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Research Article

Growth Differentiation Factor-15 and Erythroferrone are Reliable Predictors of Iron Status among Iraqi Pregnant Women with Anemia: A Case-Control Study

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Abstract

Background: It is estimated that more than half of pregnant women all over the world are anemic. The potential of erythroferrone (ERFE) and growth differentiation factor-15 (GDF15) as indicators for iron deficiency could be used to detect various types of anemia, cardiovascular and metabolic diseases. ***Objectives***: To assess whether variations in erythroferrone and Growth Differentiation Factor-15 in blood levels among pregnant women might be used as a marker for anemia. ***Methods***: A cross-sectional study recruited 120 pregnant women into a study group: 60 anemic pregnant women and 60 healthy pregnant controls. Their demographics, hematological indices, and biomarkers (growth differentiation factor-15, erythroferrone, serum ferritin and iron) were collected. ***Results***: It has been found that anemic pregnant women have statistically higher levels of Growth Differentiation-15, Erythroferrone, and other iron status compared to healthy pregnant women. The average concentration of ERFE in anemic pregnant women was 5.6 ng/mL, while in healthy pregnant women, it was 2.2 ng/mL.. For GDF-15, the average concentration was 457.27 pg/mL for anemic patients and 228.89 pg/mL for healthy pregnant women. The cutoff value of both GDF-15 and ERFE had the highest sensitivity and specificity in differentiating anemic pregnant women, 1.000 (p<0.0001) for the area under the curve in the case of healthy controls. ***Conclusions***: The markers erythroferrone and GDF-15 have a significant correlation with iron indicators and are recommended for screening anemic pregnant women.

Keywords: Anemia, Erythroferrone, Growth Differentiation Factor-15, Pregnancy.

عامل تمايز النمو -15 والإريثروفيرون من المتنبئين الموثوقين لحالة الحديد بين النساء العراقيات الحوامل المصابات بفقر الدم: دراسة الحالات والشواهد

الخلاصة

الخلفية: تشير التقديرات إلى أن أكثر من نصف النساء الحوامل في جميع أنحاء العالم يعانين من فقر الدم. يمكن استخدام إمكانات الإريثروفيرون وعامل تمايز النمو -15 كمؤشرات لنقص الحديد للكشف عن أنواع مختلفة من فقر الدم وأمراض القلب والأوعية الدموية والتمثيل الغذائي. **الأهداف**: تقييم ما إذا كانت الاختلافات في الإريثروفيرون وعامل تمايز النمو -15 في مستويات الدم بين النساء الحوامل يمكن استخدامها كعلامة لفقر الدم. **الطرائق**: شارك في دراسة مقطعية 120 امرأة حامل في مجموعة دراسة: 60 امرأة حامل مصابة بفقر الدم و 60 امرأة حامل سليمة. تم جمع التركيبة السكانية ومؤشرات الدم والمواد الكيميائية الحيوية (عامل تمايز النمو -15 ، الإريثروفيرون ، فيريتين المصل والحديد). **النتائج**: لقد وجد أن النساء الحوامل المصابات بفقر الدم لديهن مستويات أعلى إحصائيا من تمايز النمو -15 ، الإريثروفيرون ، وحالة الحديد الأخرى مقارنة بالنساء الحوامل الأصحاء. كان متوسط تركيز ERFE في النساء الحوامل المصابات بفقر الدم 5.6 نانوغرام / مل، بينما في النساء الحوامل الأصحاء، كان 2.2 نانوغرام/مل. بالنسبة لـGDF-15 ، كان متوسط التركيز 457.27 بيكوغرام/مل للمرضى المصابين بفقر الدم و 228.89 بيكوغرام/مل للنساء الحوامل الأصحاء. كانت القيمة الفاصلة لكل من GDF-15 و ERFE أعلى حساسية وخصوصية في التمييز بين النساء الحوامل المصابات بفقر الدم ، 1.000 (p<0.0001) للمنطقة الواقعة تحت المنحنى في حالة الضوابط الصحية. **الاستنتاجات**: علامات الإريثروفيرون و GDF-15 لها علاقة كبيرة بمؤشرات الحديد ويوصى بها لفحص النساء الحوامل المصابات بفقر الدم.

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INTRODUCTION

A significant public health concern that impacts 75% of women globally is anemia that develops during pregnancy. It is a widespread and worldwide issue that warrants greater focus in numerous developing nations. Severe anemia that develops during pregnancy frequently elevates the likelihood of infection, morbidity, and mortality associated with reproduction, both at birth and in the perinatal period. Moreover, it substantially contributes to maternal mortality, morbidity, and low birth weight. Iron deficiency anemia (IDA) is the chief etiology of anemia that occurs during pregnancy [1–3]. Iron supplementation during pregnancy is, in truth, vitally important for both the developing fetus and the mother. Hormones such as hepcidin, erythropoietin (EPO), and erythroferrone (ERFE) regulate the iron levels in the body [4]. The precise relationships between erythroferrone (ERFE) levels during pregnancy and other hormones as well as iron status markers remain poorly understood. Throughout pregnancy, ERFE serves a minimal role in maintaining iron homeostasis in the mother and fetus, as well as in blood cell formation, when iron levels are adequate. In order for the fetus to redistribute iron throughout its body, the hormone ERER is required for cell development [5]. Additionally, erythroferon is essential for regulating hepcidin, which is responsible for regulating the body's total iron content. Red blood cell production in the bone marrow is dependent on these hormones; therefore, a deficiency in these hormones may result in internal hemorrhaging or oxygen deprivation [6]. As a result, the EREE protein inhibits the production of hepcidin and enhances iron absorption within the body, which is essential for the synthesis of red blood cells [7]. However, the precise function that ERFE plays in clinical symptoms remains unknown. The mechanism by which ERFE disrupts the bone morphogenetic protein (BMP) signaling pathway, which regulates hepatocyte hepcidin production, is unknown. Erythropoiesis dysfunction resulting from anemia leads to the pathological production of ERFE. Despite this, its role in clinical symptoms remains unknown [7]. The precise role of growth differentiation factor 15 (GDF-15), also known as macrophage inhibitory cytokine-1, remains unknown at this time. However, scholarly investigations have demonstrated its criticality in regulating inflammatory pathways. It is widely recognized that elevated levels occur in cancer situations and under stressful conditions [8]. GDF-15 expression is minimal in the majority of tissues; however, it is abundant in the placental and maternal circulations during pregnancy. Exposure to teratogens during pregnancy is hypothesized to be reduced [9–10] due to the fact that levels are 200 times higher in the third trimester compared to levels not associated with pregnancy. It is hypothesized that trophoblast invasion contributes to the inhibition of the maternal immune response during placentation. However, the relationship between maternal iron levels and placental iron transfer and fetal nutrition remains inadequately investigated. While basement membranes and chorionic villi contain numerous iron localization transporters, the fundamental mechanisms remain unknown [11]. Current research is advancing our comprehension of the role of GDF-15 in pregnancy and body weight regulation, an area where knowledge is limited [12]. Our research introduces novel findings concerning the correlation between serum erythroferrone, growth differentiation factor-15 (GDF-15), and other iron biomarkers in anemic expectant women relative to healthy controls.

METHODS

Study design and setting

A cross-sectional investigation was undertaken at Mustansiriyah University and the National Center of Hematology in Baghdad, Iraq, spanning from October 2023 to January 2024. The purpose of the research was to examine anemia in expectant women. The study included a sample of 60 pregnant women who were present at the center during the reference period, as well as a control cohort of 60 pregnant women. The expectant participants who consented to take part were duly apprised of the study's aims and objectives. The ethical committee of Mustansiriyah University approved the research protocol. The pregnant women comprising the research group were anemic, in contrast to the control group who did not exhibit anemia.

Inclusion criteria

Women who met the followings inclusion criteria: the ages between 17 and 38 years, and having a gestational age of ≥30 weeks.

Exclusion criteria

Exempted were pregnant women who had previously undergone cesarean sections, twin pregnancies, bacterial or viral infections, hypothyroidism, gestational diabetes, or pregnancy-induced hypertension (PIH).

Outcome measurements

The confirmation of anemia in expectant women was established using the WHO criterion [13,14], which requires a Hb concentration of no more than 12 g/dL and ferritin levels of no more than 20 ng/mL. Hematological evaluations were performed on maternal peripheral blood samples obtained using an automated hematology analyzer. Iron analysis was performed using Cobas c-111. Using the ELISA method, serum ferritin, erythroferon, and GDF15 concentrations were determined.

Statistical analysis

In order to ascertain the validity of the markers as disease indicators, a number of statistical measures, including independent t-tests, correlation coefficients, and ROC curves, were applied to the data using Statistical Analysis System (SAS) version 9.1. The markers were assessed through the comparison of the area under the curve; Medical Calculate software was

utilized for the analysis. A p-value less than 0.05 was deemed to indicate statistical significance [15].

RESULTS

Table 1 revealed that 60 pregnant women had anemia, with a mean age of 27.29 and a gestational age of 32.90 weeks.

Table 1. The demographic, clinical, hematological and biochemical variables of the study groups.

Variables	Control (*n*=60)	Patients (*n*=60)	*p*-value
Age (y)	27.39±6.66	27.29±5.12	0.92
GA (y)	33.15±5.38	32.90±4.95	0.79
BMI (kg/m^2)	29.08±3.16	29.78±4.17	0.30
Hb (g/dL)	12.87±0.75	10.58±1.23	<0.0001
HCT (%)	38.50±2.41	37.97±41.22	0.92
MCV (fL)	85.49±4.90	81.05±8.00	<0.0001
MCH (pg)	28.64±2.03	26.11±3.22	<0.0001
MCHC (g/dL)	37.22±28.77	32.20±1.41	0.17
GDF-15 (pg/mL)	228.89±33.93	457.27±93.51	<0.0001
ERFE (ng/mL)	2.22±0.42	5.64±1.74	<0.0001
Ferritin (ng/mL)	19.98±3.02	2.83±1.55	<0.0001
Iron (µg/dL)	84.18±19.76	32.43±6.71	<0.0001

Values were expressed as mean±SD. GA: gestational age; BMI: body max index; Hb: Hemoglobin; HCT: Hematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; GFD-15; Growth differential factor-15; ERFE: Erythroferrone.

The control pregnant women had a mean age of 27.39 and a gestational age of 33.15. The average body max index of the patient and control groups appears to be not significantly different. The age had no statistical differences in the studied sample, as did the gestational age and BMI. Neither MCH nor HCT values were statistically significant. In contrast, Hb, MCV, GDF-15, ERF, iron, and ferritin were significantly higher (*p*-value <0.0001), as shown in Table 1. As shown in Table 2, age was significantly correlated with BMI (*r*= -0.4, *p*= 0.0001), and Hb was significantly linked to HCT (*r*= 0.8, *p*= 0.0001), as was HCT with GDF-15 (*r*= -0.28, *p*= 0.027). MCV has a statistically significant correlation with MCH and iron (*r*= 0.862, *p*= 0.0001, *r*= 0.2, *p*= 0.04, respectively). In Table 3, the correlation study of the patient group indicated a statistically significant association between age and BMI (*r*= -0.4, *p*= 0.002) and GDF-15 (*r*= 0.2, *p*= 0.04). Additionally, BMI was significantly linked to HCT (*r*= 0.3, *p*= 0.002), so there was a statistically significant positive correlation between Hb and MCV (*r*= -0.6, *p*= 0.0001), MCH (*r*= 0.6, *p*= 0.0001), MCHC (*r*= 0.4, *p*= 0.0001), and iron (*r*= 0.3, *p*= 0.005), respectively.

Table 2: Pearson correlation among the studied variables in control group.

Control		GA	BMI	Hb	HCT	MCV	MCH	MCHC	GDF	ERF	Ferritin	Iron
Age	*r*	-0.01	0.498	0.060	0.025	-0.027	0.017	-0.181	-0.128	0.067	0.080	0.144
	p	0.942	0.00	0.646	0.845	0.836	0.898	0.163	0.327	0.605	0.542	0.270
GA	r		-0.01	0.081	0.067	0.067	0.071	0.069	0.195	-0.008	-0.09	-0.239
	p		0.942	0.535	0.609	0.608	0.584	0.597	0.132	0.954	0.489	0.063
BMI	*r*			0.152	0.109	-0.181	-0.104	-0.084	-0.092	-0.041	0.117	0.049
	p			0.242	0.401	0.162	0.424	0.521	0.481	0.756	0.370	0.708
Hb (g/dL)	*r*				0.831	0.074	0.112	-0.094	0.208	0.108	-0.150	-0.064
	p				0.00	0.57	0.392	0.471	0.107	0.409	0.247	0.625
HCT%	*r*					-0.007	-0.226	-0.12	0.283	0.192	-0.098	-0.069
	p					0.96	0.080	0.357	0.027	0.138	0.454	0.597
MCV (fl)	*r*						0.862	-0.020	0.240	0.239	0.066	0.26
	p						0.00	0.879	0.063	0.063	0.614	0.043
MCH (pg)	*r*							0.009	0.117	0.121	-0.007	0.244
	p							0.945	0.37	0.352	0.957	0.058
MCHC (g/dL)	*r*								0.057	0.081	-0.085	-0.05
	p								0.663	0.537	0.513	0.701
GDF (pg/mL)	*r*									0.082	-0.119	-0.026
	p									0.529	0.360	0.841
ERF (ng/mL)	*r*										-0.230	0.156
	p										0.075	0.229
Ferritin (ng/mL)	*r*											-0.211
	p											0.103

GA: gestational age; BMI: body max index; Hb: Hemoglobin; HCT: Hematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; GFD-15; Growth differential factor-15; ERFE: Erythroferrone.

Meanwhile, MCV was significantly associated with MCH (*r*=0.9, *p*=0.0001), MCHC (*r*=0.5, *p*=0.0001), and iron (*r*=0.2, *p*=0.03), respectively. However, MCH shows a statistically significant association with MCHC (*r*=0.7, *p*=0.0001) and iron (*r*=0.2, *p*=0.04), respectively. The results of the ROC curve analysis are shown in Figure 1 and Table 4.

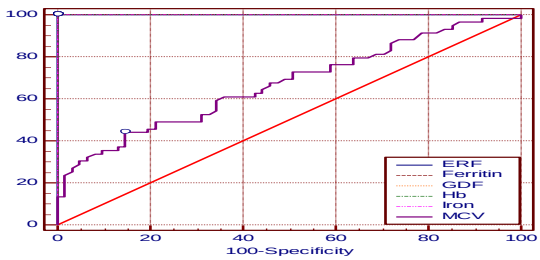

Figure 1: The ROC measures for studied markers (ERF, GDF-15, ferritin, Hb, iron, and MCV).

DISCUSSION

Anemia affects around 80% of expectant women residing in South and Southeast Asia [16].

Table 4: The cutoff value of hematological indices and biochemical markers that discriminated IDA cases from healthy controls alongside their sensitivity, and specificity.

Variable	AUC	95% CI	Cutoff point	SN	SP	*p*-value
ERF	1.00	0.97-1.0	>2.9	100	100	0.0001
Ferritin	1.00	0.97-1.0	≤9.3	100	100	0.0001
GDF	1.00	0.97-1.0	> 293.6	100	100	0.0001
Hb	1.00	0.97-1.0	≤11.9	100	100	0.0001
Iron	1.00	0.97-1.0	≤49.2	100	100	0.0001
MCV	0.67	0.578-0.75	≤80.5	44.1	85.2	0.049

GDF-15 and ERFE concentrations in the serum of anemic pregnant women are greater than those of non-anemic pregnant women, according to previous

research. In addition, anemic pregnant women have lower concentrations of serum iron and ferritin than non-anemic pregnant women. Iron deficiency anemia associated with pregnancy is a significant public health issue [17]. The IDA does not occur until three phases have passed. The first is characterized by a decrease in serum ferritin levels, which is indicated by anisocytosis and microcytosis. Later in the second stage, hemoglobin levels will decrease, and erythropoietic activity will increase as a result. Inevitably, decreased MCHC, ferritin, and hemoglobin levels manifest in the third stage [18]. Anemia tends to increase with gestational age, according to the findings of numerous studies; the third trimester has the highest prevalence (34.7%), followed by the second (19.7%) and first (14.06%).

Table 3: Pearson correlation between age, BMI and hematological parameters, biochemical parameters in the patients group.

Patient		GA	BMI	Hb	HCT	MCV	MCH	MCHC	GDF	ERF	Ferritin	Iron
Age	*r*	-0.095	0.403	0.111	-0.226	0.039	0.044	0.069	0.258	-0.005	0.150	0.199
	p	0.476	0.002	0.402	0.085	0.767	0.742	0.605	0.048	0.968	0.258	0.13
GA	r		0.237	0.232	0.025	-0.018	-0.011	0.039	-0.12	0.014	-0.071	-0.169
	p		0.070	0.077	0.851	0.894	0.936	0.77	0.364	0.915	0.593	0.202
BMI	*r*			0.218	0.397	0.187	0.219	0.225	0.242	-0.076	0.029	0.083
	p			0.097	0.002	0.155	0.095	0.087	0.065	0.566	0.830	0.532
Hb (g/dL)	*r*				0.184	0.629	0.635	0.452	-0.245	0.218	0.177	0.363
	p				0.162	0.000	0.00	0.00	0.061	0.098	0.181	0.005
HCT (%)	*r*					0.084	0.108	0.108	0.009	0.057	-0.056	0.058
	p					0.526	0.417	0.417	0.947	0.67	0.674	0.661
MCV (fl)	*r*						0.96	0.547	-0.21	0.183	0.198	0.283
	p						0.00	0.00	0.111	0.165	0.133	0.03
MCH (pg)	*r*							0.730	-0.225	0.155	0.219	0.263
	p							0.00	0.086	0.242	0.096	0.044
MCHC (g/dL)	*r*								-0.164	-0.039	0.170	0.193
	p								0.214	0.767	0.198	0.143
GDF (pg/ml)	*r*									0.085	0.063	-0.031
	p									0.523	0.638	0.815
ERF (ng/ml)	*r*										0.026	-0.068
	p										0.844	0.61
Ferritin (ng/ml)	*r*											0.107
	p											0.418

GA: gestational age; BMI: body max index; Hb: Hemoglobin; HCT: Hematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; GFD-15; Growth differential factor-15; ERFE: Erythroferrone.

The preponderance of these studies have demonstrated that hemoglobin levels decrease gradually throughout pregnancy [19,20]. Anemia during the third trimester is predominantly caused by the increased iron requirements of the fetus and placenta [21]. Moreover, the importance of physiological anemia should not be underestimated. Furthermore, extensive research conducted in the United States has substantiated the notion that the incidence of total-body iron deficiency is greater during the third trimester in comparison to the initial and second trimesters [20]. Our research indicates that expectant women who are anemic have elevated levels of GDF-15 pg/mL in comparison to those who do not have anemia. It has been confirmed that GDF-15 is expressed during the second and third trimesters of pregnancy. It is a cytokine belonging to the TGFβ superfamily and possesses anti-inflammatory properties [11]. The regulation of hepcidin during pregnancy may be affected by a variety of factors, the precise characteristics of which remain unknown at this time. Although GDF-15 levels tend to decrease in non-pregnant, healthy adults, there have been reports of elevated levels subsequent to inflammation and injury [22]. In comparison to individuals who are not expectant, serum GDF-15 concentrations increase during pregnancy and reach a peak of approximately ten times in the first trimester [8]. Some research indicates that hepcidin and GDF-15 are not statistically related. Furthermore, hepcidin inhibition is observed exclusively at exceedingly high concentrations of GDF-15, indicating that the hepcidin response to elevated GDF-15 concentrations is biphasic [23]. Therefore, it is conceivable that the mechanism by which GDF15 inhibits hepcidin during pregnancy is not known [24]. Other studies, in contrast to ours, found no significant differences in maternal GDF-15 levels in relation to malaria exposure or pregnancy-related anemia. Endogenous GDF-15 is a ubiquitous stress signal that is produced and secreted by an assortment of cell types [25]. The brainstem-based GDF-15 receptor GFRAL (glial cell-derived neurotrophic factor [GDNF] family receptor α-like) has been observed to influence weight loss and food intake. As a result, GDF-15 and its receptor have emerged as appealing therapeutic targets in the realm of metabolic disorders. The GFRAL pathway can only account for a fraction of the effects of GDF-15; thus, its complete mechanisms of action remain unknown. For instance, the function of GDF-15 is poorly understood during pregnancy. Notably, the capacity of GDF-15 to forecast biomarkers might be associated with its immune-regulatory function [8]. Since the publication of a validated human test in 2017, information regarding serum ERFE concentrations in humans has been scarce [26]. Consistent with prior investigations, including the recent study conducted by Delaney et al., which aimed to assess ERFE levels in pregnant women while evaluating its correlation with other biomarkers of iron status and regulatory hormones, our current study revealed that anemic pregnant women exhibited a greater concentration of ERFE compared to non-pregnant women. Furthermore, a positive correlation was observed between erythropoietic drive indices and ERFE, with the former being substantially elevated in pregnant women who were anemic [27]. The inaugural investigation into ERFE in pregnant individuals and its utility in identifying women susceptible to anemia, ID, or IDA was carried out by Delaney *et al*. in 2021 [4]. The study found that soluble transferrin receptor (sTfR) and erythropoietin (EPO) were significantly correlated with

ERFE in expectant women. ERFE is associated with biomarkers of iron status; however, no significant correlations have been established between ERFE and hepcidin [4]. ERFE is widely recognized for its regulatory function on hepcidin. In our study, the average ERFE concentration among control pregnant women was 2.22 ng/mL; among anemic pregnant women, it was 5.6 ng/mL, with a significance level greater than 2.9 ng/mL. A cohort of healthy expectant women had an average ERFE concentration of approximately 0.48 ng/mL throughout midgestation and delivery, according to another study [28]. ERFE concentrations have been examined in only two published studies employing the same methodology; one of these studies utilized control women who were not expectant and reported an average ERFE concentration of 0.32 ng/mL [28]. The other study, which examined female athletes aged 18 to 22, determined that the average concentration of ERFE in elite ID athletes was 1.0±1.13 ng/mL, whereas athletes with SF had a mean concentration of 3.5±5.1 ng/mL [28]. ERFE concentrations in healthy pregnant women remain significantly lower than those of elite male and female athletes [28–31] or individuals with erythropoietic stress-inducing disorders [32–35]. Finally, additional research is necessary to determine the relationship between the iron status of the neonate and the ERFE of the mother [36].

Study limitations

Our research was not without its limitations. In the first place, the number of patients was comparatively modest in relation to the prevalence of anemia among expectant women in our nation. Furthermore, we were deficient in hepcidin and erythropoietin, biomarkers that provide greater precision in the estimation of iron deficiency anemia.

Conclusion

Elevated concentrations of Erythroferrone (ERFE) and Growth Differentiation-15 (GDF-15) were found to be significantly correlated with anemia in expectant women with gestational iron deficiency, according to the study. The most prominent indicators of maternal iron deficiency and anemia are these two factors. The utilization of GDF-15 and ERFE levels as an indicator for assessing the iron requirements of expectant women is possible. Understanding the relationship between elevated maternal growth differentiation-15 (GDF-15) and ERFE in anemic expectant women requires additional study.

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Conflict of interests

No conflict of interests was declared by the authors.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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