphocyte levels in the blood can be used as a biomarker[23] In inflammation, reactive oxygen and VEGF are released from neutrophils. VEGF stimulates angiogenesis, while reactive oxygen disrupts the DNA of cells[24]. Lymphocytes are protective and regulatory components, whereas neutrophils are effective components of inflammation. There is a sensitive balance at this rate [25]. In studies by Hu et al. and Kurtul et al., blood parameters of preterm infants at a 24-hour interval after birth were recorded and it was reported that NLR was not a reliable marker for ROP prognosis[26,27]. In our study, the prenatal and perinatal or postnatal stress experienced by the infant can be seen reflected in the blood in the first week. As was mentioned in the 'Materials and Methods' section, after excluding the causes of infection, blood transfusion and so on, the data of the first week were analysed, and it was reported that NLR was not a reliable biomarker in the diagnosis and prognosis of ROP (p = 0.883).

One of the indicators of systemic inflammation in the blood is the level of monocytes[28]. In inflammation, the number of monocytes increases, while the number of lymphocytes decreases[29]. The increase in monocytes causes an increase in proinflammatory cytokines such as interleukin-1 (IL-1), 1L-6, 1L-10, tumour necrosis factor alpha (TNF-α) and formation of angiogenesis[30]. It is controversial that MLR is a reliable biomarker in the diagnosis and prognosis of ROP. A study reported that MLR was a reliable biomarker in the diagnosis and prognosis of ROP, and another study reported that it was unreliable[26,27]. Alternatively, in our study, it was reported that MLR was not a reliable biomarker in the diagnosis and prognosis of ROP (p = 0.918).

The role of platelets in abnormal angiogenesis (phase 1) and abnormal vasculogenesis (phase 2) is important[4]. Both pro- and anti-angiogenic regulatory proteins are present in α granules in the platelets. These regulatory proteins are thought to affect VEGF regulation. If the platelet count is low, it cannot fulfil this function and VEGF increases and ROP development accelerates[31]. It has been reported that a platelet count below 100,000 increases the development of aggressive posterior ROP[32]. It has also been reported that infants born with multiple pregnancies have a significant risk of developing ROP due to low platelets[33].

A study reported that PLR is not a reliable biomarker in the diagnosis and prognosis of ROP [26]. In our study also, it was reported that PLR was not a reliable marker in the diagnosis and prognosis of ROP (p = 0.082).

Although many of the harmful effects of prenatal and postnatal infection and inflammation on the development and prognosis of ROP are known, ROP risk cannot be determined using easily accessible and simple blood parameters. It is concluded that systemic inflammation parameters including NLR, MLR and PLR are not reliable markers in the diagnosis and prognosis of ROP.

Compliance with Ethical Standards:

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