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ORIGINAL ARTICLE

Relation of Serum Ferritin Level with Serum Hepcidin and Fucose Levels in Children with β-Thalassemia Major

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ABSTRACT

The aim of this study was to assess serum ferritin, hepcidin, L-fucose, and protein binding fucose levels in β-thalassemia major (β-TM) patients and to correlate the serum ferritin level with hepcidin and fucose levels. A total 70 (26 males and 44 females) children with β-TM, ages ranging from 5 to 16 years (mean age 8.3 ± 2.7 years) and 50 (25 males and 25 females) apparently healthy subjects with matching age and sex were included as a control group. An especially designed questionnaire was used to collect age, gender, body mass index (BMI), hemoglobin (Hb), serum ferritin, hepcidin-25 peptide, α-L-fucose, and protein binding fucose (PBF) levels. β-Thalassemia major patients had significantly ($p < 0.05$) higher serum ferritin, fucose and PBF levels, but the serum hepcidin level was significantly ($p < 0.05$) lower when compared to the controls, and their levels were affected by the gender of the β-TM patients, as it was significantly ($p < 0.05$) higher in female in comparison to male patients. There was no significant ($p > 0.05$) correlation between serum ferritin with hepcidin and fucose levels as a marker of iron overload in β-TM. The regulation of hepcidin, and L-fucose levels in patients with β-TM is more affected by erythropoeitic activity than by iron overload, as there was no significant correlation between serum ferritin with hepcidin and fucose levels as a marker of iron overload in β-TM.

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Introduction

β-Thalassemia (β-thal) is a heterogeneous group of hereditary blood disorders characterized by defects in the synthesis of the β chains of hemoglobin (Hb) that result in variable phenotypes, ranging from severe anemia to clinically asymptomatic individuals. β-Thalassemias have a high incidence in a large area extending from the Mediterranean Basin and parts of Africa, throughout the Middle East, and Melanesia to the Pacific Islands. It has been estimated that about 1.5% of the global population (80 to 90 million people) are carriers of β-thal, with about 60,000 symptomatic individuals born annually, the greater majority in the developing world [1,2]. There is little data on the epidemiology and burden of thalassemia, but Kadhim *et al.* [3] reported that the prevalence of thalassemia is 37.1/100.000 in the Iraqi population and β-thal major (β-TM) represents 73.9% of all types of thalassemia.

The main pathophysiology of homozygous β-thal is reduced amount (β^+) or absent (β^0) of β-globin chains result in unbalanced α- and β-globin chains where unbound α-globin chains appear to be in relative excess [2,4]. The α/β chain imbalance is responsible for the hemolysis of red blood cells (RBCs) and for apoptosis of erythroid precursors in the bone marrow and at extramedullary sites [2]. The α-globin chain aggregates form inclusion bodies within RBCs and immature developing erythroblasts responsible for oxidative stress and membrane damage. These events are followed by the premature death of many late erythroid precursors in the bone marrow and spleen resulting in ineffective erythropoiesis. The anemia and resulting hypoxia lead to a dramatic increase in serum erythropoietin levels in an attempt to compensate for the reduced oxygen-carrying capacity. The marked increase in erythropoietin stimulation, if it is not inhibited by proper transfusion therapy, can lead to uncontrolled expansion of erythroid precursors in the marrow as well as in other sites, such as the spleen and liver, leading to extramedullary hematopoiesis (EMH) [1,5]. Extramedullary hematopoiesis is particularly common in nontransfusion-dependent thalassemia (NTDT) patients (20.0%) but rarely occurs (<1.0%) in patients with transfusion-dependent thalassemia (TDT), who also exhibit underlying bone marrow suppression due to transfusion therapy [6]. Unfortunately, in Iraq, no detailed demographic data on β-thal major (β-TM) or β-thal intermedia (β-TI) patients with EMH are currently available.

Iron overload in β-thal patients due to multiple blood transfusions, hemolysis of RBCs, and the increased gastrointestinal (GI) iron absorption due to paradoxical hepcidin suppression from dyserythropoiesis, have shown that the rate of iron absorption from the GI tract in patients affected by β-thal is approximately 3-4 times greater than that in healthy individuals [5,7]. Erythropoiesis and iron

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metabolism are closely interconnected. Indeed, erythropoiesis participates in systemic iron homeostasis by regulating hepcidin [5]. Hepcidin (HAMP/H), a cysteine-rich 25 amino acid peptide synthesized in the liver from an 84 amino acid prepropeptide, is a key iron regulatory hormone, coordinating the use and storage of iron with iron acquisition. It inhibits cellular iron efflux by binding to the ferroportin, promoting its internalization and degradation, thereby negatively regulating iron absorption and iron recycling within the body [8,9]. Hepcidin is up-regulated in response to iron overload and inflammation, and down-regulated by erythropoietic stimuli such as anemia, hypoxia or erythropoietin, thereby increasing the GI absorption of iron and its release from stores [5]. In β-thalassemias, ineffective erythropoiesis induces the release of growth differentiating factor 15 (GDF15), twisted gastrulation protein homolog 1 (TWSG1), hypoxia-inducible factor and erythroferrone (ERFE), which inhibits hepcidin [10]. Determining hepcidin concentrations in subjects with iron-overload may be useful to identify the β-TM patients at higher risk of iron toxicity, and numerous studies have demonstrated that altered hepcidin expression and decreased expression of hepcidin is the cause of increased iron absorption in β-TM [5,11–17].

Fucose is a six-carbon deoxyhexose that is present in a wide variety of organisms. Fucose can obviously be found in D- and L-form, whereas L-fucose is the only form that is relevant in humans. There are two different forms of L-fucose: α-L fucose (29.5%) and β-L fucose (70.5%), in human α-L fucose, commonly incorporated into glycoproteins and glycolipids [18,19].

In mammals, α-L fucose-containing glycans have important roles in blood transfusion reactions, selectin-mediated leukocyte-endothelial adhesion, host-microbe interactions, and numerous ontogenic events, including signaling events by the Notch receptor family. α-L fucose is a component of the H, A, and B determinants of the ABO blood group and is also found in members of the Lewis series of histo-blood group antigens, including Lewis^{a}, Lewis^{b}, Lewis^{x}, and Lewis^{y} [18–20].

Thus, β-thal involve the perturbation of the balance between erythropoiesis and iron metabolism. The nature of the erythropoiesis-hepcidin-iron storage axis may differ across different thalassemia types [21], and this axis is poorly understood in patients with β-thal, specially in Iraq, where β-thal disease is often associated with high morbidity due to iron overload complications. In the current study, determination of iron overload biomarkers (hepcidin, serum ferritin), α-L fucose and protein binding fucose (PBF) were compared between β-TM patients and healthy control children, and the data were analysed to shed light on the correlations between iron overload as assessed by serum ferritin and these biomarkers in patients with β-TM. We hypothesized that the results could potentially explore the correlation of these markers as predictive biomarkers for patients at higher risk of developing iron overload.

Materials and methods

Over a 6-month period, from 1 October 2018 to 30 April 2019, a total 70 (26 males and 44 females) children with transfusion-dependent β-TM, ages ranging between 5 and 16 years (mean age 8.3 ± 2.7 years), attended the Misan Thalassemia Center, Amarah, Misan, Iraq, and 50 (25 males and 25 females) apparently healthy subjects of matching age and sex were included as a control group. All patients and control subjects provided an informed consent form to participate in the present study, that was approved by the Ethics Committee at the College of Medicine, Misan University, Amarah, Misan, Iraq. The diagnosis of β-TM was based on conventional clinical and hematological criteria [2]. A specially designed questionnaire was used to collect age, gender, body mass index (BMI), Hb, serum ferritin, hepcidin, fucose, and PBF levels. Patients with recent infection or surgery, and congestive heart failure were excluded from the study.

From patients and control, 8 ml of venous blood was drawn by venipuncture. The blood sample was separated into two tubes, one with EDTA as anticoagulant to automatically determine the Hb using ABX Micros ES 60 hematology analyzer (Horiba Medical, Montpellier, France), and the second one without anticoagulants to separate serum of the sample by centrifuge at 3000 rpm for 15 min. Serum ferritin was measured by an enzyme linked fluorescent assay (ELFA) method using VIDAS IVA 30/908, and ferritin kit, (BioMérieux, Marcy l'Étoile, France). Hepcidin-25 peptide was checked by the enzyme-linked immunosorbent assay (ELISA), using ELISA Reader dynex (Dynex Technologies Inc., Chantilly, VA, USA), and Human Hepcidin-25 (Hep-25) ELISA Kit [Shanghai Yehua Biological Technology Co., Shanghai, People's Republic of China (PRC)], according to the direction of the hepcidin kit manufacturer; the concentration of the hepcidin-25 peptide level was correlated positively depending on the color.

L-Fucose was estimated according to the Dische and Shettles method [22] as adopted by Winzler [23], using cysteine hydrochloride. This procedure depends on a direct reaction response of concentrated sulfuric acid (Sulfuric acid 95.0–98.0%, VWR Chemicals BDH®; VWR International, LLC, Radnor, PA, USA), with serum content determining the color after addition of cysteine (L-cysteine; BioMaghreb Co., Tunis, Tunisia). The color produced by hexoses under these conditions was corrected by determining absorbance at 390 and 430 nm. Standard L-fucose was procured from Sigma-Aldrich (St. Louis, MO, USA) [L-Fucose United States Pharmacopeia (USP) Reference Standard]. The PBF concentration was determined [22,23] when the protein in the serum was precipitated by adding ethanol (95.0%; Sigma-Aldrich); then the precipitate was suspended in 0.1 NaOH (Sigma-Aldrich) to solubilize the proteins. A color product was formed when fucose (in the solution) in strong acid medium, combined with the color developer (cysteine hydrochloride). The color intensity was measured at 390 and 430 nm.

Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS) version 23 for Windows (www.ibm.com/products/spss-statistics). Statistical analysis included mean ± SD; the Student's *t*-test was applied to evaluate the significance and variability of variables between

Table 1. Comparison between β-thalassemia major patients and control groups regarding body mass index and laboratory profiles.

Parameters	β-TM Group ($n = 70$)	Control Group ($n = 50$)	p Value
BMI (kg/m^2)	11.36 ± 1.46	17.29 ± 1.57	0.001
Hb (g/dL)	8.13 ± 1.60	12.2 ± 0.41	0.001
Serum ferritin (ng/mL)	3187.00 ± 155.15	128.00 ± 92.00	0.0001
Serum hepcidin (ng/mL)	12.85 ± 2.60	36.60 ± 19.00	0.001
Serum fucose (mg/dL)	20.64 ± 3.41	10.01 ± 3.27	0.0001
PBF (mg/dL)	14.06 ± 3.45	3.38 ± 2.01	0.001

n: number; BMI: body mass index; Hb: hemoglobin; PBF: protein binding fucose.
Values are presented as mean ± SD; p values were obtained using the Student's t-test.

Table 2. Comparison between male and female β-thalassemia major patients regarding body mass index and laboratory profiles.

Parameters	Males ($n = 26$)	Females ($n = 44$)	p Value
BMI (kg/m^2)	10.29 ± 1.10	12.34 ± 1.81	0.002
Hb (g/dL)	8.09 ± 1.57	8.17 ± 1.60	0.8
Serum ferritin (ng/mL)	3378.00 ± 147.10	2996.70 ± 163.19	0.001
Serum hepcidin (ng/mL)	12.09 ± 2.11	13.60 ± 3.09	0.03
Serum fucose (mg/dL)	19.68 ± 3.72	21.6.0 ± 3.09	0.02
PBF (mg/dL)	13.08 ± 3.27	15.10 ± 1.21	0.004

n: number; BMI: body mass index; Hb: hemoglobin; PBF: protein binding fucose.
Values are presented as mean ± SD; p values were obtained using the Student's t-test.

groups, and the Pearson correlation test was used to test the correlation between serum ferritin with hepcidin and fucose levels. A p values of <0.05 was considered to be statistically significant.

Results

Seventy 70 patients, with ages ranging between 5-16 years, consisted of 26 (37.1%) males and 44 (62.9%) females with β-TM patients, and 50 age-matched healthy children included 25 (50.0%) males and 25 (50.0%) females as a control group, were enrolled in this study. Table 1 demonstrated that the mean BMI (11.36 ± 1.46 kg/m^2), Hb level (8.13 ± 1.6 g/dL) and hepcidin level (3187.0 ± 155.15 ng/mL), were significantly (p values = 0.001) lower in β-TM patients as compared to the control group (17.29 ± 1.57 kg/m^2, 12.2 ± 0.41 g/dL, and 36.6 ± 19.0 ng/mL, respectively). However, mean serum ferritin (3187.0 ± 155.15 ng/mL), fucose (20.64 ± 3.41 mg/dL) and PBF (14.06 ± 3.45 mg/dL) levels were significantly (p values = 0.0001, 0.0001 and 0.001, respectively) higher in the patient group in comparison to the control group (128 ± 92 ng/mL, 10.01 ± 3.27 mg/dL and 3.38 ± 2.01 mg/dL, respectively).

When comparing between males and females in the β-TM group, our results showed that female patients have a significant ($p < 0.05$) higher BMI (12.34 ± 1.81 kg/m^2), serum hepcidin (13.6 ± 3.09 ng/mL), fucose (21.6 ± 3.09 mg/dL) and PBF (15.1 ± 1.21 mg/dL), in comparison to male patients (10.29 ± 1.10 kg/m^2, 12.09 ± 2.11 ng/mL, 19.68 ± 3.72 mg/dL and 13.007 ± 3.27 mg/dL, respectively), although mean Hb level was higher in female patients (8.17 ± 1.6 g/dL), but did not have had a significant effect ($p > 0.05$), while male patients had a significantly higher serum ferritin level (p values = 0.001), higher serum ferritin level (3378 ± 147.1 ng/mL) as compared to female patients (Table 2). Our results showed that the level of serum ferritin had a positive correlation with serum hepcidin (0.116) and fucose levels (0.108), but without significant effects (p values = 0.169 and 0.188, respectively) (Figures 1 and 2).

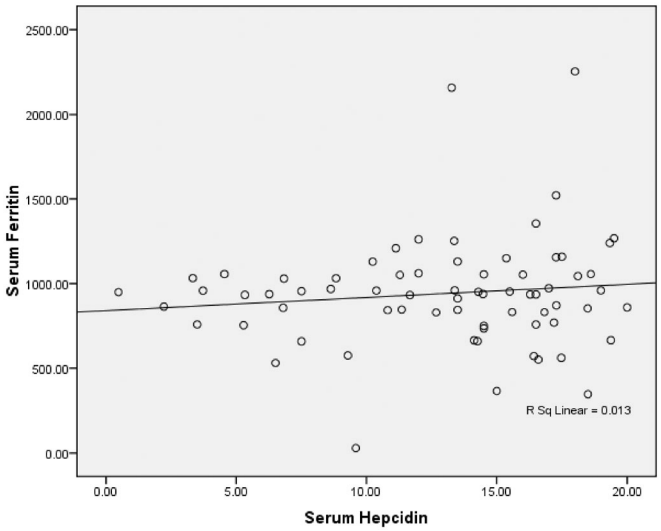

Figure 1. Pearson correlation between serum ferritin level with hepcidin level in β-TM patients.

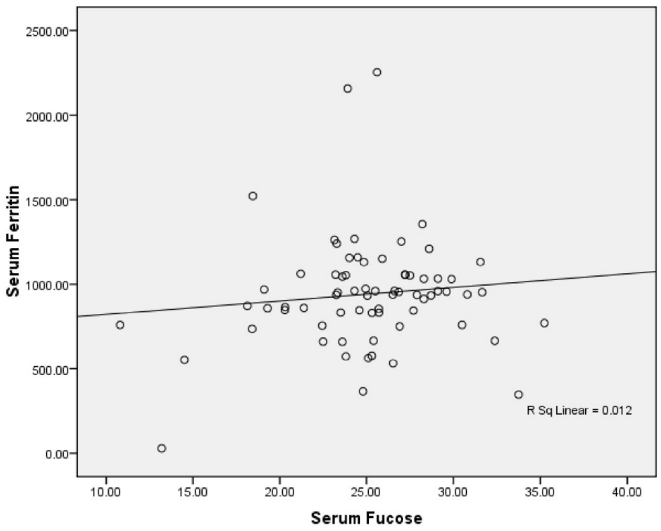

Figure 2. Pearson correlation between serum ferritin level with fucose level in β-TM patients.

Discussion

β-Thalassemia major represents the most prevalent cause of iron overload in Mediterranean countries including Iraq, and iron overload is attributed mainly to frequent blood transfusions, but it is also partly caused by increased iron absorption [2]. Hepcidin has a sophisticated regulation in β-thal patients, and its synthesis is controlled by antagonistic effects from erythropoiesis, anemia and iron overload. Hepcidin is up-regulated by increased body iron levels, infection and inflammation; it is down-regulated by ineffective erythropoiesis, hypoxia, anemia, and by increased levels

of erythropoietin [5,9]. Measuring hepcidin levels in subjects with iron loading β-TM may be useful to identify the patients at increased risk of iron toxicity due to severely increased hepcidin levels. Our study showed that β-thal patients have significantly lower serum hepcidin level compared to healthy subject. These results were in agreement with the study of Pasricha *et al.* [13] that demonstrated serum hepcidin level was lower than expected in patients with β-TM who are highly iron overloaded because of active erythropoiesis. Jones *et al.* [16] also observed 62 of 69 Sri Lankan patients with Hb E (*HBB*: c.79G > A)/β-thal with moderate or severe phenotype, hepcidin level was suppressed, while in their study, El Beshlawy *et al.* [17] revealed decreased hepcidin levels in patients with β-TM. On the other hand, previous studies [14,15] observed β-TM children with severe iron overload had a higher serum hepcidin levels compared to the controls, as well as that reported by Haghpanah *et al.* [12], who noticed that 91.7% of the Iranian subjects with β-TM had serum hepcidin levels above normal. Hepcidin levels were elevated in β-TM major, presumably due to transfusions that decrease erythropoietic drive and deliver a large iron load [11].

In the current study, serum L-fucose and PBF levels were significantly elevated in patients as compared with control group, which is similar to that reported by the Assi study [24]. Elevation of L-fucose levels among our patients was most likely due to blood transfusion reactions that may occur in transfusion-dependent β-TM patients. The ABO blood group antigens are among the most well-known fucosylated glycans, and fucose is found in members of the Lewis series of histo-blood group antigens, including Lewis^{a}, Lewis^{b}, Lewis^{x}, and Lewis^{y} [18–20].

Our data showed that serum hepcidin, fucose and PBF were affected by gender of β-thalassemia major patients. Unlike our results, previous studies by Ismail *et al.* [14], Kaddah *et al.* [15], and Beshlawy *et al.* [17], were found serum hepcidin level was not affected by gender of patients with β-TM.

The current study found no significant correlation between serum ferritin level with serum hepcidin and fucose levels as a marker of iron overload in β-TM patients. These results were in agreement with those of previous studies that demonstrated a major role of erythropoiesis compared to iron overload in regulation of hepcidin level in children with β-TM [12,14]. Moreover, Kadddah *et al.* [15] observed serum ferritin correlated significantly positive with hepcidin levels, which tended to increase by 0.002 ng/mL with each 1 ng/mL rise in serum ferritin, and also mentioned the overall predictability of serum hepcidin in severe iron overloads was statistically significant when compared to hepcidin to serum ferritin ratios. There is no data available about the role of fucose as a marker of iron overload or ineffective erythropoiesis in patients with β-TM, or other iron overload disorders. Larger trials and studies are needed to resolve the nuanced and practical utility of measuring serum hepcidin, fucose and PBF levels in β-TM, as evidenced by these markers as diagnostic or monitoring tools for iron overload in patients with β-TM is still being explored, and we need more studies as well as practical evidence.

In conclusion, based on the present study results, there were significantly higher serum ferritin, fucose, and PBF in β-TM patients, but the hepcidin level was significantly lower in patients as compared with control subjects, and their levels were affected by the gender of β-TM patients. However there was no significant correlation between serum ferritin with hepcidin and fucose levels as a marker of iron overload in β-TM, which supported the results of previous studies that regulation of hepcidin in patients with β-TM is more affected by erythropoeitic activity than by iron overload.

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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