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# Erythroferrone Hormone a Novel Biomarker is associated with Anemia and Iron Overload in Beta Thalassemia Patients.

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**Abstract.** Erythroferrone (ERFE) is a glycoprotein hormone produced by erythroblasts in response to erythropoietic activity by stimulation of erythropoietin that acts directly on the liver to inhibit production of hepcidin, lead to increases iron delivery for intensified activity of erythropoietic. Beta thalassemia are inherited disorders characterized by reduced or absent synthesis of beta globin chains in the hemoglobin (Hb) molecule. The pathophysiology has been recognized by anemia and iron overload continually with resultant of frequented blood transfusions. We intend to investigate serum ERFE level and their associations with each other. Beta Thalassemia (BT) patients, who were inspected at thalassemia center in ALzahra`a hospital teaching period transfusions blood (PTB) for each (14-30 day). Seventy patients were aged about (11-28 year) and 20 subjects healthy as control group, who matched were included in the study. **Results.** Serum ERFE levels were significantly higher in BT patients compared to control groups. There were significantly ( $p < 0.05$ ) differences in these biomarker between (BTM and BTI), (splenectomy and non splenectomy) groups. a negative correlation between ERFE levels with HB, PCV, MCV, MCH, Iron, and Ferritin while it was a not significant correlated with MCHC and PTB. High level of ERFE as new biomarker in patients with BTM and BTI is associated with mild or severe anemia and iron overload especially in patients with splenectomy.

## Introduction

Erythroferrone (ERFE), a member of TNF- $\alpha$  superfamily producing from erythroblasts during erythropoiesis. ERFE production is stimulated by endogenous or exogenous erythropoietin (EPO) <sup>(1)</sup>, thus serving to couple increased erythropoietic activity through act on hepatocytes as erythroid regulator of suppress hepcidin production, allowing for maintenance of plasma iron concentrations in the setting of increased erythropoiesis- connected with iron request <sup>(2)</sup>. Thus, ERFE might be a key factor in the control of stored iron release. <sup>(3,4)</sup>

Beta-thalassemia, one of the important genetic disease in the world, characterized by iron overload and decreased red-cell survival result in ineffective erythropoiesis resulted of defect beta-globin chain expression in the hemoglobin causes in an increase of the alfa/beta globin ratio. The extra free a chains aggregate and Precipitate in erythroblasts, leading to damage of cell membranes and reactive oxygen species (ROS) generation, leading to ineffective erythropoiesis <sup>(5,6)</sup>. However, few studies of the function of ERFE in humans because is recently discovered and remains to be investigated.



We aimed to detect associations between erythroferrone (ERFE), and iron overload. We also aimed to determine the effects of (ERFE) continuous production of RBC indexes in patients with beta thalassemia

## METHODS

The cohort study was involved 70 BT patients (36 with male, 34 female) and 20 healthy as control group age (11-28) matched in both groups (patients and controls). Who were withdraw five ml venous blood samples got from all objective, and investigated for completely automated hematology analyzer estimated complete blood count (CBC) using Analyzer Mythic 18 (RINGELSAN CO., Turkey). Serum level of iron using automated chemistry analyzer. Part of Serum samples were directly frozen and stored at  $-80^{\circ}\text{C}$  serum for measured level of ferritin (Monobind Inc., U.S.A.) and Serum ERFE were quantified using the ELISA human kit provided from (MyBioSource, San Diego, CA, USA), Compete, competitive ELISA with an intra-CV and inter-CV values of 7.6% and 10.4%, respectively. According to the manufacturer's instructions.

### Statistical analysis

The statistical significance of differences between groups was evaluated by Independent t-test in normally distribution, and non-parametric by Man-wittiny and Chi-square using (SPSS V.24). Correlations were calculated by the Pearson's correlation coefficients. All result presented as mean $\pm$ SE (standard error), p value were set at 0.05 .

## RESULTS

Statically analyzed for the hematological data, iron status, and marker of erythropoietic activity. 70 patients with B-Thalassemia mean of aged about (18.39 $\pm$ 0.72) year were indicated mild to severe anemia included RBC counts and RBC indices (Table 1). RBC, PCV, Hb, MCV, MCH and MCHC were significantly ( $p < 0.05$ ) decreased as compared with 20 control healthy group. In addition, resulted for the frequency of blood transfusion about of (18.44 $\pm$ 0.89) day for long time about of (16.33 $\pm$ 2.79) month in the majority of patients with B-Thalassemia causing the high levels of ferritin and iron to be significantly increased from the control group (Table 1).

Results the marker of erythropoietic activity, i.e. serum Erythroferrone (ERFE) was significantly elevated for patients with B-thalassemia (205.13 $\pm$ 3.11) pg/ml higher than in healthy controls (42.45 $\pm$ 1.73)pg/ml, Table 1. Although the differences between BTM 52(74.3%) and BTI 18(25.7%) were statistically significant, in all hematological parameters, iron and ferritin levels, also ERFE level was significant ( $p < 0.05$ ) decreased in BTM about (198.19 $\pm$ 3.23) pg/ml compared with BTI (225.17 $\pm$ 5.56) pg/ml (Table 2 and Fig. 1). Result of ERFE level in B-thalassemia patients for 36 (51.4%) male and 34(48.6%) female (Fig. 2) these showed decreased significantly in male (190.28 $\pm$ 3.17) pg/ml than female (220.85 $\pm$ 3.89) pg/ml. Also, B-thalassemia patients with splenectomy 51(27.1%) and non splenectomy 19(72.9%), were increased of ERFE, but decreased of iron, and ferritin level in non splenectomy (210.5 $\pm$ 3.63) pg/ml, (156.8 $\pm$ 3.64) ng/ml, and (3478.54 $\pm$ 300.39) pg/ml compared with splenectomy (190.68 $\pm$ 4.72) pg/ml, (185.78  $\pm$ 7.09) ng/ml, and (5923.78  $\pm$ 572.3) pg/ml respectively (Table 2, and Fig.3). The Results of correlations showed ERFE levels were correlated negatively significant with those of PCV, Hb, MCV, MHC, (Fig.4,5,6,7) but not significant with MCHC and PTB (Fig.8,9) respectively. In addition, ERFE level was correlated inversely with ferritin and iron levels (Fig. 10, 11).

**Table (1)** Demographic clinical features of patients with Beta thalassemia comparison with healthy control groups.

Parameters	Patients n=70			Control n= 20			p-value
	Mean	SE	Range	Mean	SE	Range	
Age (year)	18.39	0.72	25	18.15	1.234	18	0.87
11-15 n (%)	26(37.1%)			8(40%)			
16-20 n (%)	27(38.6)			7(35%)			
>20 n (%)	17(24.3)			5(25%)			
Male n (%)	36 (51.4%)			12(60%)			
Female n (%)	34(48.6%)			8(40%)			
ERFE(pg/ml)	205.13 *	3.11	98	42.45	1.73	26	0.000
Ferritin(pg/ml)	4142.26 *	296.59	9839	77.92	5.77	97	0.000
Iron (ng/ml)	164.67 *	3.60	136	50.87	3.99	62	0.000
RBC(x10 <sup>6</sup> L)	3.27 *	0.04	1.7	4.64	0.09	1.38	0.000
P.C.V%	25.02 *	0.36	13	40.11	0.38	5.1	0.000
HB(g/dL)	8.02 *	0.12	4.57	13.34	0.18	2.6	0.000
MCV (fl)	76.35 *	0.36	18.19	87.03	1.43	22.6	0.000
MCH (pg)	24.47 *	0.15	6.63	28.94	0.50	9.1	0.000
MCHC (g/dL)	32.04 *	0.10	4	33.26	0.33	5.63	0.002
FTB (month)	16.33	2.79	119	0	0	0	0
PTB(day)	18.44	0.89	53	0	0	0	0

\*significant differences at p-value 0.05, ERFE=Erythroferrone, FTB=First Transfusion Blood, PTB=Period Transfusion Blood

**Table (2)** Demographic clinical features of patients with Beta Thalassemia Major (BTM) comparison with Beta Thalassemia Intermedia (BTI) groups.

Parameters	BTM n=52			BTI n= 18			p-value
	Mean	SE	Range	Mean	SE	Range	
Age (year)	18.87	0.86	25	17.00	1.24	17	0.258
Male n (%)	27(51.9%)			9(50%)			
Female n (%)	25(48.1%)			9(50%)			
Splenectomy n(%)	14(26.9%)			5(27.8%)			
Non Splenectomy	38(73.1%)			13(72.2%)			
ERFE(pg/ml)	198.19 *	3.23	86	225.17	5.56	69	0.000
Ferritin(pg/ml)	4721.85 *	353.82	9739	2467.89	285.59	4800	0.001
Iron (ng/ml)	169.33 *	3.97	120	151.22	7.35	135	0.027
RBC(x10 <sup>6</sup> L)	3.34 *	0.04	1.60	3.06	0.07	1.20	0.001
P.C.V%	25.73 *	0.40	12.00	22.96	0.61	10.50	0.001
HB(g/dL)	8.25 *	0.14	4.57	7.36	0.20	3.33	0.001
MCV (fl)	76.85 *	0.41	18.19	74.90	0.65	8.79	0.017
MCH (pg)	24.80 *	0.17	6.63	24.02	0.26	3.47	0.047
MCHC (g/dL)	32.03	0.12	4.00	32.07	0.19	2.38	0.891
FTB(month)	17.60	3.56	118	12.67	3.45	47	0.444
PTB(day)	18.33	1.14	53	18.78	1.16	23	0.827

\*significant differences at p-value 0.05, ERFE=Erythroferrone, FTB=First Transfusion Blood, PTB=Period Transfusion Blood



Figure (1) ERF level in patients BTM, BTI, and control

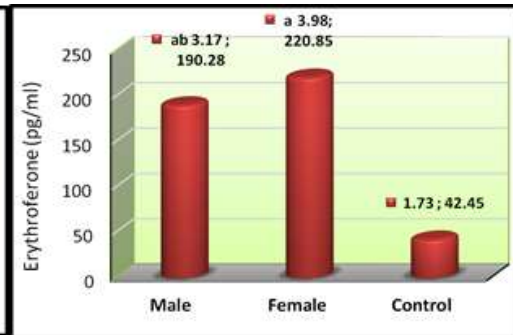


Figure (2) ERF level in male female patients, and

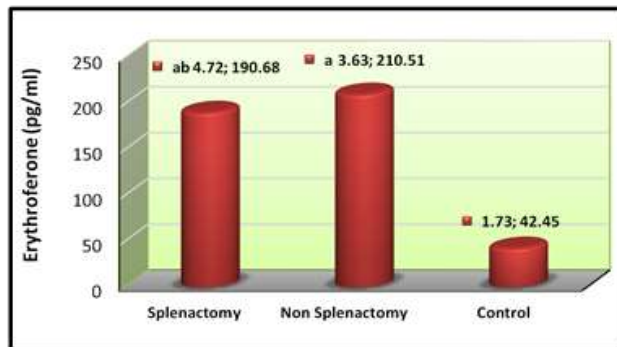


Figure (3) ERF level in Splenectomy Non Splenectomy, and control

a Significant differences between patients and control group at p-value <0.05  
 b Significant differences between them groups of patients at p-value <0.05, values SE; Mean

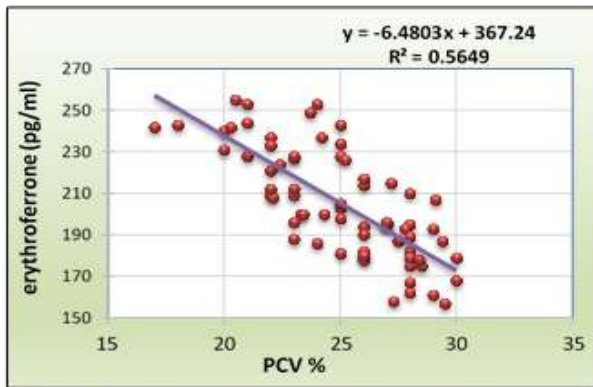


Figure (4) ERF correlated with PCV p-value 0.000

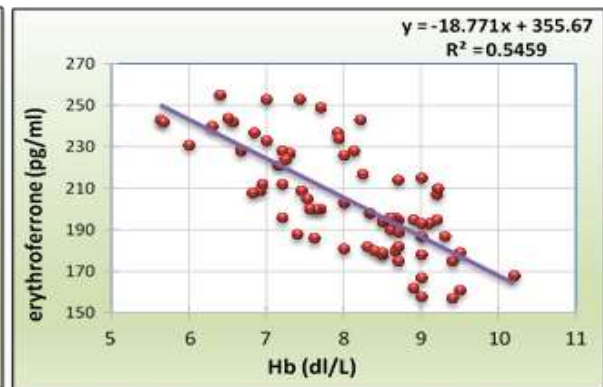


Figure (5) ERF correlated with Hb p-value 0.000

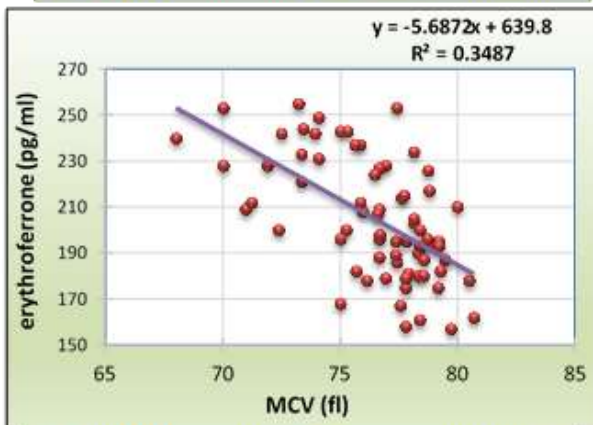


Figure (6) ERF correlated with MCV p-value 0.000

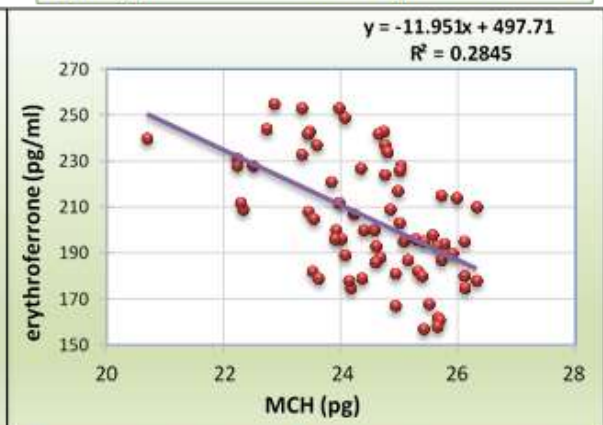


Figure (7) ERF correlated with MCH p-value 0.000

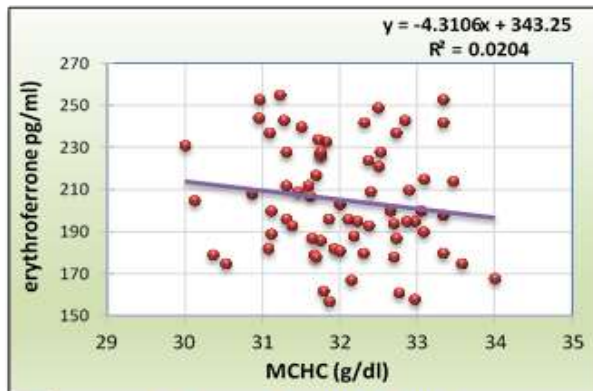


Figure (8) ERFE correlated with MCHC p-value 0.007

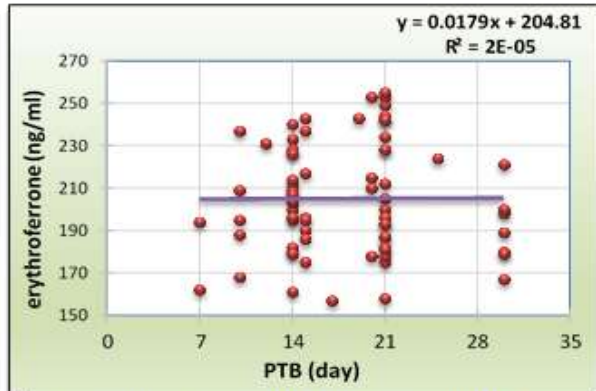


Figure (9) ERFE correlated with PTB p-value 0.87

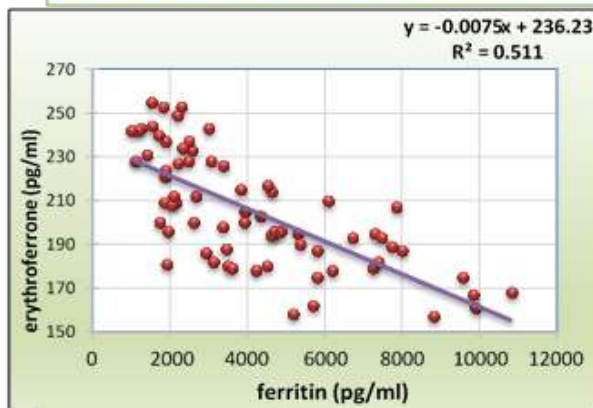


Figure (10) ERFE correlated with Ferritin p-value

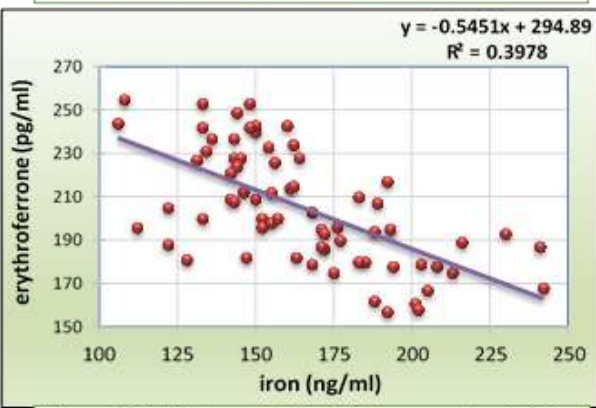


Figure (11) ERFE correlated with Iron p-value 0.000

## DISCUSSION

The current study investigated the relationship between ERFE, a hormone that secreted from erythroblast during erythropoiesis, with iron metabolism during erythropoiesis activity in patients with beta thalassemia<sup>(7)</sup>. The results of the current study were showed levels of ERFE in patients with BTM lower than BTI, and it was increased in female more than in male. In addition the patients with non-splenectomy have highest level of ERFE than splenectomy groups fig.(3). Also Levels of ferritin, iron and ERFE negatively correlated. The association of ERFE with hematological parameters Hb, PCV, and RBC indexes (MCV, MHC, and MCHC) was similar to ERFE with ferritin. Thus ERFE a biomarker significantly associated with of iron metabolism that affected of RBC indexes and occurrence sever of anemia in patients with beta thalassemia.

All hematological parameters including RBC, Hb, HCT, MCV and MCH except MCHC were found lower than the controls (table 1), Clinical data approve that the decrease of the hemoglobin level is attended by a decrease in the number of erythrocytes and reduced values of their specific indexes (MCV, MCH,HCT). Which are in accordance with the results of<sup>(8)</sup>

Several studies have confirmed that elevated iron accumulation complicates diseases with  $\beta$ -thalassemia as resultant a major constituent of ineffective erythropoiesis, in which erythroblast count are greatly expanded, but the erythroblasts undergo intramedullary apoptosis before completing differentiation<sup>(5,9)</sup>.

**Sulovska et al., 2016.** Indicated to the Erythropoiesis and iron metabolism reciprocate in the corresponding supply of iron and globin chains synthesis, critical for normal red blood cell production. Examined of varied erythroid and iron metabolism complaints have shown that disrupted

erythropoiesis reversely affects iron homeostasis and vice versa <sup>(8)</sup>. The improved erythropoiesis increases iron utilization at degrees that may become counterproductive, because reduction of spleen supplies causes excessive iron limitation, neutralizing the benefit the conflicting Hb levels of (germinal) *Tfr2*<sup>-/-</sup>-thalassemic mice decline over time, in parallel with iron overload. <sup>(10)</sup> We recently showed ERFE was highly increased in major and intermedia B thalassemia (table 2, and fig.1) these corresponding with discovered of **Ganz et. al.**, 2017 who referred to In human  $\beta$ -thalassemia, serum ERFE levels are greatly increased, are rapidly suppressed after erythrocyte transfusions, and negatively correlate with serum hepcidin concentrations, he suggesting that ERFE contributes to the pathogenesis of iron overload by a similar mechanism as in the mouse model. Thus, future therapeutic interventions inhibiting the action of ERFE could be beneficial for preventing iron overload in  $\beta$ -thalassemia and other anemias with ineffective erythropoiesis. The contribution of ERFE to the pathogenesis of human  $\beta$ -thalassemia major could even be underestimated by the *Hbb*<sup>Th3/+</sup> mouse, as this mouse is a model of  $\beta$ -thalassemia intermedia. The *Hbb*<sup>Th3/+</sup> model generates much lower serum concentrations of ERFE than those measured in human  $\beta$ -thalassemia intermedia. <sup>(7)</sup>

We recently showed ERFE negatively correlated with ferritin, iron Levels, these results (fig. 4, 10, and 11) were agreement with study of **El Gendy et.al** 2018 who indicated to Serum erythroferrone levels in iron deficiency anemia patients were significantly higher than those in control group ( $P < .001$ ). In iron deficiency anemia patients, serum erythroferrone levels correlated negatively with hemoglobin concentration ( $r = -.39$ ;  $P = .01$ ), serum iron ( $r = -.63$ ;  $P < .001$ ), transferrin saturation ( $r = -.66$ ;  $P < .001$ ), and serum ferritin ( $r = -.46$ ;  $P = .004$ ). he was conclusion The newly identified erythroferrone hormone may act as physiological hepcidin suppressor in cases with iron deficiency anemia, and so it may serve as a specific promising target of therapy in such cases. <sup>(11)</sup>

On the other hand, some recent researches discovered elevated of ERFE was important role and harmful effects in cases of inherited anemias with ineffective erythropoiesis such as  $\beta$ -thalassemia <sup>(12)</sup>. This elevated of ERFE may be resultant erythropoietin hormone this stimulated ERFE synthesis <sup>(13)</sup> that acts to suppress hepcidin in response to increased erythropoietic request, it is a major candidate to exert a similar role in conditions of dysfunctional erythropoiesis related with iron overload Anemia in  $\beta$ -thalassemia intermedia and major, resulting from defective  $\beta$ -globin synthesis, <sup>(14)</sup> is associated with decreased hepcidin expression excessive iron absorption, and systemic iron overload. <sup>(15)</sup>

As recently reported of **Kautz et. al.**, 2015 present the greatly increased erythroblasts in ineffective erythropoiesis produce large amounts of ERFE, and treatment that lessens ERFE effects would be expected to reduce inappropriate iron absorption. Erfe in thallemic mice induces a slight amelioration of ineffective erythropoiesis but does not improve the anemia<sup>(1)</sup>.

**Conclusions:** High level of ERFE as new biomarker in patients with major and intermedia Beta thalassemia is associated with mild or severe anemia and iron overload especially in patients with splenectomy.

## REFERENCES

- [1] Kautz L, Jung G, Du X, Gabayan V, Chapman J, Nasoff M, Nemeth E, and Ganz T (2015). "Erythroferrone contributes to hepcidin suppression and iron overload in a mouse model of  $\beta$ -thalassemia". *Blood*. 126 (17): 2031–7.
- [2] Coffey, Richard; Ganz, and Tomas. (2018). Erythroferrone: An Erythroid Regulator of Hepcidin and Iron Metabolism. *HemaSphere*: Volume 2 - Issue 2 - p e35
- [3] Kautz L, Jung G, Valore EV, Rivella S, Nemeth E, and Ganz T. (2014). Identification of erythroferrone as an erythroid regulator of iron metabolism. *Nat Genet*. 46: 678–684.
- [4] Kautz L, and Nemeth E. (2014). Molecular liaisons between erythropoiesis and iron metabolism. (2014). *Blood*. 24;124(4):479-82.

- [5] Ginzburg Y, and Rivella S. (2011).  $\beta$ -thalassemia: a model for elucidating the dynamic regulation of ineffective erythropoiesis and iron metabolism. . *Blood*;118:4321-4330.
- [6] Ribeil JA, Arlet JB, Dussiot M, Moura IC, Courtois G, Hermine O. (2013). Ineffective erythropoiesis in  $\beta$ -thalassemia. *ScientificWorldJournal*. 2013:394295.
- [7] Ganz T, Jung G, Naeim A, Ginzburg Y, Pakbaz Z, Walter PB, Kautz L, Nemeth E (2017). "Immunoassay for human serum erythroferrone". *Blood*. 130(10): 1243–1246.
- [8] Sulovska L, Holub D, Zidova Z, Divoka M, Hajduch M, Mihal V, Vrbkova J, Horvathova M, and Pospisilova D. (2016). Characterization of iron metabolism and erythropoiesis in erythrocyte membrane defects and thalassemia traits. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*. 160(2):231-7.
- [9] Suriapperuma T, Peiris R, Mettananda C, Premawardhena A, and Mettananda S. (2018). Body iron status of children and adolescents with transfusion dependent  $\beta$ -thalassaemia: trends of serum ferritin and associations of optimal body iron control. *BMC research notes* 11 (1), 547 *BMC Res Notes*. 11: 547.
- [10] Artuso I, Lidonnici MR, Altamura S, Mandelli G, Pettinato M, Muckenthaler M, Silvestri L, Ferrari G, Camaschella C, and Nai A. (2018). Transferrin receptor 2 is a potential novel therapeutic target for  $\beta$ -thalassemia: evidence from a murine model blood. 22;132(21):2286-2297.
- [11] El Gendy FM, El-Hawy MA, Shehata AM, Osheba HE (April 2018). "Erythroferrone and iron status parameters levels in pediatric patients with iron deficiency anemia". *European Journal of Haematology*. 100 (4): 356–360.
- [12] Russo R, Andolfo I, Manna F, De Rosa G, De Falco L, Gambale A, Bruno M, Mattè A, Ricchi P, Girelli D, De Franceschi L, and Iolascon A. (2016). Increased levels of ERFE-encoding FAM132B in patients with congenital dyserythropoietic anemia type II. *blood* 6;128(14):1899-1902.
- [13] Jiang X, Gao M, Chen Y, Liu J, Qi S, Ma J, Zhang Z, and Xu Y. (2016). EPO-dependent induction of erythroferrone drives hepcidin suppression and systematic iron absorption under phenylhydrazine-induced hemolytic anemia. *Blood Cells Mol Dis*;58:45-51.
- [14] Jarjour RA, Murad H, Moasses F, and Al-Achkar W. (2014). Molecular update of beta-thalassemia mutations in the Syrian population: identification of rare beta-thalassemia mutations. *Hemoglobin*; 38:272–276.
- [15] Kiavash F., Nabiollah A., Majid H., and Mohsen K. (2018) Ferritin and Iron Overload in Heart and Liver in Beta-Thalassemia Major Patients *National Journal of Laboratory Medicine*. Vol-7(3): IO07-IO11.