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Original Article

Hepcidin levels, markers of iron overload, and liver damage in children with β-thalassemia major

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Abstract

Background Thalassemia is a hemoglobin synthesis disorder that causes patients to need lifelong blood transfusions, leading to iron overload and alter organ function, including the liver. Hepcidin, produced by the liver, plays a role in iron homeostasis and should be increased in excess iron stores. However, the level decreases in thalassemia due to some factors, such as ineffective erythropoiesis and liver damage. Recent publications revealed that hepcidin could be associated with iron overload and also a marker of liver diseases.

Objective To analyse the correlation between hepcidin level, markers of iron overload, and liver damage in β-thalassemia major.

Methods This cross-sectional study included all β-thalassemia major age 2-18 years admitted to Dr. Mohammad Hoesin Hospital, Palembang, South Sumatera, who underwent blood transfusions from March to August 2022. We measured the level of iron overload markers, hepcidin, liver function test (LFT), and performed liver ultrasonography (USG).

Results Of 97 subjects, median hepcidin level was 10.01 ng/mL and 68% of the subjects showed a decrease. The iron overload parameters were evaluated from serum iron levels (P=0.13), ferritin levels (P=0.90), and transferrin saturation (P=0.29) and 24.7% had abnormal liver USG findings. Spearman's correlation revealed that only direct bilirubin (DB) (r=0.35; P=0.001) and liver USG (r=0.20; P=0.05) had positive correlations with decreased levels of hepcidin. Also, it had 91.7% sensitivity in predicting liver damage from ultrasound.

Conclusion The hepcidin level was not significantly associated with iron overload markers. **[Paediatr Indones. 2024;64:412-8; DOI: https://doi.org/10.14238/pi64.5.2024.412-8**].

Keywords: *β-thalassemia major; hepcidin; iron overload; liver damage*

Thalassemia is an inherited hemoglobin synthesis disorder that causes a short life span of erythrocites and leads to the need for continuous blood transfusion. The World Bank reported that 7% of the world's population was carriers of thalassemia[1] and Indonesia count for 3.8% of the entire population.[2] According to the Hematology Task Force of *Indonesian Paediatric Society*, thalassemia patients increased from 4,896 in 2012 to 9,121 in 2016, and decreased to 9,028 in 2018.[3] A study in South Sumatra reported that 15% of the population carried β-thalassemia traits.[4]

Repeated blood transfusions can cause hemosiderosis, namely iron overload, damage, and impaired organ function, including the liver.[5,6] The liver regulates iron homeostasis by storing iron and secreting the regulatory hormone; the hepcidin. It controls iron absorption from the intestinal tract and recovers iron from old erythrocytes. Hepcidin triggers internalisation of the iron protein ferroportin

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transfer to block iron release from the small intestine, macrophages, and other cells.[7,8] The discovery of hepcidin has provided a solid basis for understanding the mechanisms of systemic iron homeostasis and the aetiology of iron disorders. It also regulates the balance of circulating and stored iron levels for various physiological processes, including oxygen transport and erythropoiesis, by limiting the toxicity of excess iron. Hepatic fibrosis occurs due to excess iron in hepatocytes, which can produce hydroxy radicals (OH), which trigger the formation of collagen as the basis for fibrosis.[9,10]

The gold standard for assessing liver damage is an invasive liver biopsy. However, liver function test (LFT), including examination of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, gamma glutamate transpeptidase (GGT), bilirubin and albumin, could be performed in advance.[5,7,11] A previous study found a significant correlation between ALT and hepcidin (P=0.0001).[12] Serum bilirubin is often increased in β-thalassemia major due to hemolysis of red blood cells.13Another diagnostic tool is ultrasound (USG), especially for chronic liver disease.[13,14] The most common ultrasound appearance in patients with a diagnosis of cirrhosis is ascites, which is a combined manifestation of hepatocellular failure and portal hypertension.[15,16]

We aimed to examine the correlation between hepcidin levels, iron overload markers, and liver function in β-thalassemia patients. Additionally, a diagnostic test was conducted to seek the sensitivity and specificity of the hepcidin level to predict liver damage.

Methods

An analytical observational study with cross-sectional design was performed to analyse the correlations between hepcidin levels, markers of iron overload, and liver damage in β-thalassemia major patients. Subjects were children diagnosed with β-thalassemia major aged 2-18 years who received blood transfusions from March to August 2022 at Dr. Mohammad Hoesin Hospital (RSMH) in Palembang, South Sumatera. All parents or legal caregivers provided their written informed consent. We excluded β-thalassemia major patients who had a significant acute infection/inflammation, took antituberculosis drugs/ATD which were rifampicin, isoniazid, pyrazinamide and ethambutol, and also patients taking antiepileptic drugs/AEDs such as valproic acid and lamotrigine. This study was approved by the RSMH Ethics Review Board.

Subjects underwent history-taking, anthropometric measurements that were weight, height, and mid-upper arm circumference (MUAC), laboratory examination as followed: hemoglobin, hepcidin, ALT, AST, total bilirubin (BT), direct bilirubin (DB), serum iron (SI), ferritin, transferrin saturation, and transferrin index. For the laboratory test, we draw 6 mL of blood in the morning taken after fasting for 8 hours. Hepcidin level was examined using the DRG Hepsidin-25 (bioactive) ELISA kit at the Sriwijaya University Laboratory of the Faculty of Medicine. The normal value of the level of hepcidin ranged from 15-50 ng/mL and was classified as low or decreased when the value was <15 ng/mL. Iron overload markers consisted of serum iron, ferritin level, transferrin saturation, and transferrin index, and were classified as positive when each item increased >120 μg/dL, >1000 ng/dL, >55%, and <1.0, respectively. We checked liver injury markers that were DB, ALT and AST, and classified as positive if the value increase >1 mg/dL for DB and more than 5x for ALT and AST. Liver ultrasound was performed by a hepatology consultant and was decided abnormal based on the size and structure of the liver. Laboratory tests and ultrasound were performed before subjects received blood transfusions.

Data were processed and analysed with the statistical programme for *social sciences (SPSS) version 22* software. Bivariate analysis for categorical data was performed using the Chi-square test or Fisher's exact test. Correlations test was performed using Pearson's test or Spearman's test to obtain the correlation coefficient (r). The results with P values <0.05 were considered statistically significant. Moreover, we provided a receiver operating characteristic (ROC) curve with area under the curve (AUC) to establish the sensitivity and specificity values.

Results

There were 158 patients, 61 of them were excluded because they had hepatitis C (HCV) or human immunodeficiency virus (HIV) infection. Of 97 subjects, most were male (59.8%), 89.7% had good nutritional status, 54.6% were aged <10 years with the mean age was 9.73 (SD 4.29) years. The median duration of the illness was 52 (0-204) months; 44.3% of the subjects had a duration of the illness between 13-60 months. Most subjects received deferiprone (74.2%) and 44.3% had used iron chelation for 30-60 months with a median therapy duration of 53 (0-204) months. The median hepcidin level was 10.01 (0.02–44.42) ng/mL, 66 subjects had decreased levels. Complete subjects' characteristics are shown in **Table 1**.

Bivariate analysis of hepcidin levels and markers of iron overload (SI, ferritin, transferrin saturation, and transferrin index) and also liver injury (SGOT, SGPT, DB, and liver USG) was carried out by Chi-square and Fisher's exact tests. Those revealed no significant associations between hepcidin levels and all of iron overload markers (**Table 2**); however, decreased hepcidin level showed an odds value 7.25 times higher for abnormal liver USG (P<0.05) (**Table 3**).

Spearman's correlation test found similar results which were no significant correlation between hepcidin levels and iron overload markers, but showed a positive weak correlation with direct bilirubin and liver ultrasound (r=0.35; P=0.001 and r=0.20; P=0.05, respectively) (**Table 4**).

Table 1. Characteristics of the subjects

Characteristics	N=97
Gender, n (%)	
Male	58 (59.8)
Female	39 (40.2)
Age, n (%)	
<10 years	53 (54.6)
≥10 years	44 (45.4)
Mean age (SD), years	9.73 (4.29)
Nutritional status, n (%)	
Obese	0
Overweight	1 (1.0)
Good nutrition	87 (89.7)
Wasting	7 (7.2)
Severe wasting	2 (2.1)
Length of illness, n (%)	
1-12 months	10 (10.3)
13-60 months	44 (45.4)
> 60 months	43 (44.3)
Median length of illness (range), months	52 (0-204)
Iron chelation, n(%)	
Deferiprone	72 (74.2)
Deferasirox	25 (25.8)
Length of iron chelation therapy, n (%)	
1-12 months	11 (11.3)
13-60 months	43 (44.3)
> 60 months	43 (44.3)
Median length of therapy (range), months	53 (0-204)
Hepcidin level, n (%)	
Decreased	66 (68)
Normal	31 (32)
Median (range), ng/mL	10.01 (0.02-44.42)

Table 2. Association of hepcidin levels and markers of iron overload (N=97)

Variables	Hepcidin levels, n (%)		OR (95%CI)	P value
	Decreased	Normal		
Serum iron				
Increased	52 (72.2)	20 (27.8)	2.04 (0.79 to 5.25)	0.13*
Normal	14 (56)	11 (44)		
Ferritin levels				
Increased	56 (68.3)	26 (31.7)	1.08 (0.34 to 3.47)	0.90**
Normal	10 (66.7)	5 (33.3)		
Transferrin saturation				
Increased	55 (70.5)	23 (29.5)	1.74 (0.62 to 4.88)	0.29**
Normal	11 (57.9)	8 (42.1)		
Transferrin index				
Increased	66 (68)	31 (32)	-	-
Normal	0	0		

*Chi-square test, **Fisher's exact test

Table 3. Association of hepcidin levels and markers of liver injury (N=97)

Liver damage	Hepcidin levels, n (%)		OR (95%CI)	P value
	Decreased	Normal		
SGOT				
Increased	22 (68.8)	10 (31.2)	1.05 (0.42 to 2.61)	0.92*
Normal	44 (67.7)	21 (32.3)		
SGPT				
Increased	22 (78.6)	6 (21.4)	2.08 (0.75 to 5.82)	0.16*
Normal	44 (63.8)	25 (36.2)		
Bilirubin direct				
Increased	5 (50)	5 (50)	0.43 (0.11 to 1.51)	0.28**
Normal	61 (70.1)	26 (29.9)		
Liver ultrasound				
Abnormal	22 (91.7)	2 (8.3)	7.25 (1.58 to 33.20)	0.004*
Normal	44 (60.3)	29 (39.7)		

*Chi-square test, **Fisher's exact test

Table 4. Correlations of hepcidin and markers of iron overload and liver damage (N=97)

Markers	r	P values
Serum iron	-0.11	0.27
Ferritin	-0.17	0.09
Transferrin saturation	-0.15	0.13
Transferrin index	-0.16	0.13
AST	-0.09	0.35
ALT	-0.17	0.09
Direct bilirubin	0.35	0.001
Liver USG	0.20	0.05

*Spearman's correlation test

The receiver operating characteristic (ROC) curve was used to determine the hepcidin cut-off value to detect liver damage in β-thalassemia major patients. The area under the curve (AUC) obtained for DB was 60% with 50% sensitivity and 70% specificity (P=0.09) (**Figure 1**). Meanwhile, AUC value for liver USG abnormalities was 34% with 8% sensitivity and 91.7% specificity (**Figure 2**).

Discussion

Thalassemia is a recessive inherited disorder, which means that the chance of both genders to have the disease is equal, as shown in this study and also other study.[17] Significant growth retardation is common in thalassemia caused by chronic anemia, iron overload, and chelation toxicity. However, in our study, 89.7% of the subjects had a good nutritional status. It showed an increase compared to the 2013 study which reported that 57.6% of children with thalassemia in RSMH had good nutritional status.[18] Optimalisation of adequate nutrition in thalassemia patients is a mandatory to prevent growth and developmental disorders.[18]

A previous study in India found that the median hepcidin level was 21.89 ng/mL in males and 21.95 ng/mL in females.[17] We found that 68% of subjects had decreased hepcidin levels, but the median was lower than that reported in India. In addition, we did not compare the level between male and female subjects. Several previous studies reported lower serum hepcidin levels in β-thalassemia patients which might be related to the variability in iron overload.[13,19-21]

Decreased levels of hepcidin were found in 72.2% of those with increased serum iron, 68.3% with increased ferritin, 70.5% with increased transferrin saturation, and 68% with an increased transferrin index. In our study, the parameters for iron overload were assessed from serum iron levels (P=0.13), ferritin levels (P=0.90), and transferrin saturation (P=0.29). A study has noted no significant associations between serum hepcidin and ferritin levels in any of the three groups of thalassemia patients with beta thalassemia major, beta thalassemia/HbE, or Hb H + AE Bart.[13] Hepcidin was not significantly associated with any variables, including serum ferritin, Hb, age, labile plasma iron (LPI) or blood transfusion units among the three groups of total thalassemia patients.[193]Hepcidin regulation in thalassemia patients may be more influenced by erythropoietic activity than iron storage.[19-21]

A previous study reported lower hepcidin

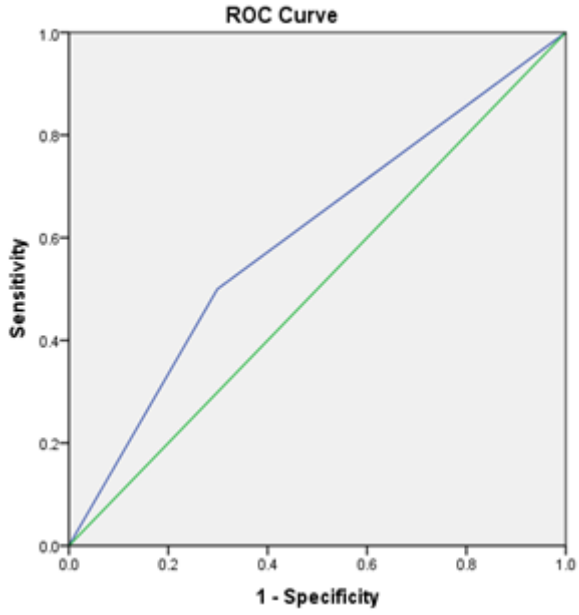

Figure 1. ROC curve of hepcidin levels and direct bilirubin

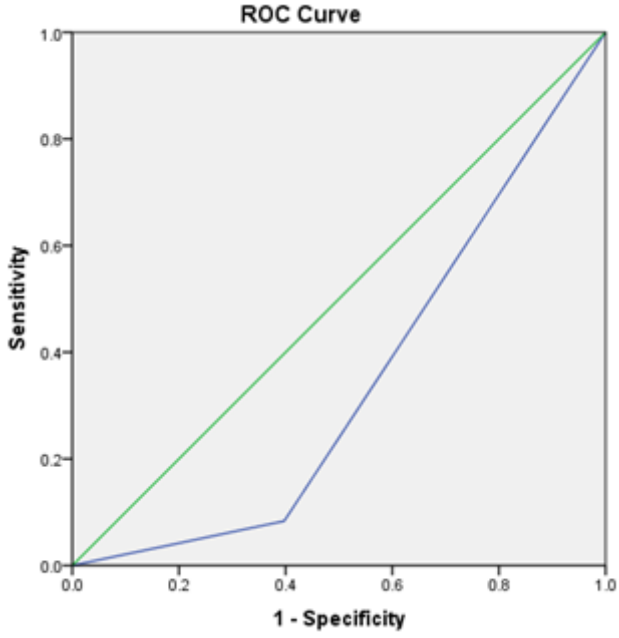

Figure 2. ROC curve of hepcidin levels and liver USG

levels in β-thalassemia patients with iron overload. Thalassemia-induced downregulation of hepcidin can result in iron overload.[22] Iron overload may occur from routine blood transfusions. Another study in India in children with β-thalassemia major, hepcidin levels decreased due to high erythroid signals.[23] On the contrary, a study stated that hepcidin levels increased in children with β-thalassemia major, with more obvious increases in patients with thalassemia major with severe iron overload. This study reported positive correlations with the frequency of transfusions and the total number of transfusions. Regular transfusion therapy can increase hepcidin production because it usually affects the degree of anemia, erythropoetic

drive, and iron load in β-thalassemia major patients.[24]

In our study, decreased hepcidin levels were observed in 68.8% of those with increased ALT, 78.6% of those with increased AST, 50% of those with increased DB, and 91.7% of those with abnormal liver ultrasounds. However, liver USG findings were solely significantly associated with decreased hepcidin. Whenever hepcidin levels decrease, the body stores more iron in body tissues, including the liver. Iron accumulation in liver cells can cause damage and inflammation to the organ, leading to cytotoxic free radical reactions which are cytotoxic in nature.[25] Some studies showed that children with thalassemia with elevated serum ferritin levels had higher levels of ALT and AST levels.[26,27]

In conclusion, more study is needed to assess the strength and usefulness of hepcidin level to predict liver injury in paediatric patients with B-thalassemia. In our study, statistical analysis confirmed that hepcidin levels were not significantly correlated with most markers of iron overload and liver damage, except for DB and liver ultrasound. Furthermore, a decreased level of hepcidin showed a high specificity to detect abnormal liver ultrasound. This fact needs to be investigated in a wider way to determine whether liver ultrasound can be used as a reliable indicator of health conditions in children with β-thalassemia major.

Conflict of interest

None declared.

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