The effects of enteral artificial amniotic fluid-containing erythropoietin on short term outcomes of preterm infants

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Necrotizing Enterocolitis (NEC) is a common devastating gastrointestinal disease, which usually develops in premature infants. Erythropoietin (EPO) as a hematopoietic hormone produced by the kidney can also be naturally found in amniotic fluid and breast milk. There is some evidence that supports the contribution of EPO in the prevention of inflammation and intestinal tissue repair. This study was aimed to determine if oral administration of artificial amniotic fluid with or without EPO would protect preterm infants against NEC and improve the certain neonatal outcomes. In this study, 150 preterm infants with gestational age 28 weeks or less and birth weight 1250 grams or less were enrolled. The infants were divided randomly into 3 groups: 1) Control group (n=50) with routine feeding protocol without any administration; 2) Amniotic fluid group (n=50) with 5mL/kg synthetic amniotic fluid; 3) EPO group (n=50) with RhuEPO dissolved in the synthetic amniotic fluid. The administrations of the study solution were started 3 days after the birth and were continued for 3 weeks (21 days). The infants in the study groups were followed up until discharge and the frequency of NEC, mortality, and other complications of the disease among the groups were compared. The mortality rate in preterm infants of the amniotic fluid and EPO groups were significantly lower than in the control group (p=0.027). We couldn't find any significant differences in the frequency of NEC and other complications among the three study groups. The administration of synthetic amniotic fluid (with or without EPO) in preterm infants may decrease the mortality rate. Use of EPO in synthetic amniotic fluid did not affect the frequency of NEC.

Key words: amniotic fluid, erythropoietin, necrotizing enterocolitis, preterm infant.

Necrotizing Enterocolitis (NEC) is a frequent gastrointestinal emergency mainly in preterm infants. It is a major cause of morbidity and mortality in infants, and is characterized by several grades of mucosal or transmural necrosis of the intestinal tissue.[1,2] The symptoms of NEC include severe abdominal distension, gastric retention, feeding difficulties, bilious or bloody emesis, and signs of disseminated infection.[3]

The symptoms of the disease are often observed suddenly in a preterm infant who has previously been healthy. Development of this disease leads to an increase in the hospitalization period and medical expenses as well. In spite of the development in neonatal care, there have been no major advances in the prevention, incidence, or mortality from NEC over the last several decades.[3] It occurs in 6-10% of all very low birth weight (VLBW) (<1500 g) infants[4] and 1-5% of all infants in neonatal intensive care units (NICU).[5] The NEC-related mortality rate is 10-30% and the possibility of its development has an inverse correlation with birth weight (BW) and

gestational age.[6,7] Despite the development of the technologies for the preservation of preterm and VLBW infants, the prevalence of NEC may rise.[8,9] Although all specific etiological factors for this disease have not yet been completely revealed, the factors which such as infectious pathogens, drugs, processes leading to hypoxia and ischemia, congenital pneumonia, decrease in birth weight, maternal age, surfactant therapy, and indomethacin therapy for the closure of patent ductus arteriosus (PDA) were associated with an increased risk of NEC.[6,10-14]

Some evidence supports the protective effect of breastfeeding, prenatal steroids, and probiotics. The newly proposed preventive treatments include oral administration of Recombinant Human Erythropoietin (RHuEPO).[15-17] Erythropoietin (EPO) is a hematopoietic hormone produced by the kidney. The amniotic fluid and breast milk also naturally contain EPO. Some research debates the contribution of EPO to the prevention of inflammation and intestinal tissue repair as well as the role of this hormone as an anti-inflammatory agent in protecting against NEC.[16,17] This study was aimed to determine if oral administration of artificial amniotic fluid with or without EPO would protect preterm infants against NEC and improve the certain neonatal outcomes.

Material and Methods

This clinical trial was conducted at Al-Zahra Teaching Hospital of Tabriz University of Medical Sciences (TUOMS) which is the main prenatal center of North West of Iran. The study was approved by Ethics Committee at TUOMS and the trial was registered on Iranian Registry of Clinical Trials (IRCT; IRCT201310164113N4). There were 243 preterm appropriate for gestational age (AGA) infants admitted to the NICU during June 21st, 2016 and February 19th, 2018. They were born with gestational age less than or equal to 28 weeks (28.0 ± 2.7, weeks), and birth weight less than 1250 g (1003.8 ± 250.7). The exclusion criteria were as follows: severe birth asphyxia, chromosome anomalies, congenital heart diseases, congenital intestinal obstruction, omphalocele, gastroschisis,

nil per oral for more than 3 weeks (NPO>3 weeks), and parents who declined consent for study. Ninety-three cases were excluded from the study (Fig. 1) and 150 infants were eventually enrolled in the present study.

The clinical risk index for babies II (CRIB II) sore was also evaluated.[18] There were no significant differences (p=0.780) in CRIB II score among the study groups (Table I). After receiving the consent of the parents, the infants were divided randomly into 3 groups: I) Control group (n=50), received routine feeding protocol without any administration; II) Amniotic fluid group (n=50), received enterally 5mL/kg/day of synthetic amniotic fluid [sodium chloride 115 mEq/L, sodium acetate 17 mEq/L, potassium chloride 4 mEq/L, Neupogen 225 ng/mL (Filgrastim, Amgen, Thousand Oaks, CA)]; III) EPO group (n=50), received RhuEPO (Epogen, Amgen) dissolved in synthetic amniotic fluid (4400 mμ/ml). Human serum albumin 5% (Baxter Healthcare, Hyland Division, Glendale, CA) was added to the infusion bag prior to addition of the RhuEPO (final concentration of albumin 0.05%). The synthetic solutions were prepared by a research pharmacist using a sterile method according to the previously reported studies.[19,20] The sample size[21] and the administration route[22] were based on the previous studies.

All infants received total parenteral nutrition started from 2nd day after the birth. Some infants also received formula. There was no significant difference in feeding protocol between the groups (Table I). The antibiotics (Ampicillin and Gentamicin) were administered in all cases according to the antibiotic therapy protocol of the unit and continued for 3-5 days. The administrations of study solution were started 3 days after the birth and were continued for 3 weeks (21 days) in the NICU.[22,23] The infants in the study groups were clinically examined and followed up until discharge every day by a neonatologist. Finally, the frequency of NEC (stage 2 or more), mortality, and other complications of the diseases were evaluated and compared statistically between the study groups.
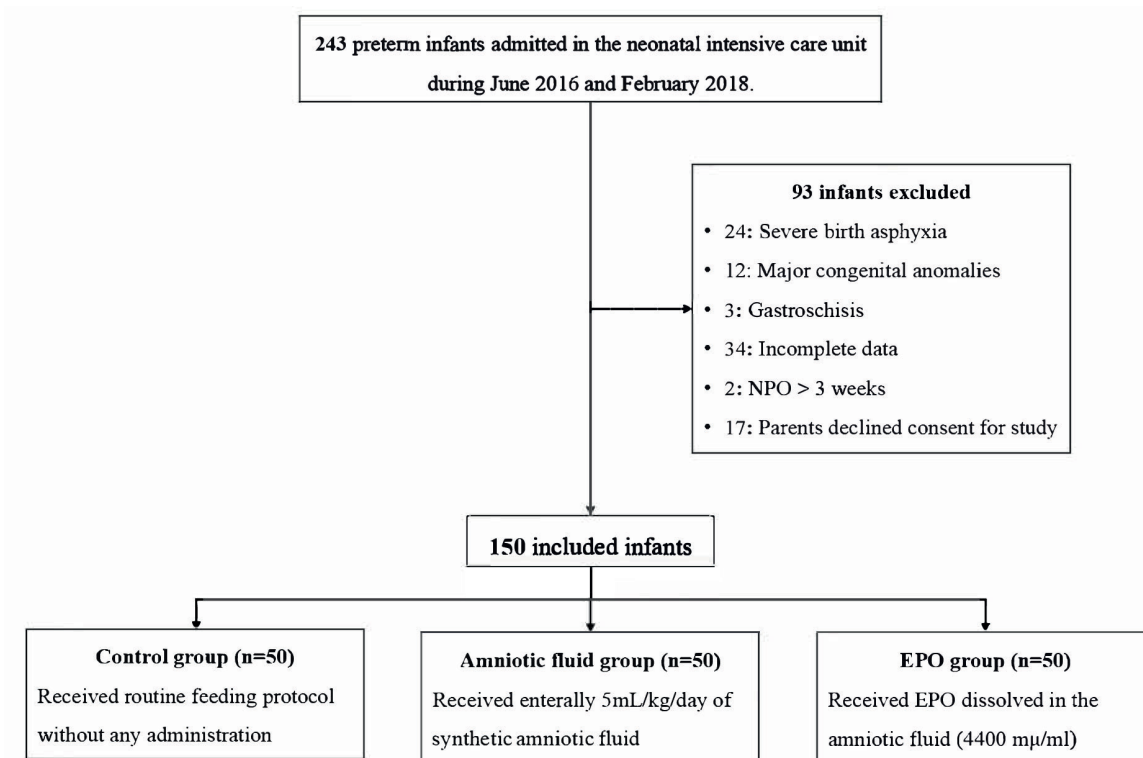
Fig. 1. The study flow chart.

Statistical Analysis

Statistical analysis was performed using SPSS software package version 16.0 for Windows. Data was shown as the mean ± standard deviation (SD) or percentages as suitable. Analysis of variance (ANOVA) test was performed for comparisons involving continuous variables between the study groups. Chi-square and Fisher's exact tests were performed to evaluate and compare categorical variables between the groups. A p-value of less than 0.05 was considered statistically significant.

Results

Of 243 infants, 93 cases were excluded from the study: 24 cases had severe birth asphyxia, 12 cases had major congenital anomalies, 3 cases had gastroschisis, 34 cases had incomplete data, 2 infants had NPO > 3 weeks, and parents declined consent for the study in 17 cases.

Demographic data and underlying medical condition of the infants' mothers in the study groups are depicted in Table I. As presented in the table, there were not any significant differences in the gestational age (p=0.054), birth weight (p=0.072), 1st minute Apgar (p=0.208), 5th minute Apgar scores (p=0.896), and prevalence of preeclampsia (p=0.833), being a twin (p=0.845) or triplet (p=0.826), chorioamnionitis[24] (p=0.805), nuchal cord (p=0.905), placenta previa (p=1), placental abruption (p=0.874), irregular vaginal bleeding (p=0.793), and antenatal steroids in pregnancy (p=0.075) among the study groups.

The frequencies of complications in preterm infants of the study groups were also evaluated. As shown in Table II, the prevalence of gastric residual (residing more than 30% of previous feeding volume) (p=0.829), vomiting (p=0.841), NEC (stage 2 or more)[25] (p=0.763), NEC (requiring surgery) (p=0.859), retinopathy of prematurity (stage 2 or 3)[26] (p=0.741), intra-ventricular hemorrhage (grade 2 or more)[27] (p=0.771), anemia of prematurity (hematocrit was<32% at 3 weeks of age)[28] (p=0.286), and late onset sepsis (positive blood culture occurring at >72-h of life)[29] (p=0.303) were not significantly

Table I. Demographic Data and Underlying Medical Condition of Mother's in the Study Groups.

Variables	Control (n=50)	AAF (n=50)	EPO (n=50)	p-value
Gestational Age (day)†	27.7±1.5	27.7±1.7	28.7±2.6	0.054*
Weight (gr)†	998.1±172.9	948.3±178.6	1065.1±189.4	0.072*
CRIB II Score† (mean±SD) (median, range)	6.9±1.8 7, 5-13	5.8±2.1 6, 5-11	6.4±1.8 7, 5-12	0.780*
Preeclampsia (n, %)	9 (18%)	11 (22%)	10 (20 %)	0.833‡
Twin (n, %)	9 (18%)	10 (20%)	7 (14%)	0.845‡
Triplet (n, %)	0	0	1 (2%)	0.826‡
Chorioamnionitis (n, %)	1 (2%)	1 (2%)	0 (0%)	0.805‡
Nuchal cord (n, %)	2 (4%)	2 (4%)	1 (2%)	0.905‡
Placenta Previa (n, %)	1 (2%)	1(2%)	1 (2%)	1‡
IVB (n, %)	5 (10%)	4 (8%)	3 (6%)	0.793‡
Placental abruption (n, %)	5 (10%)	3 (6%)	4 (8%)	0.874‡
Corticosteroid taking (n, %)	8 (16%)	17 (34%)	19 (38%)	0.075‡
Breast feeding	46 (92%)	45 (90%)	46 (92%)	0.910‡
Formula feeding	0	1 (2%)	1 (2%)	0.805‡
Breast and formula feeding	4 (8%)	4 (8%)	3 (6%)	0.890‡
1st minute Apgar (mean±SD) (median, range)	6.2±1.8 6, 2-8	5.5±2.1 6, 1-8	6.2±1.8 6, 2-8	0.208*
5th minute Apgar (mean±SD) (median, range)	7.7±1.5 8, 3-9	7.6±1.6 7, 2-9	7.8±1.2 8, 3-9	0.896*

AAF, artificial amniotic fluid; CRIB II, clinical risk index for babies II; EPO, erythropoietin; IVB, irregular vaginal bleeding
*The statistical comparison was done by Analysis of variance (ANOVA) test. †Data are shown as mean ± standard deviation (SD). ‡The statistical comparison was done by Chi-Square test.

Table II. Frequency of Complications of Preterm Infants in the Study Groups.

Variables	Control (n=50)	AAF (n=50)	EPO (n=50)	p-value
Gastric residual volume	7 (14%)	7 (14)	6 (12%)	0.829
Vomiting	15 (30%)	17 (34%)	18 (36%)	0.841
NEC stage 2 or more	4 (8%)	3 (6%)	3 (6%)	0.763
NEC requiring Surgery	0	1 (2%)	0	0.859
ROP (stage 2 or 3)	3 (6%)	3 (6%)	2 (4%)	0.741
IVH (grade 2 or more)	10 (20%)	9 (18%)	9 (18%)	0.771
Anemia of prematurity	23 (46%)	18 (36%)	20 (40%)	0.286
Late onset Sepsis	9 (18%)	12 (24%)	9 (18%)	0.303
Mortality	8 (16 %)	2 (4%)	1 (2%)	0.027*

AAF, artificial amniotic fluid; EPO, erythropoietin; IVH, intraventricular hemorrhage; NEC, necrotizing enterocolitis; ROP, Retinopathy of Prematurity. The statistical comparisons were done by Chi-Square test.
*Statistically significant (p<0.05)

different among the study groups.

The survival rate among all infants was 92.67%, therefore, 139 infants were discharged from the NICU. The mortality rate of infants in the control group (16%) was significantly higher than that among the infants receiving

synthetic amniotic fluid (amniotic fluid group) (4%) and or RHuEPO (EPO group) (2%) (p=0.027).

Discussion

In the present study, the preventive effect of EPO was studied in preterm infants. EPO is a 30.4 kDa glycoprotein that is mainly synthesized by the kidneys.[30] As a hematopoietic hormone, the main biological effect of EPO has been revealed through the improvement of differentiation and proliferation of erythroid progenitor cells leading to an increase in the number of red blood cells (RBC) in the circulation.[31] Thus, it is widely used in the treatment of neonatal anemia,[32] cancer and chemotherapy-induced anemia.[33] Apart from its hematopoietic effect, EPO receptors are broadly distributed and expressed in a variety of non hematopoietic tissues.[31] Therefore it may have different non hematopoietic biological effects such as anti inflam-mation,[31] antioxidative,[34] anti apoptosis,[35] angiogenic promotion,[36] and neuroprotection.[37]

Some studies found EPO-receptors on the luminal surface of fetal and neonatal intestinal villi.[17,38] Also, it has been shown that formula fed rodents containing EPO have preservative effect on their villous structure and function which indicate that EPO may play an important physiological role in the growth and development of the gastrointestinal tract.[39] Therefore EPO may also have a role in protection against NEC in preterm infants. Kumral et al.[15] in their study demonstrated that EPO-treated rats had decreased nitric oxide (NO) levels and limited mucosal necrosis in intestinal tissue. They stated that EPO administration might have a protective effect against NEC.

EPO probably prevents the outbreak of NEC by improvement of tight junctions (TJs) as cell-cell endothelial barriers.[17] Caplan et al.[14] in their study showed that the administration of oral EPO reduced the outbreak of NEC in neonatal mice from 45% to 23%. But in our study, there were no significant differences in NEC prevalence among EPO-treated infants and other study groups. Furthermore, we could not find any significant difference in

prevalence of anemia between the study groups. In a study by Juul et al.[40] the oral EPO consumed by infants younger than 4 months was not absorbed and no increase in the serum levels of EPO was observed within 2-4 hours after administration. In another study by Juul et al.[41] no rise in hematocrit or reticulocyte count was observed in enterally EPO-treated pups compared with those in controls after two weeks of the administration. They proposed that RHuEPO is not enterally absorbed in an intact and functional form from the intestines of neonatal rat pups. Thus, enterally dosed EPO might have no erythropoietic effects. However, in a research by Pasha et al.,[42] oral EPO was used for the treatment of anemia of prematurity, and the results reflected an increase in the plasma level of EPO in the EPO-treated group. Also, Yasmeen et al.[43] stated that the use of EPO in infants could lead to increase in hematocrit and a decrease in the frequency of transfusion required for these newborns. Romagnoli et al.[44] in their study examined the effect of EPO on the retinopathy of preterm infants. They concluded that the use of EPO could reduce the prevalence of retinopathy among preterm neonates. In our study, we did not find any significant differences in frequency of retinopathy and other complications among the study groups. The results suggested that the use of the synthetic amniotic fluid with or without EPO may contribute to the reduction of mortality rate of preterm infants with NEC, indicating that the protective effects of synthetic amniotic fluid in preterm infants might be independent of EPO. It might be due to the improving effects of synthetic amniotic fluid on the intestinal cells integrity and the immune system. Also EPO might also have preventive effects against gastrointestinal system infection.

In the present study, the ineffectiveness of EPO in protection against NEC and other complications in infants may be due to the low sample size, the inadequate dose of administered-EPO, and/or the inappropriate rout of the EPO administration (via nasogastric tube). Caffeine treatment and NEC-related mortality were not compared between the study groups which could be considered as the weaknesses of our study. Further studies with larger sample size, different doses of

EPO, and examination of other possible EPO-administration routes are needed to determine the clinical usefulness of EPO in protection against NEC in human preterm infants.

In conclusion, the results of the present study showed that the administration of synthetic amniotic fluid (with or without EPO) could decrease mortality rate in preterm infants. Use of RHuEPO in synthetic amniotic fluid did not decrease the frequency of NEC and the other possible complications.

Acknowledgements

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