INTERNATIONAL JOURNAL OF MEDICAL AND APPLIED SCIENCES
Earthjournals Publisher
E-ISSN:2320-3137
www.earthjournals.org

RESEARCH ARTICLE

"ROLE OF ERYTHROPOIETIN IN FULL TERM NEWBORNS WITH MODERATE HYPOXIC ISCHEMIC ENCEPHALOPATHY"- A PILOT STUDY

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ABSTRACT:

Objective: To investigate the role of erythropoietin to improve the neurodevelopmental outcomes at 3months of age for term new-borns with moderate HIE. **Material and methods:** A randomized, prospective clinical study was undertaken in a tertiary care centre from November 2010 to August 2012. 37 Term neonates with HIE stage 2 were randomized into Group A (n=17) who received rHu Erythropoietin 500U/kg/dose daily IV for 5 days within first 48 hours of birth. Group B (n=20) received only standard post resuscitative care. The neonates were monitored for short term outcomes such as duration of seizures, HIE stage 2, progression to stage 3, multisystem involvement and neurological tone was assessed by Amiel tison angles and EEG at 3 months of age. **Results:**Statistically significant effect was noted in the form of reduction in the duration of HIE stage 2. However there were no significant changes with other short term outcomes such as duration of seizures, multiple anticonvulsant requirements for seizure control, multisystem involvement, progression to HIE stage 3, oromotor coordination and neurological tone assessment at discharge at 3 months of age. **Conclusion:** According to our results, rHu Erythropoietin at 500 U/kg/dose daily for 5 days, intravenously starting <48 hours after birth in term babies with stage 2 HIE has shown beneficial effect in reducing the duration of HIE stage 2.
Key words: Perinatal asphyxia, recombinant human erythropoietin, neurodevelopmental outcome.

INTRODUCTION

Perinatal asphyxia is a common problem and contributes significantly to neonatal morbidity and mortality. Globally it constitutes 18% of the neonatal deaths and in India it constitutes 28.8% [1,2]
Hypoxic ischemic encephalopathy (HIE) following perinatal asphyxia is an important cause of neurodevelopmental impairment in infants. Approximately 15- 20% of asphyxiated newborns with HIE die during the newborn period and 25% of the survivors exhibit permanent deficits such as mental retardation, cerebral palsy, seizures and learning disabilities.[1,2] Given these incidence figures, pediatricians have long sought effective strategies to prevent (or) minimize the consequences of HIE.

The brain injury caused by hypoxic ischemic insult is an evolving process that begins with the initial insult and extends into a recovery period that begins less than 6hrs and extends up to 48hrs after resuscitation. This second period is referred as the "reperfusion phase" of injury. It is this that is potentially amenable to a variety of *interventions.*

Evidence suggests that this therapeutic window from 6-48hrs after a cerebral hypoxic- ischemic insult has led to the development of novel pharmaceutical products that potentially have neuroprotective properties. Such as: free radical scavengers, glutamate receptor blockers, anti-apoptotic and anti-inflammatory agents, and growth factors.[3]But none of these interventions has yet proven to limit the brain damage in newborns with HIE.

There is a need for effective treatment that can be implemented even 6 hours after the injurious event. Because of high incidence of perinatal asphyxia and poor neurological outcome following the insult, a study is required to determine the role of erythropoietin in perinatal asphyxia on the neurological outcome. And would be cost effective and less cumbersome. Hence, this study was planned.

MATERIAL AND METHODS

Study was conducted at M.S.Ramaiah HOSPITALS (NICU), Bangalore, India

- Study period – Nov 2010 to August 2012
- Type of study – Randomised control trial (pilot study)

INCLUSIONCRITERIA[10]:

Essential criteria: Term newborns with the evidence of moderate HIE within 48 hours of age of birth. Along with 2 or more of the following:

- History of fetal distress (late decelerations, decreased heart rate variability, or bradycardia (<100 beats/minute);
- 5-minute Apgar score of less than 7 ;
- Base deficit = or > 12 mEq/L in cord blood or admission arterial blood sample;
- Requirement of immediate ventilation with mask or tracheal tube after delivery.

EXCLUSIONCRITERIA:

- Twin gestation
- Congenital malformations of CNS
- Chromosomal abnormalities
- Chorioamnionitis (or) congenital infections
- Preterm, IUGR
- Postnatal age of >48 hours.

METHODS OF COLLECTION OF DATA:

37 term neonates with moderate HIE were randomized into two groups. Group A (n=18) received rHu EPO, 500 U/kg/dose daily for 5 days, intravenously starting <48 hours after birth. Neonates in control group (N=18) received standard post resuscitation care only. A written informed consent was obtained from the parents for all patients assigned to either group. Patients underwent full neurological assessment at enrolment. The severity of HIE was graded according toSarnat and Sarnat staging [11]. Only with HIE stage 2 (moderate HIE) were chosen for the study. Study was approved by the institutional ethical committee, M.S.Ramaiah Medical College and hospital.

Demographic data and data regarding duration of seizures lasted, more than one anticonvulsant needed for seizure control, duration of HIE 2 and progression to Stage 3, Amiel tison angles were

measured at the time of discharge and those who reached oral feeds by day 7, later these new-borns were closely followed up to 3 months age, again Amiel tison angles[9] were assessed, including EEG at 3 months.

Sample size chosen was 18. This is based on 10% alpha error and 90% power. Block Randomization technique was employed to distribute the study and control groups. P value: <0.05 – statistically significant.

Statistical Methods [17]**:** Descriptive statistical analysis was used in the present study. Results on continuous measurements are presented on Mean +/- SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance. Student t test has been used to find the significance of study parameters on continuous scale between two groups. Chi-square/ Fisher Exact test has been used to find the significance between two or more groups. The Statistical software namely SPSS 15.0 was used for the analysis.

RESULTS:

During the study period between November 2010 to August 2012 over a period of 21 months, 1498 babies were admitted to our NICUs. Out of 854 term babies, 69 babies (4.6% of total admissions) were diagnosed as perinatal asphyxia, of which 32 babies got excluded as described in figure 1. 37 babies were finally available for the study who wererandomized into 2 groups of which 17 babies (45.9%) received erythropoietin (Group A) and 20 babies (54.1%) control group (Group B) received no medication.

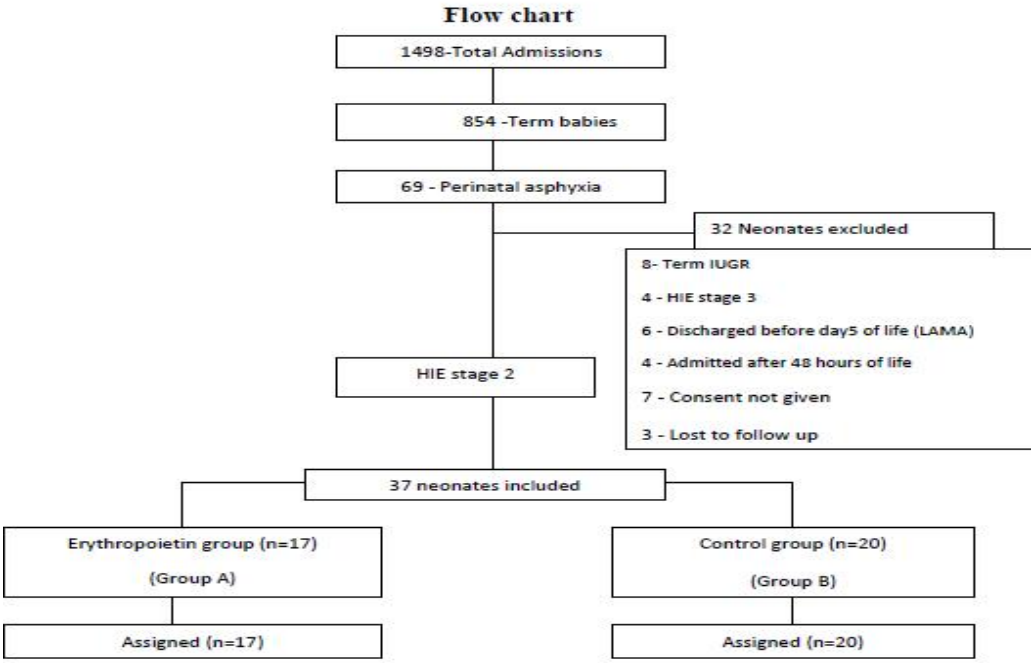

Figure 1: Trial profile

Table 1: Demographic and clinical characteristics of new-borns with HIE

Clinical characteristics	Group A (EPO) (n= 17)	Group B (without EPO) (n= 20)	p=value
Maternal details			
Age (yrs.)	26 (23, 28.5)	25.50 (22.25, 30.75)	0.9
PIH	9(52.9%)	10(50%)	0.8
GDM	2(11.8%)	3(15%)	0.77
Anaemia	1(5.9%)	3(15%)	0.37
MSAF	4(23.5%)	12(60%)	0.026
Age (hours) at admission	8 (3.5,18.5)	12 (5.25,19.5)	0.4
Inborn	3(17.6%)	1(5%)	0.21
Out born	14(82.4%)	19(95%)	
Male	13(76.5%)	9(45%)	0.052
Birth weight(kgs)	3.2 (2.95, 3.6)	3.2 (3.02, 3.47)	0.7
5 min APGAR Scores	4 (4, 5)	4.5 (3, 5)	0.7
Base excess	-15.0 (-10.25, -18)	16.25 (-12.75, -17.0)	0.17
Age of onset of seizures(hours)	8(4.5,14.0)	7(4.0,10.0)	0.59

Table.1 shows a statistically significant difference (p=0.026) in terms of babies born with meconium stained amniotic fluid. However there was no statistically significant difference in other baseline characteristics. Hence the groups were comparable.

It was seen that the median age of starting erythropoietin among the treatment group was 12 hours. The range was between 6.25 to 19 hours. Since ours is a tertiary care center, a major proportion of the babies were out born and were referred to us at varied time intervals. This could explain the range for the age of starting erythropoietin.

OUTCOMES:

The groups were measured for the following outcomes (Table 2). Of which statistically significant result ($p<0.048$) was seen with the reduction in the duration of HIE stage 2 in the treatment group. Among 17 cases in group A, HIE stage 2 lasted for 36 hours (median) ranging between 22.5 to 48 hours. However out of 20 cases in group B, HIE stage 2 lasted for 39 hours (median) ranging between 32 to 72 hours.

There was no statistically significant results with other outcomes such as progression to stage 3 HIE, multiple anticonvulsant usage, duration of seizures and several others as described in table 2.

Table 2:

	Group A (cases) (n= 17)	Group B (controls) (n= 20)	P=value
Duration of HIE 2 (hours) (median(IQR))	36 hrs (22.5, 48.0)	39 hrs (32.0,72.0)	0.048
Progressed to stage 3 HIE	2(11.8%)	3(15%)	0.77
Duration of seizures (hours)(median(IQR))	12 hrs (10.0, 21.0)	16 hrs (12.0,24.0)	0.147
More than one anti-convulsant required for seizure control	8(47.1%)	10(50%)	0.8
Reached oral feeds by day 7	15(88.2%)	13(65%)	0.1
Multisystem involvement	7(41.2%)	11(55%)	0.4
Amiel tison angles among the groups at discharge	12(80%)	11(57%)	0.17
Amiel tison angles among the groups at 3 months follow up	14(93.3%)	15(78.9%)	0.24
EEG findings at 3 months	11(73.3%)	12(63.2%)	0.52
USG cranium findings during the stay in NICU	16(94.1%)	19(95%)	0.9
CT scan findings	4(80%)	8(80%)	1.0
Deaths	2(11.8%)	2(10%)	0.86
Hyperbilirubinemia requiring phototherapy (durationin days)	2(2.0,3.0)	2(2.0,3.0)	0.53

DISCUSSION:

The current treatment for HIE is predominantly supportive, to maintain physiologic parameters. Multicenter trials have demonstrated that hypothermia improved outcomes for term neonates with moderate HIE if the infants underwent cooling within 6 hours. Selective head cooling is a cumbersome procedure, requires trained expertise and special cooling units which is not cost effective. [4]

Hence there is a need for an effective treatment that can be implemented even after 6 hours following the asphyxia event, less cumbersome, not requiring trained expertise and cost effective.

Erythropoietin (EPO) as a neuroprotective agent has been studied extensively both in animals and humans[5,6,7,8]. Erythropoietin and its receptors were upregulated following hypoxic injury and were correlated positively with outcomes [8,16]. Studies have demonstrated anti apoptotic action in neurons following hypoxic injury where NO levels were reduced with the use of EPO.[5,6,7,8]

Data regarding the role of erythropoietin in term neonates with birth asphyxia is very limited. Zhu et al (2007) conducted an RCT on 167 term newborns with moderate/ severe HIE using

Erythropoietin. The study concluded that EPO reduced the risk of disability for infants with moderate HIE, without side effects [5].

The erythropoietin dose (500IU/kg/dose) for 5 days was based on studies by Zhu et al[5] and Elmahdy et al [8]. Short term outcomes such as HIE stage 2 duration, progression to stage 3, reaching oral feeds by day 7, duration of seizure control, multisystem involvement were based on the study conducted by Bhat M et al[18].

Since this was a pilot study we selected a sample size of 18 in each group, after discussing with the statistician.

It was seen that a higher percentage babies (45%) in group B required resuscitation in the form of intubation compared to the group A (29.4%) and a significant percentage of babies (60%) in group B had a history of meconium stained amniotic fluid at birth in comparison to group A (23.5%) indicating that babies in group B could have suffered severe asphyxia which could be one of the confounding factor of the study.

Short term outcomes:

Duration of stage 2 HIE and progression to stage 3 HIE

In our study, babies underwent a thorough neurological assessment every day. Each baby was assessed for change in consciousness, tone, reflexes, autonomic functions (pupils size), and seizure activity and were staged according to Sarnat and Sarnat [11].Earlier studies by Sarnat and Sarnat have shown that HIE stage 2 duration more than 5 days is associated with poor neurodevelopmental outcome. Hence this short term outcome was used in our study to look for the benefit of EPO. We noted a statistically significant difference in reduction in the duration of HIE stage 2 among the group that received EPO ($p = <0.048$).

It was noted that results for progression to stage 3 HIE was statistically insignificant.

Duration of seizures and the requirement of more than one anti convulsant for seizure control

The age of onset of seizures among the Group A and Group B was 8 and 7 hours respectively.

In our study we observed that the seizures lasted for a longer duration in Group B (median of 16 hours) compared to Group A (median of 12 hours). The p value was not significant ($p=0.147$).

Comparison of groups in terms of seizure control showed that in Group A, 47.1% required more than one anticonvulsant for seizure control when compared to 50% in Group B. However difference was not statistically significant ($p=0.8$). This correlated with previous study done by Bhat et al where similar outcome was assessed.[18]

Oral feeds (sucking) by day 7 after asphyxia insult

Studies have shown that reaching oral feeds (sucking) at discharge was one of the short term neurological outcomes suggesting oromotor coordination in asphyxiated newborns. [18]

In our study, babies were initiated on tube feeds after assessing the gut function (absence of nasogastric aspirates or abdomen distension) which was gradually increased as per the tolerance. Once the babies were hemodynamically stable, they were assessed for oromotor coordination (suck and swallow) and were started on palladay feeds.We were able to reach oral feeds by day7 in 88.2 % neonates in group A and only 65 % neonates in group B. Result was not significant. This result is in comparison to the previous studies where this individual parameter was indicative of long term neurological outcome. [18]

Multisystem involvement among the 2 groups

Babies were monitored for organ dysfunctions such as kidneys (urine output, BUN and S.creatinine), gut involvement (feed intolerance, abdomen distension, and stool passage) hematological (thrombocytopenia), hepatic (deranged coagulation), pulmonary (PPHN) and cardiac (hypotension, inotrope requirement) during their entire stay in the NICU. Theresults were statistically not significant. We also noticed that renal system was most commonly affected. Similar results were noted in the study conducted by Elmahdy et al. [8]

Amiel tison scoring at discharge and at 3 months follow up

In the studies done by Zhu et al [5] the neurological outcome were assessed at 18 months of age using Thompson neurological assessment.In our study due to time constraint the Amiel tison angles was used for early assessment of neurological outcome at the end of 3 months. [9]

We observed after evaluating Amiel tison scoring at the time of discharge, 42%group B neonates showed abnormal findings (hypertonia, signifying neurological impairment) as against the 20% group A. However the p value was not significant.

Later the same groups were again assessed using Amiel tison angles at 3 months follow up. Results showed that 21.1 % babies among group B continued to have abnormal findings and 6.7% babies in group A had abnormal findings. Again the results were statistically not significant.

EEG at 3 months follow up

EEG was done to look for background and seizure activity before stopping anticonvulsant which correlated well with previous studies [12].

In our study, all babies were assessed at 3 months follow up visit with an EEG; it was found that 36.8% babies in group B had an abnormal EEG as against 26.7% babies in group A. The p value was not significant (p=0.52).

Neurosonogram and CT scan findings

Previous studies have shown increased cerebral echogenicity due to cerebral edema on USG cranium done within 1st week of life in asphyxiated babies[12].

All our babies had an USG cranium during first week of stay in NICU. Abnormal finding in form of cerebral edema was noted in one among each group. The p value was not statistically significant. The reason could be that USG cranium has less specificity and sensitivity in detecting the cerebral edema in previous studies [12].

Previous studies have shown that CT scan is less specific for prognosticating HIE, but has shown to be helpful in excluding IVH, structural lesions of brain[13]. In our study the CT scan brain was not performed routinely for all the subjects for the above reasons as well as cost factor and the associated risk of shifting the sick babies to the radiology room. However it was considered only when other differential diagnosis such as IVH, structural lesions in the brain, was suspected as a cause of seizures and also the individual consultant decision. Out of 5 babies in group A, 1 baby had IVH and 2 babies out of 8 in group B had IVH. The p value was not significant.

However the results would have been comparable if a larger number of babies had undergone CT scan.

Adverse effects

Erythropoietin is known to cause polycythemia, which increases the risk for hyperbilirubinemia as shown in previous studies [5,8].

In our study we monitored for hyperbilirubinemia requiring phototherapy in days, it was seen that both the groups required median of 2 days of phototherapy. Results were not statistically significant (p=0.53). Also none of the neonates who received erythropoietin develop adverse effects such as polycythemia, prolonged jaundice.

Death as an outcome

In our study we observed 2 amongst 17 neonates died in group A and 2 among 20 neonates in group B died. These babies were admitted with HIE stage 2 and later had progressed to HIE stage. However the results were not statistically significant (p=0.86). Study was comparable to the previous studies [8] and also we required a larger sample size to study death as an individual outcome.

Limitations:

This is a pilot study; we had a small sample size. We did not use amplitude EEG for monitoring HIE, relied on clinical examination for duration of HIE.

Erythropoietin levels were not measured, as the facilities were not available. Although the previous studies have shown that the surge of endogenous erythropoietin levels on day 3 of postasphyxial period would act a confounding factor. [6,8]

We could not perform MRI brain or functional MRI routinely on all babies. Although they are very sensitive techniques in detecting the changes in HIE. Previous studies have shown a better result when MRI was performed a few days after the asphyxial insult allowing time for lesions to develop and evolve[14]. Other reasons were due to financial constraints and difficulty in shifting babies for radiology room.

Long term follow up was not done owing to the time constraint of the study period. Our study was not blinded due to practical constraint during the recruitment of cases.

CONCLUSION

Recombinant human Erythropoietin at 500 U/kg/dose daily for 5 days, intravenously starting within first 48 hours of birth in term babies with stage 2 HIE has shown to reduce the duration of HIE stage 2 (in hours). However there were no significant changes with other short term outcomes such as duration of seizures, multiple anticonvulsant usage for seizure control, multisystem involvement, progression to HIE stage 3, oromotor coordination, USG cranium, CT scan brain and neurological tone assessment and EEG at 3 months of age. No adverse effects occurred in neonates receiving erythropoietin.Considering this as a pilot study, further studies with a larger sample size and long term follow up is necessary to substantiate the above results

Abbreviations:

EPO- Erythropoietin
HIE- Hypoxic ischemic encephalopathy
EEG- Electro encephalography
MSAF- meconium stained amniotic fluid

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