Original Article / Özgün Araştırma

Serum Prohepcidin Levels in Children with Thalassemia Major and Intermedia

Talasemi Majör ve İntermedialı Çocuklarda Serum Prohepsidin Düzeyleri

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Abstract

Objective: To determine the role of hepcidin hormone levels in iron accumulation in patients with thalassemia major (TM) and thalassemia intermedia (TI).

Materials and Methods: Serum prohepcidin and ferritin levels were determined in 34 patient with TM, 10 patient with TI, who attended the Department of Pediatric Hematology Adnan Menderes University Medical Faculty and the Department of Pediatrics at Aydın Atatürk State Hospital between 1 September 2006 and 30 September 2007 and 40 control patients without infection/inflammation, hepatitis or liver failure. Serum prohepcidin levels were measured using a commercial enzyme-linked immunosorbent assay kit (DRG International, Inc. Marburg, Germany); ferritin was studied with chemiluminescence method (Immulite 2000 DPC).

Results: Mean serum ferritin levels in TM, TI and control groups were 2347.97±1724.81 ng/mL (range: 144-8015 ng/mL), 1352.40±918.94 ng/mL (range: 311-3109 ng/mL), and 33.35±12.03 ng/mL (range: 20-69.1 ng/mL), respectively. Serum prohepcidin levels in the same groups were 221.78±74.38 ng/mL (range: 7l.14-446.57 ng/mL), 173.31±52.14 ng/mL (range: 100.83-267.69 ng/mL), and 218.20±50.37 ng/mL (range: 116.18-330.43 ng/mL), respectively. There was a statistically significant difference in prohepcidin levels between patients with TI and control group only (p=0.016). No correlation was found between prohepcidin and ferritin levels in all groups (r=-0.023, p=0.839).

Conclusion: Low levels of prohepcidin in patients with TI may be related to increased erythropoietic activity. Prohepcidin can be an indicator of active erythropoiesis.

Öz

Amaç: Talasemi majör (TM) ve talasemi intermedialı (Tİ) hastalarda hepsidin hormonunun öncülü olan prohepsidin düzeyleri incelenerek bu hormonun demir birikimindeki rolünü değerlendirmektir.

Gereç ve Yöntemler: Adnan Menderes Üniversitesi Tıp Fakültesi, Çocuk Hematoloji Anabilim Dalı ve Aydın Atatürk Devlet Hastanesi'nde 1 Eylül 2006 ve 30 Eylül 2007 tarihleri arasında izlenen ve enfeksiyon, enflamasyon, hepatit ve karaciğer yetmezliği olmayan 34 TM, 10 Tİ'li hastada ve 40 sağlıklı kontrol grubunda serum prohepsidin ve serum ferritin düzeyine bakıldı. Ferritin, Immulite 2000 cihazında kemilüminesans yöntemiyle, prohepsidin DRG International, Inc. Marburg, Germany kiti kullanılarak ELISA yöntemiyle çalışıldı.

Bulgular: Ortalama serum ferritin düzeyleri TM, Tİ ve kontrol grubunda sırasıyla 2347,97±1724,81 ng/mL (dağılım, 144-8015 ng/mL), 1352,40±918,94 ng/mL

(dağılım, 311-3109 ng/mL), 33,35±12,03 ng/mL (dağılım, 20-69,1 ng/mL), serum prohepsidin düzeyleri ise sırasıyla 221,78±74,38 ng/mL (dağılım, 78,14-446,57 ng/mL), 173,31±52,14 ng/mL (dağılım 100,83-267,69 ng/mL), 218,20±50,37 ng/mL (dağılım, 116,18-330,43 ng/mL) saptandı. TM, Tİ ve kontrol grubu prohepsidin düzeyleri karşılaştırıldığında yalnızca Tİ ile kontrol grubu prohepsidin düzeyleri arasında anlamlı fark saptandı (p=0,016). Üç grupta da prohepsidin ile ferritin arasında korelasyon saptanmadı (r=-0,023, p=0,839).
Sonuç: Tİ'li hastalarda prohepsidin düzeyinin düşüklüğü, artmış eritropoetik aktivite ile ilişkili olabilir. Prohepsidin eritropoezin aktif bir göstergesi olabilir.

Introduction

In patients with thalassemia major (TM) and thalassemia intermedia (TI), there is an excessive iron accumulation in the body. The main source of accumulated iron in the body is blood transfusion in patients diagnosed with TM, whereas it is the case with iron overload in patients with TI. In patients with TM, iron absorption from intestines also increases (1). Along with regular blood transfusion, ejaculating excessive iron from the body with iron chelators form a basis to the treatment of thalassemia. This protective treatment prevents excessive amount of iron accumulation in the heart, liver, and endocrine organs and minimizes the morbidity and mortality that relies on the complications (2).

In recent years, after the discovery of hepcidin, a negative regulator of iron metabolism, studies on thalassemia patients have suggested that the cause of increase in intestinal iron absorption may be the decrease in synthesis of hepcidin. It is thought that growth differentiation factor 15 excreted from late and apoptotic erythroid precursors due to ineffective erythropoiesis, twisted gastrulation protein homolog 1 excreted from early erythroid precursors and increasing hypoxia-inducible factors due to chronic anaemia and hypoxia lead to a decrease in the synthesis of hepcidin (3-5). In addition, increasing erythropoietin during stress erythropoiesis enhances erythroferrone hormone synthesis from erythroid progenitors, thus suppresses hepcidin synthesis (6).

In patients with thalassemia, the extent of ineffective erythropoiesis also determines iron distribution. If severe anaemia occurs, the level of hepcidin decreases and iron accumulation occurs rapidly and iron accumulates primarily in the liver parenchyma cells. If anaemia is not severe, iron accumulates progressively in spleen macrophages and liver Kupffer cells. Furthermore, iron accumulation in TI happens at a slower span than it does in TM (7).

In this study, the levels of prohepcidin, which is the precursors of TM and TI, are investigated to determine whether the increase in intestinal iron absorption is due to this hormone.

Materials and Methods

Thirty-four patients with TM diagnosis and 10 patients with TI diagnosis, who attended the Department of Pediatric Hematology at Adnan Menderes University, Faculty of Medicine, and the Department of Pediatrics at Aydın Atatürk State Hospital between September 1, 2006 and September 30, 2007, were included in the study. Forty patients without infection, inflammation, hepatitis or anaemia evaluated in the general pediatrics outpatient clinics were taken as control group.

Written informed consent was obtained from the parents of all children. Adnan Menderes University Medical Faculty Ethics Committee approved the study (2006/00145).

Blood samples were collected from an antecubital vein by direct venipuncture. Samples were taken before the erythrocyte transfusion in the patient group. The blood for prohepcidin was centrifuged for seven minutes at 4000 rpm in Hettich Rotina 38 R and the serums were placed in Eppendorf tubes and stored at -20 °C until assayed. Ferritin was studied on the same day the blood was taken.

Ferritin was studied by chemiluminescence method by using Immulite 2000 analyzer. Prohepcidin was worked on with DRG Hepcidin Prohormone ELISA EIA-4644 (Germany) kit by using enzyme-linked immunosorbent assay (ELISA). In this method, serum samples were placed in wells coated with prohepcidin antibody. The color of the resulting antibody-antigen complex was read at 540 nm and the results of patients were obtained based on the standard graph (ng/mL).

The data were evaluated using the SPSS 11.5 program. Descriptive and frequency analyses of all groups were done. Mean values, standard deviation, frequency, minimum and maximum values were

specified. The Kolmogorov-Smirnov test was used to assess whether the data were appropriate for normal distribution. Student's t-test and the Mann-Whitney U test were used for comparison between groups and a p value of less than 0.05 was considered statistically significant.

Results

Age, sex, and levels of ferritin and prohepcidin in TM, TI and control groups are shown in Table 1.

There was no correlation between serum ferritin and prohepcidin values of the study group (r=-0.023, p=0.839).

While no statistically significant difference in ferritin values was detected between TM and control group and between TI and control group (p=0.001) in bilateral comparisons between groups, there was a significant difference between TM and TI patients (p=0.088).

There was no significant difference in prohepcidin values between TM and control groups and between TM and TI groups (p=0.062 and 0.807, respectively) in the bilateral comparison between the groups. There was a statistically significant difference between TI and control groups (p=0.016).

Discussion

There are two regulators that provide iron balancing of an organism: erythropoietics and storage regulators. The erythropoietic regulator works with signals coming from bone marrow. When erythropoietic activity increases much, erythropoiesis in the bone marrow is absorbed to meet the iron need and intestinal iron absorption occurs even if stores are full (8,9). The storage regulator is a regulator that increases the absorption by feeling the decreasing amount of iron in the liver, skeletal muscles and blood. The erythropoietic regulator provides 20 times more active iron absorption than the storage regulator (10).

After the discovery of hepcidin, it was extensively studied in thalassemia syndromes, the most common cause of secondary iron overload. It has been suggested that hepcidin plays a central regulatory role in iron circulation and iron toxicity in thalassemia patients and hepcidin measurement may play a role in diagnostic and prognostic evaluation; exogenous hepcidin applications may be useful in providing iron homeostasis in patients with thalassemia (11,12). Adamsky et al. (13) found significantly decreased hepcidin gene expression in thalassemic rat liver as opposed to iron loading, suggesting that this decrease in hepcidin was associated with an increase in intestinal iron absorption. Franceschi et al. (14) observed excessive iron loading and defective iron regulation in elderly animals in TI animal model, and suggested that iron loading in TI was time-dependent.

Erythropoietic activity is thought to be the main regulator of hepcidin expression (15). Camberlein et al. (16) reported that 10 TM patients, 13 TI patients, and 10 controls were not significantly influenced by iron uptake of hepcidin expression, regulated by increased erythropoietic activity, and there was not a significant effect of other regulatory genes in hepcidin mRNA modulation. Weizer-Stern et al. (17) found a decrease in hepcidin and TfR2 expression in TM and TI rat models and an increase in TfR1 expression. They thought that hepcidin expression decreased in spite of iron accumulation in TM, and that this was responsible for iron absorption, the increase in sTFR1 may indicate the dominance of anemia over iron overload in the regulation of iron absorption and storage. Papanikolaou et al. (18) measured urinary hepcidin levels in 8 TM, 7 TI, and 2 adult male patients

Table 1. Thalassemia major, thalassemia intermedia and control group sex, age, ferritin and prohepcidin levels

	Sex*	Age (year)**	Ferritin (ng/mL)**	Prohepcidin (ng/mL)**
TM	18/16	12.97±8.55	2347.97±1724.81	221.78±74.38
		(1-32)	(144-8015)	(78.14-446.57)
TI	4/6	10.9±5.21	1352.40±918.94	173.31±52.14
		(4-21)	(311-3109)	(100.83-267.69)
CG	22/18	8.88±4.14	33.35±12.03	218.20±50.37
		(1-16)	(20-69.1)	(116.18-330.43)

*Boy/girl, **Mean±standard deviation (minimum-maximum)

with congenital dyserythropoietic anaemia-type 1 and they found that the level of urinary hepcidin was normal in 1 TI and 3 TM patients and decreased in others, and they thought that the difference in hepcidin concentration might be due to coexisting minor infections, other environmental factors such as inflammatory stimulus and other genetic factors. They explained low urinary levels of hepcidin by the predomination of erythroid signal in the regulation of hepcidin synthesis.

It is not clear whether the serum prohepcidin level measures the active hepcidin or whether there is a functional precursor. Changes in the level of serum prohepcidin have been reported to fail to reflect the iron status exactly (11,19,20). Serum prohepcidin levels were not found to be statistically significant difference compared to the control group in patients with high ferritin levels with hereditary hemochromatosis and sickle cell anaemia (19,20). Similarly, Kulaksiz et al. (11) did not find any relationship of serum prohepcidin levels with serum iron, transfer saturation, and serum ferritin levels. Ulukol et al. (21) reported that there was no statistically significant difference in prohepcidin levels between 16 patients with iron deficiency and 54 infants without anaemia, suggesting that prohepcidin may not reflect the true level of hepcidin and that there would be no useful biomarker for clinical trials. Tsuchihashi et al. (22) found no statistically significant difference in serum prohepcidin levels between 23 hemodialysis patients without inflammation and 10 healthy controls. They divided hemodialysis patients into two groups as iron deficiency and non-iron deficiency groups and observed that prohepcidin levels in the iron deficiency group were significantly lower. Prohepcidin levels increased in patients treated with iron administration and significantly decreasedin patients without iron supplementation. Therefore, they claimed that prohepcidin may be a functional indicator of iron deficiency in hemodialysis patients. Kijima et al. (23) found a positive correlation between prohepcidin level and sTfR1, and between hepcidin and ferritin in 45 healthy postmenopausal subjects, suggesting that prohepcidin may be associated with hypoferremia or erythropoiesis, and that hepcidin may be a marker of iron overload. In our study, we did not find any correlation between prohepcidin and ferritin. We found serum prohepcidin levels in TI patients to be significantly lower than in TM patients and controls. This suggests that prohepcidin levels are an active indicator of erythropoiesis and patients with TM have higher levels of prohepcidin than erythropoiesis patients with TI due to erythropoiesis suppression with blood transfusions.

Ethics

Ethics Committee Approval: Adnan Menderes University Medical Faculty Ethics Committee approved the study (2006/00145), Informed Consent: Written informed consent was obtained from the parents of all children.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: C.Y., A.K., L.D.K., Concept: Y.Z.A., Design: Y.Z.A., C.Y., Data Collection or Processing: C.Y., A.K., L.D.K., Analysis or Interpretation: Y.Z.A., C.Y., Literature Search: Y.Z.A., C.Y., Writing: Y.Z. A., C.Y.

Conflict of Interest: No conflict of interest was declared by the authors.

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