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Association of Blood Transfusion and Retinopathy of Prematurity in Preterm Neonate

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Abstract

Background: Retinopathy of prematurity (ROP) is a vaso-proliferative disorder of the immature retina and a leading cause of preventable childhood blindness. Multiple risk factors have been implicated in its development, including oxygen therapy, sepsis and blood transfusion. ***Objective:*** This study was conducted to determine the association between blood transfusion and ROP among preterm neonates and to assess the effect of early erythropoietin (EPO) administration on anemia correction and transfusion requirement. ***Methods:*** This randomized controlled trial was conducted in the Special Care Baby Unit (SCABU) and Neonatal Intensive Care Unit (NICU) of Dhaka Shishu (Children) Hospital from July 2018 to June 2020. A total of 60 preterm low-birth-weight neonates (<34 weeks gestation, <1800 gm birth weight) were enrolled and randomly allocated into two groups: the EPO group (n=30) and the control group (n=30). The EPO group received recombinant human erythropoietin (400 IU/kg subcutaneously, thrice weekly for two weeks), along with oral iron and folic acid supplementation. Hematologic parameters, blood transfusion requirements and ROP development were evaluated and compared between groups. ***Results:*** EPO administration significantly increased hemoglobin, hematocrit and reticulocyte counts and reduced the need for blood transfusion (23.3% vs. 53.3%; p= 0.017). The overall incidence of ROP did not differ significantly between the groups (26.7% vs. 36.7%; p= 0.405). However, a strong association was observed between blood transfusion and ROP development (p= 0.026). ***Conclusion:*** Early erythropoietin therapy effectively reduced blood transfusion requirements without increasing ROP risk. Blood transfusion was significantly associated with ROP development, suggesting that restrictive transfusion strategies and early anemia management may help reduce ROP incidence among preterm neonates.

Keywords: Retinopathy of prematurity (ROP); Blood transfusion; Preterm neonate; Erythropoietin (EPO); Anemia of prematurity; Low birth weight (LBW); Neonatal intensive care unit (NICU).

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INTRODUCTION

Preterm birth interrupts the normal intrauterine maturation of the retina and its vasculature, placing very preterm and very low birth weight (VLBW) infants at substantial risk of retinopathy of prematurity (ROP), a vaso-proliferative retinal disorder that remains a leading cause of avoidable childhood visual impairment worldwide. ROP is characterized by a biphasic pathophysiology in which early suppression of physiologic retinal vascular growth is followed by hypoxia-driven pathological neovascularization that, if unchecked, may progress to tractional retinal detachment and permanent vision loss [1-3]. The incidence and severity of ROP vary with neonatal survival rates, local NICU practices (particularly oxygen management) and the demographic mix of infants served; in low- and middle-income settings the burden of ROP may be large because of expanding neonatal survival without corresponding ROP screening and treatment capacity [1,4]. Well-established risk factors for ROP include lower gestational age and lower birth weight (LBW); however, ROP is a multifactorial disease and multiple

postnatal exposures prolonged oxygen therapy, respiratory instability, sepsis, poor weight gain and dysregulated growth factor signaling modify risk [2,3]. Among hematologic factors, both anemia of prematurity and the practice of red blood cell (RBC) transfusion have been repeatedly implicated in observational studies as associated with increased ROP risk, but distinguishing the causal role of transfusion itself from confounding by severity of illness remains challenging [5-7]. There are several plausible biological mechanisms linking RBC transfusion to ROP. Transfusion of adult packed red blood cells replaces circulating fetal hemoglobin (HbF) with adult hemoglobin (HbA), altering oxygen delivery characteristics at the retinal level and potentially increasing retinal oxygen exposure after transfusion; this sudden change in oxygen delivery could exaggerate the phase-I vaso-obliterative effects or precipitate abnormal angiogenic signaling [8]. In addition, transfusions increase exposure to iron and may amplify oxidative stress and inflammation; both oxidative injury and inflammatory mediators can exacerbate retinal vascular pathology in susceptible preterm infants [6,9]. These mechanistic considerations are supported by meta-analytic and large observational data that report an association between greater transfusion exposure (number, volume or earlier timing) and higher odds of ROP, although the strength of association is attenuated in some analyses after multivariable adjustment for confounders such as gestational age and illness severity [5,7,10].

Because transfusion is often used to treat clinically important anemia that itself may be harmful, strategies that reduce transfusion exposure such as restrictive transfusion thresholds, careful blood-sampling minimization and erythropoiesis-stimulating therapy have been studied. Recombinant human erythropoietin (rHuEPO) and related agents stimulate erythropoiesis and can decrease transfusion requirements in preterm infants; yet, early concerns were raised that erythropoietic stimulation (or higher circulating EPO concentrations) might promote retinal angiogenesis and thus increase ROP risk [11-13]. Large, randomized trials and secondary analyses have therefore explicitly evaluated ROP outcomes in infants treated with EPO: contemporary high-quality trials, including the PENUT (Preterm Erythropoietin Neuroprotection) trial program and subsequent analyses, have not demonstrated a consistent increase in overall ROP incidence or severity attributable to EPO administered in neuroprotective or erythropoietic regimens [12,13]. Nevertheless, heterogeneity across dosing regimens, timing of initiation and populations studied makes it important to consider transfusion- ROP relationships separately from EPO safety questions [12,13]. Given the persistent uncertainty about whether transfusion exposure contributes independently to ROP development (or is mainly a marker of disease severity), further well-characterized studies are needed especially in middle- and low-income country settings where transfusion practices and neonatal case-mix differ from high-income settings [5,7]. Clarifying the association between blood transfusion and ROP and identifying modifiable transfusion-related factors (timing, volume, HbF depletion, or iron load) would inform neonatal transfusion policy and ROP prevention strategies. The present study therefore examines the relationship between blood transfusion and ROP among preterm neonates, leveraging prospectively collected clinical and hematologic data to adjust for key confounders and to explore transfusion timing and dose in relation to ROP outcomes.

METHODOLOGY

Study Design and Settings

This study was a randomized controlled trial conducted to evaluate the association between blood transfusion and the development of retinopathy of prematurity (ROP) among preterm neonates and to assess the effect of early administration of recombinant human erythropoietin (EPO) on the correction of anemia of prematurity and the subsequent need for blood transfusion. The study was conducted in the Special Care Baby Unit (SCABU) and Neonatal Intensive Care Unit (NICU) of Dhaka Shishu (Children) Hospital, Dhaka, the largest pediatric hospital in Bangladesh, over a period of two years, from 1st July 2018 to 30th June 2020. All preterm, low-birth-weight (LBW) neonates admitted to SCABU and NICU of Dhaka Shishu Hospital during the study period were considered eligible for enrollment.

Sample Size Determination

Sample size was calculated using the following formula:

$$n = \frac{(Z_\alpha + Z_\beta)^2 \times 2\sigma^2}{(\mu_1 - \mu_2)^2}$$

Where,

- $Z_\alpha = 1.96$(For 95% confidence level)
- $Z_\beta = 1.28$(For 90% power)
- $\sigma = 1.7$(SD of control group)
- $\mu_1 = 11.6$(Expected control mean)
- $\mu_2 = 9.83$(Expected experimental mean)

The calculated sample size was 24 neonates in each group. To enhance reliability, 30 neonates per group (total 60) were finally included**.**

Sampling Method

Participants were selected through simple random sampling among eligible preterm neonates (<34 weeks of gestational age) admitted during the study period. Randomization was done by lottery method: sealed envelopes containing group assignments ("Control" or "Early EPO") were placed in a box and drawn one at a time during enrollment. This ensured unbiased allocation until the target sample size was achieved.

Selection Criteria

Inclusion Criteria

- Gestational age <34 completed weeks
- Birth weight <1800 grams
- Age <8 days

Exclusion Criteria

- Gestational age >34 weeks
- Birth weight >1800 grams
- Syndromic infants or those with major congenital anomalies
- Neonates with other ocular disorders (cataract, glaucoma, corneal opacity, or retinal vascular disease)

Data Collection Instrument

Data were collected using a pretested structured questionnaire containing sections on demographic information, maternal history, neonatal course and laboratory findings.

Study Procedure

After obtaining approval from the Ethical Review Committee (ERC) of the Bangladesh Institute of Child Health (BICH) and informed written consent from parents or legal guardians, eligible neonates were enrolled.

- Assessment: Gestational age was determined by the Modified New Ballard Score and maternal last menstrual period (LMP).
- EPO Group: Received recombinant human EPO (Epoetin) 400 IU/kg subcutaneously every alternate day (three times a week) for two weeks (total six doses), along with daily oral iron (6 mg/kg/day) and folic acid (1 mg/kg/day) supplementation.
- Control Group: Received only standard oral iron and folic acid supplementation.
- Drug verification: Epoetin (Recombinant human erythropoietin- Incepta Pharmaceuticals Ltd., Bangladesh) was used after confirming expiry date and regulatory approval.

Blood samples were collected at three time points- before EPO initiation, at the end of the second week and at the end of the fourth week- to assess hemoglobin (Hb), hematocrit (Hct) and reticulocyte counts. Daily follow-up included monitoring of oxygen therapy, respiratory distress syndrome (RDS), apnea, sepsis and other complications. ROP screening was performed by an experienced ophthalmology team using indirect ophthalmoscopy at 4 weeks of age, then weekly or biweekly if abnormal, or monthly if normal.

Statistical Analysis of Data

Data were entered, cleaned and analyzed using Statistical Package for Social Sciences (SPSS) version 26.0. Categorical variables were presented as numbers and percentages; continuous variables as mean with standard deviation (±SD). Comparison of categorical variables were done by Chi-square or Fisher's exact test. Continuous variables were compared using paired t-tests and unpaired t-tests. A p-value <0.05 was considered statistically significant.

Ethical Issues

Participation in the study did not affect ongoing treatment or add financial burden. Parents were informed of their right to withdraw at any stage without loss of standard care. Confidentiality of all participant data was strictly maintained, in accordance with the Declaration of Helsinki.

RESULTS

A total of 60 preterm low birth weight infants (<34 weeks gestational age, <1800 gm birth weight) were enrolled in this study and equally divided into two groups- those who received early erythropoietin (EPO) and those who did not. The baseline demographic and clinical characteristics were comparable between the groups (p>0.05).

The mean gestational age of the EPO group was 31.77±1.50 weeks and that of the control group was 32.03±1.88 weeks. Mean birth weights were 1410.3±261.1 gm and 1427.3±269.9 gm, respectively. Male-to-female distribution was similar in both groups (p= 0.796). No significant differences were found in maternal factors such as hypertension, prolonged rupture of membrane or multiple births (p>0.05 in all instances) (Table- 1).

Table 1: Baseline characteristics of all the infants enrolled in study (N=60)

Variables	EPO Group (n=30)	Control Group (n=30)	p-value
Gender			
Male	16(53.3%)	15(50.0%)	0.796
Female	14(46.7%)	15(50.0%)	
Gestational age (weeks)	31.77±1.50	32.03±1.88	0.547
Birth weight (gm)			
LBW (1500-2499 gm)	15(50.0%)	14(46.7%)	
Very LBW (1000-1499 gm)	14(46.7%)	14(46.7%)	0.547
Extreme LBW (<1000 gm)	1(3.3%)	2(6.7%)	
Mean±SD birth weight	1410.3±261.1	1427.3±269.9	
Maternal age (years)	24.3±5.93	26.0±5.28	0.237

Variables	EPO Group (n=30)	Control Group (n=30)	p-value
Maternal hypertension	9(30.0%)	3(10.0%)	0.104
Prolong rupture of membrane	7(23.3%)	6(20.0%)	0.754
Twin/triplet	11(36.7%)	6(20.0%)	0.152

Among neonatal comorbidities, sepsis was significantly lower in the EPO group (40.0%) compared to controls (66.7%) (p= 0.038). The incidence of respiratory distress syndrome (RDS), apnea and hyperglycemia did not differ significantly between the groups (p>0.05 in all instances) (Table- 2).

Table 2: Associated disease with prematurity in between two groups (N= 60)

Associated disease	EPO Group (n=30)	Control Group (n=30)	p-value
RDS	3(10.0%)	5(16.7%)	0.448
Apnea	3(10.0%)	4(13.3%)	0.688
Sepsis	12(40.0%)	20(66.7%)	0.038
Hyperglycemia	3(10.0%)	2(6.7%)	0.640

At baseline, hemoglobin (Hb), hematocrit (Hct), and reticulocyte counts were similar in both groups (p>0.05). After two weeks, reticulocyte counts were significantly higher in the EPO group (3.30±0.62%) compared to controls (1.63±0.69%) (p<0.001). At four weeks, Hb (13.99±2.48 gm/dL vs. 9.98±2.76 gm/dL), Hct (40.16±7.63% vs. 29.34±7.37%), and reticulocytes (3.71±0.71% vs. 1.19±0.32%) were all significantly higher in the EPO group (p<0.001 in all instances) (Table- 3).

Table 3: Hematological parameters at different time points (N= 60)

Parameter	Time Point	EPO Group (Mean±SD)	Control Group (Mean±SD)	p-value
Hemoglobin (gm/dL)	Baseline	14.53±2.44	15.73±3.02	0.096
	14 days	12.40±1.99	12.77±1.85	0.460
	28 days	13.99±2.48	9.98±2.76	<0.001
Hematocrit (%)	Baseline	41.86±8.50	46.83±12.32	0.074
	14 days	37.64±6.60	38.24±5.47	0.708
	28 days	40.16±7.63	29.34±7.37	<0.001
Reticulocyte (%)	Baseline	1.15±0.40	1.70±0.41	0.085
	14 days	3.30±0.62	1.63±0.69	<0.001
	28 days	3.71±0.71	1.19±0.32	<0.001

A significant difference was found in transfusion requirements: only 23.3% of infants in the EPO group required transfusion compared with 53.3% in the control group (p= 0.017). This finding suggests that early EPO therapy substantially reduced the need for red cell transfusion among preterm neonates (Table- 4).

Table 4: Requirement of blood transfusion (N= 60)

Blood Transfusion	EPO Group (n= 30)	Control Group (n= 30)	p-value
Yes	7 (23.3%)	16 (53.3%)	0.017
No	23 (76.7%)	14 (46.7%)	

Blood transfusion was significantly lower among infants who received EPO.

It was observed that, ROP developed in 26.7% of the EPO group and 36.7% of the control group; this difference was not statistically significant between the groups (p= 0.405) (Table- 5).

Table 5: Development of retinopathy of prematurity (ROP) among the study population (N= 60)

ROP Status	EPO Group (n=30)	Control Group (n=30)	p-value
Yes	8 (26.7%)	11 (36.7%)	0.405
No	22 (73.3%)	19 (63.3%)	

The distribution of retinopathy of prematurity (ROP) stages was comparable between the EPO and control groups. In the EPO group (n=8); 2 infants (25.0%) had Stage 1 ROP, 3 (37.5%) had Stage 2 and 3 (37.5%) had Stage 3 ROP. In the control group (n=11); 3 infants (27.3%) had Stage 1, 3 (27.3%) had Stage 2 and 5 (45.5%) had Stage 3 ROP. Although Stage 3 ROP was proportionally more frequent in the control group than in the EPO group (45.5% vs. 37.5%), while Stage 2 was somewhat more common in the EPO group (37.5% vs. 27.3%), these differences were not statistically significant (p= 0.890) (Table- 6).

Table 6: Severity of ROP among the study groups (n= 19)

Stage of ROP	EPO Group (n= 8)	Control Group (n= 11)	p-value
Stage 1	2 (25.0%)	3 (27.3%)	0.890
Stage 2	3 (37.5%)	3 (27.3%)	
Stage 3	3 (37.5%)	5 (45.5%)	

A statistically significant association was observed between blood transfusion and ROP development. Among ROP-affected infants, 63.6% in the control group required transfusion compared with only 12.5% in the EPO group (p= 0.026). This indicates that infants receiving transfusions were more likely to develop ROP (Table- 7).

Table 7: Association between blood transfusion and ROP (n= 19)

Blood Transfusion	EPO Group (n= 8)	Control Group (n= 11)	p-value
Yes	1 (12.5%)	7 (63.6%)	0.026
No	7 (87.5%)	4 (36.4%)	

Infants who received transfusions were significantly more likely to develop ROP.

Sepsis incidence was significantly lower in the EPO group (40.0% vs. 66.7%; p= 0.038). Other complications- including necrotizing enterocolitis, intraventricular hemorrhage, need for mechanical ventilation and mortality, did not differ significantly between groups (p>0.05 in all instances) (Table- 8).

Table 8: Complications observed in both groups (N= 60)

Complication	EPO Group (n= 30)	Control Group (n= 30)	p-value
Sepsis	12 (40.0%)	20 (66.7%)	0.038
Necrotizing enterocolitis	1 (3.3%)	1 (3.3%)	1.000
Intraventricular hemorrhage	2 (6.7%)	4 (13.3%)	0.718
Mechanical ventilation	4 (13.3%)	5 (16.7%)	0.718
Neonatal mortality	3 (10.0%)	4 (13.3%)	0.687

DISCUSSION

This randomized controlled study conducted among preterm neonates admitted to the NICU of Dhaka Shishu Hospital, Dhaka, Bangladesh. We evaluated the association between blood transfusion and the development of retinopathy of prematurity (ROP) and the potential modifying effect of early erythropoietin (EPO) therapy. Our results revealed that early administration of recombinant human EPO significantly improved hematological parameters- hemoglobin, hematocrit and reticulocyte counts; while reduced the requirement for red blood cell transfusions. However, there was no statistically significant difference in the overall incidence of ROP between the EPO and control groups. Importantly, a significant association was observed between blood transfusion and ROP development, suggesting that transfusion exposure may independently contribute to ROP pathogenesis in preterm infants.

The mean gestational age and birth weight of participants in both groups were comparable, consistent with similar baseline characteristics seen in previous neonatal ROP studies [14]. These parameters are established determinants of ROP risk, as retinal vascular development is inversely related to gestational age and weight at birth [15]. Despite comparable demographic profiles, a lower frequency of transfusion in the EPO-treated group was evident, reflecting EPO's erythropoietic efficacy and its role in reducing transfusion dependency. Similar findings were documented by Aher SM and Ohlsson A, who demonstrated that EPO effectively reduces donor blood exposure in preterm neonates without increasing mortality or major morbidity [16].

Our study found that only 23.3% of infants receiving early EPO required blood transfusion compared to 53.3% in the control group, a difference that was statistically significant (p= 0.017). This aligns with findings from Maier *et al.,* who observed a 40–60%

reduction in transfusion requirement following early EPO therapy in preterm infants [17]. Increased reticulocyte production observed in our EPO group at two and four weeks further confirms effective erythropoiesis, supporting the hematological benefits of early EPO administration.

However, the incidence of ROP was not significantly different between the two groups (26.7% in the EPO group versus 36.7% in controls, p= 0.405). This suggests that EPO itself neither prevents nor exacerbates ROP when used within the studied dosage and duration. Large multicenter trials, including the Preterm Erythropoietin Neuroprotection (PENUT) trial, similarly reported no significant difference in ROP incidence between EPO-treated and placebo groups, indicating that EPO does not increase the risk of ROP [18].

The distribution of ROP severity (stages 1-3) was also similar across groups (p>0.05). Stage 3 ROP was found in 37.5% of affected infants in the EPO group and 45.5% in the control group. The findings suggest that EPO administration was not significantly associated with the distribution or severity of ROP stages in this study population. However, the small sample size should be considered when interpreting the absence of a statistically significant association.

The key finding of this study was the statistically significant association between blood transfusion and ROP (p= 0.026). Among neonates who developed ROP, 63.6% had received transfusions compared to only 12.5% who did not. This finding supports the hypothesis that blood transfusion may play a contributory role in ROP development. The mechanism behind this association may be multifactorial. Adult packed red cells contain hemoglobin A (HbA) with lower oxygen affinity compared to fetal hemoglobin (HbF), leading to increased retinal oxygen exposure after transfusion and subsequent oxidative injury to developing retinal vessels [19]. Additionally, transfused blood increases free iron levels and oxidative stress, potentially aggravating endothelial damage and retinal neovascularization [20].

Our results corroborate previous observational and meta-analytic evidence. Zhu Z *et al.,* conducted a systematic review and found that transfused preterm infants had 1.8 times higher odds of developing ROP compared to non-transfused peers [5]. Similarly, Hengartner T *et al.,* reported that early and multiple transfusions were independently associated with severe ROP after adjusting for gestational age and oxygen exposure [7]. Teofili L *et al.,* further suggested that early transfusions reduce circulating fetal hemoglobin and thereby increase susceptibility to ROP through altered oxygen dynamics [8].

Despite these associations, it remains debated whether transfusion is a true causal factor or a proxy for disease severity. Critically ill neonates requiring transfusion often have higher oxygen exposure, sepsis and prolonged ventilation- all known risk factors for ROP [21,22].

The absence of a significant difference in ROP incidence between EPO and control groups also indicates that early EPO administration, within the studied regimen, is not detrimental to retinal vascular development. This finding agrees with Fauchère JC *et al.,* who showed that early high-dose EPO did not increase ROP severity or the need for laser therapy [23]. Our findings thus reinforce the safety of early EPO use for anemia correction in preterm neonates while highlighting the need for vigilant monitoring of transfusion practices.

From a public health perspective, these findings are especially relevant for resource-limited neonatal units in Bangladesh and South Asia, where transfusion protocols are often liberal and oxygen monitoring facilities may be inconsistent. The findings of this study suggest that, early management of anemia using EPO along with restrictive transfusion protocols and careful neonatal monitoring, can contribute to the prevention of transfusion-related complications such as ROP, thereby improving neonatal outcomes in resource-limited settings like Bangladesh.

CONCLUSIONS

The study demonstrated that early use of EPO significantly improved hematological parameters hemoglobin, hematocrit and reticulocyte count and markedly reduced the need for blood transfusion among preterm neonates. However, the overall incidence and severity of ROP were not significantly different between neonates who received EPO and those who did not. A statistically significant association was found between blood transfusion and the occurrence of ROP. Lower gestational age, respiratory distress syndrome (RDS) and hyperglycemia were identified as additional risk factors for ROP development. Therefore, while early EPO therapy appears to safely reduce transfusion requirements without increasing ROP risk, judicious use of blood transfusion and strict monitoring of oxygen therapy are essential to minimize the occurrence of ROP in preterm neonates.

Recommendation

Reducing unnecessary transfusions through restrictive transfusion thresholds, EPO therapy and judicious oxygen use could minimize ROP risk and improve long-term visual outcomes in preterm neonates. Further larger, well-controlled studies are recommended to confirm the association between early use of EPO and ROP.

Strengths and Limitations

The strengths of this study include its randomized controlled design, standardized

ophthalmologic assessments and uniform laboratory evaluations. However, the study was limited by its single center setting and relatively small sample size; which may affect the generalizability of results. The follow-up period was confined to early post-natal weeks, so late-onset ROP progression could not be fully assessed.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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