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Original Article

Oral Vs Subcutaneous Erythropoietin in Prevention of Anemia of Prematurity

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Md. Jamshed Alam [1], Md. Kamrul Ahsan Khan [2], Md. Shafiqul Islam [3], Kamrul Ahsan [4], Sanjoy Kumer Dey [5], Md. A. Mannan [6], Mohammad Shahidullah [7]

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ABSTRACT

Introduction: *Anemia of prematurity (AOP) is a common problem of very low birth weight (VLBW) babies. Blood transfusion is a necessity when it occurs in moderate to severe form putting the child in to the risk of transfusion related complications. Erythropoietin, a potent stimulator of hemopoesis is available in breast milk in good amount and absorbed intact under physiologic condition. In this background oral recombinant human erythropoietin (rhEPO) can be a useful alternative to its subcutaneous administration in prevention of AOP.* ***Methods:*** *This randomized controlled study was conducted in the NICU of BSMMU from Jan-15 to Dec-15. Total 60 preterm (<34 weeks) VLBW (<1500g) infants were enrolled and randomly divided into Oral (group-A) and Subcutaneous (Group-B). Group-A received rhEPO 400 IU/Kg, 3 times weekly in oral route Group-B the same subcutaneously (S/C) and continued for 2 weeks (Total 6 doses), starting 14 days after birth, when baby achieved oral feeding of at least 50 ml/kg/day of breast milk. All infants received oral iron and folic acid supplementation up to 12 weeks of postnatal age. Transfusion data were recorded. Anthropometric and hematological assessments were done at 2, 4, 6 and 12 weeks of age.* ***Results:*** *Out of 60, total 57 babies completed study, 28 vs 29 babies. Baseline clinical characteristics and hematological values were comparable in both groups. Mean Hb were*

1. *Associate Professor (Pediatrics), Mymensingh Medical College. Mymensingh.*
2. *Assistant Professor (Neonatology), Sheikh Sayera Khatun Medical College, Gopalgonj.*
3. *Assistant Professor (Pediatrics), Tangail Medical College. Tangail.*
4. *Assistant Professor (Pediatrics), Sahid Tazuddin Ahmed Medical College, Gazipur.*
5. *Professor Neonatology. BSMMU.*
6. *Professor Dept. of Neonatology. BSMMU.*
7. *Professor & Chairman. Dept. Neonatology & Ex. Pro VC. BSMMU.*

11.88±0.54gm/dl & 12.12±1.32gm/dl, the mean HCT was 35.66±1.65% & 36.38±3.97% and the mean reticulocyte 9.85±1.50% & 9.22±3.11% in the oral and subcutaneous group respectively at 12 weeks follow up (p>0.05). Similar weight gain was recorded in both groups. One neonates in each group required blood transfusion. ***Conclusion:*** *Administration of oral rhEPO in preterm VLBW infants after the first two weeks of life along with iron and folic acid supplementation stimulated erythropoiesis, increased weight gains and the need for red cell transfusions similar to that of subcutaneous rhEPO.*

Key words: Preterm very low birth weight, Anemia of prematurity, oral rhEPO.

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INTRODUCTION

Anemia in neonates is defined as more than 2 SD below the mean value of the amount of hemoglobin or HCT for a given age [1]. It is very common in very low birth weight (VLBW) infant [2]. Anemia of prematurity (AOP) is a hypo regenerative anemia usually appearing after the second week, reaching its highest intensity in the second month of life. It is normocytic and normochromic anemia with a low reticulocyte count and has been attributed to erythropoietin (EPO) deficiency. The pathogenesis of AOP is not fully elucidated but contributory factors include the reduced life span of the fetal erythrocytes, the relatively low EPO concentration and rapid body growth. [3] It, sometimes has clinical manifestations like pallor, tachycardia, tachypnea, apnea, decreased activity, no weight gain that necessitates blood transfusion [1, 4]. The mainstay of management of AOP is blood transfusion but it is not without the risk of transfusion transmitted infections, volume overload, alloimmunization etc. [5]. So reduction of blood transfusion in the treatment of AOP is always desired.

EPO is the major factor in erythropoiesis in the fetal and neonatal period as well as in puberty by inhibition of apoptosis of progenitors in erythroid lineage and provoking their proliferation and differentiation to normoblasts[6]. Serum level of EPO in premature neonates is lower than mature neonates and after birth its level decreases even more [7]. The low EPO levels detected in premature infants and proper response to synthetic erythropoietin suggested that EPO administration in premature infants could be of benefit in trying to maintain or increase the hematocrit levels. Studies showed that the severity of AOP and the need for red cell transfusion can be reduced by a combination therapy of recombinant human erythropoietin (rhEPO), iron and folic acid and by limiting iatrogenic blood loss[8, 9, 10]. As therapy with rhEPO is found to be effective in the prevention of AOP, its use is increasing gradually. However, the optimal therapeutic dosage, duration, time and route of administration of rhEPO are still remaining uncertain. A wide range of rhEPO doses and administration schedules have been evaluated in preterm infants since the first pilot study [11]. Though the dose and administration schedules were variable but the results were almost similar.

EPO does not cross the placenta. Jull *et al.* has observed that EPO found in mother's milk, is produced by mammary glands [12,

[13]. Evidence shows important developmental roles for these milk-borne growth factors. A significant proportion of milk-borne EPO resists proteolytic degradation. EPO receptors have been found on gastric and intestinal mucosa, and in mesenteric vascular endothelium. It has also been observed that the level of rhEPO, added to mother's milk or standard formula milk under conditions similar to that of the stomach or intestine remaining constant, but undergoes digestion when added to 5% dextrose or saline [14].

While parenteral rhEPO has been shown to enhance erythropoiesis and decrease the need for late blood transfusion in preterm infant, its enteral administration provided some conflicting results [13]. Britton et. al. and Ballin et. al. showed increased erythropoiesis without any change in blood transfusion rate[1, 15]. In this study, we have compared the effectiveness of oral rhEPO versus subcutaneous rhEPO in prevention of AOP. Fe and Folic Acid supplementation was received by three groups. So far to our knowledge, no such study has yet been conducted in Bangladesh to evaluate the efficacy of oral Erythropoietin in prevention of AOP and to compare with injectable EPO. We have evaluated a simple and more suitable way for prevention of anemia of prematurity.

METHODS & MATERIALS

This randomized control trial (RCT) has been carried out in the Department of Neonatology of Bangabandhu Sheikh Mujib Medical University (BSMMU), over a period of one year from Feb13 to Jan 14. Calculated sample size was 73 but a total of 60 newborns have been enrolled purposively. Newborn babies admitted in the NICU of BSMMU were the study population. Babies with gestational age <34 weeks, having birth weight <1500 gm, with stable cardiopulmonary status and oral feeding has been started with breast milk and an amount of at least 50 ml/kg/day is achieved, has been included. Infant with congenital anomalies, intra-ventricular hemorrhage**,** neonatal seizures, immune-mediated hemolytic anemia or having evidence of acquired or congenital infection or babies requiring ventilator support were excluded. After enrollment of each newborn, group allocation was done with sealed envelope method in to two groups' 30 each. Oral erythropoietin Group-A and Subcutaneous erythropoietin as Group-B. Before randomization neither the parents nor the study physician was aware of the group the baby belongs.

After enrolment detailed history of each patient was taken from nearest/close relative to the mother. Then babies were examined thoroughly. Gestational age was determined by maternal record (Maternal recall of LMP or available ultra-sound reports) and further confirmed by New Ballard Scoring system. All relevant information was recorded in the predesigned questionnaire. Before intervention weight was measured by an electronic weighing scale (SALTER Model- 914 UK) with accuracy of ±5g, keeping the baby undressed and before feeding by the neonatal nurse. OFC was measured with an inelastic standard plastic measuring tape to the nearest 1 mm. Head circumference is the maximum circumference around the head at the level of the point just above the glabella anteriorly and the occipital protuberance posteriorly. At about 12-16 days of postnatal age when babies were found

stable and feeding is advanced to at least 50 ml/kg/day with breast milk, base line investigations like CBC (Hb%, HCT), Reticulocyte count and Serum Ferritin level were done. **Group-A** has received oral rhEPO 400IU added to breast milk three times a week for up to 2 weeks, total six dose with daily administration of oral $FeSO_4$ (6mg/kg/day) & folic acid (1mg/kg/day) up to 12weeks. **Group-B** has received rhEPO 400IU subcutaneously in the anterior thigh, three times a week for up to 2 weeks, total six dose with daily administration of oral $FeSO_4$ (6mg/kg/day) & folic acid (1mg/kg/day) up to 12weeks.

Recombinant human Erythropoietin (rhEPO) was available as Injection Epoitin (1000 IU in 0.5ml) in a prefilled syringe manufactured by a local Pharmaceutical company (Incepta Pharmaceuticals Limited). It was diluted 10 times with normal saline & calculated in drops for oral administration. S/C administration was done by the prefilled syringe without dilution. Iron and folic acid was given orally from day 1 of intervention in empty stomach before feeding. Care giver, nurses & doctors were made aware of the adverse events that may happen. Adverse events such as feeding intolerance, necrotizing enterocolitis, circulatory instability, temperature instability, thrombocytopenia leucopenia and others if any were recorded.

After discharge from the hospital parents were advised to attend the follow up clinic at 4weeks, 6 weeks and 12 weeks of age. During all visits anthropometric measurements like weight, length, OFC, side effects of drugs and hematological parameters like CBC (Hb%, Hct.), Reticulocyte count (corrected with hematocrit) and Serum ferritin level done. All the babies were exclusively breastfed after discharge. Any patient with Hb level of <7gm/dl found in any follow up, was evaluated and managed with blood transfusion and the number of which required in each group was recorded.

Blood samples were collected in two different test tubes, one containing EDTA for CBC, Reticulocyte count and other in plain test tube for Serum Ferritin level estimation. CBC (Hb%, Hct.) was determined by photometric method using automated blood analyzer, ABX Pentra Dx 120. Reticulocyte count was done by (FAC) Fluorescent activated flow cell cytometer. Serum Ferritin estimation was done by ARCHITECT Ferritin assay using the reagent is ARCHITECT Ferritin Reagent Kit (7k59). All the investigation was done in the department of Biochemistry of BSMMU.

Data were processed and analyzed by using the Statistical Package for Social Sciences-20 (SPSS-20) software for Windows. Student's T test and Chi square test were used where applicable. Results were considered statistically significant at p value <0.05.

RESULTS

Among the enrolled 60 new born, after oral administration of iron, 4 babies had vomiting once or twice and abdomen seems to be distended mildly. These problems were managed by keeping the oral iron off for one to two days. During whole study period 2 patients required blood transfusions (1 in oral and 1in subcutaneous group), 5 patients developed feeding intolerance, and three patients had septicemia and DIC (Disseminated Intravascular Coagulation). One patient

died, 1patient denied further blood sampling after the first baseline investigations, 1 patient was dropped out after discharge keeping no further communication, so ultimately 57 patients completed the study, Group-A 28 and in Group-B 29 patients. Among them 38(66%) were male and 19 (34%) were female.

Table-I: Baseline characteristics of the babies at enrollment

Variables	Group-A (Mean ±SD)	Group-B (Mean ±SD)	P Value[#]
GA (weeks)	30.75±2.17	31.55±1.39	.315
BW (gm)	1235.50±144.50	1280.50±142.25	.356
PNA	12.9±1.27	12.2±3.46	.466
Weight at enrolment	1140.50±140.61	1187.89±134.60	.186

[#] T test.

Baseline characteristics of the babies at enrollment were comparable in both groups.

Table-II: Hematological parameters at 14 days and 28 days of age

Variables	Time of	Group-A (Oral) (Mean ±SD)	Group-B(S/C) (Mean ±SD)	P value[#]
HCT %	14 days	42.27±5.36	44.53±5.80	.378
	28 days	39.70±6.20	39.83±5.31	.412
Hb (gm/dl)	14 days	14.19±1.82	14.81±1.93	.484
	28 days	13.29±1.89	13.30±1.75	.456
Serum Ferritin	14 days	294.18±75.28	294.45±81.05	.303
(µgm/L)	28 days	181.56±61.50	217.64±77.24	.065
Reticulocyte %	14 days	4.65±0.969	6.25±3.43	.112
	28 days	7.81±1.02	7.11±3.39	.051

[#] T test

Hematological parameters (Hb level, Hct, Reticulocyte count and Serum ferritin level) in both groups at 14 days and 28 days of postnatal age were not statistically significant. Similarly, serum Ferritin level and reticulocyte count were also comparable.

Table-III: Hematological parameters at 6th week and 12th weeks of age

Variables	Time	Oral group (Mean ±SD)	S/C group (Mean ±SD)	P value[#]
HCT %	6 weeks	33.78±3.01	36.05±6.53	.056
	12 weeks	35.66±1.65	36.38±3.97	.059
Hb (gm/dl)	6 weeks	11.21±1.05	12.05±2.26	.062
	12 weeks	11.88±0.54	12.12±1.32	.054
Serum Ferritin	6 weeks	89.43±30.79	124.76±44.28	.043
(µgm/L)	12 weeks	77.90±27.60	111.10±41.53	.036
Reticulocyte %	6 weeks	9.30±1.68	8.50±3.42	.055
	12 weeks	9.85±1.50	9.22±3.11	.057

[#] T test

HCT%, Hb gm/dl was slightly more in the subcutaneous group than Oral group, but was not significant. Serum Ferritin levels were more in group B ($p<0.05$) than

group-A, there was increased erythropoiesis at 6 and 12 weeks of age, in both groups as indicated by increase in Reticulocyte count, but is not significant. Two patients in oral group and one in subcutaneous group required blood transfusion, which was not significant (X^2-0.39, p-0.532). Adverse events observed were apnea, abdominal distension, vomiting, hyperkalemia and lethargy in both groups which were not significant statistically.

Table-IV: Comparison of Weight between groups:

Parameters Weight (g)	Group-A (Oral) Mean ± SD	Group-B(S/C) Mean ± SD	P Value[#]
Enrollment (14days)	1140.50±140.61	1187.89±134.60	.186
At 28 days	1495.26±138.37	1513.68±120.33	.521
At 6 weeks	1912.63±140.03	1852.05±185.56	.511
At 12 weeks	2793.15±109.39	2772.10±110.98	.691

[#] T test

Both the groups had comparable weigh gain.

DISCUSSION

Critically ill preterm infants experience daily phlebotomy losses within the first 1 to 2 weeks after birth which may equal 5-10% of their total blood volume [5]. Such losses typically result in multiple RBC transfusions. This iatrogenic anaemia is commonly followed by AOP, necessitating additional transfusions. A limited capacity to increase the EPO concentration renders preterm infants less capable of compensating for either of this anaemia. Human recombinant erythropoietin (rhEPO) has been studied as an alternative to transfusions in preterm infants [16]. The goal of therapy is to reduce the number of blood transfusions, exposure to different donors, and reducing the risk of transmission of CMV, Hepatitis B, HIV and other transfusion associated infection and also associated risk of retinopathy of prematurity and bronchopulmonary dysplasia.[17, 18]. In this study we have assessed the efficacy of rhEPO through oral versus subcutaneous route.

A wide range of dosing schedule has been used by different authorities.[2, 11, 19, 20, 21, 22]. In this study, preterm VLBW neonates received rhEPO 400IU/kg either orally or subcutaneously, three times weekly for 2 weeks resulting in a total of 2400IU in group-A and Group-B respectively. All the infants in both groups were supplemented with iron and folic acid daily. Fifty-seven neonates completed the study. Neonates of all groups were similar in gestational age, postnatal age, birth weight, and weight at enrollment. At time of enrollment, majority of them were healthy but some presented with neonatal jaundice and infection. Baseline hematological values (Hb, Hct, reticulocyte count) were estimated and no significant differences were found between the groups. During whole study period 2 patients required blood transfusions (1 in oral and 1in subcutaneous group. Similar amount of blood was withdrawn from both the groups during phlebotomy for investigations.

This study showed a gradual increase in hematological values in 3rd and 4th follow up and weight gain in subsequent follow up after enrollment. Baseline hematological values were within the normal levels but gradually reduced in

both groups in 2nd and 3rd follow up, but this reduction was not significant.

Carneilli et. al. was the first to evaluate the effect of EPO on prevention of AOP[23]. Khatami et al. concluded that early rhEPO and iron administration stimulated erythropoiesis [24]. A multi centric European study in VLBW infants showed no benefit in the first two weeks after birth [25]. These findings are consistent with our study, where intervention and hematologic evaluation was done after two weeks of post-natal age. Similar results were also found in other studies [2, 20, 22, 26]. In most studies, EPO staring soon after birth has shown a lack of useful effect, may be related to the effects of illness and transfusions on erythropoiesis, protein deficiency and high phlebotomy losses.

In this study Hb level at 28 days, 6 weeks and 12 weeks was 13.29±1.89gm/dl, 11.21±1.05gm/dl, 11.88±5.54gm/dl in oral group and 13.30±1.75gm/dl, 12.05±2.26gm/dl and 12.12±1.32gm/dl in subcutaneous group, which was not significant, which similar to the study of Molavi et.al[27]. Similarly HCT, S. Ferritin and Reticulocyte count in two groups at subsequent three follow ups were comparable between the groups with no statistical significance, which is similar to Molavi et.al [27] and Khatami et. al [24]. In this study, during follow up for 6 weeks, 1 neonate in both the group required blood transfusion, were statistically not significant. Transfusion requirement has been reported at 7-90% for premature infants [28]. The results of this study were comparable with the study Saeidi et al. Most of the babies in this study had a stable clinical and cardio-respiratory condition. Soubasi et al. concluded that EPO administration reduces the need for transfusion in uncomplicated premature neonates, but not in complicated neonates requiring mechanical ventilation [29]. Similarly Darveau et al. also concluded that rhEPO could not reduce the need for RBC transfusions in critically ill anemic patient [30]. No critically ill neonates were evaluated in this study.

Studies published to date have administered a wide range of oral or parenteral iron doses. In the present study, iron was administered orally at a dose of 6 mg/kg/day to all infants in an effort to maximize the amount of iron available for erythropoietin. Doses of iron were adjusted weekly according to changes in body weight. Studies that evaluated EPO, reported limited erythropoiesis with inadequate iron supplementation [31]. Shannon et al. administered iron at 3mg/kg/day for all infants who were tolerating full enteral feedings [30]. Maier suggested that iron supplements in a dose of 2mg/kg/day might have been inadequate for optimal erythropoiesis [32]. Serum ferritin concentration presumably reflects adequate administration of iron and do not provide complete information about iron storage [32]. As of Whitwhall study, serum iron level was not measured in the present study [33].

No differences were noted regarding the side effects or morbidities between the groups. Some studies have reported hyperkalemia, convulsion, neutropenia, arthritis and exanthema but this did not occur in this study[9, 22, 31]. Some studies also reported hypertension but blood pressure could not be measured in the present study due to lack of resource [31, 33].

There was no significant difference in weight between the groups during enrollment and at subsequent follow up, but showed a gradual increase in weight. As high hemoglobin level and absence of anaemia has a profound role on tissue oxygenation, cellular growth and proliferation as well as metabolic function, so an increment of weight was found from enrolment to the 12th week follow up in both oral and S/C group respectively. This finding is consistent with a study done by Khatami et al [24].

Many controversial questions regarding the use of rhEPO in attempts to either diminish the severity of or to treat AOP, still remain unanswered. Strauss stated that rhEPO has efficiency in stimulating erythropoiesis in preterm infants, but the success in elimination or marked reduction in the need for RBC transfusion has not been definitively demonestrated[34]. But Turker et al. concluded that rhEPO combined with enteral iron is effective and safe in preventing AOP, and reduced transfusion need [35].

Cochrane Collaboration Review of 23 studies with enrollment of 2074 preterm infants, revealed that parenteral early administration of EPO reduces the no of red blood cell transfusion, volume transfused, and the number exposure of donors [36]. While parenteral rhEPO has been shown to enhance erythropoiesis and decrease the need for late blood transfusion in preterm infant, the effects of its oral administration provided conflicting results. The result of this study is discussed for evaluation of the effects of enteral rhEPO on erythropoiesis in stable preterm infants.

CONCLUSION

From this study it may be concluded that either oral or subcutaneous administration of rhEPO along with iron and folic acid supplementation from second week of life stimulates erythropoiesis, maintains hematocrit and hemoglobin at higher level, has increased weight gain, and is safe in the preterm very low birth weight infants.

RECOMMENDATION

Before routine use of oral rhEPO in prevention of anemia of prematurity further large scale, multi-center, randomized, controlled clinical trials are needed to validate its beneficial effects.

LIMITATIONS OF STUDY

Single center study with a small sample size. Oral preparation was not available in our market; parenteral preparation was used orally. Serum iron and serum erythropoietin level could not be done.

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