

# Evaluation of Iron Overload in the Heart and Liver Tissue by Magnetic Resonance Imaging and its Relation to Serum Ferritin and Hepcidin Concentrations in Patients with Thalassemia Syndromes

Volkan Karakus<sup>1,6</sup> · Ayşegül Kurtoglu<sup>2</sup> · Dilek Ersil Soysal<sup>3</sup> · Yelda Dere<sup>4</sup> · Selen Bozkurt<sup>5</sup> · Erdal Kurtoglu<sup>6</sup>

Received: 26 May 2016 / Accepted: 29 September 2016 / Published online: 7 October 2016  
© Indian Society of Haematology & Transfusion Medicine 2016

**Abstract** Iron overload is one of the major prognostic factor in thalassemia patients. We aimed to evaluate iron accumulation in the heart and liver by MRI in thalassemia major, thalassemia intermedia, and S-β thalassemia patients and to examine its association with ferritin and hepcidin levels. Serum ferritin and hepcidin levels were recorded. Iron overload (IOL) in the heart and liver parenchyma was determined based on the standardized T2\* and R2 values measured by MRI. The results were evaluated considering the tissue iron overload, serum ferritin and hepcidin levels. Comparing the 109 patients with the 30 healthy controls revealed the mean age: 24.4 ± 11 versus 31.2 ± 5 years, median levels of serum ferritin: 1693 versus 40 ng/mL, and hepcidin: 1.94 versus 0.355 ng/mL;  $p < 0.001$ , respectively. Comparison of age, serum ferritin and hepcidin levels and MRI findings of the patients with or without IOL revealed that, ferritin and T2\* were significantly different in the patients with IOL in cardiac

tissue ( $p = 0.004$  and  $p < 0.001$ ), and, age, ferritin and R2 were significantly different in the patients with IOL in liver tissue ( $p = 0.036$ ,  $p < 0.001$  and  $p < 0.001$ ). The MRI-based T2\* and R2 values were moderately and inversely correlated with serum ferritin ( $r = -0.37$ ;  $p < 0.001$  and  $r = -0.46$ ;  $p < 0.001$ ). No correlations were found between the MRI-based T2\*, R2 values and serum hepcidin. A moderate and positive correlation existed between serum ferritin and hepcidin ( $r = 0.45$ ;  $p < 0.001$ ). We considered that, enhanced intestinal iron absorption characterized by decreased serum hepcidin levels in the intervals between successive transfusions were resulted in iron accumulation in our patients.

**Keywords** Magnetic resonance imaging · Ferritin · Hepcidin · Heart · Liver

## Introduction

The thalassemias are a group of inherited disorders that are caused by altered or absent hemoglobin chain synthesis leading to ineffective erythropoiesis and subsequent anemia [1, 2]. Transfusion therapy for thalassemia major prevents many of the complications introduced by ineffective erythropoiesis, but produces toxic iron accumulation. Hence, the inevitably pursuant complications are from iron excess in various organs such as the heart, and the liver [3].

Serum ferritin is the most common, convenient, least expensive and regularly used method for assessing iron overload (IOL) [4]. The correlation between serum ferritin and liver iron has been established in β-thalassemia major patients [2].

The anemia and hypoxia resulting from ineffective erythropoiesis in thalassemia syndromes influence the

✉ Volkan Karakus  
dr\_v\_karakus@yahoo.com

<sup>1</sup> Department of Hematology, Muğla Sıtkı Koçman University Training and Research Hospital, 48000 Muğla, Turkey

<sup>2</sup> Department of Biochemistry, Antalya Research and Training Hospital, 07000 Konyaalti, Antalya, Turkey

<sup>3</sup> Department of Internal Medicine, Faculty of Medicine, Izmir University of Economics, 35360 Balçova, Izmir, Turkey

<sup>4</sup> Department of Pathology, Muğla Sıtkı Koçman University Faculty of Medicine, 48000 Muğla, Turkey

<sup>5</sup> Department of Biostatistics and Medical Informatics, Akdeniz University Faculty of Medicine, 48000 Antalya, Turkey

<sup>6</sup> Department of Hematology, Antalya Research and Training Hospital, 07000 Konyaalti, Antalya, Turkey

expression of the serum protein hepcidin. Hepcidin negatively regulates iron absorption because it downregulates the expression ferroportin, a transmembrane protein responsible for exporting intracellular iron into circulation and for iron absorption from the gastrointestinal tract [2].

The direct assessment method of tissue iron is the liver biopsy. The optimal liver iron concentration is 3.2–7.0 mg/g dry liver weight in patients with thalassemia major [5]. Indirect assessment methods include the measurement of serum free iron, ferritin and hepcidin levels [6]. Hepatic R2 and cardiac T2\* values calculated with MRI provide the quantitative assessment of iron overload in the tissues. Hepatic MRI R2 above 6.3 ms and cardiac MRI T2\* above 20 ms indicate normal iron concentrations in the hepatic and cardiac tissues respectively [2, 7, 8].

The aim of the present study is to evaluate iron accumulation in the heart and liver parenchyma of patients with thalassemia major, thalassemia intermedia, and S $\beta$  thalassemia and to examine its association with serum ferritin and hepcidin levels.

## Materials and Methods

### Patient and Control Groups

This is a cross-sectional descriptive study conducted in 109 patients who were followed in the Thalassemia Division of the Department of Hematology at Antalya Research and Training Hospital. Of these patients, 95 were  $\beta$ -thalassemia major, 11  $\beta$ -thalassemia intermedia and 3 were S $\beta$ -thalassemia. They all consented to the use of their medical data in the study.

Of the 109 patients included in the study, 94 had comorbid conditions; 43 (39.4 %) had osteoporosis, 22 (20.2 %) had hypothyroidism, 9 (8.3 %) had diabetes mellitus, 8 (7.4 %) had hypertension, 6 (5.5 %) had vitamin B12 deficiency, 2 (1.8 %) had major depression, and 1 (0.9 %) had heart failure, arrhythmia, chronic obstructive pulmonary disease, and Down syndrome. Genotypes of the patients were also recorded. In the order of frequency, 49.1 % had IVS.I.110.G > A mutation, 7.3 % had IVS.II.1.G/A mutation, 6.9 % had IVS. I.6.T/C mutation, 5.5 % had –30.T > A mutation, 3.7 % had IVS.I.1 and IVS.II.745.C/G mutation, 3.2 % had FSC.44.(–C) mutation, 1.8 % had Cod.8. (–AA) mutation, and 0.9 % had HbS, Cod.44, IVS.I.130, and Cod.27 mutations. Other mutations were observed with a frequency of approximately 0.5 %.

All patients were receiving blood transfusions, zinc, folic acid and calcium supplements in accordance with their requirements and were under chelation therapy with deferoxamine (DFO) at the time of the study. None of the patients had any degree of renal insufficiency. Patients

were further categorized as compliant and noncompliant according to their attendance at the appointments and the appropriate use of chelating agents and other drugs in recommended doses. Of these patients, 27.8 % were found to be noncompliant to the given therapy and some of them to splenectomy, although necessary. Hypersplenic patients in this group that rejected an operation were monitored closely with transfusions according to their requirements.

Blood counts and serum ferritin and hepcidin levels were recorded. Because serum hepcidin levels are affected by transfusions and chelation therapy all blood samples were drawn in the morning in fasting state before the transfusions. Cardiac and hepatic MRI-based standardized T2\* and R2 values were used to indicate tissue iron accumulation. T2\* >20 ms indicated normal iron concentration, T2\* between 15–20 ms indicated mild, T2\* between 10–14 ms indicated moderate, and T2\* <10 ms indicated severe cardiac iron accumulation. R2 >6.3 ms indicated normal iron concentration, R2 between 2.8–6.3 ms indicated mild, R2 between 1.4–2.7 ms indicated moderate, and R2 <1.4 ms indicated severe hepatic iron accumulation [2, 6–8].

The control group consisted of 30 healthy individuals without a known disease or drug use for the comparison of results, in which age, serum ferritin and hepcidin levels were measured and recorded.

By considering the tissue IOL, the results were evaluated between IOL and serum ferritin and hepcidin levels in the patients with thalassemia syndromes.

### Laboratory

Serum hepcidin levels were measured with ELISA using the Human Hepcidin Kit (catalog no: E1979 h) manufactured by Wuhan Eiaab Science Co. Ltd., and the results were expressed as ng/mL. Serum ferritin level was measured using the chemiluminescence method in a Beckman Coulter Unicel Dx1 800 device, and the results were expressed as ng/mL. Blood count was measured using the Coulter LH 780 device based on flow-cytometry methods. Routine biochemistry was measured using a Beckman Coulter AU5800 device based on spectrophotometric methods.

Written informed consent was obtained from each patient and all subjects in the control group before the study. The study protocol was approved by Antalya Research and Training Hospital Ethics Committee.

### Statistical Analysis

Statistical analysis was carried out using SPSS (Statistical Package for Social Sciences) for Windows 16.0. Data from the patients and the control subjects were summarized and

tabulated. In addition to descriptive statistics, Student's *t* test was used to compare quantitative data between the groups, and Pearson's Chi square test, Fisher's Chi square test, and the Mann–Whitney U test were used to compare qualitative data between the groups. The patients were divided into two groups according to the presence of tissue IOL in the heart and liver, and these groups were compared to the control group. The Kruskal–Wallis test was used in this comparison and post hoc analysis was performed with the Bonferroni correction if more than two groups were compared and a *p* value <0.016 was considered statistically significant. Spearman's correlation analysis was used to analyze the correlations between the patients' data. A 95 % confidence interval was selected, and a *p* value <0.05 was considered to be statistically significant.

## Results

Of the 109 patients included in the study, 95 (87.2 %) had  $\beta$ -thalassemia major, 11 (10.1 %) had  $\beta$ -thalassemia intermedia, and 3 (2.8 %) had S $\beta$ -thalassemia. The study included 50 males (45.9 %) and 59 females (54.1 %), and the control group consisted of 30 healthy adults, 14 of which were males (46.7 %) and 16 were females (53.3 %). There was no significant difference between the groups in terms of gender distribution (*p* = 0.938). However, the mean age was higher in the control group (min:19, max:42) than in the patients group (min:9, max:59) ( $31.2 \pm 5.6$  vs.  $24.4 \pm 11.4$  years; *p* < 0.001).

All patients were receiving blood transfusions in accordance with their requirements. The frequency of transfusions according to the diagnoses was 11 days (6–28 days) in the patients with  $\beta$ -thalassemia major, 18.5 days (15–47 days) in the patients with  $\beta$ -thalassemia intermedia, and 25 days (21–25 days) in the patients with S $\beta$ -thalassemia.

Of these patients, 44 (46.3 %) in the thalassemia major group, 10 (90.9 %) in the thalassemia intermedia group, and 1 (33.3 %) in the S $\beta$ -thalassemia group had undergone splenectomy.

The median value of serum ferritin levels was 1693 ng/mL (208–9160) in the patient group and 40 ng/mL (20–43) in the control group (*p* < 0.001), and the median value of serum hepcidin levels was 1.94 ng/mL (0.1–19.64) in the patient group and 0.355 ng/mL (0.12–1.12) in the control group (*p* < 0.001).

According to the MRI findings, 51.37 % of the patients had excessive iron accumulation in the hepatic tissue and 27.52 % of the patients had excessive iron accumulation in the cardiac tissue. The lower the MRI-based T2\* and R2 values were, the higher the IOL and associated tissue stiffness would be. The distribution of these values

according to the diagnoses was as follows: Thalassemia major; T2\*: 25.3 ms (8.7–69.1) and R2: 4.49 ms (0.7–208); Thalassemia intermedia; T2\*: 31.4 ms (20.4–56.4) and R2: 8.14 ms (2.9–14.3), and S $\beta$ -thalassemia; T2\*: 28.2 ms (21.8–40.9) and R2: 10.35 ms (1.5–12.03). None of the patients in  $\beta$ -thalassemia intermedia or S $\beta$ -thalassemia groups had cardiac IOL (Table 1).

Table 2 and 3 revealed the comparison of patients with or without IOL in the cardiac and hepatic tissues in terms of age, serum ferritin and hepcidin levels, and the MRI-based T2\* and R2 values respectively. Data were not available for 7 patients.

The comparison between three groups (patients without cardiac/hepatic IOL versus patients with cardiac/hepatic IOL versus control group) yielded a significant difference if *p* < 0.016. We found the median age of control group was higher than that of both patients groups with and without cardiac IOL (*p* < 0.001), and the difference between the three groups arose from the control group. A significant difference existed in age between patients with and without hepatic IOL and the control group (25 vs. 30 years; *p* = 0.014 and 19.5 vs. 30 years; *p* < 0.001 respectively), and the difference between the three groups arose from the patient group with hepatic IOL.

Patients with or without cardiac IOL had significantly higher serum ferritin and hepcidin levels compared to the control group (serum ferritin: 2858 ng/ml vs. 1402 ng/ml vs. 40 ng/ml; *p* < 0.001 and serum hepcidin: 1.5 ng/ml vs. 1.89 ng/ml vs. 0.35 ng/ml, *p* < 0.001). The differences between the three groups arose from the control group.

Similarly, patients with or without hepatic IOL had significantly higher serum ferritin and hepcidin levels compared to the control group (serum ferritin: 2578 ng/mL vs. 975.5 ng/ml vs. 40 ng/ml; *p* < 0.001 and serum hepcidin: 1.60 ng/ml vs. 1.97 ng/ml vs. 0.35 ng/ml; *p* < 0.001). The differences between the three groups arose from the control group.

MRI based T2\* and R2 values were positively correlated to each other (*r* = 0.49; *p* < 0.001) as well as the correlation between age and T2\* valve (*r* = 0.24; *p* = 0.016). However, inverse correlation was detected between age and serum ferritin level (*r* = 0.45; *p* < 0.001) and hepcidin level (*r* = -0.21; *p* < 0.011), respectively.

The MRI-based T2\* and R2 values were moderately and inversely correlated with serum ferritin levels (*r* = -0.37; *p* < 0.001 and *r* = -0.46; *p* < 0.001, respectively). There was a moderate and positive correlation between the severity of cardiac IOL and serum ferritin level (*r* = 0.39; *p* < 0.001), and a strong and positive correlation between the severity of hepatic IOL and serum ferritin level (*r* = 0.57; *p* < 0.001). No correlations were found between the MRI-based T2\*, R2 values and serum hepcidin levels.

**Table 1** Evaluation of iron accumulation by MRI- based T2\* and R2 in the cardiac and hepatic tissues of the patients with Thalassemia syndromes

Degree of iron accumulation	Thalassemia major n (%)		Thalassemia intermedia n (%)		Sβ-Thalassemia n (%)	
	T2*	R2	T2*	R2	T2*	R2
Normal	62 (65.3)	40 (42.1)	7 (63.6)	3 (27.3)	3 (100)	3 (66.7)
Mild	18 (18.9)	17 (17.9)	0 (0)	3 (27.3)	0 (0)	0 (0)
Moderate	10 (10.5)	20 (21.1)	0 (0)	0 (0)	0 (0)	1 (33.3)
Severe	2 (2.1)	15 (15.8)	0 (0)	0 (0)	0 (0)	0 (0)
Unknown	3 (3.2)	3 (3.2)	4 (36.4)	4 (45.4)	0 (0)	0 (0)
Total	95 (100)	95 (100)	11 (100)	11 (100)	3 (100)	3 (100)

MRI-based standardized T2\* (cardiac) and R2 (hepatic) values were used to indicate tissue iron overload

**Table 2** Age, serum ferritin and hepcidin levels in a total of 102 patients with or without IOL in the heart

	Without IOL n = 72 (70.6 %) median (min–max)	With IOL n = 30 (29.4 %) median (min–max)	p
Age (years)	22 (9–59)	20 (9–34)	0.132
Ferritin (ng/mL)	1402 (208–8985)	2858 (264–9160)	0.004*
Hepcidin (ng/mL)	1.89 (0.1–16.28)	1.50 (0.28–19.64)	0.537
MRI-based T2* values (msec)	30 (20.4–69.1)	15.3 (8.7–19.3)	<0.001*

IOL Iron overload

Mann–Whitney U test, \*  $p < 0.05$  are significant

**Table 3** Age, serum ferritin and hepcidin levels in a total of 102 patients with or without IOL in the liver

	Without IOL n = 46 (45.1 %) median (min–max)	With IOL n = 56 (54.9 %) median (min–max)	p
Age (years)	25 (9–59)	19.5 (9–39)	0.036*
Ferritin (ng/mL)	975 (208–8985)	2578 (260–9160)	<0.001**
Hepcidin (ng/mL)	1.97 (0.12–19.64)	1.60 (0.1–9.44)	0.909
MRI-based R2 values (msec)	7.17 (0.80–208.6)	1.99 (0.70–59.7)	<0.001**

IOL Iron overload

Mann–Whitney U test, \*  $p < 0.05$  are significant

A moderate and positive correlation between serum ferritin and hepcidin levels ( $r = 0.45$ ;  $p < 0.001$ ) was found.

## Discussion

Iron overload in patients with thalassemia is a common feature which requires continuous chelation therapy and monitoring. Serum ferritin determination is widely accepted as a simple method for following iron load in patients with primary hemochromatosis; however, several reports on thalassemic patients emphasize that ferritinemia is not accurate and that other methods such as magnetic resonance imaging is more precise [9].

The severity of the clinical manifestations in β-thalassemia varies widely, ranging from patients that are almost asymptomatic to individuals who suffer from severe anemia and require regular blood transfusion to sustain life [10].

The disease occurs with equal frequency in males and females [11], and the present study reported results similar to those in the literature. Thalassemia is one of the most common hereditary diseases in the world. Our study showed that IVS.I.110.G > A was the most common mutation with a frequency of 49.1 %, followed by the mutations IVS.II.1.G/A in 7.3 % and IVS.I.6.T/C in 6.9 % of the patients in accordance with the most common β-thalassemia genotypes in Turkish population [12, 13].

As a part of the therapy, the timing of splenectomy which must be determined with meticulous assessment [11, 14] had been performed in 90.9 % of our patients with  $\beta$ -thalassemia intermedia and 46.3 % of the patients with  $\beta$ -thalassemia major. Hypersplenic patients in this group that rejected an operation were monitored closely with transfusions according to their requirements.

Iron overload is the principal and multifaceted complication of  $\beta$ -thalassemia. Physiologically, it is caused by an increased absorption of iron from the gastrointestinal tract as a consequence of ineffective erythropoiesis, and is greatly aggravated by chronic transfusion therapy [10]. The aim of blood transfusion is to provide Hb levels that would maintain tissue oxygenation and suppress ineffective erythropoiesis in the bone marrow and would not inhibit normal growth in patients with thalassemia [15]. The patients with  $\beta$ -thalassemia major are known to exhibit higher demand for blood transfusions compared to other thalassemia types, and the need for transfusion often occurs in the first year of life. Moderate and mild forms may require sporadic blood transfusions [11, 14]. In the present study, the frequency of transfusions according to the requirements of the patients was significantly shorter in patients with  $\beta$ -thalassemia major compared to patients with  $\beta$ -thalassemia intermedia and S $\beta$ -thalassemia.

Organ dysfunction caused by iron accumulation in the body is the most important cause of mortality and morbidity in thalassemia patients. Thus, transfusion-independent individuals with thalassemia intermedia have a slower progression of iron overload and generally develop complications later in life compared to patients with thalassemia major who are chronically transfused [2, 10]. Whilst non-transfusion-dependent thalassemia (NTDT) patients receive no or only occasional transfusions, their intestinal iron absorption is continuously upregulated, leading to slow accumulation of iron in tissues, particularly in the liver [2]. Magnetic resonance imaging using either R2 (1/T2) or R2\* (1/T2\*) pulse sequences is a reliable and noninvasive method for assessing liver iron concentration, and has been validated against liver biopsy measurements [2]. Magnetic resonance imaging evaluation of iron overload using T2\* imaging is used to quantify iron loading, before clinical manifestations, in all organ systems, including the heart [3]. T2\* MRI may be used to accurately measure iron concentration in the heart; however, cardiac iron overload is not typically seen in NTDT patients [2]. Thus, our results revealed the liver tissue was affected more than the cardiac tissue in occasionally transfused thalassemia intermedia and S $\beta$ -thalassemia patients compared to frequently transfused thalassemia major patients. As a remarkable impact was observed on the liver, this finding suggested that factors other than transfusion that were involved in iron balance could have been implicated in IOL.

In addition to iron accumulation due to repeated transfusions, enhanced gastrointestinal iron absorption in anemic thalassemia patients also increases iron accumulation. Hepcidin, the key regulator of intestinal iron absorption, negatively regulates iron absorption because it downregulates the expression ferroportin, a transmembrane protein responsible for exporting intracellular iron into circulation and for iron absorption from the gastrointestinal tract. The by-passed ferroportin mechanism in the intestinal epithelium partly explains enhanced iron absorption. The increased erythropoietic activity and hypoxia have been also shown to enhance intestinal iron absorption [2, 16, 17].

In  $\beta$ -thalassemia patients serum hepcidin levels are expected to be high in those receiving repeated blood transfusions and with IOL. In the present study, as expected, serum hepcidin levels of the patients were found to be significantly higher than serum hepcidin levels of the control group. However, there was no significant difference in serum hepcidin levels between patients with IOL in the heart and liver and patients without IOL. We assumed that decreased hepcidin concentrations in the intervals between successive transfusions of patients with IOL caused enhanced iron absorption and resulted in iron accumulation [18], considering that all patients were receiving blood transfusions.

In the present study, in which most of the patients were adults, increased serum ferritin levels were correlated with IOL in the heart and liver. In the study by Wood et al. [19], yearly iron intake did not correlate with serum ferritin levels and iron overload in the liver and heart of the patients. They reported their results on children, however, in the 15–18 year old age group an increased tissue damage was observed. In another study by Mazza [9], analyses of serum ferritin levels and MRI data was performed in the patients with mild to moderate siderosis and those with severe and very severe siderosis of the liver. Although, MRI provided a practical grading of the hepatic iron load based on the severity of the picture appeared to be in accordance with our findings, no significant correlation was seen with ferritinemia levels when different severities of siderosis were compared. A trend toward significance was evident only when the ferritin values of patients with less severe iron load were compared with those of thalassemic patients with greater iron overload [9]. As would be expected, the present study showed an inverse correlation between serum ferritin levels and MRI-based T2\* and R2 values, and a positive correlation was shown with the severity of IOL indicating iron accumulation in the heart and liver. No correlation was found between serum hepcidin levels and MRI-based iron accumulation. In addition, there was no obvious

increase in serum hepcidin levels that would reach statistical significance in patients with IOL than in patients without IOL in the cardiac and hepatic tissues. This is considered to have caused enhanced intestinal iron absorption and tissue iron accumulation. In healthy individuals, serum hepcidin that has an important role in body iron hemostasis is known to increase in response to increased serum iron and iron stores and inhibit export of iron from the intestines and macrophages into the circulation [11, 20]. In patients with thalassemia, hepcidin levels lower than expected has been associated with ineffective erythropoiesis and hypoxia [18].

The effects on intracellular iron on hepcidin mechanism have not been fully understood. Recently, Growth Differentiation Factor 15 (GDF15) and Twisted Gastrulation Protein 1 (TWSG1), secreted from the increased amounts of erythroid precursors as a result of ineffective erythropoiesis, are considered to play a role in hepcidin regulation and act as a suppressor for hepcidin in  $\beta$ -thalassemia patients [18, 21].

The main limitations of the current study were the smaller size of the studied cohort and the age differences between control and patient groups.

## Conclusion

Iron intake with repeated transfusions and enhanced intestinal iron absorption are the two important reasons for iron accumulation in transfusion-dependent thalassemia patients. The decrease in hepcidin levels has been suggested as the most important cause of enhanced iron absorption and impaired regulation of the release of iron in the macrophages. Assessment of iron overload (IOL) in the liver and cardiac tissue can be determined by the standardized R2 and T2\* values calculated on MRI as well as the simple laboratory method by measuring serum ferritin and hepcidin levels in these patients. Correlations between tissue IOL, serum ferritin and hepcidin concentrations may help us to predict organ dysfunction before clinical manifestations.

## Compliance with Ethical Standards

**Conflicts of interest** All authors declare no conflicts of interest.

**Informed Consent** Informed consent was obtained from all the individual participants included in this study.

**Ethical Approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

## References

1. Weatherall DJ, Clegg JB (1980) The clinical and molecular heterogeneity of the thalassemia syndromes. *Ann N Y Acad Sci* 344:83–100
2. Taher AT, Viprakasit V, Musallam KM, Cappellini MD (2013) Treating iron overload in patients with non-transfusion-dependent thalassemia. *Am J Hematol* 88:409–415
3. Hamed AA, Elguindy W, Elhenawy YI, Ibrahim RH (2016) Early cardiac involvement and risk factors for the development of arrhythmia in patients with  $\beta$ -thalassemia major. *J Pediatr Hematol Oncol* 38(1):5–11
4. Kolnagou A, Yazman D, Economides C, Eracleous E, Koutoghriorghes GJ (2009) Uses and limitations of serum ferritin, magnetic resonance imaging T2 and T2\* in the diagnosis of iron overload and in the ferritkinetics of normalization of the iron stores in thalassemia using the international committee on chelation deferoxamine/deferrioxamine combination protocol. *Hemoglobin* 33(5):312–322
5. Olynk JK, O'neil R, Britton RS, Bacon BR (1994) Determination of hepatic iron in fresh and paraffin embedded tissue: diagnostic Implications. *Gastroenterology* 106:674–677
6. Fisher R, Harmatz PR (2009) Non-invasive assessment of tissue iron overload. In: Gewirtz AM, Keating A, Thompson AA et al (eds) *Hematology 2009*, vol 1. American Society of Hematology Education Program Book, New Orleans, pp 215–221
7. St. Pierre TG, Clark PR, Chua-Anusom W (2005) Measurement and mapping of liver iron concentration using magnetic resonance imaging. *Ann N Y Acad Sci* 1054:379–385
8. Anderson LJ, Holden S, Davis B et al (2001) Cardiovascular T2-star (T2\*) magnetic resonance for early diagnosis of myocardial iron overload. *Eur Heart J* 22:2171–2179
9. Mazza P, Giua R, De Marco S, Bonetti MG, Amurri B, Masi C et al (1995) *Haematologica* 80:398–404
10. Gardenghi S, Grady RW, Rivella S (2010) Anemia, ineffective erythropoiesis and hepcidin: interacting factors in abnormal iron metabolism leading to iron overload in  $\beta$ -Thalassemia. *Hematol Oncol Clin North Am* 24(6):1089–1107
11. Borgna-Pignatti C, Galanello R (2009) Thalassemias and Related Disorders: Quantitative disorders of hemoglobin synthesis. In: Greer JP, Foester J, Rodgers GM et al (eds) *Wintrobe's clinical hematology*, 12th edn. Lippincott Williams & Wilkins, Philadelphia, pp 1083–1131
12. Yılmaz B, Balım Z, Ozkınay Gunduz C, Altıntaş N, Aydınok Y et al (2000) Molecular genetic analyses of Beta-Thalassemia patients. *Ege Tıp Dergisi* 39(3):153–157
13. Ghazi O, Tadmouri A, Başak N (2001)  $\beta$ -thalassemia in Turkey: a Review of the clinical epidemiological molecular and evolutionary aspects. *Hemoglobin* 25(2):227–239
14. Gümrük F (2012) Hemoglobin ve Hemoglobinopatiler. In: İliçin G, Biberoglu K, Süleymanlar G, Ünal S (eds) *Hastalıkları*. 3. Baskı. Güneş Kitabevi, Ankara, pp 1634–1644
15. Karakas Z (2010) Principles of transfusion in Thalassemia. *Türkiye Klinikleri J Hem Onc Special Topics* 3(1):50–57
16. Cunningham MJ, Sankaran VG, Nathan DG, Okrin SH (2009) The Thalassemias. In: Orkin SH, Nathan DG, Ginsburg D, Look AT, Fisher DE, Lux SE et al (eds) *Nathan and Oski's hematology of infancy and childhood*, 7th edn. Saunders Elsevier, Philadelphia, pp 1015–1107
17. Peters TJ, Raja KB, Simpson RJ, Snape S (1988) Mechanisms and regulation of intestinal iron absorption. *Ann N Y Acad Sci* 48:541–547
18. Nemeth E (2010) Heparidin in  $\beta$ -Thalassemia. *Ann N Y Acad Sci* 1202:31–35
19. Wood JC, Origa R, Agus A, Matta G, Coates TD, Galanello R (2008) Onset of cardiac iron loading in pediatric patients with thalassemia major. *Hematologica* 93(6):917–920

20. Tokgöz H, Çalışkan Ü (2010) Iron regulation, metabolism and measurement of overload in thalassemia. *Türkiye Klinikleri J Hem Onc Special Topics* 3(1):70–75
21. Ganz T, Olbina G, Girelli D, Nemeth E, Westerman M (2008) Immunoassay for human serum hepcidin. *Blood* 112:4292–4297