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Effect of short term recombinant human erythropoietin (rHuEPO) therapy in the prevention of anemia of prematurity (AOP) in very low birth weight (VLBW) neonates

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Abstract

Premature infants especially those with birth weight <1500 g suffer from Anaemia of prematurity (AOP) and associated problems. Erythropoietin therapy is a safe effective way to prevent and to treat anaemia of prematurity. To evaluate the effect of short term administration of recombinant human erythropoietin (rHuEPO) with iron and folic acid in very low birth weight (VLBW) neonates in the prevention of anaemia of prematurity. A randomized controlled trial was carried out at Dhaka Shishu Hospital. Sixty preterm very low birth weight (PTVLBW) babies were enrolled in this study. Thirty were assigned to rHuEPO group and 30 as control. Baseline haematologic values were estimated before administration of rHuEPO. From day 7 of life rHuEPO-200 IU/kg/dose subcutaneously every alternate day for 2 weeks was administered to rHuEPO group. All infants in both groups have received oral iron, folic acid from day 14. Clinical and haematological assessment was done at 6 and 10 weeks of life. Baseline clinical characteristics and haematologic values were almost similar in both groups. This study has shown increase in haematological values(haemoglobin and haematocrit) and reduction in the number of blood transfusions during both the 1st and 2nd follow up in rHuEPO group in comparison to control group ($p<0.01$). Short term rHuEPO appears to be very effective in prevention of Anaemia of prematurity.

Introduction

Prematurity and low birth weight is a common global problem. PTVLBW causes high morbidity and mortality but the survival of PTVLBW has increased due to advancement of modern facilities in investigations, medications and Neonatal ICU care. This also contributes to the increase in numbers of Anaemia of prematurity (AOP) patients. Anemia of prematurity is characterized by low serum erythropoietin (EPO) level with a remarkably low hemoglobin concentration and low reticulocyte count[1]. AOP may cause symptoms such as poor feeding, inadequate weight gain, apnea, tachycardia, tachypnoea etc[2]. The mainstay of management of AOP is blood transfusion but it is hazardous for preterm very low birth weight (PTVLBW) neonates viz transmission of infection, volume overload, alloimmunization etc. Recombinant erythropoietin therapy-offers promise in ameliorating the anemia of prematurity[3].

During last two decades many clinical trials have been conducted to evaluate the role of recombinant human erythropoietin in preventing and treating anemia of prematurity. Most of the studies showed that the severity of anemia of prematurity and the need for red cell transfusion can be reduced by a combination therapy of rHuEPO, iron and folic acid and by limiting iatrogenic blood loss[4-8]. As therapy with recombinant human erythropoietin is found to be effective in the prevention of anemia of prematurity, its use is increasing gradually.

However, the optimal therapeutic strategy of dosage, duration and time of administration of recombinant erythropoietin still remain uncertain. A wide range of recombinant human erythropoietin doses and administration schedules have been evaluated in preterm infants since the first pilot study published by Halpérin et al. in 1990[9]. Though the dose and administration schedules were variable in the previous studies but the results were almost similar.

So far no such study has been conducted in Bangladesh to see the effect of administration of rHuEPO in addition to Iron, folic acid in prevention of AOP in PTVLBW neonates. This study was carried out to see the effect of short term

administration of rHuEPO with iron and folic acid in prevention of AOP and reduction in the number of transfusions in PTVLBW neonates.

Materials and Methods

The randomized controlled trial study was conducted from April 2007–May 2008 at Neonatal Unit and Intensive Care Unit (ICU) of Dhaka Shishu (children) Hospital, Dhaka, Bangladesh. It is a 500 bedded tertiary care children hospital. There is around 2000 neonatal admission per year of which around 18% are preterm very low birth weight neonates (hospital admission register - Jan.-Dec. 2006).

In this study we included both male & female preterm neonates of less than 7 days of age, <35 weeks of gestation and <1500gm weight. Neonates with IUGR, anemia due to other causes, gross congenital anomalies and acutely ill patients were excluded. The purpose and procedure of the study were explained and written consent were obtained from the parents or guardian who agreed to participate. Sixty neonates were selected and divided in to two groups by simple randomization (lottery method). Thirty neonates (group-I) were supplemented with rHuEPO, iron and folic acid and thirty neonates (group-II) were supplemented with iron and folic acid only.

Study procedure: After registration of the patient, detailed history was taken from the mother or relatives and a physical examination was done. Gestational age was determined by maternal record (maternal recall of LMP or available ultra sonogram reports) and by New Ballard Score system. When there was discrepancy between maternal record and New Ballard Score system then Ballard scoring system was used to estimate the gestational age. Before intervention weight, Crown- heel length, Occipito-frontal circumference (OFC) were measured and recorded.

Hematological Assessment: Hematological Assessment was done for both groups on day 7 before administration of rHuEPO (for baseline investigation) and during the follow up at 6 weeks and 10 weeks of age at the hospital pathology department. Baseline and follow up investigations (Hb concentration, Reticulocyte count, Platelet count, and Hct value) were determined by automated blood analyzers. Reticulocyte count was estimated microscopically using brillant-cresyl-blue staining. Hematological assessment was also done at any time if clinical anemia was found.

rHuEPO, Iron and Folic acid Administration: Neonates assigned to group I received rHuEPO 200 IU/kg/dose[10] subcutaneously three times /week for 2 weeks started on day 7 of life. Patients of both groups received oral Iron 6 mg/kg/day[11] and Folic acid 0.5mg every alternate day[12] up to 12 weeks of life. Both Iron and Folic acid administration started from day 14 of life or as soon as enteral feeding was initiated after day 14.

During discharge from the hospital parents were advised to attend the follow up clinic at 6 weeks and 10 weeks of age. A follow up clinic is regularly maintained by the Neonatal Unit of Dhaka Shishu (children) Hospital for all discharged neonates. During both visits breast feeding counseling was given, anthropometric measurements (weight, length, OFC) were recorded and hematological investigations (Hemoglobin concentration, Hematocrit value, Reticulocyte count) were checked. Any patient with Hb level of ≤7 gm/dl patient was readmitted in the hospital and managed with packed cell transfusion. The study protocol was approved by the Ethical Review Committee of Dhaka Shishu (children) Hospital and Bangladesh Institute of Child Health, Dhaka.

Statistical Analysis : Appropriate statistical analysis was done with SPSS version 12.0 (Statistical package of social service Inc, Chicago, USA). The results were presented in tables. For significance of difference unpaired Student 't' test and chi-square test were done. A probability 'p' value <0.05 was considered as significant.

Results

Total 60 preterm very low birth weight neonates were enrolled in the study. Thirty patients in each group. Group I supplemented with rHuEPO, iron and folic acid and Group II supplemented with iron and folic acid. In group I, 4 infants died during hospital stay and 1 patient did not come in 1st follow up and 1 in 2nd follow up. In Group II, 5 infants died during hospital stay and 2 did not come in 2nd follow up. Finally 24 infants in Group I and 23 infants in Group II completed the follow up until 10 weeks of age. These 13 dropped out infants were excluded from the analysis. No adverse effect of erythropoietin were found.

Neonates of both groups were matched and similar in age, sex, gestational age, birth weight, length and OFC during the admission. This study showed slight male preponderance. (in group I number of male was 18 i.e. 60% and in group II male was 19 i.e. 63.3%). Baseline clinical and hematological values (Hb, Hct, Reticulocyte count) were estimated and no significant differences (p>0.05) were found between the two groups (Table-I, II).

Table I: Base line clinical characteristics at the time of admission

	Group I (n=30) Mean±SD	Group II (n=30) Mean±SD	P value
Gestational age	30.4±0.9	30.2±1.2	0.395
Birth weight	1348.3±50.1	1365.1±68.4	0.282
Length	40.1±1.2	39.8±1.2	0.333
OFC	29.4±0.9	29.0±0.8	0.061

Table II: Base line hematological values at day 7 of life

	Group I (n=30) Mean±SD	Group II (n=30) Mean±SD	p value
Hb gm/dl	14.9±1.1	15.4±1.3	0.102
Hematocrit %	45.±3.3	47.3±3.2	0.102
Reticulocyte count %	1.8±0.8	2.2±0.7	0.069
MCV fl	106.0±3.5	107.8±4.0	0.064
Platelet K/µL	214.2±49.5	212.0±45.4	0.861

During hospital stay in the first 3 weeks of their life, total number of neonates (7 vs 8), number of transfusions (2.1±0.6 vs1.9±0.6) and age at which blood transfusions required (11.4±2.8 vs 12.2±1.8 days) were almost similar in both groups (Table-III, IV).

Table III: Blood transfusion (BT) status during hospital stay

	Group I (n=24)		Group II (n=23)		P value
	n	%	n	%	
No. of neonate required BT	7	29.2	8	34.8	0.679
No. of. neonate with out BT	17	70.8	15	65.2	

Table IV: Blood transfusion status during hospital stay

	Group I (n=24) Mean±SD	Group II (n=23) Mean±SD	P value
Age at which transfusion required	11.4±2.8	12.2±1.8	0.230
No. of transfusion	2.1±0.6	1.9±0.6	0.152

Hemoglobin levels: Baseline hemoglobin levels were within the normal limit (14.9 ± 1.1 vs. 15.4 ± 1.3) but gradually these levels were reduced in both groups. Reduction of hemoglobin level was more visible in Group II than in Group I, which was statistically significant (P value is <0.05, Table V).

Requirements of blood transfusions: No blood transfusions were required in group I during the 1st and 2nd follow up. However in group II, three (13%) infants in the 1st follow up and two (8.7%) infants in the 2nd follow up required blood transfusions (Table-VI).

Growth pattern: Growth pattern of both the groups were compared. There was no significant difference in weight during the enrollment but an increment of weight was found from baseline to the 1st follow up (from 1348.3±50.1gm to 2203.1±74.5gm) in group I and (from 1365.1±68.4gm to 2136.6±110.4 gm) in group II respectively. A similar trend in weight gain was also found in the 2nd follow up (Table VII).

The linear growth was higher in the rHuEPO recipient group than the control group (P<0.05). Mean increasing length at the 1st follow up from 40.1±1.2cm to 46.4±1.1cm in group I and from 39.8±1.2cm to 45.7±1.3cm in group II respectively. These differences were statistically significant between the two groups. A similar trend in length increment was found in the 2nd follow up (Table VII).OFC increment in both groups during the 1st & 2nd follow up was not statistically significant.

Table V: Hematological data during follow-up at 6 weeks and 10 weeks of life

Parameters	1st follow up at 6weeks			2nd follow up at 10 weeks		
	Group I (n=24) Mean±SD	Group II (n=23) Mean±SD	P value	Group I (n=24) Mean±SD	Group II (n=23) Mean±SD	P value
Hb gm/dl	10.9±1.0	8.1±1.2	0.001	11.6±0.6	9.6±1.3	0.001
HCT %	33.1±2.5	26.7±4.2	0.001	35.1±2.3	27.7±3.4	0.001
Reticulocyte count%	1.7±0.5	1.2±0.5	0.001	2.0±0.4	1.4±0.6	0.001

Table VI: Distribution of patient during follow-up in terms of Hemoglobin concentration

	1st follow up at 6weeks				2nd follow up at 10 weeks			
	Group I (n=24)		Group II (n=23)		Group I (n=24)		Group II (n=23)	
	n	%	n	%	n	%	n	%
≤7 gm/dl	0	0.0	3	13.0	0	0.0	2	8.7
>7- <10 gm/dl	5	20.8	12	52.2	0	0.0	7	30.4
≥10 gm/dl	19	79.2	8	34.8	24	100	14	60.9

Table VII: Weight gain and Linear growth of the study infants

	Weight (gm)			Length (cm)		
	Group I (n=24) Mean±SD	Group II (n=23) Mean±SD	P value	Group I (n=24) Mean±SD	Group II (n=23) Mean±SD	P value
Baseline	1348.3±50.1	1365.1±68.4	0.282	40.1±1.2	39.8±1.2	0.333
1st follow up	2203.1±74.5	2136.6±110.4	0.031	46.4±1.1	45.7±1.3	0.037
2nd follow up	3135.7±141.4	2969.2±152.3	0.001	51.2±1.1	50.4±1.4	0.022

Discussion

Recombinant human erythropoietin (rHuEPO) is capable of increasing erythropoiesis with very little adverse effects in preterm infants[1]. There is also no evidence of erythropoietin insensitivity therefore rHuEPO helps in reducing the number of red cell transfusion requirements in preterm infants[4-6,8].

Since the first pilot study conducted by Halpérin et al. in 1990[13] numerous clinical trials have been conducted in the last two decades with a wide range of doses and administration schedules of rHuEPO to evaluate the role of it in the prevention and treatment of anemia of prematurity. This is the first documented study has been conducted in Bangladesh to evaluate the effect of short term therapy of rHuEPO in the prevention of AOP in PTVLBW neonates.

There were a total of 13 infants who were dropped out from this study, 6 infants were from group I and 7 infants from group II. In group I out of 6, 4 infants died, due to neonatal sepsis (3) and NEC (1) during the hospital stay, 2 did not come to the follow up. In group II, 5 patients died. Four due to neonatal sepsis & 1 due to IVH. Two infants of this group did not come to the follow up.

Baseline hemoglobin levels were within the normal limit but gradually these levels were reduced in both groups. Reduction of hemoglobin level was more visible in Group II than in Group I, which was statistically significant (P<0.001).This results were consistent with the other studies[7].

Fall of Hb level after birth is a physiological process for all neonate and it is exaggerated in preterm babies leading to various morbidities. One of the reasons for decrease Hb level is low concentration of erythropoietin (EPO). With this background studies are being conducted throughout the world in the use of erythropoietin in neonate to prevent anemia of prematurity. Ohls RK et al found that reticulocyte count and hemoglobin concentration was significantly higher in the rHuEPO treated group and the high level of hemoglobin concentration was maintained throughout the rHuEPO treatment period, this level was observed in all pre term infants and they maintained their Hb level same as the levels of term infants[10]. AG Bechensteen et al also reported that corrected reticulocyte count increased (P<0.001) and Hematocrit increased (P<0.05) in rHuEPO recipient group[5]. These findings were consistent with our study.

Number of neonates who required blood transfusions in the first 3 weeks of their life was almost similar in both the groups. No patient required blood transfusions in group I during the 1st and 2nd follow up. By this way hazards of blood transfusions could be avoided in group I. In group II, however, 3(13%) infants in the 1st follow up and 2(8.7%) infants in the 2nd follow up required blood transfusions.

Prevention of anemia in early neonatal (2weeks) period is not effective with EPO therapy was found in our study and also in other studies. Maier R et al found VLBW infants showed no benefit in the reduction of blood transfusion in first 2 weeks after birth[3]. Donato et al studied infants of birth weight <1250 gm found that the number of transfusions remained unchanged in infants who has received rHuEPO treatment before 2 weeks of age[14]. The first evidence of a response to the three times weekly administration of rHuEPO is an increase in the reticulocyte count within 10 days, followed by increase red cell count, hemoglobin and hematocrit level, usually within 2 to 6 weeks[15,16] Because of the length of time required for erythropoiesis, changes in hematocrit levels is usually not observed in less than 2 weeks of time and may require up to 6 weeks in some patients. In contrast with these results to similar studies conducted by Ohls R et al noted a significant decrease in number of blood transfusion in 1st two weeks[17].

Response of dose and duration: Meyer and colleagues compared transfusion requirements within the 80 preterm infants (<32 weeks gestation and <1500g) receiving SC human recombinant erythropoietin at a dose of 200 units/kg given three times weekly or placebo for 6 weeks. Iron supplementation consisted of 2 to 3 mg/kg/day oral iron. This was increased to 6 mg/kg/day if feritin concentrations were <65µg/dl. Erythropoietin recipients required fewer transfusions (0.17 versus 0.52 transfusions per infant, p=0.002)[18]. In our study dose of EPO is similar and the duration is shorter to this study but we found the same effect. But Shannon K.M. et al found there was no significant difference in the number of transfusions required between the rHuEPO and the placebo groups in a double blinded study[11].

In this study iron and folic acid was provided to the infants from day 14 of their life or as soon as enteral feeding was initiated. Infants were predominantly breast fed during the study period. Compliance with iron and folic acid supplementation was good in both groups during the follow up. In this study hematological values were higher and found statistically significant in group I compared to group II which indicates iron supplementation was needed along with EPO.In other studies, AG Bechensteen et al and Rolf F. Maier et al 2002 showed similar results from iron supplementation[5,7].

~~In this~~ study growth pattern of both the groups were compared, there was no significant difference in weight during the enrollment but soon a difference was found at the 6 and 10 week follow up ($p<0.05$). As high hemoglobin level and absence of anemia has a profound role on tissue oxygenation, cellular growth and proliferation as well as on metabolic functions, so an increment of weight was found during follow up. Significant increase in linear growth was found during both follow up in the rHuEPO recipient group than the control group ($P<0.05$). Rolf F Maier et al found that there was no difference in the terms of weight gain and linear growth between rHuEPO and control group, most probably because of the patients were Extreme low birth weight infants[12].

This study showed OFC increment in both group during the 1st and 2nd follow up was not statistically significant. This result was similar to the results of Rolf F.Maier et al[7].

Conclusion: Though the sample size is small but it is evident from this study that despite giving a moderate dose of rHuEPO for a relatively shorter duration the regime was equally effective as higher doses and longer duration to prevent AOP in PTVLBW. It has also improved weight gain and linear growth in these babies. There fore short term therapy of rHuEPO could be practiced along with iron and folic acid in preterm very low birth weight babies to prevent AOP.

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