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Evaluate the level of Serum Erythropoietin in Premature Babies

Ribaz S. Asaad [(1)]*

Abbas A. Rabaty [(2)]

(1) Rapareen Pediatric Hospital, Erbil
Iraq

(2) Department of Pediatric College of Medicine
Hawler Medical University
Erbil
Iraq

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ABSTRACT

Background Erythropoietin is the growth hormone of erythropoiesis that produced mainly in the liver during fetal life and in the kidney during infancy. It's low level considered being the main cause of anemia in prematurity. The aim was to measure serum erythropoietin concentrations in premature babies and correlate it to different hematological parameters.

Patients & Methods: This was a cross-sectional study conducted for four months starting from 1st December 2018 to 1st March 2019, included 25 premature neonates admitted in neonatal care unit of Rapareen pediatric hospital & maternity hospital, Erbil, Iraq. All premature neonates whose gestational age was between 28 -34 weeks were included in the study regardless of their birth weight. Hemoglobin, hematocrit, reticulocyte count and serum erythropoietin levels were measured in premature babies at first and at the end of the fourth week.

The Results: Serum erythropoietin measured in total 25 premature babies, in which serum erythropoietin in 52% of them was within the normal range (2-20 mIU/ml) while in 48% were below this range. serum erythropoietin concentration positively correlated to gestational age (r=0.47) in contrast it was negatively correlated to the Hb (r=0.51) and PCV(r=0.34).

Conclusion: The results of this study suggest that about half of premature neonates had low serum EPO level while other half had normal serum EPO level in the 1st week of life, also we identified a strong negative correlation between serum EPO and Hb in the 1st month of life, therefore, there was a statistically significant difference between serum EPO in 1st week and 1st month.

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**Corresponding author E mail :* rebazsardar84@yahoo.com

Introduction

The World Health Organization (WHO) defines preterm birth as any birth before 37 completed weeks of gestation, This is further subdivided on the basis of gestational age (GA):

- Extremely preterm (<28 weeks);
- Very preterm (28–<32 weeks);
- Moderate or late preterm (32–<37 completed weeks of gestation) [1].

Almost all infants experience various degrees of anemia or physiologic anemia of infancy. Typically, hemoglobin (Hb) concentration decreases to between 9.5-11 g/dL around 10-12 weeks in healthy term infants[2]. Preterm infants experience more profound and earlier onset of anemia than physiologic anemia of infancy deemed anemia of prematurity (AOP)[3]. APO is hypo-regenerative and normocytic-normochromic anemia that is characterized by the presence of a low serum erythropoietin (EPO) level with a remarkably low hemoglobin concentration[4]. Blood hemoglobin concentration falls to lower levels in infants born prematurely — to approximately 8 g/dL in infants with birth weights of 1.0 to 1.5 kg and to approximately 7 g/dL in infants with birth weights <1 kg [5].Preterm infants are often anemic and typically experience heavy blood losses from frequent laboratory testing in the first few weeks of life. Although their anemia is multifactorial, repeated blood sampling and reduced erythropoiesis with extremely low serum levels of EPO are major determining factors[6] [7] [8]. EPO is an essential hormone for red blood cell production, is mainly produced in the liver before birth and in the kidney after birth[9]. The primary source of EPO is the liver Kupffer cells of the fetus, this function is exercised postnatal by the peritubular renal cells [10]. The shift from the liver to kidney EPO-production starts in the last gestational week and continues to the 4th-6th postnatal week [11]. Serum level of EPO in premature neonates is lower than mature neonates and after birth, its level decreases even more[12]The primary site of EPO production in preterm infants is in the liver, rather than the kidney. This dependency on hepatic EPO is important because the liver is less sensitive to anemia and tissue hypoxia — hence, there is a relatively sluggish EPO response to the infant's falling hematocrit (HCT) level [13].

Aim: The aim of this study is

1- to measure serum EPO concentrations in premature babies and correlate it to different hematological

parameters like packed cell volume (PCV)and Hb.

2- To compare Hb and serum EPO in the first week and at a 1st month.

Material & Method:

This study is a cross-sectional one, it measures the serum erythropoietin levels in premature babies. It is conducted during the period of December 2018 to March 2019, on 25 neonates who admitted to the Department of Neonatal Intensive Care at(Rapareen pediatric teaching hospital & maternity hospital) in Erbil city. It included all premature neonates whose gestational age was between 28 -34 weeks regardless of the birth weight. Exclusion criteria were those neonates with hemolytic anemia, congenital infections, congenital malformations, severe asphyxia, intraventricular hemorrhage or those who were in need of exchange transfusion or in need to undergo surgery. Hb, PCV(hematocrit), reticulocyte count and serum EPO levels were measured in premature babies at the 1st week and the end of the 4th week. A detailed record including gender, gestational age, Birth weight, mode of delivery was registered.

The level of EPO was measured by enzyme-linked immunosorbent assay (ELISA) using human EPO ELISA kit E-EL-H364096T belong to Elabscience company which depends on the sandwich-ELISA principle. The amount of EPO is expressed by milli-international units per milliliter (mIU/ml) and it is reference value is 2 - 20 mIU/ml. The measurement process involves the following steps:

1- Add 100 ml standard or sample to each well. Incubate 90 minutes at 37°c
2- Remove the liquid.Add100ml Biotinylated detection Ab. Incubate 1 hour at 37°c
3- Aspirate and wash 3 times
4- Add 100 ml HRP conjugate. Incubate 30minutes at37°c
5- Aspirate and wash 5 times
6- Add 90 ml substrate Reagent. Intubate 15 minutes at 37°c
7- Add 50ml stop solution. Read at 450nm immediately
8- Calculation of results

Data management and statistical analysis: Data recorded on a specially designed questionnaire, collected and entered in the computer then analyzed using appropriate data system which is called Statistical Package for Social Sciences (SPSS) version 24 and the results compared between patients with different variables, with a statistical significance level of < 0.05. The results presented as frequencies, percentages in tables and figures and analyzed using correlation and T-tests.

Ethical considerations: This study submitted to the scientific and research ethics committees of the

Kurdistan Board of Medical Specialties for scientific and ethical approval. This study was explained for each patient's parents and verbal consent obtained from each babies parents. Confidentiality of data was ensured too.

Results

A total of 25 patients have been enrolled in the study, 15 of them were male. The male: female ratio was 1.5:1. Around half (48%) of the babies had abnormal or lower than normal readings (Figure 1).

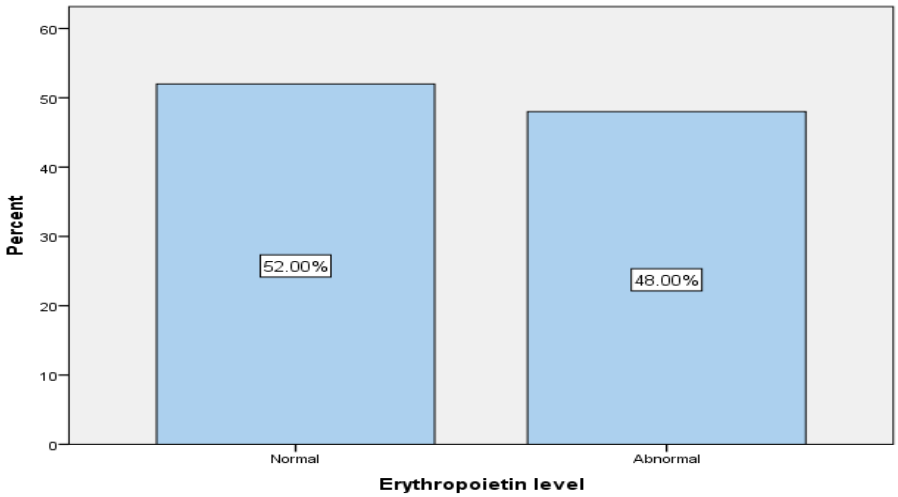

Figure 1: Erythropoietin levels (first reading) of the patients.

According to data of Table 1 show that there was no statistically significant association between gender of the newborns and erythropoietin levels whether first or second reading.

Table 1: Comparison between male and female erythropoietin levels (mIU/ml) in the first and second readings.

Readings	Gender	N	Mean	S.D	P-value
First	Male	15	3.47	2.36	0.21
	Female	10	2.23	2.34	
Second	Male	15	8.13	4.11	0.65
	Female	10	9.05	5.89	

Out of the total 25 newborns, for 14 of them, the mode of delivery was Caesarean section and their average EPO level in the first reading was 3.94mIU/ml which was higher than the mean EPO of the normal vaginal group (1.75mIU/ml). This difference was statistically significant and P-value was 0.02 (Table 2).

Table 2: Association between erythropoietin (first reading) and mode of delivery.

Variable	Mode of delivery	N	Mean	S.D	P-value
S. erythropoietin first reading (mIU/ml)	Normal vaginal	11	1.75	2.28	0.02
	Caesarean section	14	3.94	2.07	

The results of Table 3 indicate a weak positive correlation between serum erythropoietin (first reading) and birth weight i.e. with an increase in birth weight the serum erythropoietin level will increase slightly. The correlation coefficient was 0.24. The correlation coefficient between serum erythropoietin (first reading) and gestational age was moderately positive and equal to 0.47.

Table 3: Correlation between erythropoietin (mUI/ml) and, hemoglobin, PCV first readings, birth weight, and gestational age.

Variable	Mean	Std. Deviation	Correlation coefficient
S. Erythropoietin (mIU/ml)	2.97	2.39	
Birth weight (kg)	1.43	0.48	0.24
Gestational age	30.36	2.17	0.47

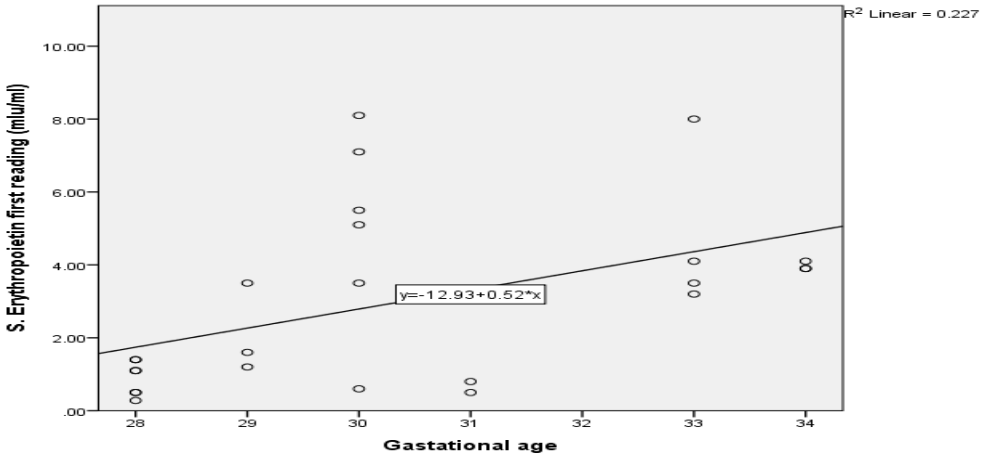

Figure 2: Correlation between erythropoietin (mUI/ml) first reading and gestational age.

The findings of Table 4 reveal that there was a strong negative correlation between serum erythropoietin and hemoglobin during the second measurement. If the hemoglobin level increase, the s. erythropoietin measurements will diminish significantly. The correlation coefficient was - 0.51. There was a moderate negative correlation between serum erythropoietin and PCV, which means when PCV level among newborns start to increase, the serum erythropoietin level will decrease moderately. The correlation coefficient was - 0.34.

Table 4: Correlation between erythropoietin (mlu/ml) and hemoglobin and PCV second readings.

Variable	Mean	S.D	Correlation coefficient
S. Erythropoietin (mIU/ml)	8.50	4.80	**- 0.51**
Hb (g/dl)	9.72	1.79	
PCV (%)	30.26	8.03	**- 0.34**

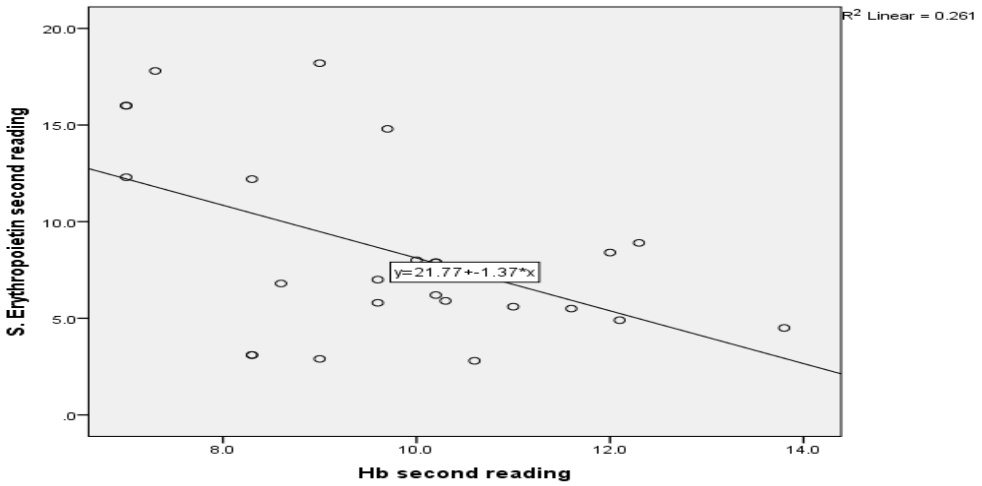

Figure 3: Correlation between erythropoietin (mUI/ml) and Hb second readings.

The results of Table 5 show that the average s. erythropoietin level for the second reading increased dramatically in comparison to the first measurement. The first reading was 2.97mUI/ml which tripled to 8.50mUI/ml in the second measurement. This increase was statistically significant. Hb level in the first reading was 15.5 g/dl and then dropped to 9.72g/dl in the second reading. This decrease was statistically significant. In both cases, T-test was done to compare between the two averages and P-values were 0.001.

Table 5: Comparison of EPO, Hb level in the first & second reading

Variables	Range	Mini.	Max.	Mean	S.D	P-value
EPO, first reading	7.82	0.28	8.10	2.97	2.39	0.001
EPO, second reading	15.4	2.80	18.2	8.50	4.80	
Hb, first reading	8.7	10.7	19.4	15.50	1.85	0.001
Hb, second reading`	6.8	7.0	13.8	9.72	1.79	

Discussion

In this study, we investigated premature neonates for serum EPO concentration & some hematological parameters like Hb, PCV and we established a correlation of serum erythropoietin to gender, mode of delivery, birth weight, gestational age, & PCV with Hb, with some limitations in this study due to small sample size, we faced many obstacles during the period of making this research, few premature neonates died before completing the follow-up, difficulty in taking blood sample from these premature and lack of some parents interest regarding the follow up of the serum EPO level in the second sample taking.

Regarding the measurement of serum EPO concentration we found that serum EPO in premature babies that done in both Rapareen pediatric teaching hospital and maternity

hospital in 52% were within normal range (2-20 mIU/ml) while the remaining 48% were below normal value , this study is near to the result of a study done in Leiden University, Netherland which showed that 37% of their samples, EPO levels were below the detection limit of their assay of 1.4 mU/mL (35% in the transfused group and 48% of the samples in the non-transfused group). 14

While other studies had shown a higher level of erythropoietin as in Garcia JF et al & Meyer J et al 15, 16

There was no association in serum EPO level between male and female gender in current study & this resembles a study done in Germany revealed that there was no difference between the mean erythropoietin concentrations in boys and girls 17.While in another study done in Australia by the Australian National Health and Medical Research Council in 2005 revealed that the geometric mean EPO was higher in boys than in girls.18

In our study we observed that serum EPO was higher in first reading in those delivered by cesarean section (c/s) than those born vaginally which agreed with the study done in Romania that showed serum EPO correlated with the type of delivery; in infants delivered by caesarian section the serum EPO was higher, which suggests a relation between increased levels of EPO and fetal distress that imposes C-section 19 while in another study showed that In uncomplicated term human pregnancies with vigorous newborn infants, EPO levels in umbilical cord blood at birth are higher after uncomplicated vaginal deliveries than after elective cesarean sections without labor contractions 20 This suggests that even normal labor and vaginal delivery results in sufficient fetal tissue hypoxia stimulate fetal EPO synthesis 21

Regarding the relation of serum erythropoietin level to the birth weight, we found that there was a weak positive correlation between serum EPO & birth weight (r = 0.24), however, in other study found evidence of a negative association between cord blood EPO and birth weight for gestation and sex 18.While in another study done in Berlin in 1998 showed that there was no significant relationship found between EP levels and birth weight. 22

In the current study, there was a positive correlation between erythropoietin level with gestational age, the r was 0.47, this came in agreement with a study done in 2019 by Helsinki University Hospital, Finland done on term babies & revealed that serum erythropoietin levels in umbilical cord correlated

with gestational age among vaginal deliveries (r = 0.250) 18,23 while in another research done in University of Iowa, USA found that plasma EPO levels did not correlate with gestational age 24

Finally, as we expected that there was evidence of a negative correlation between serum EPO concentrations in the first month to the Hb (r=0.51) and PCV (r=0.32) which was similar to a study done in Japan25, however, another study confirmed weak negative correlation between serum EPO and Hb 26

As we noted in our study, the Hb mean dropped down in the first month of life, and because of this negative correlation between EPO and Hb, there was a significant difference in serum EPO level in the 1st week and 1st month of life (p=0.001.(

Conclusion:

The results of this study suggest that about half of premature neonates had low serum EPO level while other half had normal serum EPO level in the 1st week of life ,also we identified a strong negative correlation between serum EPO and Hb in the 1st month of life, therefore, there was a statistically significant difference between serum EPO in 1st week and 1st month . neonates delivered by cesarean suction had a higher level of serum EPO.

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