The Egyptian Journal of Hospital Medicine (July 2024) Vol. 96, Page 3217-3226

Study of Cord Blood Erythroferrone Levels and Its Relation To The Iron Status in High-Risk Neonates

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ABSTRACT
Background: Currently, erythroferrone (ERFE) has been discovered to be involved in iron control and erythropoiesis.
Objective: To investigate cord blood erythroferrone level and iron status in neonates with high risk factors and correlate these factors to the development of iron deficiency anemia at birth.
Patients and methods: This is a prospective study that was conducted on neonates recruited from the Obstetrics and Gynecology Department, Faculty of Medicine, Menoufia University and Neonatal Intensive Care Unit (NICU) during the period from January 2023 to March 2024. The participants underwent full history taking, clinical examination, and laboratory investigations including iron profile and erythroferrone.
Results: The mean serum iron and ferritin levels exhibited significant differences among the groups (p<0.001), with the lowest mean values were shown in the neonates of iron deficient mothers. Total iron binding capacity (TIBC) and cord blood erythroferrone levels showed a statistically significant difference among the groups (p<0.001), with the highest mean value was shown in the neonates of iron deficient mothers. ROC analysis showed that ERFE had high area under the curves (AUCs) to differentiate neonates of iron-deficient mothers (0.974, p <0.001) and low-birth-weight neonates (0.953, p<0.001) from the control group.
Conclusion: Our findings indicate that neonates of iron-deficient mothers and those with low birth weight exhibit elevated ERFE levels, highlighting the potential of ERFE as a biomarker for diagnosing iron deficiency anemia in high-risk neonates, as demonstrated by the excellent performance of ERFE levels in distinguishing affected neonates from healthy controls. This underscores the clinical relevance of ERFE in neonatal care and its potential as a therapeutic target.
Keywords: High risk neonates, Iron profile, Erythroferrone.

INTRODUCTION
Biochemical iron deficiency represents an initial phase of iron deficiency characterized by the absence of erythropoietic dysfunction or anemia. This stage precedes more advanced forms of iron deficiency, such as limited erythropoiesis and iron deficiency anemia. Identifying and addressing biochemical iron deficiency in neonates could prevent the progression to severe neurological impairments associated with iron deficiency, including enduring cognitive deficits that may persist even after iron deficiency is remedied [(1)].

It has been anticipated that the fetus mirrors the iron status of the mother. A thorough comprehension of the connection between neonatal and maternal iron parameters can guide devising protocols necessary for enhancing neonatal and maternal outcomes. Exploring the impact of maternal iron levels on fetal iron parameters holds significant promise, enabling early interventions to mitigate the adverse impact of iron deficiency on development long before they reach a severe stage [(2)].

Several risk factors predisposing infants to iron deficiency in early infancy have been recognized, including being born small for gestational age and being infants of diabetic mothers. Notably, cord blood ERFE has emerged as a key player in iron regulation and erythropoiesis [(3)]. Cord blood erythroferrone has quickly become a key player in the intricate field of iron regulation. It is secreted by erythroblasts in response to erythropoietin (EPO) stimulation and plays a central role in modulating hepcidin production, a hormone synthesized in the liver that governs iron absorption and utilization [(4)].

Despite our growing understanding of ERFE's role in iron regulation, there are critical gaps in our knowledge regarding its role in neonatal iron regulation. Neonates, with their rapidly expanding body mass, undergo substantial erythropoietic demands, increasing the need for iron uptake. The evolution of the low-oxygen intrauterine environment, characterized by extramedullary erythropoiesis, to air-breathing life presents unique challenges in aligning erythropoiesis with dietary iron needs [(5)].

This study aimed to investigate cord blood erythroferrone level and iron status of neonates with high risk factors and correlate these factors to the presence of iron deficiency anemia at birth.

SUBJECTS AND METHODS
Subjects:
This is a prospective study that was conducted on neonates recruited from the Obstetrics and Gynecology Department, Faculty of Medicine, Menoufia University and Neonatal Intensive Care Unit (NICU) during the period from January 2023 to March 2024. The laboratory investigations were performed at the Clinical Pathology Department, Menoufia University Hospital.

Received: 04/04/2024
Accepted: 12/06/2024

This study included neonates with high risk factors (n = 70) including low birth weight neonates (n = 25), infants of diabetic mother (n = 20), and neonates of iron deficient mothers (n = 25), recruited from the NICU of Menoufia University Hospital as well as control group (n = 20) including gender- and age-matched healthy neonates recruited from the Obstetrics and Gynecology Department, Menoufia University Hospital.

A multi-step approach was used to identify diabetic and iron-deficient mothers for our study. Regarding diabetic mothers, a thorough review of electronic medical records was conducted to identify patients with a documented history of either gestational or pre-existing diabetes. Additionally, laboratory tests including measurements of blood glucose levels, HbA1c levels, and oral glucose tolerance tests were employed to confirm the presence of diabetes in cases where medical records were inconclusive or unavailable. Iron-deficient mothers were identified through a combination of clinical assessments and laboratory tests. Clinical assessments involved evaluating symptoms such as fatigue, weakness, and pale skin, while laboratory tests included complete blood counts, and measuring serum iron and ferritin levels. Hemoglobin levels below 11 g/dL in pregnant women suggest anemia, while serum ferritin levels below 15 ng/mL are indicative of depleted iron stores. Additionally, serum iron levels under 50 μg/dL further confirm iron deficiency.

Neonates whose parents refused to participate in the study and those with different genetic abnormalities were excluded from the study.

All enrolled neonates were subjected to full history taking from the parents including personal history, obstetric history, and any medical history, as well as general clinical assessment.

Laboratory Investigations: in the form of complete blood count (CBC), serum iron (Normal reference range for neonates is 50-120 mcg/dL), total iron-binding capacity (TIBC: Normal reference range for neonates is 250-450 μg/dL), and serum ferritin analysis (Normal reference range for neonates is 25-200 ng/dL).

Methods:

Six milliliters of cord blood were drawn under aseptic conditions and distributed as follows:

(a) Two milliliter of whole blood was taken in an EDTA vacutainer and mixed gently and used to measure CBC.

(b) Four milliliters of blood were taken in plain test tubes. After coagulation, the samples were centrifuged at 1500 rpm for 10 min. The separated serum was used for the assay of iron parameters and erythroferrone.

Serum cord blood erythroferrone levels were analyzed using ELISA technique (ERFE, Abbexa, USA). The analysis was performed according to the manufacturer instructions: Serum samples were first prepared and added to wells coated with specific capture antibodies. After blocking non-specific sites, detection antibodies were introduced to bind the captured erythroferrone. An enzyme-conjugated secondary antibody was then applied, followed by the addition of a substrate solution that produces a color change measurable by a microplate reader. This sequence of steps ensured precise and reproducible quantification of cord blood erythroferrone levels.

Ethical approval:

This study has been approved by the Menoufia Faculty of Medicine's Ethics Committee. Following receipt of all information, informed written consents were obtained from parents of the included neonates. The Helsinki Declaration was adhered to at every stage of the investigation.

Statistical analysis:

Using IBM SPSS software version 28.0, the data were entered into the computer and examined. Range, mean, standard deviation (SD), median, and inter quartile range (IQR) were used to present quantitative data. One-way ANOVA was used to compare numerical data and if the difference was significant, the post-hoc Tukey test was used for pairwise comparisons. Categorical data were presented by frequency and percentage and were compared by the X^2-test and Monte Carlo test. Correlating numerical data was done using the Pearson correlation test. To evaluate how well ERFE levels discriminated among high-risk newborns, a receiver operating characteristic (ROC) curve was used. Statistics were deemed significant if the P value was less than 0.05.

RESULTS

Regarding the complications during pregnancy, maternal age, gravidity and mode of delivery, there was no significant difference between the groups. Post-partum complications represented by hemorrhage, blood transfusion, and- or septic wound, showed significantly different rates among the groups. Iron-deficient mothers had a notably higher rate of post-partum hemorrhage (60%) and required blood transfusion (Table 1).

Table (1): The maternal data in the studied groups

	Control (n = 20)		Neonates of iron deficient mothers (n = 25)		Low birth weight neonates (n = 25)		Infants of diabetic mother. (n = 20)		Test of Sig.	p
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**		
Any complication during delivery										
No	20	100	22	88.0	23	92.0	19	95.0	χ^2= 4.442	^{MC}p= 0.761
Bleeding	0	0	2	8.0	2	8.0	1	5.0		
Atony	0	0.0	1	4.0	0	0.0	0	0.0		
Maternal age (Years)										
Min. – Max.	20.0 – 36.0		20.0 – 41.0		20.0 – 42.0		19.0 – 40.0		F= 1.665	0.181
Mean ± SD.	27.15 ± 4.36		27.56 ± 5.50		30.56 ± 6.34		28.25 ± 6.49			
Median (IQR)	26.0 (24.0 – 30.50)		26.0 (23.0 – 31.0)		30.0 (24.50 – 35.0)		27.0 (23.50 – 35.0)			
Primigravida or multiparous										
Multiparous	14	70.0	9	36.0	13	52.0	9	45.0	χ^2= 5.400	0.145
Primigravida	6	30.0	16	64.0	12	48.0	11	55.0		
Type of delivery										
Normal	13	65.0	12	48.0	11	44.0	8	40.0	χ^2= 2.957	0.398
Caesarean	7	35.0	13	52.0	14	56.0	12	60.0		
Post-partum complications										
No	20	100.0	5	20.0	10	40.0	11	55.0	χ^2= 94.05	^{MC}p= <0.001*
Post-partum hemorrhage	0	0.0	10	40.0	0	0.0	0	0.0		
Anemia/Need for blood transfusion	0	0.0	10	40.0	15	60.0	0	0.0		
Septic wound	0	0.0	0	0.0	0	0.0	9	45.0		

IQR: Inter quartile range, SD: Standard deviation, F: F for One way ANOVA test, χ^2: Chi square test, MC: Monte Carlo test

Gestational age, birth weight analysis and neonates' height at birth revealed significant differences among the groups. Post hoc analyses confirmed significant differences between all groups. Post-birth complications were assessed, including the need for an incubator, blood transfusion, and jaundice. Significant differences were observed among the groups. Notably, a high proportion of neonates of iron-deficient mothers required an incubator (52%) and blood transfusion (40%). Incubation due to jaundice was the highest in the low-birth-weight neonates (68%) (Table 2).

Table (2): The neonatal data in the studied groups

	Control (n = 20)		Neonates of iron deficient mothers (n = 25)		Low birth weight neonates (n = 25)		Infants of diabetic mother. (n = 20)		Test of Sig.	P
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**		
Gender										
Male	11	55.0	14	56.0	17	68.0	11	55.0	χ^2= 1.193	0.755
Female	9	45.0	11	44.0	8	32.0	9	45.0		
Gestational age (weeks)										
Min. – Max.	37.0 – 40.0		34.0 – 40.0		29.0 – 40.0		37.0 – 40.0		F= 13.611	<0.001*
Mean ± SD.	38.70 ± 0.98		37.52 ± 1.78		35.08 ± 3.67		38.75 ± 0.85			
Median (IQR)	39.0 (38.0 – 39.0)		38.0 (36.0 – 39.0)		35.0 (32.0 – 38.0)		39.0 (38.0 – 39.0)			
p_0			0.302		<0.001*		1.000			
P between groups.			p_1 = 0.001*, p_2=0.267, p_3<0.001*							
Weight (gm)										
Min. – Max.	2700.0 – 4000.0		2500.0 – 3900.0		1061.0 – 2900.0		3200.0 – 5000.0		F= 130.703	<0.001*
Mean ± SD.	3409.5 ± 384.3		3052.0 ± 294.2		1940.8 ± 460.4		4240.0 ± 430.9			
Median (IQR)	3500 (3100 – 3700)		3000.0 (2900 – 3200)		2000 (1500 – 2193.5)		4200.0 (4050 – 4500)			
p_0			0.018*		<0.001*		<0.001*			
P between groups.			p_1 = 0.001*, p_2=0.001*, p_3<0.001*							
Height (cm)										
Min. – Max.	46.0 – 60.0		39.00 – 60.0		32.0 – 62.00		46.0 – 58.0		F= 21.347	<0.001*
Mean ± SD.	53.0 ± 4.18		51.84 ± 5.19		42.69 ± 6.57		52.05 ± 3.49			
Median (IQR)	53.0 (50.0 – 55.50)		51.0 (49.0 – 56.0)		41.0 (39.50 – 45.0)		51.0 (50.0 – 55.0)			
p_0			0.873		<0.001*		0.935			
P between groups.			p_1,<0.001*, p_2=0.999, p_3<0.001*							
Any complication after birth										
No	17	85.0	0	0.0	0	0.0	7	35.0	χ^2= 111.288	<0.001*
Incubator	3	15.0	13	52.0	7	28.0	0	0.0		
Blood transfusion	0	0.0	10	40.0	0	0.0	0	0.0		
Jaundice	0	0.0	2	8.0	1	4.0	0	0.0		
Jaundice/ incubator	0	0.0	0	0.0	17	68.0	13	65.0		

IQR: Inter quartile range, SD: Standard deviation, χ^2: Chi square test, F: One way ANOVA test, p_0: p value for comparing between control and each other group, p_1: p value for comparing between neonates of iron deficient mothers and low birth weight neonates, p_2: p value for comparing between neonates of iron deficient mothers and infants of diabetic mother, p_3: p value for comparing between low birth weight neonates and infants of diabetic mother, *: Statistically significant

A comparison between different study groups based on CBC showed that hemoglobin levels, RBC count, hematocrit levels, MCV and MCH exhibited significant differences among the groups. As regards HB levels, post hoc analyses revealed significant differences between all neonatal groups. While RBCs count showed significant differences between neonates of iron deficient mothers and the control group. Platelet count (PLT) and white blood cell count (WBCs), showed no significant differences among the groups or between individual groups (Table 3). Statistically significant differences were observed among all the studied groups regarding serum iron levels and ferritin. Post hoc analysis used for more detailed comparison between each neonatal group and control group and in-between neonatal groups revealed significant differences between all groups apart from the neonates of diabetic mothers that were not significantly different from the control group. TIBC values showed a statistically significant levels difference among the groups. The highest mean value is shown in the neonates of iron deficient mothers (Table 3). The mean of cord blood erythroferrone level in control group was 6.36 ± 1.55 ng/ml and there was a significant difference in cord blood erythroferrone levels among different groups. Neonates of iron-deficient mothers had a significantly higher mean of 41.52 ± 9.55 ng/ml compared to control group. Post hoc analysis revealed statistically significant increased levels of cord blood erythroferrone in neonates of iron deficient mothers group compared to low-birth-weight neonates and neonates of diabetic mothers. While no significant difference was observed between low-birth-weight neonates and neonates of diabetic mothers (Table 3).

Table (3): Comparison between the different studied groups according to CBC parameters, iron profile, and erythroferrone levels

Parameter	Control (n = 20)	Neonates of Iron Deficient Mothers (n = 25)	Low Birth Weigh Neonates (n = 25)	Infants of Diabetic Mothers (n = 20)	F	P
Hb (g/dl), Mean ± SD.	16.5 ± 1.8	12.70 ± 1.19	13.23 ± 2.38	14.33 ± 2.35	13.816	<0.001*
p0		<0.001*,	<0.001*,	0.003*		
P between groups.		p1=0.344,	p2=0.007*,	p3=0.067		
RBCs (10^{12}/L), Mean ± SD.	5.1 ± 0.5	3.95 ± 0.60	4.58 ± 0.71	4.88 ± 0.79	9.677	<0.001*
p0		<0.001*	0.608	0.992		
P between groups.		p1=0.006*,	p2<0.001*,	p3=0.421		
HCT (%), Mean ± SD.	55.0 ± 5.5	35.30 ± 5.39	45.22 ± 6.36	42.29 ± 6.02	18.461	<0.001*
p0		<0.001*	0.970	0.162		
P between groups.		p1 <0.001*,	p2<0.001*,	p3=0.298		
MCV (fl/cell), Mean ± SD.	110.0 ± 7.5	86.28 ± 9.34	98.68 ± 17.18	88.25 ± 16.61	3.657	0.016*
p0		0.049*	0.992	0.149		
P between groups.		p1 = 0.069,	p2=0.0982,	p3=0.207		
MCH (pg/cell), Mean ± SD.	30.0 ± 2.0	27.60 ± 1.58	29.08 ± 1.12	31.15 ± 2.03	57.561	<0.001*
p0		<0.001*	<0.001*	<0.001*		
P between groups.		p1 =0.006*	p2<0.001*	p3<0.001*		
MCHC (g/dl), Mean ± SD.	34.0 ± 1.0	27.44 ± 1.87	29.0 ± 1.56	33.02 ± 1.70	64.429	<0.001*
p0		<0.001*	<0.001*	0.928		
P between groups.		p1 =0.010*	p2<0.001*	p3<0.001*		
PLT (10^3/uL), Mean ± SD.	275 ± 50	218.4 ± 53.32	258.0 ± 62.82	255.6 ± 61.82	1.536	0.211
WBCs (10^3/uL), Mean ± SD.	12.0 ± 1.5	10.97 ± 7.67	15.10 ± 3.42	8.91 ± 1.01	6.450	0.092
Iron – Serum (mcg/dL) Mean ± SD.	75.0 ± 10.0	39.88 ± 9.93	60.56 ± 7.83	70.30 ± 2.0	98.86	<0.001*
p0		<0.001*	<0.001*	0.454		
P between groups.		p1, p2, p3<0.001*				
TIBC (Ug/dl), Mean ± SD.	300.0 ± 50.0	276.0 ± 43.61	258.40 ± 49.32	118.45 ± 5.81	158.23	<0.001*
p0		<0.001*	<0.001*	0.101		
P between groups.		p1=0.299,	p2, p3<0.001*			
Ferritin (ng/dl), Mean ± SD.	125.0 ± 25.0	39.10 ± 9.59	72.36 ± 17.90	104.97 ± 25.78	55.43	<0.001*
p0		<0.001*	<0.001*	0.306		
P between groups.		p1=0.001*,	p2<0.001*,	p3=0.015*		
Cord blood erythroferrone(ng/ml) Mean ± SD.	6.36 ± 1.55	41.52 ± 9.55	10.76 ± 2.60	7.31 ± 1.80	226.779	<0.001*
p0		<0.001*	0.040*	0.971		
P between groups.		p1 <0.001*,	p2<0.001*,	p3=0.154		

The correlation between cord blood erythroferrone levels and various parameters in the studied neonates showed that significant negative correlation was observed with serum iron (r=-0.372) in neonates of iron deficient mothers, a statistically significant negative correlation with WBCs (r=-0.567) in low-birth-weight neonates, and a positive insignificant correlation with weight (r=0.414) in infants of diabetic mother. No statistically significant other correlations were found (Table 4).

Table (4): Correlation between cord blood erythroferrone(ng/ml) and different parameters in the study neonates

	Neonates of iron deficient mothers (n = 25)		Low-birth-weight neonates (n = 25)		infants of diabetic mother (n = 20)	
	R	P	r	p	r	p
Maternal age	-0.045	0.832	0.202	0.332	-0.117	0.624
Gestational age	-0.099	0.636	0.136	0.518	0.087	0.715
Weight (gm)	0.269	0.193	-0.109	0.603	0.414	0.070
Height (cm)	-0.133	0.525	0.016	0.939	-0.414	0.069
Hb (g/dl)	0.074	0.724	0.118	0.575	-0.302	0.196
RBCs (10^{12}/l)	0.049	0.817	0.027	0.897	-0.247	0.295
HCT %	0.072	0.732	0.018	0.932	-0.333	0.151
MCV (fl/cell)	-0.112	0.595	0.034	0.871	-0.098	0.682
MCH (pg/cell)	0.260	0.210	-0.212	0.309	-0.238	0.311
MCHC (g/dl)	-0.250	0.228	-0.180	0.389	0.081	0.735
PLT ($10\times^3$/ul)	0.267	0.197	-0.159	0.448	0.302	0.196
WBCs ($10\times^3$/ul)	-0.153	0.465	-0.567	0.003*	0.145	0.543
Iron –Serum (mcg/dL)	-0.372	0.067	0.335	0.101	-0.059	0.806
TIBC(Ug/dl)	-0.072	0.732	-0.056	0.792	0.294	0.209
Ferritin (ng/dl)	0.262	0.206	0.085	0.685	-0.045	0.851
r: Pearson coefficient						

The following figures show that a cut-off value for cord blood erythroferrone of >8 ng/ml had a high AUC of 0.974 (p <0.001) to differentiate neonates of iron deficient mothers from control, with high sensitivity (96.0%), specificity (95.0%), PPV (96.0%), and NPV (95.0%) (Fig. 1). While the cut-off value of >7.4 ng/ml was identified for significant discrimination between low-birth-weight neonates and the control group, (p<0.001) with sensitivity (96.0%), specificity (85.0%), PPV (88.9%), and NPV (94.4%) (Fig. 2). The diagnostic performance of cord blood erythroferrone (ng/ml) in discriminating infants of diabetic mothers from the control group exhibited a modest AUC of 0.554, with a non-significant p value (0.561) (Fig. 3).

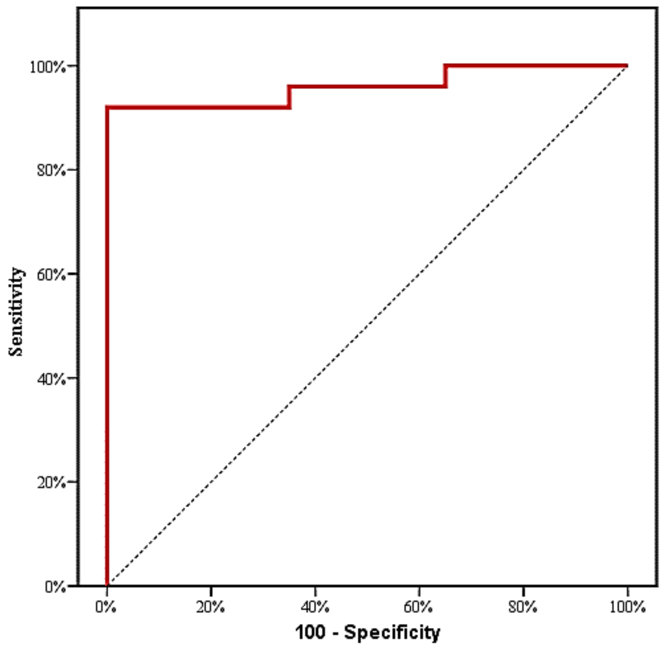

Figure (1): ROC curve for cord blood erythroferrone(ng/ml) to discriminate neonates of iron deficient mothers (n = 25) from control (n = 20).

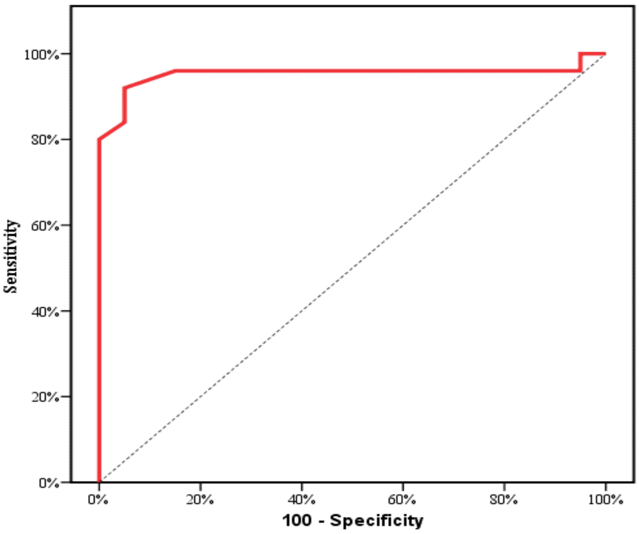

Figure (2): ROC curve for cord blood erythroferrone(ng/ml) to discriminate low birth weight neonates (n = 25) from control (n = 20).

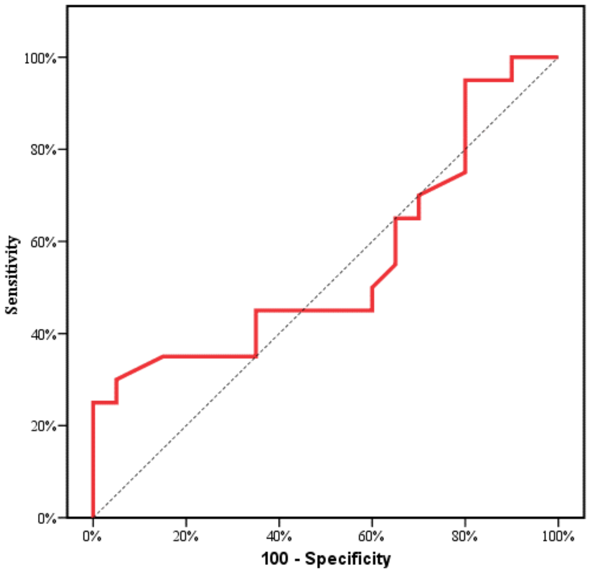

Figure (3): ROC curve for cord blood cord blood erythroferrone(ng/ml) to discriminate infants of diabetic mother (n = 20) from control (n = 20).

DISCUSSION

In newborns, iron deficiency may be present from birth even if a baby is born with enough iron. It can cause significant and long-lasting neurocognitive impairment. As a result, initiatives are required to either prevent newborn iron deficit or to effectively treat it [(5)]. Neonatal iron deficiency cannot always be prevented or treated with enteral iron supplementation; the patient's iron homeostatic mechanisms must remain intact for enteral iron dose to be successful. While such processes are extensively documented in adults, less is known about them in newborns [(6)].

The iron-regulating hormonal axis consists of EPO, ERFE, hepcidin, and ferroportin. The human fetus produces both hepcidin and EPO, yet there is currently limited data on the ERFE status of neonates [(5)]. It is imperative to gather data to characterize ERFE concentrations at birth and investigate it with newborn iron status biomarkers for a comprehensive understanding of its role in neonatal health.

This study aimed to investigate cord blood erythroferrone level and iron status of neonates with high risk factors and correlate these factors to iron deficiency anemia at birth. Assessment of the CBC parameters in the included neonates showed significant differences in the hemoglobin levels, RBC count, hematocrit levels, MCV and MCH, with neonates of iron-deficient mothers having the lowest levels of blood indices. This is in accordance with previous studies conducted by **Qaiser *et al.*** **[(7)], Terefe *et al.*** **[(8)] and Timilsina *et al.*** **[(9)]** that revealed the association between maternal and offspring hematological indices.

The present study encompassed a detailed comparison of iron profiles among the different studied groups. The lowest mean of serum iron and ferritin levels and the highest mean of TIBC were exhibited in the neonates of iron deficient mothers. Similar results were reported by **El-Farrash *et al.* [10], Basu *et al.* [11] and Sanni *et al.* [12].**

In the current study, cord blood ERFE levels were assessed in the included neonates, and the mean of cord blood ERFE in control term group was 6.36 ± 1.55 ng/ml.

To the best of our knowledge this study is one of the fewest studies that investigated the level of cord blood erythroferrone in term and high-risk neonates. Previous research done by **Delaney *et al.*** [13], who investigated cord blood in neonate born to woman according to their age group ERFE in neonates born to women carrying multiples (age: 21-43 y; n = 127) or teens (age: 14-19 y; n = 164), the mean was 0.73 ng/mL (95% CI: 0.63, 0.85 ng/mL).

Another study carried out by **Bahr *et al.*** [5] found that cord blood erythroferrone level ranged between 0.9–2.3 ng/ml with a mean of 1.6 ± 1.3 in term neonates.

Unfortunately, until now there has been no documented national or international reference range regarding cord blood ERFE levels and these controversial results may arise from the variety of sample sizes, ethnicity or population.

Our results showed significantly higher mean of cord blood ERFE in neonates of iron-deficient mothers (41.52 ± 9.55 ng/ml), followed by LBW neonates (10.76 ± 2.60 ng/ml) compared to normal neonates (6.36 ± 1.55 ng/ml) while neonates of diabetic mothers did not exhibit significant difference. These data were further confirmed in the current work by ROC curve analysis that revealed performance of cord blood ERFE levels to discriminate neonates of mothers with iron deficient anemia and LBW neonates from normal healthy neonates.

Our findings align the study of **El Gendy *et al.*** [14], indicated indicated that serum ERFE levels were significantly higher in patients with iron deficiency anemia than those in control group and declared the negative association between ERFE levels and iron stores. Closer to the context of the current study, **Bäckström *et al.*** [15] reported a significantly higher ERFE levels in LBW neonates compared to normal ones and also found a negative correlation between serum ERFE and hemoglobin and iron levels. The authors, in agreement with our study, suggested an earlier onset of increased transcription of ERFE in LBW babies.

These data suggest a potential adaptive response to maternal anemia and compromised fetal growth. In the context of maternal anemia, the increased cord blood ERFE levels may reflect the fetus's attempt to optimize iron availability for erythropoiesis and support red blood cell production. This response could be considered a compensatory mechanism to overcome the challenges posed by maternal iron deficiency, ensuring an adequate supply of iron for fetal hematopoiesis [16].

Similarly, the observed higher cord blood erythroferrone levels in neonates with low birth weight might indicate an adaptive response to fetal growth restriction. In conditions of intrauterine growth restriction, the fetus may prioritize iron availability for critical functions such as oxygen transport, thereby adjusting cord blood erythroferrone production to maintain optimal iron utilization [17].

On the other hand, contradictory results were reported by **Bahr *et al.*** [5] who found significantly lower cord blood ERFE levels in neonates of diabetic mothers and preterm neonates, and the study of **Delaney *et al.*** [13] who reported significant negative associations between iron status biomarkers and cord blood ERFE at birth suggesting that cord blood ERFE supports intrauterine erythropoietic iron demand of the fetus.

Fetal adaptation mechanisms to optimize iron availability for erythropoiesis may respond differently to specific maternal and intrauterine conditions, influencing cord blood ERFE regulation [18]. The conflicting findings on cord blood ERFE levels and their association with participants characteristics among different studies may be attributed to the diversity of study populations, encompassing distinct maternal health conditions and neonatal characteristics. Variations in the timing of cord blood ERFE assessment, along with differences in laboratory methods and techniques, could contribute to the observed discrepancies. Moreover, genetic, and environmental factors, not uniformly accounted in the studies, may contribute to variations in outcomes [17].

In this study, in the group of LBW neonates, a statistically significant negative correlation was observed between cord blood ERFE levels and WBCs. To the best of our knowledge, this association was not previously investigated. It's plausible that alterations in iron availability, influenced by cord blood ERFE levels, impact the functionality of immune cells, leading to changes in WBC counts [18]. However, a comprehensive understanding of the specific mechanisms underlying this correlation would require further research and exploration of the complex interactions between iron regulation and immune function.

In this study, the correlation between cord blood ERFE levels and various parameters in infants of diabetic mothers demonstrated a positive insignificant correlation with weight p=0.07. One possible explanation could be related to the intricate relationship between iron metabolism and growth. Cord blood ERFE, as a regulator of iron homeostasis, may play a role in supporting erythropoiesis and overall physiological processes associated with growth and weight gain. Alternatively, the correlation might be reflective of broader metabolic and hormonal changes in infants of diabetic mothers, where variations in glucose metabolism and insulin levels could impact both iron regulation and growth [19].

In the current work, we observe varying diagnostic performances of cord blood erythroferrone (ng/ml) across different neonatal conditions. Cord blood erythroferrone at a cut-off value of >8 ng/ml and at a cut-off value of >7.4 ng/ml with highly significant PPV and NPV can effectively distinguish neonates of iron-deficient mothers and LBW neonates from the control group respectively. However, when discriminating infants of diabetic mothers from the control group, cord blood erythroferrone demonstrated limited discriminative ability.

These analyzes emphasize that erythroferrone at these high levels can be used as a marker to detect neonatal anemia in neonates born to iron deficient mothers or low birth weight neonates even before evident appearance of anemia in lab results.

Additionally, these findings underline the assumption that erythroferrone plays a critical role in the pathogenesis of iron deficiency and low birth weight anemia in neonates. Furthermore, it suggests an adaptive response to enhance iron availability for erythropoiesis. As this hormone suppresses hepcidin production, thereby increasing iron absorption and mobilization. Given this, erythroferrone could be a therapeutic target for protection against and managing iron deficiency anemia and anemia in low-birth-weight neonates. Interventions aimed at modulating erythroferrone levels may help improve iron availability and support better hematologic and developmental outcomes in affected neonates. Especially in neonates who are not responding to enteral iron. Further research is needed to explore the therapeutic potential of adding erythroferrone as a treatment option in neonatal care.

CONCLUSION

This study provides significant insights into the role of cord blood erythroferrone (ERFE) in neonatal health, particularly in the context of iron deficiency anemia and low birth weight. Our findings indicate that neonates of iron-deficient mothers and those with low birth weight exhibit elevated cord blood ERFE levels, suggesting an adaptive response to enhance iron availability for erythropoiesis. This response may help to support red blood cell production and overall fetal growth despite maternal iron deficiency or growth restrictions.

Furthermore, the study highlights the potential of ERFE as an early biomarker for diagnosing iron deficiency anemia in high-risk neonates, this underscores the clinical relevance of ERFE in neonatal care and its potential as a therapeutic target.

- **Conflict of interest:** None declared.
- **Fund:** Non-fundable.

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