ORIGINAL STUDY

Predictive Value of Erythroferrone and Hepcidin for Blood Transfusion Frequency and Comorbidities in β-thalassemia

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Abstract

Objectives: **To investigate the significance of erythroferrone (ERFE) and hepcidin in β-thalassemia (β-TM) patients in prediction of frequency of transfusions, iron overload, and its impact on comorbidity and disease severity.**

Background: **Repeated transfusions are used to treat anemia, the main concern of β-TM patients. They cause iron to accumulate, which, if left untreated, results in significant morbidity. ERFE was found to alter hepcidin release, thus increasing iron availability. This study investigated its usefulness as a predictor of iron overload status and its impact on severity and prognosis.**

Methods: **A case−control study was done on 90 patients, divided into two groups: group A: patients' group included 60 β TM patients, further classified according to serum ferritin level at cutoff 2000.8 ng/ml into groups A1 and A2, and group B: 30 apparently healthy controls. A thorough clinical examination included complete blood count (CBC), liver functions, kidney functions, serum iron, ferritin, and total iron binding capacity (TIBC). Serum levels of hepcidin and ERFE were assayed by ELISA.**

Results: **There are significantly higher hepcidin and ERFE levels in groups A1 and A2 than in group B. Serum ERFE increased in groups A1 and A2 suffering from myocardial dysfunction, diabetes mellitus, and gall bladder (GB) stones, and in patients with hepatomegaly in group A1. There is a negative correlation between ERFE and frequency of blood transfusions in groups A1 and A2. A positive correlation is found between frequency of blood transfusions and hepcidin in group A1 and serum iron in group A2.**

Conclusion: **ERFE can be an efficient biomarker of iron overload state, disease severity, and comorbidities better than hepcidin. It can also be used to predict the frequency of blood transfusions in β-TM patients.**

Keywords: Erythroferrone, Hepcidin, Iron overload, Thalassemia major

1. Introduction

A genetic disorder called β-thalassemia (β-TM) is characterized by a diminished or lack of synthesis of β-globin chain. 1−5% of people worldwide carry β-TM allele [1]. As a result, α-globin chains will accumulate excessively in erythroid precursors, leading to globin chain imbalance and a state called 'ineffective erythropoiesis' which, in an attempt to generate more erythrocytes and compensate for the imbalance, maturing nucleated erythroid cells undergo apoptosis. This leads to chronic hemolytic anemia and increased iron absorption [2].

Additionally, severe erythroid hyperplasia in bone marrow, extramedullary hematopoiesis, and hepatosplenomegaly are caused by ineffective erythropoiesis, anemia and accelerated hemolysis in these patients [3]. To correct anemia and suppress endogenous, ineffective erythropoiesis, regular blood transfusions are needed [4]. Moreover, years of repeated blood transfusions that result in iron

Received 10 August 2023; revised 6 September 2023; accepted 12 September 2023.
Available online 23 January 2024

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https://doi.org/10.59204/2314-6788.1103

overload can significantly increase the incidence of morbidity and mortality if left untreated [5].

The frequency of blood transfusions a thalassemic patient receives affects how much iron they accumulate. 1 gm of iron will be present in 4 units of blood. When body iron exceeds 400–1000 mg/kg body weight, signs of clinical toxicity start to appear. After 10–12 transfusions, signs of iron overload can usually become apparent. Hyperactive bone marrow will encourage greater intestinal iron absorption in addition to iron delivered by blood transfusions, which will contribute to the overall body load [6].

By assessing liver iron concentration via liver biopsy or magnetic resonance imaging (MRI), researchers have investigated the laboratory's role in predicting the development of tissue iron deposition. In several studies, serum ferritin has been identified as a reliable biomarker of iron overload severity, and its predictive role in thalassemia has been emphasized and linked to cardiac death [7].

Serum ferritin levels and transferrin saturation (TS%) were not sensitive enough to be used as screening measures for iron overload. Studies have criticized its poor ability to predict iron accumulation because of some conditions that compromise its effectiveness, including inflammatory states, liver disorders, rapid cell turnover, and vitamin C deficiency cases [7,8].

Erythroferrone (ERFE), an essential erythroid regulator, was discovered to be a major negative regulator of hepcidin in conditions of stress or ineffective erythropoiesis, increasing iron availability for developing erythroid progenitors [7]. It is produced by erythroid precursors in the bone marrow and spleen under control of renal erythropoietin. Further researches regarding the ERFE/hepcidin pathway may identify more precise targets for medical treatment to treat iron overload in β-TM, other anemias linked to ineffective erythropoiesis (such as congenital anemia and myelodysplastic syndromes), and primary iron overload illnesses [7].

The aim of this study was to determine the relationship between ERFE and transfusion frequency in β-TM major patients. This study was conducted to investigate the significance of ERFE and hepcidin in Egyptian β-TM patients in the prediction of iron overload states, frequency of transfusions, and their impact on severity and comorbidities.

2. Methods

This case–control study was conducted at the Clinical Pathology and Internal Medicine Departments, Faculty of Medicine, Menoufia University, during the period from March 2022 to February 2023. The least sample size calculated using statistics and the sample size programmed is 81 participants based on a review of past literature by Saad et al. [9], who reported that there was a significant positive correlation between ERFE, hepcidin, and serum iron. The power of the study is 80 %, and the confidence level is 95 %. We added 9 patients, so the total number of participants became 90, divided into three equal groups.

Group A: 60 β-TM major patients recruited from hematology inpatient wards and outpatient hematology clinics of the Internal Medicine Department. The serum ferritin cutoff was set at 2000.8 ng/ml to distinguish significant from insignificant iron overload.

Group A1: 30 β-TM patients (13 males and 17 females aged between 21 and 53 years old) with insignificant iron overload (Ferritin level less than 2000.8 ng/ml).

Group A2: 30 β-TM patients (12 males and 18 females aged between 18 and 50 years old) with significant iron overload (ferritin level more than 2000.8 ng/ml).

Group B: 30 healthy controls (11 males and 19 females aged between 22 and 45 years old).

The study was approved by the Research Ethical Committee of the Menoufia Faculty of Medicine, and written informed consent was obtained from each participant with IRP approval number C PATH 50.

Sample collection: 6 ml of venous blood were collected under complete aseptic conditions and divided as follows: 4 ml of blood were delivered to a vacutainer plain tube, left for 30 min to clot, and then centrifuged for 10 min at 3000 rpm. Sera were separated into two aliquots, one for measurement of serum ferritin, serum iron, liver function, and kidney function tests, and the other for measurement of serum hepcidin, serum ERFE, and total iron binding capacity (TIBC). 2 ml were collected into an EDTA-containing tube for complete blood count (CBC).

Thalassemia major diagnosis was based on clinical presentation, peripheral blood evaluation, and HB electrophoresis. All studied patients were subjected to a full history taking and thorough clinical examination. The following laboratory investigations were performed: CBC was measured by the hematology autoanalyzer Sysmex XN-1000 (Sysmex Corporation, Kobe, Japan), Liver functions and kidney function tests were done by the autoanalyzer AU 680 (Beckman Coulter, Fullerton, USA). Serum iron and ferritin were measured by the immunoassay analyzer Architect i1000SR (Abbot

Diagnostic, Illinois, U.S.A.). TIBC was measured using colorimetric assay kits.

Serum levels of hepcidin and ERFE were assayed using an ELISA kit supplied by Sunred Co (Sunred Co., Ltd, Shanghai, China) (Cat. No. 1020, 5646), based on the principle that certain antibodies can recognize the presence and level of antigen binding by binding to a specific target antigen. The plate needs to be coated with high affinity antibodies in order to improve the assay's sensitivity and accuracy.

2.1. Statistical analysis

Data collected were tabulated and analyzed by the Statistical Package of Social Science (SPSS, version 20; SPSS Inc., Chicago, Illinois, USA) on an IBM personal computer. Qualitative data were described using number and percentage and assessed using χ^2 test. Quantitative data were represented using the mean, SD, median, and interquartile range (IQR). The difference between two normally distributed groups was determined by Student's *t*-test. A

Table 1. Comparison between the different studied groups according to studied parameters.

	Group A1 ($n = 30$)	Group A2 ($n = 30$)	Group B ($n = 30$)	Test of sig.	*P*
Hb (g/dl)					
Mean ± SD.	7.03 ± 1.21	6.96 ± 0.89	13.53 ± 0.99	F = 393.50[a]	<0.001[a]
Median (Min–max.)	7 (3.9–9.5)	7.15 (5.3–8.4)	13.25 (12–16.7)		
Sig. bet. grps.	$P_1 = 0.967, P_2 < 0.001$[a], $P_3 < 0.001$[a]				
MCV (fl)					
Mean ± SD.	65.60 ± 8.45	71.10 ± 8.34	85.21 ± 2.66	F = 62.159[a]	<0.001[a]
Median (Min–max)	63.25 (54.5m–85.9)	3.19 (2.19–4.06)	5 (4.5–5.93)		
Sig. bet. grps.	$P_1 = 0.009$[a], $P_2 < 0.001$[a], $P_3 < 0.001$[a]				
RDW (%)					
Mean ± SD.	28.71 ± 6.76	27.04 ± 6.52	12.93 ± 0.80	F = 76.108[a]	<0.001[a]
Median (Min–max.)	31.15 (13.8–38.1)	29 (13.6–36.3)	13 (11.6–14.7)		
Sig. bet. grps.	$P_1 = 0.465, P_2 < 0.001$[a], $P_3 < 0.001$[a]				
ALT (U/l)					
Mean ± SD.	41.07 ± 21.08	62.53 ± 40.88	23.47 ± 9.79	H = 28.879[a]	<0.001[a]
Median (Min–max.)	49 (6–86)	51 (16–203)	22.5 (7–45)		
Sig. bet. grps.	$P_1 = 0.057, P_2 = 0.001$[a], $P_3 < 0.001$[a]				
AST (U/l)					
Mean ± SD.	46.27 ± 21.64	82.13 ± 43.59	24.30 ± 8.83	H = 45.463[a]	<0.001[a]
Median (Min–max.)	50 (11–93)	72.5 (24–208)	22 (13–46)		
Sig. bet. grps.	$P_1 = 0.002$[a], $P_2 < 0.001$[a], $P_3 < 0.001$[a]				
Serum iron (μg/dl)					
Mean ± SD.	191.33 ± 65.54	194.33 ± 70.28	70.20 ± 15.58	H = 55.346[a]	<0.001[a]
Median (Min–max.)	1725 (83–411)	192.5 (78–406)	65.5 (52–101)		
Sig. bet. grps.	$P_1 = 0.913, P_2 < 0.001$[a], $P_3 < 0.001$[a]				
TIBC (μg/dl)					
Mean ± SD.	265.27 ± 68.02	285.55 ± 72.15	307.40 ± 26.12	H = 6.271[a]	0.043[a]
Median (Min–max.)	275 (91.67–366.67)	300 (116.6–408.33)	300 (274.80–375.0)		
Sig. bet. grps.	$P_1 = 0.123, P_2 = 0.013$[a], $P_3 = 0.347$				
Ferritin (ng/ml)					
Mean ± SD.	1459.5 ± 573.0	6592.4 ± 4172.5	73.26 ± 55.51	H = 79.122[a]	<0.001[a]
Median (Min–max.)	1727.9 (332.4–2000.8)	5135.1 (3146.1–24737.2)	43 (23.54–206.7)		
Sig. bet. grps.	$P_1 < 0.001$[a], $P_2 < 0.001$[a], $P_3 < 0.001$[a]				
Hepcidin (ng/ml)					
Mean ± SD.	332.4–2000.8	3146.1–24737.2	23.54–206.7	H = 263.932[a]	<0.001[a]
Median (Min–max.)	805 (700–1400)	975 (740–1400)	122.5 (80–175)		
Sig. bet. grps.	$P_1 = 0.403, P_2 < 0.001$[a], $P_3 < 0.001$[a]				
Erythroferrone (ng/ml)					
Mean ± SD.	12.74 ± 1.80	11.78 ± 2.30	1.02 ± 0.39	H = 438.290[a]	<0.001[a]
Median (Min–max.)	13.5 (9.0–15.20)	12 (8.0–15.20)	0.90 (0.50–1.80)		
Sig. bet. grps.	$P_1 = 0.294, P_2 < 0.001$[a], $P_3 < 0.001$[a]				

ALT, alanine transaminase; AST, aspartate transaminase; **F, F for One way ANOVA test**, Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (Tukey)**; **H, H for Kruskal Wallis test**, Pairwise comparison bet. each 2 groups was done using Post Hoc Test; MCV, mean corpuscular volume; RDW, red cell distribution width; **SD**, Standard deviation.

P: *P* value for comparing between the three studied groups.

*P*1: *P* value for comparing between Group A1 and Group B.

*P*2: *P* value for comparing between Group A2 and Group B.

*P*3: *P* value for comparing between Group A1and Group A2.

[a] Statistically significant at *P* less than or equal to 0.05.

Mann–Whitney test was used for two nonparametric variables between two groups. The difference between more than two groups that are normally distributed and not normally distributed was determined by analysis of variance and Kruskal–Wallis tests, respectively, followed by post-hoc test. The association between two quantitative variables was shown by Correlation coefficient (r). The receiver operating characteristic (ROC) curve was used to assess the diagnostic value of biomarkers and the most appropriate cutoff value by calculating the area under the curve (AUC). Multivariate regression analysis was used to predict the value of one dependent variable from the values of two or more independent variables. A P value less than or equal to 0.05 is considered significant [10].

3. Results

This study was carried out on 90 patients in three groups. A comparison between group A1 and A2 regarding hematological parameters revealed significantly elevated mean corpuscular volume (MCV) in group A2 compared with group A1 ($P = 0.009$). Liver function tests depicted an elevation of aspartate transaminase (AST) in group A2 than in group A1. Serum iron and ferritin were significantly higher in thalassemic patients than controls, while TIBC was significantly lower in group A1 only compared with group B ($P_1 = 0.013$). Patient groups showed significantly higher levels of ferritin in group A2 than group A1 ($P_3 < 0.001$). Serum hepcidin and ERFE are significantly higher in thalassemic patients than controls (Table 1).

A ROC curve for the studied parameters was done to discriminate between group A1 and group A2. It revealed that ferritin, at a cutoff 2000.8 ng/ml, had the highest significant sensitivity and specificity ($P < 0.001$). Serum hepcidin, ERFE, and TIBC showed a statistically significant difference only when combined with ferritin with high sensitivity and specificity (100 %), $P < 0.001$ (Table 2, Fig. 1).

The frequency of blood transfusions significantly increased in group A2 compared with group A1 ($P < 0.001$). This significant increase was also seen in patients complaining of myocardial dysfunction ($P = 0.011$), gall bladder (GB) stones ($P = 0.011$), diabetes mellitus ($P < 0.011$), ($P = 0.026$) in groups A1 and A2 and hepatomegaly in group A1 ($P = 0.011$) (Fig. 2). Significant positive correlation was found between frequency of blood transfusion and hepcidin in group A1 ($r = 0.338$, $P < 0.001$) and serum iron in group A2 ($r = 0.464$, $P = 0.010$). While, significant negative correlation was observed with ERFE in group A2 ($r = -0.818$, $P < 0.001$) (Fig. 3).

Furthermore, frequency of blood transfusion was the single independent factor affecting ERFE. Multivariate linear regression analysis in groups A1 and A2 for parameters affecting the frequency of blood transfusions revealed that ERFE is the single independent factor that affects the frequency of transfusions (Table 3).

4. Discussion

Since iron overload is the most prevalent factor in thalassemia major morbidity and mortality, it represents a potential treatment target [7]. ERFE was found to alter hepcidin release, thus increasing iron availability. This study investigated its usefulness as a predictor of iron overload status, blood transfusion frequency, and its impact on severity and prognosis.

The hematological parameters in this study depicted a significant decrease in thalassemic patients except for red cell distribution width (RDW)%, which was significantly increased. These came in accordance with previous research by Saad et al. [9]. Whereas, MCV was significantly increased in group A2. In addition, ERFE correlated significantly with RDW% in group A2.

Table 2. Validity (area under a curve, sensitivity, specificity) for different parameters to discriminate group A2 (n = 30) from group A1 (n = 30).

	AUC	*P*	95 % C.I	Cut off	Sensitivity	Specificity	PPV	NPV
Hepcidin (ng/ml)	0.594	0.212	0.446–0.742	>875	63.33	66.67	65.5	64.5
Erythroferrone (ng/ml)	0.618	0.117	0.474–0.761	≤13	70.0	53.33	60.0	64.0
TIBC (μg/dl)	0.605	0.162	0.461–0.749	>275	60.0	53.33	56.2	57.1
Ferritin (ng/ml)	1.000	<0.001[a]	1.0–1.0	2000.8	100.0	100.0	100.0	100.0
Hepcidin and Erythroferrone	0.644	0.055	0.505–0.784		60.0	60.0	60.0	60.0
Hepcidin and TIBC	0.599	0.186	0.455–0.744		56.67	53.33	54.84	55.17
Hepcidin and Ferritin	1.000	<0.001[a]	1.0–1.0		100.0	100.0	100.0	100.0
Erythroferrone and TIBC	0.644	0.055	0.500–0.789		60.0	70.0	66.67	63.64
Erythroferrone and Ferritin	1.000	<0.001[a]	1.0–1.0		100.0	100.0	100.0	100.0
TIBC and Ferritin	1.000	<0.001[a]	1.0–1.0		100.0	100.0	100.0	100.0

AUC, Area Under a Curve; CI, Confidence Intervals; NPV, Negative predictive value; *P* value, Probability value; PPV, Positive predictive value.

[a] Statistically significant at *P* less than or equal to 0.05.

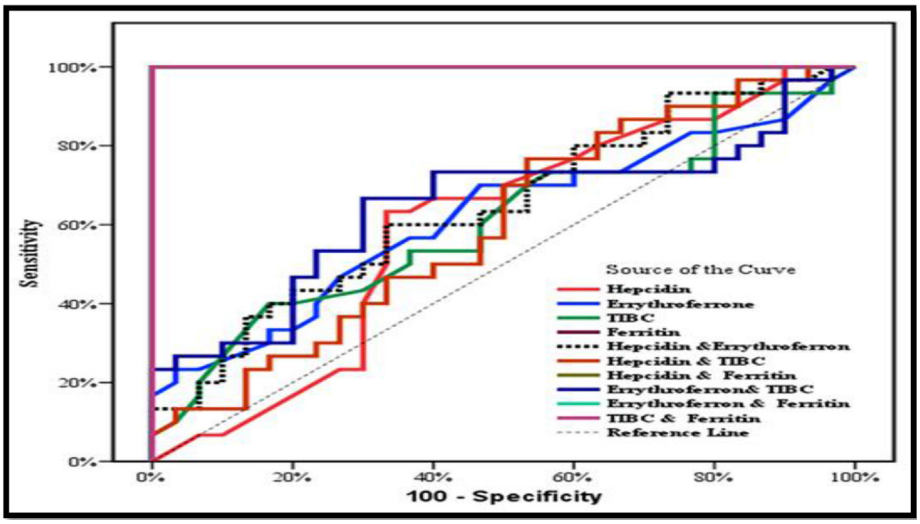

Fig. 1. Receiver operating characteristic curve for different parameters to discriminate group A2 (n = 30) from group A1 (n = 30).

As regards liver functions as a reflection of disease severity, AST, particularly in group A2, was significantly higher than group A1. In addition, group A2 had a significantly higher incidence of hepatomegaly than group A1. These findings agreed with Faiq et al. [11] and Abd et al. [12], who explained that AST was more prevalent than alanine transaminase (ALT) in the heart, liver, skeletal muscles, and renal tissue. When patients' serum level of iron increase, it is deposited in these organs and causes their damage, releasing AST. On the other hand, Al-Ghanimi et al. [13] reported a decrease in serum levels of ALT and AST in thalassemic patients compared with controls.

The iron profile in the current study came in accordance with the results of Saad et al. [9], which showed that group A2 had considerably higher serum ferritin levels than group A1 (5135.1 vs. 1727). Additionally, El Gamal et al. [7] reported that, compared with normal controls, patients' serum ferritin levels (median 2575 ng/ml) were substantially higher than those of controls (median 51 ng/ml). Diverse levels of ferritin were reported in many studies [14]. Adequacy and frequency of blood

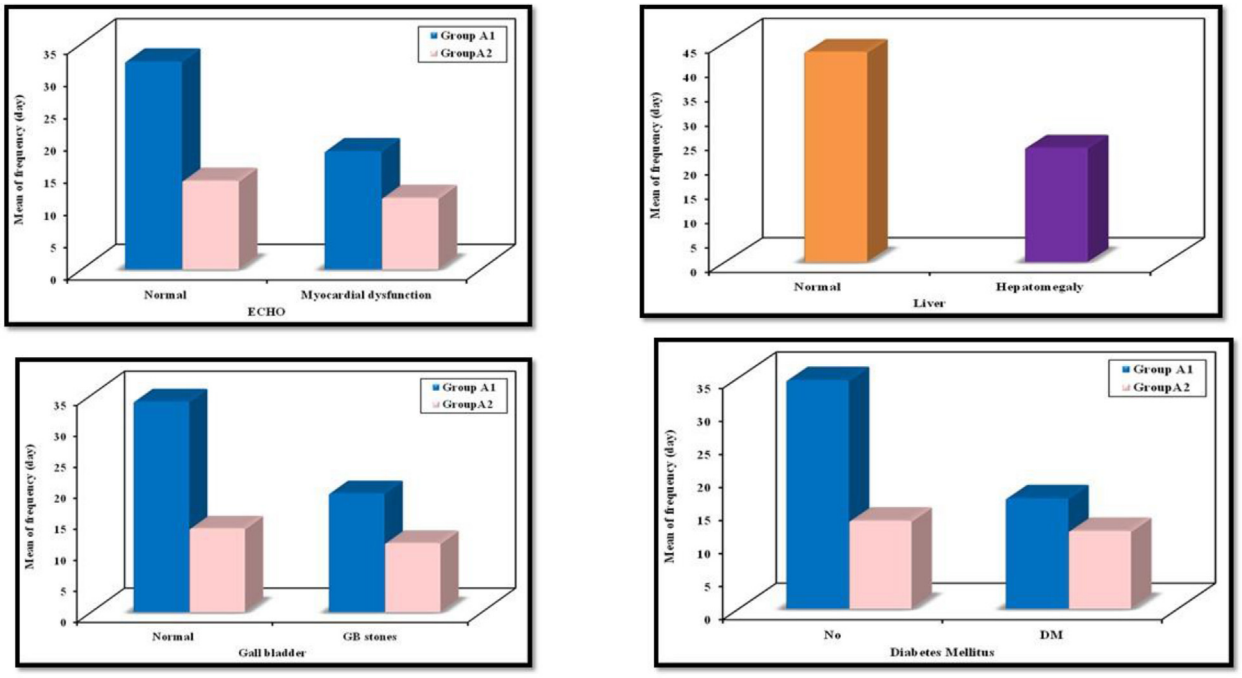

Fig. 2. Relation between frequency (day) and comorbidities.

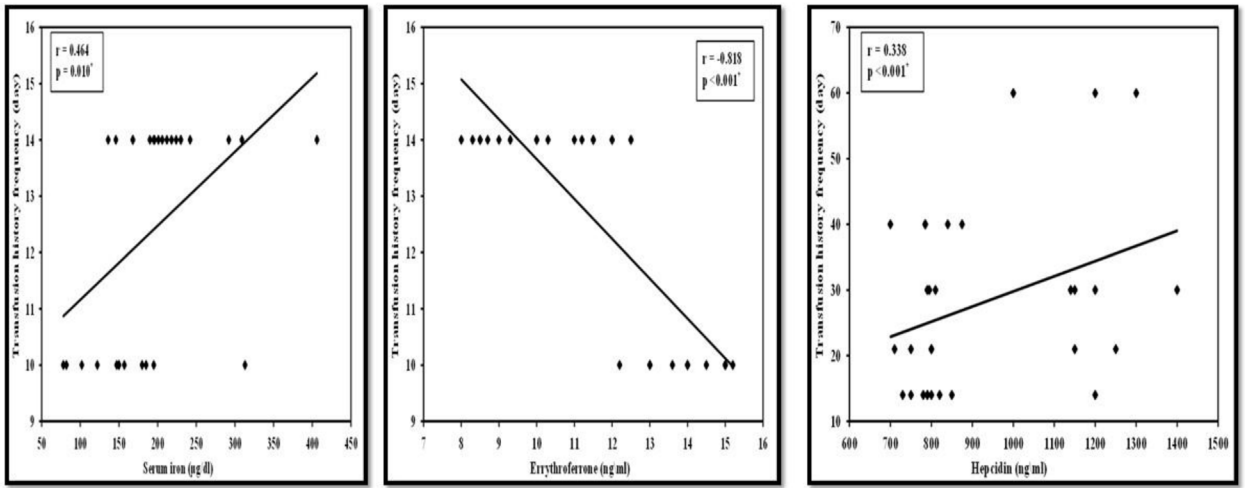

Fig. 3. Correlation between frequency of blood transfusion (day) and different parameters.

transfusions, patients' ages, and effectiveness of chelation protocols may be the main sources of this diversity [7].

The elevated levels of serum ERFE, in thalassemic patients came in accordance with the results of Saad et al. and Al Rawi et al. [9,15]. Also, El-Gamal and colleagues demonstrated significantly higher levels of ERFE gene expression in β-TM human patients compared with controls using qRT-PCR [7]. It also agreed with earlier research by Makis et al. [16] and Olivera et al. [17], though their experiments were constructed on β thalassemic mouse models, not humans. Furthermore, Huang and colleagues [18] added that ERFE may be the cause of the iron-loading phenotype seen in ineffective erythropoiesis and the enhanced iron absorption during times of stress-induced erythropoiesis in order to increase iron availability for hemoglobin synthesis. Besides, Current and colleagues [19] reported that ERFE suppresses hepcidin synthesis, restoring ferroprotein function, enhancing intestinal iron absorption, and mobilizing iron from stores. Also, Arezes et al. [20] concluded from a study on murine thalassemia that ERFE exerts its effects on hepcidin by attaching to bone morphogenetic proteins and thus blocking mechanisms that regulate hepcidin expression.

Interestingly, the serum hepcidin level in this current study was significantly higher in thalassemic patients than in control group. Our results came in accordance with those of Ismail et al. [21], Ozturk et al. [22], and Zaman et al. [23]. Moreover, Huang et al. [18] concluded that hepcidin levels were correlated with ineffective erythropoiesis but, were not correlated with iron overload. Yet, Aboul-Enein et al. [24] have concluded no change in hepcidin expression. On the other hand, a few previous studies reported lower levels of hepcidin than controls [7,9]. Contrarily, the only study that proved an inverse relationship between ERFE and hepcidin in humans was performed on chronic kidney disease patients on hemodialysis [25].

All these controversies in serum hepcidin can be explained by many factors; higher levels of serum hepcidin in our study were due to regular transfusion therapy and splenectomy. A case that is not present in thalassemia major mouse models [7]. Moreover, splenectomy enhances infections in thalassemic patients [21]. Studies have also shown that hepcidin rises in acute and chronic inflammatory conditions [26]. Furthermore, hepcidin can also be influenced by inter-individual variations [27].

Findings concerning the ROC curve in this study agree with those of Smesam et al. [28] in a study done on Iraqi thalassemic patients, who reported that ROC analysis demonstrated that the z-score of a composite of ERFE and ferritin has full diagnostic potential for β-TM. They even added that they are an effective predictor of iron overload. El Gammal et al. [7] had also emphasized the role of serum ferritin as a reliable biomarker of the severity of iron overload and its predictive value for cardiac death. Therefore, it can be rationally viewed that ERFE can be a more reliable biomarker for disease severity and comorbidities than serum hepcidin. ERFE displayed a significant positive relationship with comorbidities such as myocardial dysfunction, GB stones, and diabetes mellitus. While hepcidin was only positively related to diabetes mellitus in group A1 or those who underwent splenectomy in group A1 and group A2. In agreement with us, Spoto et al. [29] emphasized that serum ERFE is associated with mortality and cardiovascular events in chronic kidney and heart disease patients independently from inflammation markers like CRP, serum iron, and ferritin.

Table 3. Univariate and multivariate linear regression analysis for the parameters affecting frequency of blood transfusion (day) in each group.

	Group A1				Group A2			
	Univariate		[a]Multivariate		Univariate		[a]Multivariate	
	P	B (LL − UL 95%C.I)	*P*	B (LL −UL 95%C.I)	*P*	B (LL −UL 95%C.I)	*P*	B (LL −UL 95%C.I)
Frequency	**0.340**	5.716 (−6.34−17.77)			1.000	0.0		
Onset	**0.373**	−0.089 (−0.29−0.11)			**0.589**	0.015 (−0.04−0.07)		
Hb (g/dl)	**0.485**	−1.553 (−6.05−2.94)			**0.915**	0.046 (−0.83−0.92)		
MCV (fl)	**0.385**	−0.227 (−0.92−0.37)			**0.584**	0.025 (−0.07−0.12)		
RDW (%)	**0.631**	−0.192 (−1.0−0.62)			**0.420**	−0.047 (−0.16−0.07)		
Serum iron	**0.528**	−0.026 (−0.11−0.06)			**0.010**[b]	0.013 (0.003−0.02)	**0.158**	0.005 (−0.0−0.01)
TIBC (μg/dl)	**0.674**	−0.017 (−0.10−0.06)			**0.960**	0.0 (−0.010−0.01)		
Ferritin (ng/ml)	**0.410**	0.004 (−0.01−0.01)			**0.719**	0.0 (0.0−0.0)		
ALT (U/L)	**0.580**	0.071 (−0.19−0.33)			**0.178**	0.012 (−0.01−0.03)		
AST (U/L)	**0.857**	0.023 (−0.23−0.278)			**0.469**	0.006 (−0.01−0.02)		
Hepcidin (ng/ml)	**0.069**	0.023 (−0.002−0.05)			**0.208**	0.003 (−0.0−0.01)		
Errythroferrone	**<0.001**[b]	−7.380 (−8.48 to −6.28)	**<0.001**[b]	−6.887 (−8.55 to −5.23)	**<0.001**[b]	−0.708 (−0.90 to −0.56)	**0.001**[b]	−0.589 (−0.90 to −0.28)
Myocardial dysfunction	**0.011**[b]	−13.905 (−24.43 to −3.38)	0.111	4.981 (−1.24−11.20)	**<0.001**[b]	−2.667 (−3.78 to −1.56)	**0.620**	−0.370 (−1.89−1.15)
Gall bladder stones	**0.003**[b]	−14.833 (−24.29 to −5.38)	0.058	−4.448 (−9.05−0.16)	**<0.001**[b]	−2.357 (−3.57 to −1.14)	**0.056**	−0.975 (−1.98−0.03)
Diabetes Mellitus	**<0.001**[b]	−17.904 (−26.76 to −9.05)	0.765	−0.941 (−7.36−5.48)	**0.034**[b]	−1.556 (−2.98 to −0.13)	**0.171**	0.817 (−0.38−2.01)
Hepatomegaly	**0.001**[b]	−19.665 (−29.97 to −9.36)	0.206	−3.369 (−8.72−1.98)	–	–		
Splenomegaly	**0.684**	−2.190 (−13.09−8.71)			**0.379**	0.667 (−0.86−2.19)		
Onset (month)	**0.410**	−0.084 (−0.29−0.12)			**0.589**	0.015 (−0.04−0.07)		
Duration (y)	**0.099**	0.598 (−0.12−1.312)			**0.101**	−0.062 (−0.14−0.01)		

ALT, alanine transaminase; AST, aspartate transaminase; B, Unstandardized Coefficients; C.I, Confidence interval; LL, Lower limit; MCV, mean corpuscular volume; RDW, red cell distribution width; UL, Upper Limit.

[a] All variables with *P* less than 0.05 was included in the multivariate.

[b] Statistically significant at *P* less than or equal to 0.05.

Correlating ERFE and blood transfusion frequency in groups A1 and A2 revealed a significant negative correlation in both groups. Furthermore, in multivariate analysis, ERFE was identified as the only independent predictor affecting the frequency of transfusions, and the frequency of transfusions has its own independent impact on ERFE too. To the best of our knowledge, this is one of the first studies that directly correlated ERFE and transfusion frequency. Emphasizing its role as a reliable marker to predict and monitor the effectiveness of blood transfusions. Hence, it can be considered a potential therapeutic target to effectively manage blood transfusion strategies and control iron overload status.

The limitations of this study were that it was conducted in a single center with relatively small sample size. Additionally, MRI scans could not be performed in all cases, so we relied on serum ferritin to define iron overload state.

4.1. Conclusion

ERFE can be an efficient biomarker of iron overload, especially when it is combined with ferritin. It also represents a more potent biomarker of organ damage and comorbidities than hepcidin. It can also provide a promising predictive tool for early iron accumulation and frequency of blood transfusion.

4.2. Recommendations

It is recommended that further studies on human β-TM with larger sample sizes will clarify the role of ERFE in β-TM disease. Incorporating ERFE testing in the workup of β-TM patients in early phases of blood transfusion is recommended.

Conflicts of interest

There is no conflict of interest.

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