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Original Article

Hemostatic abnormalities in children with thalassemia major and liver iron overload

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Abstract

Background Thalassemia major (TM) patients are susceptible to liver dysfunction due to iron deposition. Pediatric TM patients often present with bleeding. Blood loss necessitates transfusions, leading to increased iron absorption from the gut.

Objective To study hemostatic abnormalities in children with TM and iron deposition in the liver.

Methods This cross-sectional study involved 190 non-splenectomized children with TM. Liver iron deposition was evaluated using T2* MRI. Prothrombin time (PT), activated partial thromboplastin time (aPTT), and platelet counts were assessed from blood specimens.

Results Most subjects were diagnosed with β-thalassemia and β-thalassemia/HbE. The majority of subjects were on deferiprone (DFP) treatment. Approximately 89.5% of subjects had liver iron overload. Prolongation of PT and aPTT, as well as thrombocytopenia were observed in 60%, 27.9%, and 19.5% of subjects, respectively. Prolonged aPTT and thrombocytopenia were observed three times more frequently in subjects with moderate-severe liver iron overload than in subjects with normal-mild liver iron overload (P=0.04 and 0.001, respectively).

Conclusion Most TM subjects have liver iron overload ranging from mild to severe. Prothrombin time and aPTT prolongation, as well as thrombocytopenia are easily found in TM children. There were significantly more moderate-severe liver iron deposition patients with aPTT prolongation and thrombocytopenia than normal-mild patients with these conditions. Hence, we suggest that pediatric TM patients undergo liver iron deposition evaluations and use iron chelators in an optimal manner, in order to limit the risk of bleeding. **[Paediatr Indones. 2018;58:175-9; doi: http://dx.doi.org/10.14238/pi58.4.2018.175-9]**.

Keywords: *prothrombin time; activated partial thromboplastin time; platelet; liver iron overload*

Thalassemia is an inherited blood disorder characterized by diminished production of one or more globin chains. The degree of thalassemia severity is affected by several factors, such as type of globin mutation, presence of hemoglobin variants, hereditary persistence of fetal hemoglobin (HPFH) mutation, and other mutations inside the β-globin cluster.[1]

Thalassemia major (TM) is the most severe form of thalassemia, characterized by severe anemia. Patients are totally dependent on regular blood transfusions throughout their entire lives. Red cell transfusions lead to accumulation of iron in several organs. This condition is worsened by the fact that the patient's chronic state of anemia causes increased absorption of iron from gastrointestinal tract. At the cellular level, accumulated iron increases production of reactive oxygen species, which subsequently damage the cells.[2] Therefore, iron chelation therapy plays a crucial role in the survival of TM patients, although in most conditions it is not

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sufficient to prevent iron deposition in tissues.[1,2] The liver, as the predominant iron storage organ, may be damaged earlier compared to other organs.[3]

Although a chronic hypercoagulable state has been observed in many thalassemia studies, it was mostly reported in adult subjects.[4-5] A pediatric study reported that 29.6% of thalassemia patients had bleeding manifestations, such as epistaxis and gum bleeding.[6] Loss of blood aggravates the anemic condition, and leads to increasing required volumes of blood transfusion and intensifying iron absorption in the gastrointestinal tract. Therefore, we aimed to explore liver iron deposition and hemostatic abnormalities in thalassemic children with liver iron overload, based on their laboratory values.

Methods

This cross-sectional study included 190 non-splenectomized children with TM. Spleen size was classified using Schuffner grading, which an imaginary line that connect right costal arch to spina iliaca anterior superior sinistra (SIAS). The line was divided into 8 segments equally: Schuffner 1 lies in right costal arch, Schuffner 4 lies in umbilicus, and Schuffner 8 lies in SIAS. The degree of liver iron deposition was determined using *MRI 1.5 Tesla* scanner (Siemens Avanto, Germany) with T2* gradient echo (GRE) sequence. The T2* values were analyzed using *CMRtoolsTM* software (Thalassemia-Tools, London, United Kingdom). Subjects were asked to hold their breath for 11-13 seconds (s), while scanned images were collected at 12 different echo times (1.3-23 milliseconds/ms). Liver iron overload was categorized as normal >6.3 ms, mild 2.7-6.3 ms, moderate 1.4-2.7 ms, or severe <1.4 ms.[7] The normal reference laboratory values for PT, aPTT, and platelet counts in our center were 9.8-11.2s, 31-47s, and 150,000-450,000 cells/μL, respectively. Platelet values for each subject were acquired after calculating mean platelet counts for 1 year.

Results

The mean age of subjects was 13 (SD) years. The numbers of male and female subjects were almost equal. Most subjects were diagnosed with β-thalassemia (51.1%) and β-thalassemia/HbE (45.8%). The majority of subjects were treated with deferiprone (DFP) monotherapy (65.8%), followed by combined DFP+deferasirox (DFX) (15.8%), and DFX monotherapy (9.5%). Approximately 73.2% of subjects had spleen size of Schuffner II or less, and only 10.5% of subjects had normal liver iron deposition. The PT prolongation was observed in 60% of subjects, while aPTT prolongation was observed in 27.9% of subjects. A total of 19.5% of subjects had thrombocytopenia (**Table 1**).

In order to fulfill the conditions for statistical analysis, patients were grouped into the normal-mild iron overload (group A) or the moderate-severe iron overload (group B). There was only slight difference of mean PT between group A and group B (P=0.587).

Table 1. Demographic distribution of subject characteristics

Characteristics	N=190
Mean age (SD), years	13 (2.45)
Sex, n (%)	
Male	96 (50.5)
Female	94 (49.5)
Diagnosis, n (%)	
α-thalassemia	5 (2.6)
β-thalassemia	97 (51.1)
β-thalassemia/HbE	87 (45.8)
α-β-thalassemia/HbE	1 (0.5)
Iron chelation, n (%)	
Monotherapy	
DFO	0 (0)
DFP	125 (65.8)
DFX	18 (9.5)
Combination therapy	
DFO+DFP	12 (6.3)
DFO+DFX	4 (2.1)
DFP+DFX	30 (15.8)
No chelation	1 (0.5)
Spleen size, n (%)	
Normal	34 (17.9)
Schuffner I-II	105 (55.3)
Schuffner III-IV	44 (23.2)
> Schuffner IV	7 (3.6)
Liver iron overload/Liver T2* MRI, n (%)	
Normal	20 (10.5)
Mild iron overload	55 (28.9)
Moderate iron overload	65 (34.2)
Severe iron overload	50 (26.3)
PT prolongation, n (%)	114 (60)
aPTT prolongation, n (%)	53 (27.9)
Thrombocytopenia, n (%)	37 (19.5)

DFO=deferoxamine, DFP= deferiprone, DFX= deferasirox

Interestingly, mean aPTT in group B [44.94 (6.66) s] was significantly longer than in group A [41.68 (4.92) s] (P=0.036). A significant difference was also observed in mean platelet counts between groups, with a mean difference of 59,337 cells/μL (P=0.001) (**Table 2**).

Table 3 shows that PT prolongation was observed in around 60% of subjects in both groups (P=0.915). However, aPTT prolongation was significantly higher in group B (37.2%) than in group A (13.6%); (P=0.04) (**Table 4**).

Table 5 shows that platelet counts were also significantly different between the liver iron overload groups. Thrombocytopenia was more common in group B (27.2%) compared to group A (8.1%); (P=0.001).

Discussion

In our study, PT prolongation was observed in 60% of subjects. Prothrombin time is a test to determine deficiency of clotting factors, which are involved in the extrinsic coagulation pathway, including factors II, V, VII, and X. Clotting factors, with the exception of factor VIII, are primarily synthesized in the liver. Liver dysfunction may lead to decreased bile secretion into the duodenum and subsequent impairment of vitamin K absorption. Vitamin K acts as a co-factor in the production of factors II, VII, IX, and X.[6,8] Interestingly, no significant mean PT difference was observed between subjects with normal-mild iron overload and those with moderate-severe iron overload. These results suggest that production of clotting factors and bile salt secretion were impaired at an earlier stage of liver iron deposition.

In contrast, aPTT prolongation was observed in only 27.9% of subjects. Prolonged aPTT in thalassemia may be caused by liver dysfunction due to iron deposition and chronic activation of the intrinsic coagulation cascade.[6] One study suggested that chronic blood transfusions and hemolysis-induced activity of kallikrein-esterase, result in increased usage

Table 2. Mean PT, aPTT, and platelet counts in in the liver iron overload groups

Liver iron status	Mean (SD)					
	PT, s	P value	aPTT, s	P value	Platelet count, cells/μL	P value
Normal-mild iron overload (group A)	11.47 (0.89)	0.587	41.68 (4.92)	0.036	274,530 (104,215)	0.001
Moderate-severe iron overload (group B)	11.75 (1.55)		44.94 (6.66)		215,193 (90,730)	

Table 3. PT prolongation in the liver iron overload groups

Liver iron status	PT		P value
	Normal	Prolongation	
Normal-mild iron overload, n (%) (group A)	31 (40.9)	44 (59.1)	0.915
Moderate-severe iron overload, n (%) (group B)	45 (39.5)	70 (60.5)	

Table 4. aPTT prolongation in the liver iron overload groups

Liver iron status	aPTT		P value
	Normal	Prolongation	
Normal-mild iron overload, n (%) (group A)	65 (86.4)	10 (13.6)	0.04
Moderate-severe iron overload, n (%) (group B)	72 (62.8)	43 (37.2)	

Table 5. Thrombocytopenia in the liver iron overload groups

Liver iron status	Platelet counts		P value
	Normal	Thrombocytopenia	
Normal-mild iron overload, n (%) (group A)	69 (91.9)	6 (8.1)	0.01
Moderate-severe iron overload, n (%) (group B)	84 (72.8)	31 (27.2)	

of factors XII and XI.[9] Prolonged aPTT was observed in more patients with moderate-severe liver iron overload than those with normal-mild status. Factor VIII is synthesized mainly by endothelial cells, not hepatocytes.[10] As such, most patients with normal-mild liver iron overload had normal aPTT, although their PT was abnormal. It is important to note that technical factors may cause false positive results of PT and aPTT prolongation. Those factors include ratio of blood volume to citrate anticoagulant, variation in citrate concentration, and the difficulty of blood drawing in children.[11]

From those explanations, we assume that PT and aPTT prolongation were consequences of liver dysfunction and chronic activation of clotting factors. One study found that the tendencies of bleeding and thrombosis are different between children and adults. Bleeding is commonly found in children. The risk of bleeding rises with increasing age until they reach 11-16 years, which is the period of hemostatic balance between thrombophilic and anti-thrombotic. In contrast, the tendency of thrombosis is more common in adults.[6]

Thrombocytopenia is common in thalassemia patients. Two mechanisms that explain this condition are increased platelet destruction and decreased thrombopoietin (TPO) level. Increased platelet destruction is primarily caused by splenomegaly, which can be found easily in thalassemia patients due to extramedullary erythropoiesis process and extensive extravascular hemolysis.[12] In our study, 26.8% of subjects had spleen size greater than Schuffner II.

Transfusion-dependent thalassemia patients are also prone to liver iron overload, which later damages normal liver cells.[3] Chronic liver disease may also contribute in platelet destruction due to increased shear stress, fibrinolysis, and immunologic destruction mediated by anti-glycoprotein antibodies. Thrombopoietin, which is predominantly synthesized in the liver, plays important roles in megakaryocytopoiesis and platelet maturation.[13]

In conclusion, most children with TM have abnormal liver iron deposition. PT prolongation is more common compared to aPTT prolongation and thrombocytopenia. Patients with moderate-severe liver iron deposition are susceptible to aPTT prolongation and thrombocytopenia. Prolonged PT is not affected by the degree of liver iron overload. In conclusion. We suggest that children with thalassemia major undergo liver iron overload evaluation and iron chelation therapy, in order to minimize the risk of bleeding.

Conflict of interest

None declared.

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