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Assesment of erythroferrone levels in neonates with anaemia of prematurity

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***Abstract*---**Background: The anemia of prematurity is caused by untimely birth occurring before placental iron transport and fetal erythropoiesis are complete. Objective: To determine the serum levels of erythroferrone and their relation to the anaemia of prematurity. Patients and methods: This study was case control study carried out in the the Pediatric Department, Faculty of Medicine, Menoufia University. This study included two groups; the first group included newborns of both sexes with anemia of prematurity admitted to the NICU during the study period, the 2nd group included group of healthy newborns. all participants were subjected to history taking, general examination, Local examination, Abdominal examination and Laboratory investigations including complete blood count (CBC), and blood film, C reactive protein (CRP), Blood culture when needed, Kidney functions tests: including serum creatinine and urea, Liver function tests: including serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT), Hepcidin level and Erythroferrone. Results: There was no statistically significant difference in liver, renal functions, CRP. Hepcidin showed significant decrease in diseased group when compared to the control group. Free erythroferrone levels showed significant increase in diseased group when compared to the

control group. Free erythreoferrone showed significant positive correlation with age, and significant negative correlation with HB (hemoglobin), HCT (hematochrite). Conclusion: Hepciden concentration is decreased and erythroferrone concentration is increased in children with anemia of prematurity. Therefore, both markers can be used in early diagnosis of anemia of prematurity.

***Keywords*---**anaemia, erythroferrone, hepcidin, prematurity.

Introduction

Preterm infants with birth weight <1.0 kg (commonly designated as extremely low birth weight, or ELBW, infants) have completed ≤29 weeks of gestation, and nearly all will need red blood cell (RBC) transfusions during the first weeks of life. Every week in the United States, approximately 10,000 infants are born prematurely (i.e., b37 weeks of gestation), with 600 (6%) of these preterm infants being ELBW [1].

In pre-term infants RBCs have a shorter life span (40–60 days) compared with those of adults (120 days) with fewer precursor red cells found in the bone marrow. In addition, pre-term infants have relative deficiencies in the key micronutrients required for erythropoiesis (iron, folate, protein, vitamins E and B12) resulting from low stores at birth with poor post-natal accretion due to gastrointestinal immaturity, limiting enteral feeding and micronutrient bioavailability [2].

Phlebotomy loss is therefore a significant iatrogenic factor contributing to persistence of anemia of prematurity, with ELBW infants routinely losing approximately 11–22 ml/kg of blood each week (neonatal total circulating volume is approximately 80 ml/kg) for the first 6 weeks of life [3]. Hepcidin, a small peptide mainly produced by the liver, is absolutely required for the maintenance of systemic iron homeostasis in basal conditions. Hepcidin controls serum iron levels by binding to ferroportein (FPN) and inducing its degradation. Low hepcidin stabilizes FPN at the cellular membrane, promoting dietary iron absorption, increasing the release of iron from macrophages, and enabling iron mobilization from hepatocytes. Likewise, hepcidin is suppressed in conditions associated with accelerated erythropoiesis (e.g., anemia due to bleeding, hemolysis, or iron deficiency) [4].

Erythroferrone is released by erythroid precursors in the marrow and the spleen in response to erythropoietin (EPO) stimulation. Erythroferrone induces hepcidin suppression during increased erythropoietic activity, and thereby increase iron availability for new erythrocytes synthesis [5]. By suppressing hepcidin, increases the absorption and mobilization of iron to provide an adequate iron supply during stress erythropoiesis such as during rapid growth or blood loss. production is stimulated by endogenous or exogenous (EPO), thus serving to couple increased erythropoietic activity with decreased hepcidin, allowing for maintenance of plasma iron concentrations in the setting of increased erythropoiesis-associated

iron demand [5]. Therefore, the aim of this work was to determine the serum levels of erythroferrone and their relation to the anaemia of prematurity.

Patients and methods

This study was case control study carried out in the the Pediatric Department, Faculty of Medicine, Menoufia University during the period from March 2021 to March 2022. An informed consent was taken from the guardian of each patient or control before participation. All procedures were carried out in accordance with the ethical standards. Approval from the ethics committee of the Faculty of Medicine, Menoufia University was taken. IRB approval number and date 1/2021 PEDI.

This study included 2 groups of neonates: the first group included newborns of both sexes with anemia of prematurity admitted to the NICU during the study period, the 2nd group included group of healthy newborns matching the cases in the age and sex with no anemia for estimation of normal levels of serum erythroferrone. All newborns presented with anemia of prematurity in the study period were included in our study. Parental refusal or newborns with major congenital anomalies, Inherited metabolic disorders, Hemoglobinopathies were excluded from our study.

For all participants subjected to the following Personal, present, past and family history: such as age, gender, prenatal, natal and postnatal history including the type of feeding, iron supplementations and blood transfusion. General examination including anthropometric measurements and vital signs, Local examination: including central nervous system (CNS), cardiovascular system (CVS), chest and Abdominal examination and Laboratory investigations including complete blood count (CBC), and blood film, C reactive protein (CRP), Blood culture when needed, Kidney functions tests: including serum creatinine and urea, Liver function tests: including serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT), Serum sodium (Na) and serum potassium (K), Serum Hepcidin level.

Specific investigations including erythroferrone analyzed using ELIZA kits supplied by (SunRed) synthesized in france catalogue number 201-12-5646 and hepcidin analyzed using ELIZA kits supplied by (SunRed) synthesized in france catalogue number 201-12-1020.

Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp) Qualitative data were described using number and percent. The Shapiro-Wilk test was used to verify the normality of distribution Quantitative data were described using range (minimum and maximum), mean, standard deviation, median and interquartile range (IQR). Significance of the obtained results was judged at the 5% level. Chi-square test was used for categorical variables, to compare between different groups, Fisher's Exact used for correction for chi-square when more than 20% of the cells have expected count less than 5. Student t-test used for normally distributed

quantitative variables, to compare between two studied groups. Mann Whitney test used for abnormally distributed quantitative variables, to compare between two studied groups. Pearson coefficient used for to correlate between two normally distributed quantitative variables.

Results

HB and HCT showed significant decrease in diseased group when compared to control (p <0.001). However, no significant difference between studied groups regarding WBCs and platelet (P> 0.05). (Table 1)
Hepcidin levels showed significant decrease in diseased group when compared to the control group (p <0.001). No significant difference was found between studied groups regarding CRP, blood culture and PH. (Table 2)
Free erythroferrone levels showed significant increase in diseased group when compared to the control group (p<0.001). (Table 3)
Free erythreoferrone showed significant negative correlation with HB in both diseased and control group (p< 0.001). (Table 4)
This table shows Correlation between ERFE and different parameters in disease group (erythroferrone showed significant positive correlation with age (p=0.001), and significant negative correlation with HB, HCT TB and hepcidin (p= <0.001, <0.001, 0.026 and <0.001 respectively). (Table 5)

Discussion

The present study was case control study carried out in the the Pediatric Department, Faculty of Medicine, Menoufia University on 2 groups the cases group included newborns of both sexes with anemia of prematurity and a control group. There was no statistically significant difference in liver, renal functions, CRP, blood culture and PH among studied groups. Therefore, no systemic illness affecting the scope of the study in studied subjects.

The present study showed that birth weight showed significant decrease in anemia of prematurity group when compared to control group. This data is supportive for the fact that anemia is significantly associated with being born small [9]. In Anemia Of Prematurity (AOP), mean Hb concentration reportedly falls to approximately 8 g/dL in infants with a birth weight of 1000-1500 g and to 7 g/dL for infants weighing less than 1000 g [10]. Also, previous studies showed that up to 40% of VLBW infants [11] and 90% of ELBW infants require one or more RBC transfusions during their birth hospitalization [12].

The present study revealed that the need for ventilation and sepsis showed significant increase in cases group when compared to control group. However, nasal O2 was more in control group when compared to cases group. In line with our finding, noticed that infants with anemia have high risk of comorbidities such as respiratory diseases (respiratory distress syndrome, pneumonia) or respiratory diseases combined with other infections (sepsis, skin infection) (40.7% and 43.4%, respectively) [13]. Due to the prematurity of respiratory and immunity system in preterm infants, it is comprehensive that the associated diseases mentioned above appeared frequently in this study [14]. It has been reported that

the correction of infant anemia is associated with a reduction in the increased morbidity (fever, respiratory tract infections and diarrhea) [15].

The present study showed that no significant difference was found between studied groups regarding Placenta previa, Placental abruption, Preeclampsia, PROM, Anemia, DM, HTN, MgSO4 and Iron ($p>0.05$). Contrarily, Raffaeli et al showed that maternal conditions such as obesity, gestational diabetes, hypertension, and placental insufficiency in IUGR also limit iron transfer to the fetus [11]. Nearly 17% of preterm infants are iron deficient at birth [16].

However, steroid intake showed significant increase in cases group when compared to control group. The present study revealed that respiratory rate and heart rate were increased in diseased group when compared to the control group, but the difference not statistically significant. Also, no significant difference between studied groups regarding temperature. However, blood pressure showed significant increase in diseased group when compared to control group. A study of Wardrop et al., showed that 50% of the preterm neonates have abnormal clinical signs related to anemia such as pallor, tachycardia, rapid breathing and delayed reflexes [17]. This abnormality is due to the function of Hb – the main oxygen-carrying protein in blood. Lacking of Hb could lead to the insufficient support of oxygen delivery to tissue, pallor, respiratory stress and tachycardia [18].

Premature infants have a more significant anemia that occurs earlier than in term infants, with the nadir at an average Hb concentration of 7–8 g/dL by a postnatal age of 4–6 weeks. Anemia of prematurity is a normocytic, normochromic, and hypoproliferative process that may lead to vital sign abnormalities, clinical instability, and the need for allogeneic red blood cell (RBC) transfusion(s) [19]. The present study showed that HB and HCT showed significant decrease in diseased group when compared to control. However, no significant difference between studied groups regarding WBCs, platelet and reticulocytic count.

The present study showed that hepcidin showed significant decrease in diseased group when compared to the control group ($p <0.001$). this may be explained by Hepcidin mRNA transcription in the liver is inhibited in the presence of hypoxia, iron deficiency, and anemia, resulting in increased iron absorption from the duodenum and iron release from the macrophages [12]. The present study showed that free erythroferrone showed significant increase in diseased group when compared to the control group. Free erythreoferrone showed significant negative correlation with HB in both diseased and control group. The increased erythroferrone level and the reported correlations with iron parameters can be explained as regulatory mechanism in cases with anemia of prematurity to induce hepcidin suppression and so increases iron availability.

Moreover, erythroferrone showed significant positive correlation with age ($p=0.001$), and significant negative correlation with HB, HCT, TB and hepcidin. No significant correlation was found between erythroferrone and gestational age, Birth Weight, Body Weight, Maternal Age, Temp, B/P (M), RR, HR, WBC, PLT, Retics, ALT, AST, Urea, Creatinine, direct bilirubin,CRP and PH. In line with our finding, demonstrated that ERFE directly inhibits the induction of hepcidin expression by BMP5, BMP6, and BMP7. Also, El Gendy et al showed that

erythroferrone hormone may act as physiological hepcidin suppressor in cases with iron deficiency anemia and serum erythroferrone concentrations correlated negatively with Hb concentration serum iron, transferrin saturation, and serum ferritin [20].

Lenhartová et al showed that ERFE was positively correlated with the reticulocyte hemoglobin content at 2 ($r^2 = 0.2374$) and 4 weeks ($r^2 = 0.6005$). An assumed negative correlation between ERFE and hepcidin was not determined during the neonatal period [21]. Delaney et al showed that after controlling for neonatal EPO or sTfR [2 indicators that were also significantly positively associated with gestational age at birth], the relation between ERFE and gestational age was no longer significant (both P = 0.5) [22].

Our study provides the first estimation of serum ERFE levels in human model with anemia of prematurity. Further studies of hepcidin-erythroferrone axis may help to provide more particular target for treatment of refractory cases with anemia of prematurity. Our further recommendations are to extent the study to include larger number of patients of different age groups, and the simultaneous estimation of other serum iron status parameters. Also, the development of validated accurate assay for human serum ERFE levels estimation and the standardization of reference range in healthy individuals of different age group will be of fundamental challenge in the near future.

Conclusion

Hepciden concentration is decreased and erythroferrone concentration is increased in children with anemia of prematurity. Therefore, both markers can be used in early diagnosis of anemia of prematurity.

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Table 1: Comparison between the two studied groups according to CBC

CBC	Diseased group (n = 30)	Control group (n = 30)	p
Hb (g/l)			
Min. – Max.	8.0 – 10.0	13.10 – 15.90	<0.001*
Mean ± SD.	8.94 ± 0.64	14.57 ± 0.90	
HCT			
Min. – Max.	24.30 – 32.90	41.90 – 47.70	<0.001*
Mean ± SD.	27.67 ± 2.37	44.55 ± 1.75	
WBC (10^9/L)			
Min. – Max.	2.50 – 24.0	8.0 – 17.0	0.215
Mean ± SD.	10.53 ± 4.67	11.77 ± 2.78	
PLT (10^9/L)			
Min. – Max.	84.0 – 548.0	90.0 – 311.0	0.135
Median (IQR)	217.0 (147.0 – 311.0)	180.0 (130.0 – 222.0)	

IQR: Inter quartile range SD: Standard deviation
p: p value for comparing between the two studied groups
*: Statistically significant at p ≤ 0.05

Table 2: Comparison between the two studied groups according to CRP, blood culture, hepcidin and PH

Variables	Diseased group (n = 30)	Control group (n = 30)	p
CRP			
Min. – Max.	6.0 – 12.0	6.0 – 6.0	0.078
Median (IQR)	6.0 (6.0 – 6.0)	6.0	
Blood Culture			
No	28(93.3%)	30(100.0%)	FEp= 0.492
Yes	2(6.7%)	0(0.0%)	
Hepcidin			
Min. – Max.	7.80 – 60.30	17.10 – 114.80	<0.001*
Median (IQR)	27.20 (17.70 – 47.80)	55.30 (35.90 – 75.40)	
PH			
Min. – Max.	7.35 – 7.40	7.35 – 7.40	0.201
Mean ± SD.	7.37 ± 0.02	7.38 ± 0.03	

IQR: Inter quartile range SD: Standard deviation
FE: Fisher Exact
p: p value for comparing between the two studied groups
*: Statistically significant at p ≤ 0.05

Table 3: Comparison between the two studied groups according to serum ERFE level

ERFE	Diseased group (n = 30)	Control group (n = 30)	p
Min. – Max.	4.27 – 14.75	2.35 – 8.54	<0.001*
Mean ± SD.	10.60 ± 2.86	5.56 ± 1.95	

SD: Standard deviation
p: p value for comparing between the studied groups
*: Statistically significant at p ≤ 0.05

Table 4: Correlation between ERFE and HB (g/dl)

Hb(g/dl)	ERFE	
	r	p
Total sample	-0.864*	<0.001*
Disease	-0.964*	<0.001*
Control	-0.982*	<0.001*

r: Pearson coefficient
*: Statistically significant at p ≤ 0.05

Table 5: Correlation between ERFE and different parameters in the diseased group (n = 30)

	ERFE	
	r	p
GA (weeks)	-0.228	0.225
Age (Days)	0.569	0.001*
Birth Weight (kg)	-0.332	0.073
Body Weight (kg)	-0.086	0.653
Maternal Age (years)	0.027	0.889
Temperature (°C)	0.144	0.447
B/P (M)	-0.182	0.337
RR (breath per minute)	-0.230	0.221
HR(beat per minute)	-0.299	0.109
Hb (g/l)	-0.964	<0.001*
HCT	-0.889	<0.001*
WBC	-0.271	0.148
PLT	-0.279	0.136
Retics	0.005	0.978
ALT (IU/L)	-0.117	0.537
AST (IU/L)	0.089	0.639
Urea(Mg/dl)	-0.018	0.925
Cr(Mg/dl)	-0.007	0.972
TB (Mg/dl)	-0.407	0.026*
DB(Mg/dl)	-0.186	0.326
CRP(Mg/dl)	0.040	0.835
Hepcidin	-0.964	<0.001*

PH	0.081	0.670

r: Pearson coefficient
*: Statistically significant at $p \leq 0.05$
RR: respiratory rate HR: heart rate TB: total bilirubin DB: direct bilirubin
CRP: C reactive protein ALT: Alanine transferase AST: aspartate transferase
PLT: platelet HB: hemoglobin HCT: hematochrite CR: creatinine