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Anthropometric outcome in low birth weight infants treated with erythropoietin; a randomized clinical trial

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Abstract

Introduction: Erythropoietin (EPO) is a glycoprotein hormone, which has a key role in the number of red blood cells in mammalian blood.
Objectives: The aim of this study was to evaluate whether EPO is associated with anthropometric outcomes in low birth weight infants.
Patients and Methods: This study was conducted on 90 premature neonates aged under 35 gestational weeks, with the weight of less than 2000 g, and selected through convenience sampling. The subjects were assigned to EPO (n=45) and control groups (n=45) by random allocation with the aim of evaluating the relationship between EPO and anthropometric outcome, hemoglobin, and hematocrit in low birth weight infants. The weight and head circumstance of the infants were measured on their birthdays, and days 14, 28, and 42. Additionally, hemoglobin and hematocrit were measured on days 7 and 42. From day 14, EPO injection was given to the EPO group three times a week for one month (12 times). The dosage for each baby was 100 U/kg of 2000 unit ampules which was given as a subcutaneous injection in the baby's arm.
Results: The mean weights on birthday and day 42 in the EPO group were 1397±270 g and 2614±739 g, respectively, while in the placebo group they were 1280±281 g and 1486±208 g, respectively. In addition, the mean head circumstance on birthday and day 42 in the EPO group was 28.6±1.7 cm and 33±2.5 cm, respectively, while in the placebo group it was 27.8±2.3 cm and 29.8±2.2 cm, respectively.
Conclusion: According to the results of the current study, weight gain and head circumstance gain rose significantly in the EPO group compared with the placebo group ($P<0.001$).
Trial Registration: The trial protocol was approved by the Iranian Registry of Clinical Trials website (identifier: IRCT20160511027853N2, https://en.irct.ir/trial/47069, ethical code; IR.QUMS.REC.1398.133).

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Introduction

Erythropoietin (EPO) is the hormone that regulates hematopoiesis and contains 165 amino acids and 40% carbohydrates. In the fetus, hematopoiesis persists by inhibiting the apoptosis of erythroid precursors (1) which differentiate and proliferate into normoblasts and increase the lifespan of red blood cells. In the fetus, EPO is produced by the liver, whereas in adults it is mainly produced by renal peritubular cells and a very small amount of it is produced by the liver (1,2). EPO was initially thought to be the only effective cytokine in blood. However, it was revealed that its receptors are very widespread so that it can also lead to the development of vascular endothelial cells, the brain, and the gastrointestinal tract (3-6). It also helps angiogenesis, hypertension, narrowing of arteries, and spontaneous healing of the wounds. Preterm anemia, which is seen in very low birth weight infants (7), is a hypogenerative anemia that usually appears from the second week of life. This anemia is normochromic (8). Premature anemia which is characterized by decreased reticulocytes and an inadequate response to EPO in many preterm infants causes them to require frequent blood transfusions (9). Preterm infants have a low EPO production capacity, a low tolerance to anemia, and a short RBC lifespan, leading to comorbidities. They often require blood transfusions during hospitalization and after discharge from the hospital (10). After a few days of EPO

Key point

Erythropoietin is an effective factor in weight gain and head circumference in premature infants.

injection, blood transfusion may be repeated due to the persistence of the baby's initial problem such as its clinical signs (apnea, bradycardia, tachycardia, poor feeding, and being underweight) which may be due to other illnesses and may not be considered as accurate signs of anemia (11). EPO is usually injected subcutaneously into the baby's arm. EPO is effective on the hemoglobin level, general condition, consciousness, and nutrition.

The most important anthropometric indices of neonates are weight and head circumference (12). The birth weight is always expressed according to gestational age and gender. The baby's weight depends on several factors including the mother's weight, weight gain during pregnancy, general health, and the father's weight (13). The baby's head circumference is measured by measuring the largest circumference of the head that passes between the ridge on the back of the head and the forehead.

Objectives

Due to limited data regarding the role of EPO in anthropometric outcomes in low birth weight infants, we decided to investigate whether there is a relationship between EPO and anthropometric outcomes in low birth weight infants.

Patients and Methods

Study design

This study was a clinical trial, which was conducted on 90 premature neonates in the neonatal unit of Kowsar educational, research, and medical center of Qazvin from 2019 to 2020 after obtaining informed consent from their parents. The researchers paid close attention to ethical considerations in all steps of the study. The study was conducted on 90 premature neonates aged less than 35 gestational weeks, with the weight of less than 2000 g, and selected through convenience sampling.

Two combined methods were used for selecting the case and control groups. First, the infants were visited by a neonatologist and their health was confirmed. Based on a random system, the infants were divided into two groups to carry out the project. The subjects were assigned to EPO (n = 45) and control groups (n = 45) by random allocation. EPO was used in 0.5 mL ampoules (2000 IU) (Pouyesh Darou Biopharmaceutical Co.). For placebo, sterile distilled water containing 5 ml per ampoule (Shahid Ghazizadeh Pharmaceutical Co.) was used. The inclusion criteria were the birth weight of less than 2000 g, the gestational age of under 35 weeks (fertilization age was 27 weeks and did not exceed 35 weeks), having no diseases (healthy babies who had no problems or illnesses in the first week of life), and Iranian nationality. The exclusion criteria were the parents' dissatisfaction with continuing to participate in the research, the neonates' death or critical conditions, the neonates' discharge before the end of the study, congenital anomalies, sepsis, thrombocytopenia, diastolic pressure of greater than 60 mm Hg, shock, hydrops fetalis, inflammation, seizures, and hemolytic diseases. The researchers began the intervention after explaining the research goals and methods to the neonates' parents and obtaining their consent. The data collection tools included a demographic information record sheet, an EPO checklist, and the neonates' anthropometric monitoring checklist.

First, birthday weight and head circumference were measured in both groups. Then, one week after birth, the CBC diff was checked in both groups. Second, from day 14, the EPO injection was given to the EPO group three times a week for one month (12 times). The dosage for each baby was 100 U/kg of 2000 unit ampules which was given as a subcutaneous injection in the baby's arm. In the control group, placebo was not injected into the neonates due to their prematurity as well as the injection problems (especially infection); rather, the placebo was fed to them. All the complications of the injection including allergic reactions, swelling, itching, skin rash, respiratory problems, swelling of the arms and legs, cough, nausea, vomiting, and urination (infant restlessness) were checked. Two weeks after the last injection, the CBC diff test were checked again. At last, on days 14, 28, and 42, the weight and head circumference were measured and compared before and after the intervention.

Data analysis

The collected data were analyzed by SPSS software. To compare the relationship between EPO and anthropometric outcomes, independent t test and ANOVA were used. A *P* value of less than 0.05 was considered as a significant level.

Results

The CONSORT diagram for the study is depicted in Figure 1. Ten infants were withdrawn from the analysis and 90 preterm infants (45 in the EPO group and 45 in the placebo group) remained in the study. The EPO and placebo groups were comparable in age and gender and received the same appropriate management except for the use of recombinant human EPO (rhEPO) or placebo. Table 1 summarizes the demographic and clinical characteristics as well as the neonatal outcomes of the rhEPO and the placebo control groups. The average age of the participants was 30.25±2.2 weeks and the majority of the neonates were male (N = 48, 50.5%). The study showed significant differences between the two groups (repeated measurement and independent t-tests) concerning weight and head circumstance gain on days 7, 14, 28, and 42. In addition, the median hemoglobin and hematocrit levels were comparable in both groups ($P < 0.001$; Table 1).

The data were analyzed using 2-way ANOVA for repeated measures between the groups and an independent t-test for continuous variables. The p-values of less than 0.05 were considered significant. The present study investigated the effects of rhEPO and non-rhEPO on premature neonates' weight, head circumference, hemoglobin, and

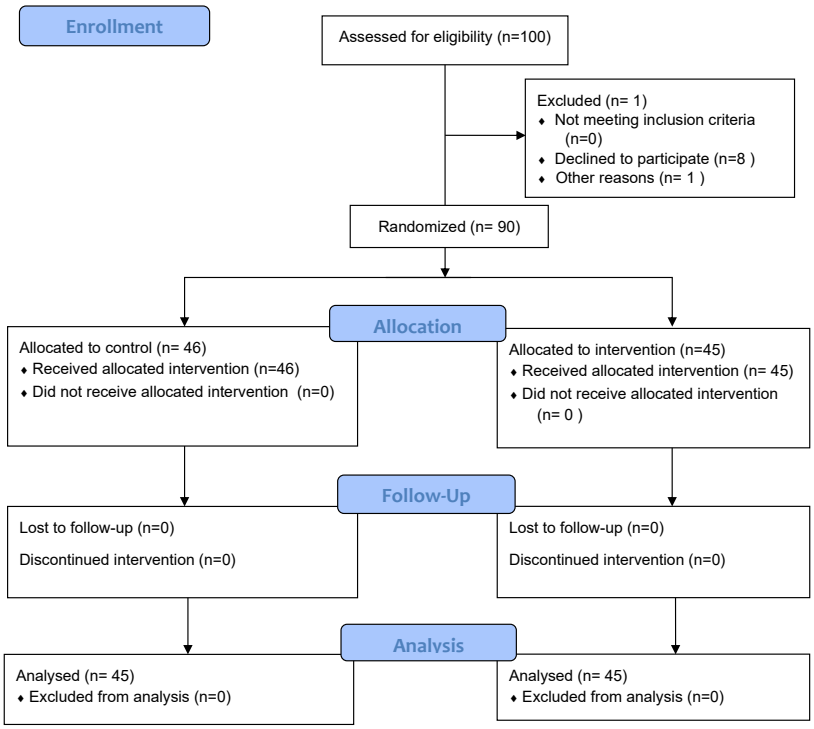

Figure 1. The flowchart of the study.

hematocrit. The results showed that weight gain and head circumference gain were significantly lower in the non-rhEPO group than in the rhEPO. Mean weight change and head circumference in the neonates were significantly higher in the rhEPO group on days 14, 28, and 42, compared to the non-rhEPO group. Neonatal weight gain at 42 days was 1217 g in the EPO group and 206 g in the non-rhEPO group. The weight gain was almost six times higher in the EPO group than in the non-rhEPO group (Figure 2). On day 42, the neonatal head circumference gain was 4.4 cm in the EPO group, while it was 2 cm in the placebo group. The head circumference gain in the EPO group was 2.4 times higher than that of the placebo group (Figure 3).

Discussion

The median hemoglobin and hematocrit levels decreased in both groups on day 42 compared to those on birthday

Table 1. Demographic characteristics and outcomes of the patients in erythropoietin and placebo group (N = 90)

Variable	EPO (n = 45)	Placebo (n = 45)	P-value*	P-value**
Male, % (N)	19 (42.2)	29 (64.4)	0.548	-
Female, %(N)	26 (57.8)	16 (35.6)		
Age (wk), mean ± SD	30.89±2	29.62±2.3	0.548	-
Birth weight (g), mean ± SD	1397±270	1280±281	0.749	<0.001
Weight day 14 (g), mean ± SD	1596±304	1266±228	0.058	
Weight day 28 (g), mean ± SD	2034±489	1359±261	<0.001	
Weight day 42 (g), mean ± SD	2614±739	1486±208	<0.001	
Birth head (cm), mean ± SD	28.6±1.7	27.8±2.3	0.153	<0.001
Head day 14 (cm), mean ± SD	29.9±2.2	28.1±2.1	<0.001	
Head day 28 (cm), mean ± SD	31.2±2.3	28.8±2.1	<0.001	
Head day 42 (cm), mean ± SD	33±2.5	29.8±2.2	<0.001	
Birth hemoglobin (g/dL), mean ± SD	14.8±4.8	17.3±2.9	0.464	0.728
Hemoglobin day 42 (g/dL), mean ± SD	10.3±2	13±1.8	0.315	
Birth hematocrit (g/dL), mean ± SD	40.5±7	48.1±6.6	0.898	0.477
Hematocrit day 42 (g/dL), mean ± SD	31.2±4.8	37.7±4.5	0.720	

*Independent *t* test
**Repeated measurement.

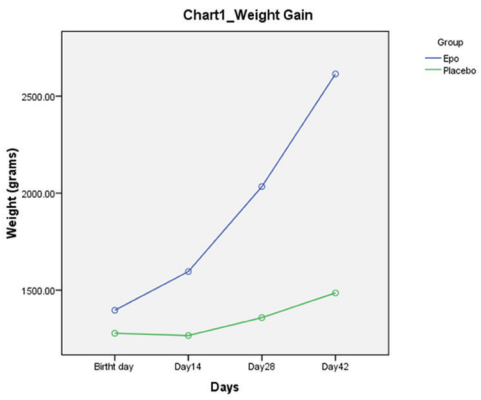

Figure 2. The comparison between erythropoietin group and placebo group weight.

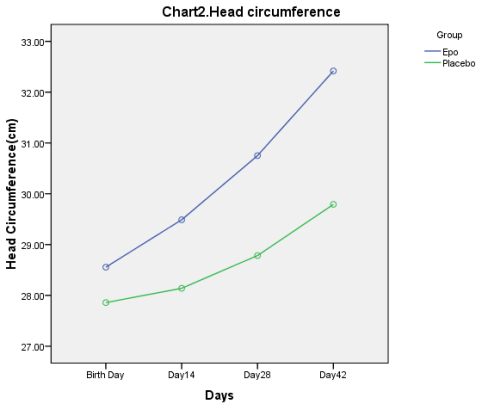

Figure 3. The comparison between erythropoietin group and placebo group head.

($P<0.001$; Table 1). This may be due to a significant increase in the body weight (six fold) in the rhEPO group compared to an increase in their hemoglobin and hematocrit levels. In a clinical trial in premature infants, Pasha et al concluded that EPO can decrease hemoglobin and hematocrit levels in the bloodstream and increase the reticulocyte count. EPO stimulates hematopoiesis without increasing hemoglobin and hematocrit in premature infants. In their study, hemoglobin decreased from nine to seven mg/dL and hematocrit decreased from 32.9 to 21.3 mg/dL in the case group. In addition, in the control group, hemoglobin decreased from 11.5 to 8.4 mg/dL and hematocrit decreased from 34.3 to 24.8 mg/dL (14). Therefore, there was no significant difference between the two groups. The results of the above study are in line with those of the present study. Moreover, in the study of Reinhardt et al on lean mass and body weight regulation, it was found that intra-EPO caused weight loss in men and weight gain in women (15).

These changes were based on the EPO levels of men and women and their hormonal changes. In addition, in the study of Teng et al, weight gain occurred in both genders of mice which is in line with the current study (16). However, in two studies by Zhang et al and Lee et al, EPO caused weight loss in male mice. In these studies, weight loss occurred which is not in line with the present study (17, 18).

Therefore, the authors suggest that more studies be conducted on infants to further investigate their general conditions and to further study the effects of EPO on them. It will also be useful to examine the results of evolutionary changes as well as the reasons for anthropometric changes.

Limitations of the study

The main limitation of the current study was the decrease in births in recent years. As a result, sample collection took a lot of time.

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Authors' contribution

FM and SHM were the main researchers of the study. AK collected the samples. AASH reviewed the manuscript and critically evaluated the intellectual material. All authors participated in writing the final draft of the manuscript, revised the manuscript, and critically evaluated the intellectual material. All authors read and verified the content of the manuscript and checked the accuracy and integrity of each part of the study.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical issues

This research considered and observed all the principles of the Helsinki Declaration and was approved by the Ethics Committee of Qazvin University of Medical Sciences (#IR.QUMS.REC.1399.133). The Iranian Registry of Clinical Trials (#IRCT20160511027853N2, https://en.irct.ir/trial/47069,) approved the trial protocol. Moreover, ethical issues (including plagiarism, data fabrication, double publication) were completely observed by the authors. The neonates' parents gave their consent to publish the research.

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