Relation Between Absolute Nucleated Red Blood Cell Count at Birth and Retinopathy of Prematurity

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ABSTRACT

Purpose: The aim of this study is to prove the association between potential fetal hypoxia and retinopathy of prematurity (ROP) development and absolute nucleated red blood cell (aNRBC) is used to evaluate it in premature infants without any hypoxic or ischemic history.

Methods: Medical records of 43 premature infants with ROP who were admitted to the neonatal intensive care unit at Wonkwang University Hospital from January 2004 to December 2014 were analyzed retrospectively. We excluded 15 infants who had a confounding medical condition that could have increased the aNRBC count. Finally, 28 premature infants affected by ROP were enrolled and compared with 28 pair-matched controls. The aNRBC counts at birth in these infants were compared. Statistical analysis was performed with a paired *t*-test for continuous data, and a Fisher's exact test for categorical data. $P < 0.05$ was considered significant.

Results: There were no significant differences in perinatal characteristics such as gestational age (GA), birth weight (BWt), Apgar scores, premature rupture of membrane (>24 hours), prenatal betamethasone, surfactant or respiratory distress syndrome between the ROP and the control infants. In addition, neither group differed in major morbidities such as patent ductus arteriosus, periventricular leukomalacia, intraventricular hemorrhage ($\geq$ Grade 2), or bronchopulmonary dysplasia. Regardless of the severity of ROP, the aNRBC counts at birth in premature infants with ROP were not higher than in the control infants.

Conclusion: The aNRBC counts at birth may not be related directly to the development of ROP.

Key Words: Absolute nucleated red blood cells, Fetal hypoxia, Retinopathy of prematurity

INTRODUCTION

Retinopathy of prematurity (ROP) is a postnatal disorder of the retinal vessels that develops only in the incompletely vascularized retina of premature infants¹⁾.

The incidence of ROP has been reported in 20.3% of infants with a birth weight of less

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than 1,500 g, and in 33.2% of infants with a birth weight of less than 1,000 g²⁾. The incidence of ROP among very low birth weight infants has been reported to be 36.9% in our hospital³⁾.

ROP progresses in two phases. The first phase begins with cessation of retinal vascular growth and vessel regression due to hyperoxia, followed by the second phase in which insufficient vascularization of the developing retina creates hypoxia and hypoxia-induced pathological retinal vessel growth⁴⁻⁷⁾. Thus, hypoxia of retinal cells from secondary and perinatal events may be one of the possible reasons for ROP⁴⁻⁷⁾.

In some reports, fetal hypoxia-ischemia induced an increased rate of ROP in conditions such as fetal growth restriction and severe neonatal asphyxia, conditions that are affected by a compromised retinal blood flow and/or oxygen supply⁶⁻⁹⁾. These conditions are also noted in maternal pregnancy-induced hypertension, pre-eclampsia, maternal diabetes mellitus, maternal smoking and Rh isoimmunization¹⁰⁻¹⁴⁾.

Absolute nucleated red blood cell (aNRBC) called erythroblasts or normoblasts are rarely found circulating in older children but are commonly seen in the blood of newborns, due to the fact that aNRBCs are primarily produced in the fetal bone marrow in response to erythropoietin, and stored in the bone marrow as precursors to reticulocytes and mature erythrocytes. Many acute and chronic stimuli increase the circulating aNRBC counts from an increased erythropoietic activity^{15,16)}.

Lubetzky et al.¹⁷⁾ reported a case matched analysis showing that there was an elevation of aNRBC counts at birth in premature infants who later developed ROP. In this report, maternal and fetal hypoxic or ischemic conditions were excluded

because they may influenced the counts of aNRBC at birth. They suggested that an increased aNRBC count at birth which is associated with relative fetal hypoxia followed by erythropoietin, and potential or long-lasting fetal hypoxia and/or ischemia may play a role in the pathogenesis of ROP.

Thus, the aim of this study is to evaluate potential relationship between fetal hypoxia and ROP development for premature infants without hypoxic or ischemic medical history, who later developed ROP, had higher aNRBC counts at birth compared with control infants.

MATERIALS AND METHODS

The medical records of 43 premature infants with ROP who were admitted to the neonatal intensive care unit at Wonkwang University Hospital from January 2004 to December 2014 were analyzed retrospectively. We included only who were gestational age (GA) <34 weeks and their birth weight (BWt) <2000 g. We excluded 15 infants with intrauterine growth retraction (<10 percentile in Lubechencho curves)^{18,19)}, pregnancy induced hypertension²⁰⁾, gestational diabetes mellitus²¹⁾, placental abruption or placenta previa²²⁾, Apgar scores <4 in 1 or 5 minutes, perinatal infection (eg, maternal fever and white blood cells >15,000/mm³) or chorioamnionitis (eg, maternal fever, abdominal tenderness, maternal tachycardia [>100/min], fetal tachycardia [>160/min] and foul amniotic fluid)²³⁾, and blood group incompatibility with positive Coombs' test. Because these variables are known to affect neonatal aNRBC counts, we

Figure 1. Study subjects. Of 43 infants with ROP, 15 infants who had a confounding condition that can increase aNRBCs, and the 4 infants who had a combined confounding condition, were excluded. Finally 28 infants with ROP and 28 matched control infants with the same gestational age (GA), birth weight (BWt), and 1, 5-minute Apgar score were compared. Abbreviation: ROP, retinopathy of prematurity.

exclude them, and only evaluate a potential aNRBC effect to development of ROP. Finally, 28 premature infants with ROP were enrolled and compared with 28 pair matched controls who had the same GA, BWt and 1, 5-minute Apgar score (Figure 1). The perinatal characteristics, major morbidities, and hematologic parameters including aNRBC counts at birth were also compared.

For ROP screening, all infants with a BWt $\leq 1,500$ g or a GA of ≤ 32 weeks and selected patients with a BWt of 1,500-2,000 g, who required oxygen therapy, underwent an indirect ophthalmoscope 4-6 weeks after birth according to the 2006 American Academy of Pediatrics (AAP) guidelines²⁴.

Statistical analysis was performed with a paired *t*-test for continuous data, and a Fisher's exact test for categorical data. *P*-value <0.05 was considered statistically significant.

RESULTS

A total of 28 infants with ROP were compared with a matched control group. There was no significant difference between the groups with respect to perinatal characteristics according to the Apgar score, premature rupture of membrane (PROM),

Table 1. Perinatal Characteristics of Infants with Retinopathy of Prematurity and Their Matched Controls

	Infants with ROP (n=28)	Control (n=28)	P-value
GA (wks) (range)	29.1 $\pm$ 2.0 (26-33)	28.4 $\pm$ 1.7 (25-32)	0.164
BWt (g) (range)	1,272 $\pm$ 251 (810-1,850)	1,204 $\pm$ 280 (540-1,890)	0.339
Apgar			
1 min	4.1 $\pm$ 1.0	4.2 $\pm$ 1.3	1.000
5 min	6.3 $\pm$ 1.0	6.4 $\pm$ 1.5	0.770
PROM (≥ 24 hrs), n (%)	11 (39.3)	9 (32.1)	0.781
Administration of betamethasone, n (%)	13 (46.4)	16 (57.1)	0.593
Respiratory distress syndrome, n (%)	17 (60.7)	23 (82.1)	0.138
Surfactant administration, n(%)	12 (42.9)	19 (67.9)	0.106
Patent ductus arteriosus, n(%)	8 (28.6)	5 (15.2)	0.528
Ibuprofen administration, n(%)	8 (28.6)	5 (15.2)	0.528

Plus-minus values are mean $\pm$ SD.

Abbreviations: ROP, Retinopathy of prematurity; GA, gestational age; BWt, birth weight; PROM, premature rupture of membrane.

prenatal administration of betamethasone, respiratory distress syndrome, surfactant administration, patent ductus arteriosus (PDA) or ibuprofen (brufen syrup[®]) administration (Table 1). By design, the case and control group had a similar GA, BWt, and 1, 5-minute Apgar score.

In addition, we compared the major morbidities and oxygenation supplements of ROP infants with the matched control group. We found no significant difference in major morbidities such as periventricular leukomalacia (PVL), intraventricular hemorrhage (IVH) ($\geq G2$) and bronchopulmonary dysplasia (BPD) defined as treated with more than 21% oxygen for at least 28 days postnatally between the two groups²⁵. And, oxygen supplementation such as the duration of ventilator therapy, duration of oxygen therapy, required maximal fraction of inspired oxygen (FiO₂), and duration of total parenteral nutrition (TPN) was also no different in both groups (Table 2).

In the 28 ROP infants, 11 infants (39.3%) were in Stage I, 3 infants (10.7%) were in Stage II, and 14 infants (50.0%) were in Stage III. Regarding the severity of ROP, 17 infants (60.7%) were graded at a more severe stage than Stage II, and 14 infants (50.0%) at a more severe stage than Stage III. Thirteen infants (46.4%) needed surgery.

We compared the hematologic parameters including aNRBC of ROP infants with the matched control group. There were no significant differences between the infants with ROP and the control group in terms of hematocrit, absolute lymphocyte, and platelets counts. And, to evaluate whether infants who developed ROP had higher aNRBC counts at birth compared with the control infants, we examined their aNRBC counts.

Table 2. Major Morbidities and Oxygenation Supplementation in Infants with Retinopathy of Prematurity and Their Matched Controls

	Infants with ROP (n=28)	Control (n=28)	P-value
Ventilator duration (d) (n=21 vs. 23)	8.7 $\pm$ 10.1	6.4 $\pm$ 9.1	0.427
Duration of oxygen (d)	37.8 $\pm$ 27.5	36.4 $\pm$ 30.7	0.858
Maximal FiO ₂ (%)	54.5 $\pm$ 25.7	45.4 $\pm$ 20.7	0.153
Duration of TPN (day)	20.1 $\pm$ 11.2	16.5 $\pm$ 12.8	0.268
Periventricular leukomalacia, n	3	2	1.000
Intraventricular hemorrhage ($\geq G2$), n	2	5	0.422
Bronchopulmonary dysplasia, n	17	16	1.000

Plus-minus values are mean $\pm$ SD.

Abbreviations: ROP, retinopathy of prematurity; FiO₂, fraction of inspired oxygen; TPN, total parenteral nutrition.

Table 3. Comparison of Hematologic Findings Including between Infants with Retinopathy of Prematurity and Their Matched Controls

	Infants with ROP (n=28)	Control (n=28)	P*
aNRBC (/mm ³)			
Total ROP (n=28)	4,181±590	4,244±390	0.639
Stage I (n=11)	4,109±526		0.384
≥Stage II (n=17)	4,229±643		0.923
≥Stage III (n=14)	4,323±666		0.630
with operation (n=13)	4,342±636		0.546
Hematocrit (%)	45.6±6.5	47.9±4.4	0.127
Platelets (10 ³ /μL)	235.8±76.1	209.5±73.2	0.193
Absolute lymphocytes (10 ³ /μL)	3.8±2.7	4.0±2.4	0.771

Values are mean±SD.

Abbreviations: aNRBC, absolute nucleated red blood cell; ROP, retinopathy of prematurity.

*paired *t*-test: infants with ROP vs. control group.

There was no significant difference in the aNRBC counts of ROP infants with Stage I ($P=0.384$), a more severe stage than Stage II ($P=0.923$) or a more severe stage than Stage III ($P=0.630$) compared with the control infants. Moreover, no difference was observed in aNRBC counts in infants who underwent surgery compared with the control infants ($P=0.546$) (Table 3). Regardless of the severity of ROP, the aNRBC counts at birth in premature infants with ROP were not higher than in the control infants.

DISCUSSION

ROP is a major ocular disorder developed in the incompletely vascularized retina of premature infants, and is associated with significant sequelae that result in blindness. It was described by Lutbetzky et al.¹⁷⁾ that when defining a subgroup of preterm infants at risk for ROP, elevated aNRBC counts at birth may help.

ANRBCs clear rapidly after birth in normal neonates, with the aNRBC count falling by approximately 50% by 12 hours of age, 20-30/mm³ by 48 hours of age, and no remaining aNRBCs after 3-4 days of life²⁶⁻²⁸⁾, however in premature infants, aNRBCs may persist in small numbers for up to 1 week^{27,28)}. The mean normal values of aNRBCs in the first few hours of life in healthy term newborn infants is approximately 500-1,000/mm³, however these values are normally up to 10,000/mm³ in small pre-

mature newborns^{21,26,27,29)}.

Increased aNRBC counts are seen in combination with long-standing erythropoietin caused by fetal hypoxia and/or ischemia, which have been found in pregnancies complicated by intrauterine growth restriction, maternal hypertension, pre-eclampsia, maternal smoking, Rh isoimmunization and maternal diabetes¹⁰⁻¹⁴⁾. When adding chronic hypoxia to the above, raised aNRBCs have also been found physiologically in preterm newborns^{22,27,29)}. In the present study, we excluded conditions known to compromise retinal blood flow and/or oxygen supply such as the above-mentioned variables because these factors are known to affect neonatal aNRBC counts.

It is not yet clearly between increased circulating neonatal aNRBC counts and ROP mechanism. However, it is accepted that relative fetal hypoxia results in increased levels of erythropoietin, which in turn leads to stimulation of erythropoiesis and increased numbers of circulating aNRBCs. Furthermore, the need for an oxygen supply, due to repeated hypoxic and hyperoxic episodes, may be an important factor in the pathogenesis of ROP^{15,18,22,30)}. A few studies have been performed to determine the correlation between high aNRBC levels at birth and ROP^{17,31-33)}.

Lutbetzky et al.¹⁷⁾ found that an elevation in the aNRBC count of premature infants, relative to fetal hypoxia, developed ROP, which is supported by the fact that long-lasting fetal hypoxia and/or ischemia may act an important portion of ROP pathophysiology. In their report, the relationship between hypoxia and ROP was not explained entirely, however hypoxic condition increase vascular endothelial growth factor production, which may stimulate neovascularization secondary. Also, it is possible that hypoxic condition stimulate insulin-like growth factor binding protein-1 production which may decrease free insulin-like growth factor concentrations, prevent normal vessel growth as a result. Niranjana et al.³¹⁾ also reported that the aNRBC at birth could be considered as a screening tool because an increase in the aNRBC count correlated with fetal hypoxia. However, Gotru et al.³²⁾ also reported the relationship between ROP and the aNRBC at birth, in which they hypothesized that infants with ROP over stage II have a higher aNRBC count at birth. They concluded that the aNRBC count at birth was not related to the development of severe ROP. Here, we also carried out a case-matched study similar to the 3 above-mentioned reports, in which we could not find any relationship between the aNRBC count at birth and the development of

ROP. Furthermore, regardless of the severity of ROP, including surgery cases, the aNRBC count at birth in premature infants with ROP was not higher than in the control infants. So, we agree with the Gotru's report that the aNRBC count at birth was not related to the development of severe ROP. However, Christensen et al.³³⁾ reported that aNRBC count over the 95% limit at birth is related with severe ROP ($\geq$ Stage 3). Moreover, Niranjana et al.³¹⁾ reported that the more severity of ROP, the higher aNRBC count significantly. Hence aNRBC count in ROP is more significant in the sensitivity, specificity, and positive predictive value than other hematological parameters. In fact, extreme increases in aNRBCs are seen physiologically in premature infants and infants with labour and vaginal birth, and may be found without asphyxia¹⁵⁾. Even increase of aNRBC is seen as idiopathic¹⁵⁾. Thus, we cannot support the suggestion that an increased aNRBC count at birth is related to the development of ROP.

Other hematologic indices as a hematocrit, absolute lymphocyte count, and platelets also known to be an indicator of fetal hypoxia. We founded these indices are not good indicator to detect the fetal hypoxia in this report, because which might indicate acute rather than chronic hypoxia. Lubetzky et al.¹⁷⁾ also founded that these indices are not elevated in chronic hypoxia infants.

However, to conclude that the aNRBC affect the development of ROP, more analyses are needed. That is because many infants are excluded in this study for several reasons and the sample size is too small.

We conclude that the aNRBC count at birth was not higher in premature infants who developed ROP compared with that in the control infants. Therefore aNRBC counts at birth may not be related directly to the development of ROP.

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