Journal of the Egyptian Society of Haematology & Research, Vol. 10, No. 1, March: 15-19, 2014

Regulation of Iron Metabolism Through GDF-15 and Hepcidin in ß-Thalassemia Major Patients

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ABSTRACT

Background: Transfusion iron overload is the most important complication of ß-thalassemia and is a major focus in its management. Extensive iron deposition is associated with cardiac hypertrophy, dilatation and degenerative disorders of the myocardial fibers.

Aim of the Work: This work was done to investigate the role of hepcidin and growth differentiation factor15 (GDF-15) and their interaction in the progressive iron overload in ß-thalassemia major thus helping in prevention of iron overload in those patients.

Patients and Methods: The study was done on 60 patients with ß-thalassemia major, their age ranged from two to thirteen years with a mean of 7.50±3.21 and a median of 8 years, as well as 20 apparently healthy controls, their age ranged from two to twelve years with mean of 6.04±3.390 and a median of 6 years . The hepcidin hormone, GDF-15, ferritin and routine investigations were done for all patients and controls. The hepcidin hormone and GDF-15 were done using ELISA.

Results: There was high significant difference in serum ferritin between patients and controls (2180.75± 1438.858 VS 91.55±60.42ng/ml; *p*=<0.001). Significant difference was also found in serum GDF-15 between patients and controls (33880.33±7208.83 VS 451.05± 47.56pg/ml; *p*=<0.001). Furthermore, a high significant difference in serum hepcidin was found between patients and controls (22.81±14.353 vs. 92.87±53.373ng/ml; *p*=<0.001). Serum GDF15 level showed significant positive correlation with ferritin and significant negative correlation with hepcidin in patients with ß-thalassemia (*r*: 0.545 and –0.609 respectively, *p*<0001). Splenectomized patients showed significantly higher levels of serum ferritin and serum GDF-15 and significantly lower levels of hepcidin as compared to non-splenectomized patients (1763.43± 1327.97 vs. 1621.08 ±1297.27ng/ml,; *p*=0.008; 33255.71± 7753.62 vs. 22770.69±17081.887pg/ml, *p*<0.001 and 23.19±15.118 VS 46.42±46.792ng/ml, *p*=0.001 respectively).

Conclusion: GDF-15 hyper-expression occurs in unison with ineffective erythropoiesis and positively correlates with ferritin levels in patients with ß-thalassemia. In patients with ß-thalassemia, Hepcidin level decreases as a result of GDF-15 hyper-expression.

Key Words: *Hepcidin – GDF-15 – Iron overload – ß-Thalassemia major.*

INTRODUCTION

The thalassemia are heterogonous genetic disorders of hemoglobin synthesis, occurring more frequently in the Mediterranean region, the Indians, south east Asia and west Africa, divided according to their severity into major which is severe and transfusion dependent, intermediate and minor forms of illness **[1]**. In patients with ß-thalassemia, deficient ß-globin-chain production and accumulation of α-chains causes apoptosis of red blood cell precursors, which results in ineffective erythropoiesis and anemia of variable severity that is aggravated by reduced red blood cell survival secondary to hemolysis **[2,3]**. Transfusion iron overload is the most important complication of ß-thalassemia and is a major focus in its management, which can be prevented by adequate iron chelation therapy. Extensive iron deposition is associated with cardiac hypertrophy, dilatation and degenerative disorders of the myocardial fibers **[4]**. Hepcidin, a 25 amino acid iron peptide hormone, inhibits iron influx into plasma from duodenal enterocytes and macrophages, which recycles iron from erythrocytes and hepatocytes and stores it by inactivation of iron export pump ferroprotien **[5,6]**. This protective mechanism is suspended and iron uptake is enhanced despite normal or even increased body iron in patients with genetic ferroprotien defects and iron loading anemia **[7]**. Growth differentiation factor 15 (GDF-15) has been identified as a bone marrow

derived factor that abrogates hepcidin-mediated protection from iron overload under conditions of increased erythropoiesis **[8]**. In this work, we investigated the recently identified regulators of iron metabolism, hepcidin and GDF-15 in patients with ß-thalassemia major in order to understand their role and interaction in the progressive iron overload in this type of chronic anemia thus helping in the prevention of iron overload in those patients.

PATIENTS AND METHODS

Patients:

The present study included sixty patients with ß-thalassemia major (thirty six males and twenty four females, twenty one of them had been splenectomized). Their age ranged from two to thirteen years with mean of 7.50±3.21 and a median of 8 years. All patients were recruited from Pediatrics Department, Sohag University Hospital. Also, the study included twenty age and sex matched apparently healthy children who attend the pediatric outpatient clinic for different complaints (e.g. cough, diarrhea, etc.) as a control group. The study was approved by the Ethical committee and a written informed consent was obtained from parents of all cases in accordance with Sohag University Hospital Ethical Committee Guide Lines.

Methods:

Blood was drawn into standard EDTA vacutainers for the assay of CBC and Hb electrophoresis and plain tubes for the assay of ferritin, hepcidin and GDF-15. Separated serum was divided in aliquots, one aliquot was used for ferritin estimation, and the rest of aliquots were stored at –70ºC to be used for hepcidin and GDF-15 estimation.

Laboratory investigations:

Complete blood count (CBC) was done by the use of cell dyne-3700 (Abbott Diagnostics, Dallas, USA). Hemoglobin electrophoresis was done by the use of Genio Electrophoresis (Interlab S.R.l. Company, Roma, Italy). Serum ferritin was done by the use of Architect 2000 system (Abbott Diagnostic, Dallas, USA). Hepcidin was measured using hepcidin ELISA kit (Wuhan EIA-ab Science co; Ltd. Wuhan, China). Growth differentiation factor-15 (GDF-15) was measured using BioVendor GDF-15/MIC-1 ELISA kit (BioVendor Research and Diagnostic Products co., Candler, North Carolina, USA).

Statistical analysis:

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS-version 17). All data was expressed as mean±SD. For statistical evaluation, Student *t*-test was used. Correlation Coefficient (*r*) was used for showing positive and negative correlation between variables. *p*-value was considered significant if less than 0.05.

RESULTS

The study was carried out on sixty children with ß-thalassemia major (thirty-six males and twenty-four females, twenty-one of them had been splenectomized), as well as twenty apparently healthy controls. Hematological data of patients and controls are represented in Table (1).

Serum ferritin and GDF-15 levels were significantly higher while serum hepcidin was significantly lower in patients than controls (Table 1).

Serum GDF-15 level in patients showed significant positive correlation with ferritin and significant negative correlation with hepcidin (Figs. 1,2) and Hb level; no correlation was encountered with age. In the control group, it showed significant negative correlation with hepcidin and age, insignificant negative correlation with Hb level and no correlation with ferritin (Table 2).

In patients group, hepcidin showed significant positive correlation with Hb level, significant negative correlation with ferritin and no correlation with age. In the control group, it showed significant positive correlation with age, significant negative correlation with ferritin and no correlation with Hb (Table 3).

Splenectomized patients showed significantly higher levels of serum ferritin and serum GDF-15 and significantly lower levels of hepcidin as compared to non-splenectomized patients (Table 4).

Table (1): Hematological data of 60 ß-thalassemia major and 20 healthy children.

Parameter	Patients Mean±SD (range)	Controls	*p*
WBCs x10^9/L	10.69±5.24 (1.5-21.6)	7.96±2.80 (4-13.2)	0.001
RBCs x10^{12}/L	3.23±0.56 (2-4)	4.27±0.58 (3-5)	<0.001
Hb (g/dl)	7.03±1.75 (2.9-9.3)	11.68±0.81 (9.2-12.7)	<0.001
Plat. x10^9/L	364.95±183.32 (70-856)	241.05±72.54 (148-463)	<0.001
Hb A1%	33.16±22.05 (0-79)	97.18±0.39 (96-98)	<0.001
Hb A2%	3.91±1.60 (1-8)	2.52±0.29 (2-3)	<0.001
Hb F%	62.59±22.41 (16-99)	0.24±0.264 (0-1)	<0.001
Ferritin: ng/ml	2180.75±1438.86 (110-5800)	91.55±60.42 (8-210)	<0.001
Hepcidin: ng/ml	22.81±14.353 (2-63)	92.87±53.37 (41-230)	<0.001
GDF-15: pg/ml	33880.33±7208.84 (20100-44700)	451.05±47.56 (385-520)	<0.001

Table (2): Correlation between GDF15 and other variables in 60 ß-thalassemia patients and 20 controls.

Group	GDF15 X Hepcidin		GDF15 X Age		GDF15 X Hb		GDF15 X Ferritin	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Patients	–0.609	<0.001	–0.185	<0.157	–0.33	0.010	0.545	<0.001
Control	–0.718	<0.001	–0.451	0.046	–0.356	0.124	0.525	0.018

Table (3): Correlation between hepcidin and other variables in 60 ß-thalassemia patients and 20 controls.

Group	Hepcidin X Age		Hepcidin X Hb		Hepcidin X Ferritin	
	r	*p*	*r*	*p*	*r*	*p*
Patients	0.096	0.466	0.261	0.041	–0.288	0.025
Control	0.459	0.042	0.111	0.643	–0.529	0.016

Table (4): Comparison between splenectomized and non splenectomized ß-thalassemic patients as regards factors affecting iron homeostasis.

Parameter	Splene-ctomy	Mean±SD (range)	*p*
S. Ferritin (ng/ml)	Yes	1763.43±1327.97 (270-4567)	0.008
	No	1621.08±1297.27 (110-5800)	
GDF15 (pg/ml)	Yes	33255.71±7753.62 (21000-44700)	<0.001
	No	22770.69±17081.887 (20100-44500)	
Hepcidin (ng/ml)	Yes	23.19±15.118 (2-63)	0.001
	No	46.42±46.792 (5-62)	

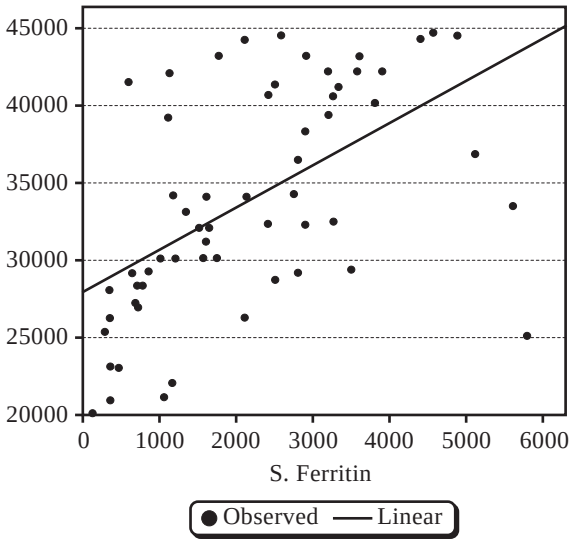

Fig. (1): Correlation between serum GDF15 and ferritin levels in patients group.

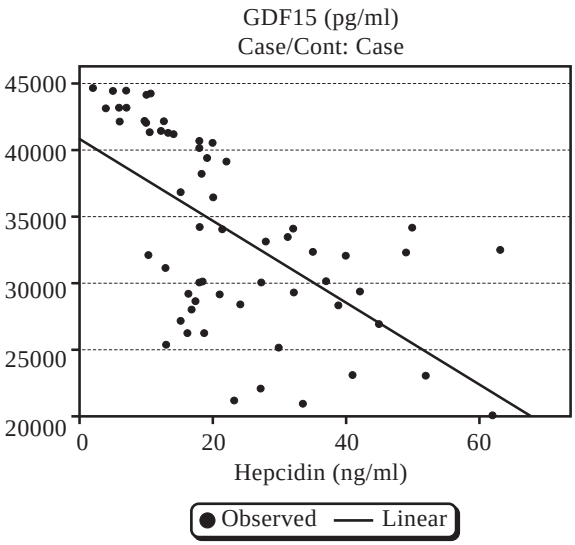

Fig. (2): Correlation between serum GDF15 and hepcidin levels in patients group.

DISCUSSION

ß-thalassemia is the most common chronic hemolytic anemia in Egypt (85.1%). A carrier rate of 9-10.2% has been estimated in 1000 normal random subjects from different geographical areas of Egypt **[9]**. ß-thalassemia is common in Mediterranean countries constituting a major public health problem **[9]**.

Iron absorption is increased in patients with congenital anaemias characterized by ineffective erythropoiesis. Clinically, increased intestinal iron absorption compounds the effects of transfusion iron overload in patients with thalassemia syndromes **[10]**.

Our results revealed highly significant elevation of serum ferritin in ß-thalassemia patients. These results agree with others **[11-13]**, who reported that even non-transfusion-dependent thalassemia patients often develop lethal iron overload.

The results of the present study revealed reduction of serum hepcidin level in ß-thalassemia patients. This observation was compatible with others **[14-16]**, who clearly demonstrated that even in ß-thalassemia major patients, who are highly iron overloaded, serum hepcidin levels are lower than would be expected because of the exuberant erythropoiesis. The main effect of hepcidin is negative regulation of cellular iron export from macrophages, duodenal enterocytes, and hepatocytes by promoting degradation of ferroportein, a transmembrane iron exporter.

In this work, there was marked elevation of serum GDF-15 in ß-thalassemia patients. These data agree with Zhao & Chang **[17]**, who reported that ineffective erythropoiesis is recognized as the principal reason of non-transfusion iron overload. In the process of expanded erythropoiesis, the apoptosis of erythroblasts induces the up-regulation of GDF15.

GDF15 suppresses hepcidin production by hepatocytes, subsequently low hepcidin level increases iron absorption from the intestine resulting in iron overload. Physiological dose of GDF15 can promote the growth and differentiation of erythroid progenitors, but a high dose of GDF15 can suppress the secretion of hepcidin. The regulation of GDF15 may also be related to iron level, epigenetic regulation and hypoxia **[18-21]**.

Erythropoietin-stimulated erythroblasts produce secreted mediators that act on the liver to suppress hepcidin production. Dying erythroblasts or erythroblasts that fail to mature appropriately may further contribute to secretion of hepcidin suppressors, perhaps explaining the paradoxical lack of iron overload in patients with expanded erythroblasts but normal maturation, such as in un-transfused chronic hemolytic anemia; GDF15 is one of these suppressors **[18-21]**.

In the present work, there was increase in serum ferritin level in splenectomized thalassemic patients than non splenectomized; this agree with Pootrakul **[22]**.

Our study showed increased serum GDF-15 in the splenectomized thalassemic patients than non splenectomized, consequently decreasing the serum hepcidin level in the splenectomized group.

In conclusion, GDF-15 hyper-expression occurs in unison with ineffective erythropoiesis and positively correlates with ferritin levels in patients with ß-thalassemia. In patients with ß-thalassemia, hepcidin level decreases as a result of GDF-15 hyper-expression. GDF-15 level decreases in splenectomized thalassemic patients than non-splenectomized, consequently decreasing the serum hepcidin level in the splenectomized patients. In the future, therapeutic use of hepcidin and hepcidin agonists may help to restore normal iron homeostasis in patients with ß-thalassemia who develop secondary iron overload.

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