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Original Article

Phosphatidylserine-exposed red blood cells and ineffective erythropoiesis biomarkers in patients with thalassemia

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Abstract: Objective: The degree of ineffective erythropoiesis is known to be associated with clinical severity among individuals with thalassemia. The association of ineffective erythropoiesis biomarker levels with different thalassemia genotypes, however, remains limited. The aim of this study was to explore the level of phosphatidylserine-exposed red blood cells (PS-exposed RBCs) and ineffective erythropoiesis biomarkers (growth-differentiation factor-15 and soluble transferrin receptors) in patients with different genotypes. Methods: A cross-sectional study was conducted on 139 patients of age 18 years and above with different genotypes at Srinagarind Hospital, Khon Kaen University, Thailand. The levels of PS-exposed RBCs were determined using flow cytometry. Measurements of growth-differentiation factor-15 (GDF-15) and soluble transferrin receptors (sTfR) were evaluated by the ELISA method. Results: The PS-exposed RBCs levels were found to be significantly higher in splenectomized beta-thalassemia patients. Patients with beta-thalassemia had the highest GDF-15 levels, followed by patients with non-deletional alpha-thalassemia. Patients with non-deletional alpha-thalassemia showed elevated hemoglobin levels and reduced GDF-15 levels after splenectomy. Patients with beta-thalassemia and non-deletional alpha-thalassemia had the highest levels of PS-exposed RBCs and ineffective erythropoiesis biomarkers, which correlated with the clinical severity of thalassemia. Conclusions: The levels of ineffective erythropoiesis biomarkers were different across thalassemia genotypes. Splenectomy may improve clinical symptoms of patients with non-deletional alpha thalassemia but not of patients with beta-thalassemia. These findings demonstrate differences in the degree of ineffective erythropoiesis in thalassemia, which emphasizes the need for different treatment approaches among patients with different thalassemia genotypes.

Keywords: Phosphatidylserine exposed red blood cells, ineffective erythropoiesis biomarkers, thalassemia

Introduction

An imbalance of alpha-globin and beta-globin chains in thalassemia resulting in early apoptosis of maturing nucleated erythroid cells, leads to hematopoietic expansion as a compensatory mechanism. Early apoptosis of erythroid cells, referred to as "ineffective erythropoiesis", causes chronic anemia [1]. The degree of unbalanced globin chains is a key factor for determining the disease severity of thalassemia. Clinically, thalassemia disease is classified as 1) transfusion-dependent-thalassemia (TDT) and 2) non-transfusion-dependent-thalassemia (NTDT). While TDT is characterized by a lifelong requirement for blood transfusions, NTDT may require only occasional transfusions for the survivor [2].

Phosphatidylserine (PS) is a negatively charged phospholipid that is commonly located in the inner monolayer of RBC membranes. In patients with thalassemia, RBC membranes are oxidized and express negatively charged phosphatidylserine (PS) from the inner monolayer to the outer monolayer of RBC membranes. These are called, "phosphatidylserine-exposed red cells (PS-exposed RBCs)". The expression of

phosphatidylserine in the outer layer of RBCs causes premature cell death within the bone marrow (ineffective erythropoiesis) or peripheral circulation (hemolysis) [3, 4]. Moreover, the increased PS-exposed RBCs promote the hypercoagulable state in patients with thalassemia by activating coagulation pathways and stimulating platelets [5]. These complications occur more prevalently in NTDT patients who underwent splenectomy [6, 7].

Hepcidin is well established as a central regulatory molecule of iron homeostasis. Growth-differentiation factor-15 (GDF-15) is a member of the transforming growth factor-β (TGF-β) superfamily secreted from erythroid precursors into the circulation that suppresses hepcidin activity [8]. Studies have shown that a high GDF-15 level in patients with thalassemia was associated with iron overload, ineffective erythropoiesis, and clinical severity [9-12].

The soluble serum transferrin receptor (sTfR) is a reliable marker of enhanced erythropoietic activity. Higher levels of sTfR have been found in patients with thalassemia compared to healthy controls. Previous studies in patients with thalassemia have also demonstrated an association of high sTfR levels with extramedullary erythropoiesis and iron overload [13].

In southeast Asian countries, thalassemia disease is common and presents with complex genotypes. In Thailand, the prevalences based on phenotypes are estimated at 2.5-10% for α^0-thalassemia, 1-8% for hemoglobin (Hb) Constant Spring (CS) and Hb Paksé, 15-20% for α^+-thalassemia and 3-9% for β-thalassemia. Hemoglobin E (Hb E) can be found in between 30-50% of the population, especially in the northeastern region [14, 15]. Different thalassemia genotypes may have distinct degrees of ineffective erythropoiesis and iron homeostasis. Although several studies have addressed the association between the levels of PS-exposed RBCs, GDF-15, and sTfR and severity of thalassemia, none have simultaneously examined these parameters, which might help to improve the understanding of different severities of patients with different genotypes. This study was aimed to evaluate the PS-exposed RBCs levels as ineffective erythropoiesis biomarkers, and iron homeostasis in different thalassemia genotypes.

Patients and methods

The PS-exposed RBCs, GDF-15, and sTfR levels in patients with thalassemia, aged ≥18 years old, were evaluated at Srinagarind Hospital, Khon Kaen University, Thailand from April 2019 to January 2020. The medical records and laboratory data of all enrolled patients were collected. Liver iron concentrations (LIC) and cardiac iron concentrations were evaluated by the MRI-T2* technique. All blood samples were collected before patients received the next blood transfusion therapy.

All participants gave written informed consent. The research protocol was approved by the Institutional Review Board (IRB) for Human Research of Khon Kaen University (HE611361).

Hematologic values and DNA analysis

Hematologic data were recorded using Sysmex XN-9000 (Sysmex Co., Kobe, Japan). Serum ferritin levels were determined using the Roche Cobas e801 autoanalyzer (Roche Diagnostics, Mannheim, Germany). Identification of alpha-thalassemia-1 mutations (SEA and THAI deletions), alpha-thalassemia-2 mutations (3.7 and 4.2 kb deletions), Hb Constant Spring (Hb CS), and Hb Paksé (Hb PS) were performed routinely using multiplex-gap PCR and allele-specific PCR assays. Identification of β-thalassemia mutations and the Hb E gene were performed using allele-specific PCR as described [16].

PS-exposed RBCs measurements by flow cytometry

RBC staining and PS-exposed RBC level measurement were performed according to the previous study described by Pattanapanyasat et al. [8]. Fixed RBCs were measured using FACSCanto II flow cytometry (BD Biosciences, San Jose, CA) and analyzed with the BD FACSDiva version 6.1.3 software (BD Biosciences). The numbers of positive cells with both FITC-Annexin V and PE-glycophorin A were enumerated, and isotype control-positive cells were restricted to <0.3%.

GDF-15 and sTfR measurements

GDF-15 and sTfR concentrations were determined with standard commercially available enzyme-linked immunosorbent assay (ELISA)

kits: ab155432-GDF-15 Human ELISA (Abcam, Cambridge, UK) and Human sTfR ELISA (Bio-Vendor, Brno, Czech Republic).

Genotype groups

The patients were divided into 3 groups including: 1) beta-thalassemia, 2) non-deletional alpha thalassemia, and 3) deletional alpha thalassemia. The beta-thalassemia group was a group of patients with beta-globin gene mutations with or without Hb E. The non-deletional alpha thalassemia group included patients with Hb H-CS or Hb H-PS disease with or without Hb E, while the deletional alpha thalassemia group was a group of patients with Hb H disease without Hb CS or Hb PS.

Red blood cell transfusion

Transfusion-dependent thalassemia (TDT) is defined as the patients who receive RBC transfusions at less than a 6-week interval. Non-transfusion-dependent thalassemia (NTDT) is defined as those patients who received RBC transfusions at more than a 6-week interval.

Statistical analyses

Data were analyzed using SPSS 26.0 (IBM., IL, USA). Data for continuous variables were reported as mean ± standard deviation or median (range); data for categorical variables were reported as frequency and percentage. The Student's t-test was used to compare continuous data from the two groups. For data in a normal distribution, differences among groups were assessed using one-way analysis of variance (ANOVA) as post-hoc analysis. Non-parametric tests were performed using the Kruskal-Wallis test or Mann-Whitney U test. A *P*-value <0.05 was considered significant.

Results

The 139 patients were classified into three groups of thalassemia including: 1) beta-thalassemia (n=88, 63.3%), 2) non-deletional alpha-thalassemia with or without compound heterozygous Hb E (n=43, 30.9%), and 3) deletional alpha-thalassemia with or without compound heterozygous Hb E (n=8, 5.8%). A summary of baseline clinical characteristics is shown in **Table 1**. The mean age in this cohort was 34.5±14.5. Splenectomy was more common among patients with beta-thalassemia as compared to those with alpha-thalassemia. More than half of patients with beta-thalassemia were TDT. On the contrary, 81.4% of patients with non-deletional alpha thalassemia were NTDT. **Table 2** shows the beta-gene and alpha-gene mutations observed in this cohort. The most common beta-gene mutation is codon 41/42 (-TCTT) with codon 26 (GAG→AAG) or Hb E (47 patients, 33.8%) followed by codon 17 (A→T) with Hb E (18 patients, 12.9%) and codon 71/72 (+A) with Hb E (7 patients, 5%). In patients with non-deletional alpha-thalassemia, the most common genotype was alpha^0-thalassemia (SEA deletion) with Hb CS and heterozygous Hb E (21 patients, 15.1%) followed by alpha^0-thalassemia (SEA deletion) with Hb CS (18 patients, 12.9%). The most common mutation found in patients with deletional alpha thalassemia is SEA deletion with a 3.7 kb deletion (6 patients, 4.3%). The quantitative levels of PS-exposed RBCs, GDF-15, and sTfR are summarized in **Table 3**. The levels of PS-exposed RBCs were not different among the three types of thalassemia. Patients with beta-thalassemia had a significantly higher level of GDF15 (53,757.2±36,220.6) compared to those with non-deletional alpha thalassemia (20,179.0±15,117.5). sTfR was significantly elevated in patients with non-deletional alpha thalassemia (24.4±20.4) as compared to patients with beta-thalassemia (18.3±15.1) and deletional alpha-thalassemia (9.6±9.2). Box plots of PS-exposed RBCs, GDF-15, and sTFR levels in these groups of patients are shown in **Figure 1**.

Effect of splenectomy on the levels of PS-exposed RBCs, GDF-15, and sTFR

The level of PS-exposed RBCs was significantly higher among patients with beta-thalassemia and non-deletional alpha-thalassemia who underwent splenectomy. In patients with non-deletional alpha-thalassemia, the GDF-15 and LIC levels were significantly decreased and Hb levels trended to increase after splenectomy. On the contrary, among those patients with beta-thalassemia, no significant differences in the GDF-15 and Hb levels were observed between splenectomized and non-splenectomized patients. The levels of GDF-15 were significantly higher in patients with beta-thalassemia than in those patients with alpha thalas-

Table 1. Baseline clinical characteristics of 139 patients with thalassemia

Characteristic	All patients (n=139)	Beta-thalassemia group (n=88)		Non-deletional alpha thalassemia group (n=43)		Deletional alpha thalassemia group (n=8)	
		NTDT N=40	TDT N=48	NTDT N=35	TDT N=8	NTDT N=7	TDT N=1
Sex (n, %)							
Male	57 (41.0)	18 (45)	19 (39.6)	14 (40)	2 (25)	4 (57.1)	0
Female	82 (59.0)	22 (55)	29 (60.2)	21 (60)	6 (75)	3 (42.9)	1
Splenectomy (n, %)							
Yes	51 (36.7)	15 (37.5)	26 (54.2)	9 (25.7)	1 (12.5)	-	-
No	88 (63.3)	25 (62.5)	22 (45.8)	26 (74.3)	7 (87.5)	7 (100)	1
Age at enrollment (yrs.) Mean ± SD	34.5±14.5	32.9±11.5	34.1±13.2	32.4±14.8	36.8±16.5	56.3±18.7	18.1
Time after splenectomy (yrs.) Mean ± SD	10.6±7.8	3.8±6.5	6.9±8.9	1.3±2.9	1.5±4.2	-	-
RBC (10^6/μL)	3.65 (2.14-6.99)	3.4 (2.4-5.3)	3.3 (2.1-4.8)	4.5* (3.2-7.0)	3.9 (3.4-5.5)	4.9 (3.7-6.3)	4.2
Hb (g/dL)	7.6 (4.6-12.6)	7.3 (5.1-9.1)	7.3 (4.6-10.2)	8.3 (5.5-11.3)	7.6 (6.0-8.8)	9.0 (7.5-12.6)	7.8
MCV (fL)	69.1 (47.9-88.1)	67.3 (47.9-84.9)	70.25 (51-88.1)	68.7 (52.1-85.7)	68.5 (55.9-77.6)	66.0 (54.2-78.7)	70.5
Nucleated RBCs	5 (0-1682)	15.5 (0-1682)	89 (0-927)	0 (0-105)	0 (0-9)	0	0
Serum ferritin (ng/mL)	1007 (145-6515)	936* (145-6202)	1665 (428-6515)	621 (185-3292)	1371 (316-4329)	856 (488-2000)	896
Liver iron concentration (LIC) (mg/g dry weight)	9.3 (1.5-35.8)	10.1 (3.2-33.0)	11.4 (1.5-35.8)	4.4 (1.7-32.7)	9.7 (5-20)	3.7 (2-5.7)	5.2
Cardiac T2* (msec.)	39.1 (7.4-72.1)	38.5 (11.9-56.6)	39.5 (7.4-55.7)	39.0 (27.5-72.1)	40.6 (34.5-47.4)	43.3 (31.5-61.4)	33.1

NTDT = non-transfusion-dependent thalassemia; TDT = transfusion-dependent thalassemia.

Table 2. Thalassemia mutations by the genotype groups

Genotype groups	n, (%)
Beta-thalassemia group	
$\beta^{\text{codon 41/42 (-TTCT)}}$/Hb E	47 (33.8)
$\beta^{\text{codon 17 (A→T)}}$/Hb E	18 (12.9)
$\beta^{\text{codon 71/72 (+A)}}$/Hb E	7 (5.0)
$\beta^{\text{IVSII\#654 (C→T)}}$/Hb E	6 (4.3)
$\beta^{\text{IVSI\#1 (G→T)}}$/Hb E	3 (2.2)
$\beta^{\text{IVSI\#5 (G→C)}}$/Hb E	3 (2.2)
$\beta^{\text{codon 26 (G→T)}}$/Hb E	1 (0.7)
$\beta^{\text{codon 95 (+A)}}$/Hb E	1 (0.7)
$\beta^{\text{codon 123-125 (-ACCCCACC); Khon kaen}}$/Hb E	1 (0.7)
Homozygous β-thalassemia ($\beta^{\text{codon 41/42 (-TTCT)}}$)	1 (0.7)
Non-deletional alpha thalassemia	
EA Bart's disease with Hb Constant Spring ($--^{SEA}/\alpha\alpha^{CS}$, β/β^{E})	21 (15.1)
Hb H disease with Hb Constant Spring ($--^{SEA}/\alpha\alpha^{CS}$, β/β)	18 (12.9)
EA Bart's disease with Hb Pakse' ($--^{SEA}/\alpha\alpha^{PS}$, β/β^{E})	4 (2.9)
Deletional alpha thalassemia	
Hb H disease ($--^{SEA}/\alpha^{-3.7}$, β/β)	6 (4.3)
EA Bart's disease ($--^{SEA}/\alpha^{-3.7}$, β/β^{E})	1 (0.7)
Hb H disease ($--^{SEA}/\alpha^{-4.2}$, β/β)	1 (0.7)

semia for both splenectomized and non-splenectomized patients. The level of sTfR seemed to be higher in splenectomized patients although not reaching statistical significance (**Figure 2**).

Effect of RBC transfusion on the levels of PS-exposed RBCs, GDF-15, and sTFR

Box plots of the levels of PS-exposed RBCs, GDF-15, and sTfR in three groups of thalassemia according to RBC transfusion are shown in **Figure 3**. In beta-thalassemia, the patients who were NTDT had a lower Hb level and higher GDF-15 level compared to those with TDT. The Hb level was significantly higher in patients with non-deletional alpha-thalassemia who were NTDT as compared to those with TDT. The GDF-15 levels were not significantly different among patients with non-deletional alpha-thalassemia in both TDT and NTDT groups. None of the patients in the deletional alpha-thalassemia group were TDT (**Figure 3**). The levels of PS-exposed RBCs in TDT were higher than in NTDT, but the differences were not significant.

Discussion

The levels of GDF-15 and sTfR were different among thalassemia genotypes. These findings demonstrated differences in underlying ineffective erythropoiesis and iron homeostasis between patients with different genotypes. This study showed that the patients with beta-thalassemia and non-deletional alpha-thalassemia had a greater degree of ineffective erythropoiesis and iron overload compared to those with deletional alpha-thalassemia.

Phosphatidylserine-exposed red cells are abnormal red blood cells found in patients with thalassemia. This is a consequence of a migration of the negatively-charged phospholipids from the inner to the outer red blood cell membranes. An expression of phosphatidylserine on these red blood cells initiates coagulation pathways resulting in a hypercoagulable state in patients with thalassemia [5]. Previous studies have demonstrated an increase in the level of PS-exposed red cells in patients with thalassemia who underwent splenectomy which is a major risk factor for pulmonary hypertension in these patients [17, 18]. In this cohort, the levels of PS-exposed RBCs were not different among the three groups of thalassemia. These findings are inconsistent with the previous studies that found apoptosis of erythroid precursors in beta-thalassemia was significantly higher than in Hb H-CS disease. Accumulations of alpha-globin chains (either α^{A} in beta-thalassemia or α^{CS} in the patients with Hb CS) in erythroid precursors, however, are particularly effective in causing apoptosis. Nevertheless, beta-globin accumulation may also cause a moderate increase in apoptosis observed in marrow erythroid precursors in Hb H disease [19]. Notably, the levels of PS-exposed RBCs seem to be similar among patients with beta-thalassemia and those patients with non-deletional alpha-thalassemia. This suggests that the severity of non-deletional alpha thalassemia patients might be similar to patients with beta-thalassemia. In patients who underwent splenectomy, the level of PS-exposed RBCs was signifi-

Table 3. Level of PS-exposed RBCs, GDF15 and sTfR by genotype group

	Beta-thalassemia (n=88)	Non-deletional alpha thalassemia (n=43)	Deletional alpha thalassemia (n=8)	Normal Control [34, 35] (Chansai S *et al*. Huang Y *et al*.)	*P*-value
PS-exposed RBCs (%) Median, range	1.2 (0.1-4.1)	1.2 (0.1-4.0)	0.9 (0.4-2.5)	0.21 (0.16-0.27)	*0.765*
Mean ± SD	1.32±0.8	1.32±0.9	1.12±0.7		*0.813*
GDF15 (pg/mL) Median, range	43268.0[*,†] (6857.0-160500.0)	15928.6[*] (1219.5-60571.4)	10000.0[†] (7000-41928.6)	176.02 (58.57-953.62)	<0.001
Mean ± SD	53757.2±36220.6[*,†]	20179.0±15117.5[*]	15178.6±11906.0[†]		<0.001
sTfR (μg/mL) Median, range	14.4 (2.2-68.2)	20.0 (2.2-73.0)	7.0 (2.3-28.4)	2.63 (0.32-5.34)	*0.073*
Mean ± SD	18.3±15.1[*]	24.4±20.4[*,θ]	9.6±9.2[θ]		0.033

[*]significant different between Beta-thalassemia and Non-deletional alpha thalassemia. [†]significantly different between Beta-thalassemia and Deletional alpha thalassemia. [θ]significantly different between Non-deletional alpha thalassemia and Deletional alpha thalassemia.

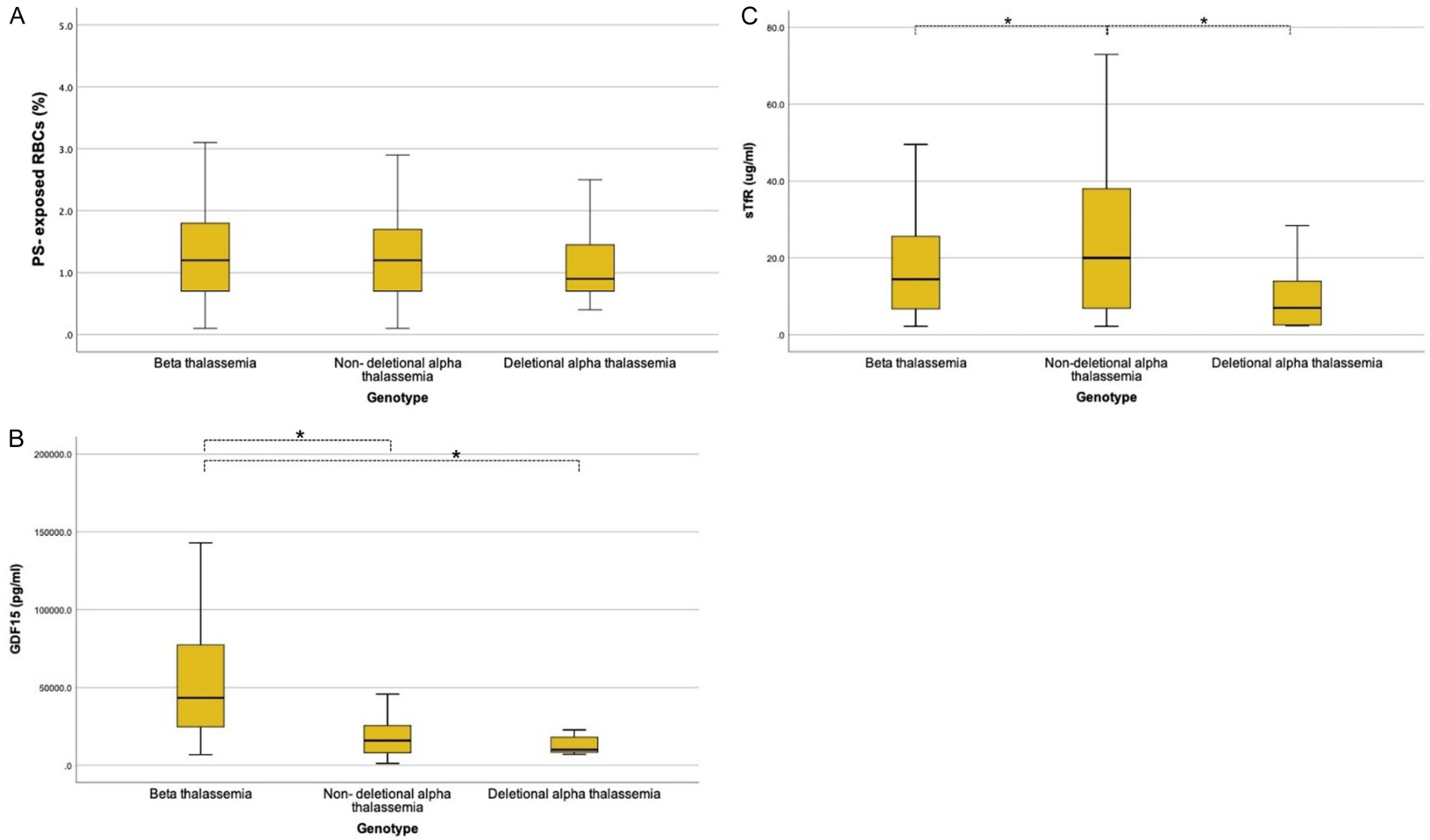
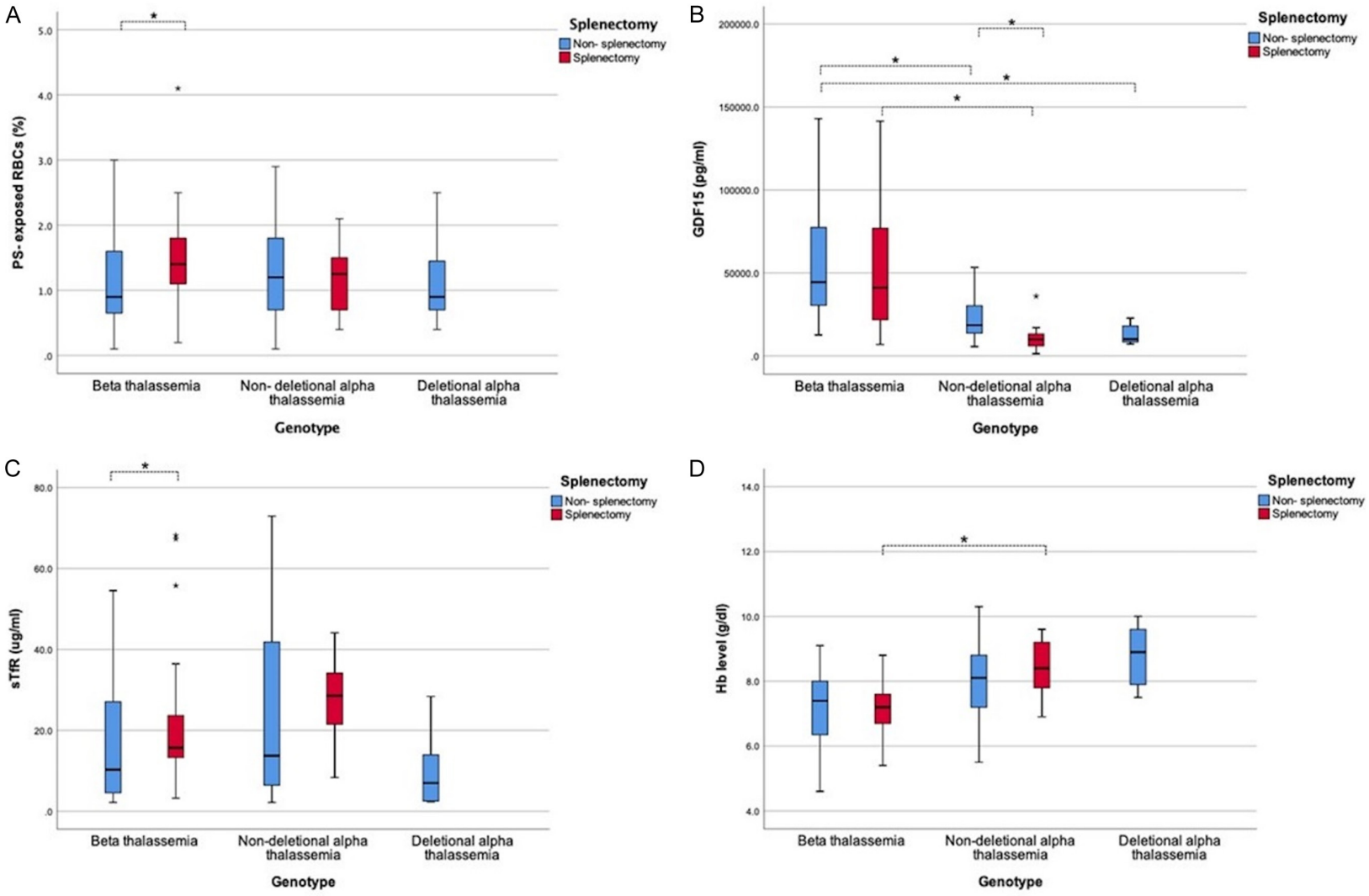


Figure 1. Graph box of phosphatidylserine-exposed red blood cells (PS-exposed RBCs), growth-differentiation factor-15 (GDF15), and soluble transferrin receptors (sTFR) levels in patients with thalassemia by genotype group.


A
PS- exposed RBCs (%)
5.0
4.0
3.0
2.0
1.0
.0
Splenectomy
Non- splenectomy
Splenectomy
Beta thalassemia
Non- deletional alpha thalassemia
Deletional alpha thalassemia
Genotype
B
GDF15 (pg/ml)
200000.0
150000.0
100000.0
50000.0
.0
Splenectomy
Non- splenectomy
Splenectomy
Beta thalassemia
Non-deletional alpha thalassemia
Deletional alpha thalassemia
Genotype
C
sTfR (ug/ml)
80.0
60.0
40.0
20.0
.0
Splenectomy
Non- splenectomy
Splenectomy
Beta thalassemia
Non-deletional alpha thalassemia
Deletional alpha thalassemia
Genotype
D
Hb level (g/dl)
14.0
12.0
10.0
8.0
6.0
4.0
Splenectomy
Non- splenectomy
Splenectomy
Beta thalassemia
Non-deletional alpha thalassemia
Deletional alpha thalassemia
Genotype

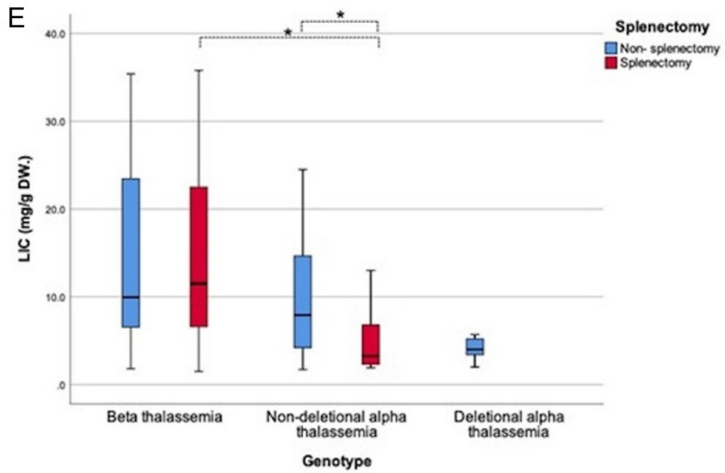
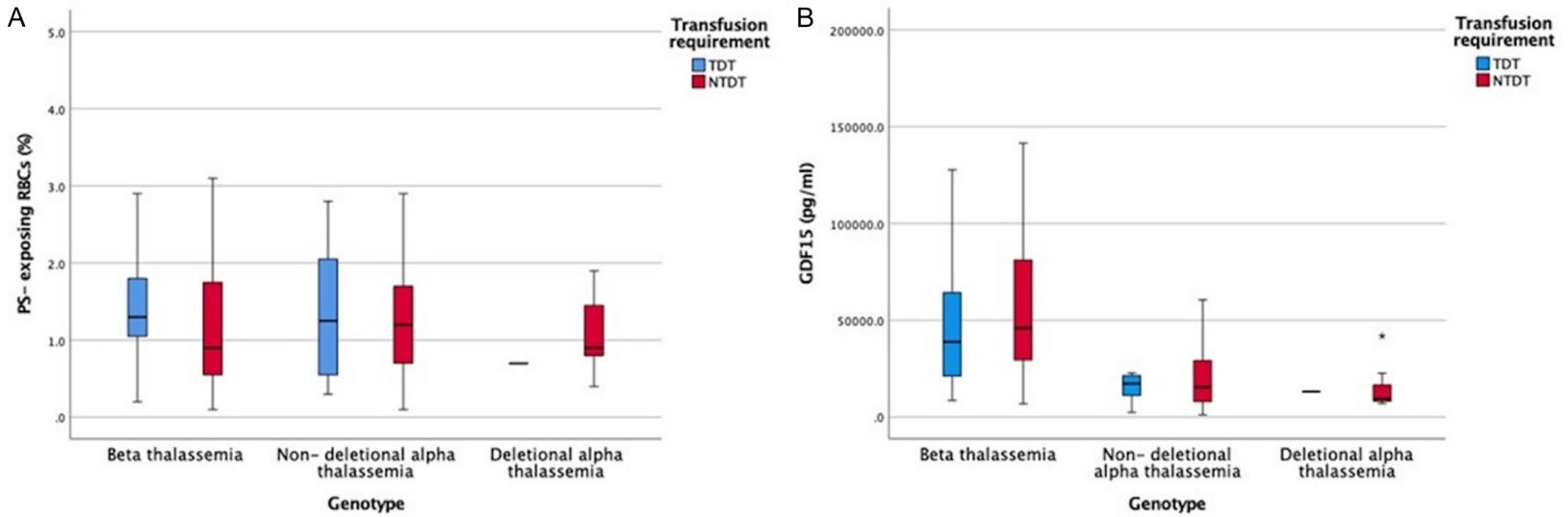


Figure 2. Graph box of PS-exposed RBCs, GDF15, sTFR, Hb level, and liver iron concentration (LIC) in patients with thalassemia by splenectomy status. (A) PS-exposed RBCs levels by splenectomy status, (B) GDF15 levels by splenectomy status, (C) sTFR levels by splenectomy status, (D) Hb levels by splenectomy status, and (E) LIC by splenectomy status.



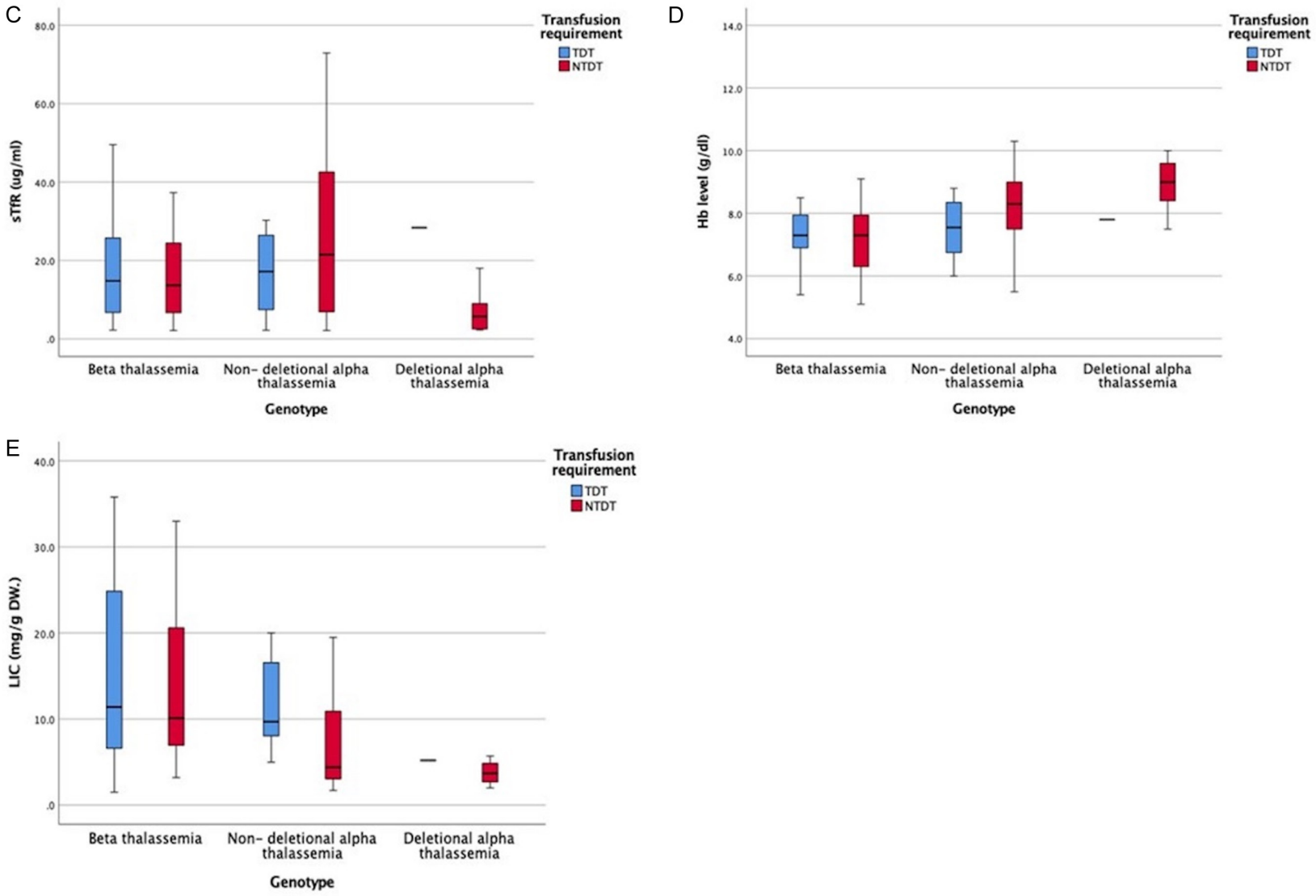


Figure 3. Graph box of PS-exposed RBCs, GDF15, sTFR, Hb levels and LIC in patients with thalassemia by RBC transfusion status. (A) PS-exposed RBCs level by RBC transfusion status, (B) GDF15 level by RBC transfusion status, (C) sTFR level by RBC transfusion status, (D) Hb level by RBC transfusion status, and (E) LIC by RBC transfusion status.

cantly elevated in beta-thalassemia compared to non-splenectomized patients. These findings are consistent with previous studies that found an increased expression of phosphatidylserine in abnormal RBCs in patients with beta-thalassemia/Hb E who underwent splenectomy [17]. The level of PS-exposed RBCs was not significantly different among patients with TDT or NTDT. These results may be explained by a regular RBC transfusion that can suppress the level of PS-exposed RBCs in splenectomized patients [20]. Moreover, adequate RBC transfusions in these patients also ameliorate clinical symptoms of pulmonary hypertension [20, 21].

GDF-15 is, among other functions, a marker of ineffective erythropoiesis secreted by erythroid progenitors. It functions to inhibit erythroid differentiation and suppress hepcidin activity. Suppression of hepcidin promotes duodenal iron absorption that contributes to iron overload in patients with thalassemia [4, 22]. In this study, patients with beta-thalassemia had the highest level of GDF-15 as compared to other genotypes. Moreover, the levels of GDF-15 in both splenectomized and non-splenectomized patients with beta-thalassemia were significantly higher than in alpha-thalassemia patients. These findings suggest that patients with beta-thalassemia had a higher degree of ineffective erythropoiesis than those with alpha-thalassemia [23, 24]. Moreover, a recent study found that GDF-15 levels correlated with severity in patients with beta-thalassemia. The study showed significant increases in GDF-15 levels as follows: 26-fold (in TDT), 6-fold (in NTDT), and 2-fold (in beta-thalassemia carriers) as compared to normal controls. Therefore, GDF-15 may be a biomarker that represents underlying ineffective erythropoiesis in those patients with thalassemia [25]. An interesting result is that the patients with non-deletional alpha thalassemia who underwent splenectomy had significantly higher Hb levels and lower GDF-15 levels than non-splenectomized patients. Zhou *et al.* found that splenectomy improved anemia in patients with hemoglobin H Constant Spring (Hb H-CS) disease, but the GDF-15 levels were not reduced in splenectomized patients [26]. Nevertheless, the results from this study imply that splenectomy may have some benefit in patients with non-deletional alpha-thalassemia. On the contrary, the level of GDF-15 was not different among patients with beta-thalassemia based on their splenectomy status. This evidence, therefore, suggests that splenectomy should not be recommended in these patients. In addition, the therapeutic rationale for splenectomy in beta-thalassemia major (TM) is to decrease the blood transfusion requirement and reduce iron overload [27]. Splenectomy, however, increased the risk of venous thrombosis and pulmonary hypertension, alongside infections after splenectomy in thalassemic patients [28]. The level of GDF-15 was slightly increased in patients with beta-thalassemia who were NTDT, but there was no statistical significance. This result may be due to almost half of the patients with beta-thalassemia in this cohort receiving regular blood transfusions.

The soluble serum transferrin receptor is a marker that represents active erythropoiesis and is secreted by erythroid precursors. The level of sTfR is intensified in particular conditions including erythroid hyperplasia and depletion of body iron stores [29]. Patients with non-deletional alpha-thalassemia had a significantly higher level of sTfR than patients with beta-thalassemia and deletional alpha thalassemia. These findings are inconsistent with those of a previous study reporting that in NTDT patients, sTfR levels were significantly higher in beta-thalassemia than in Hb H disease [30]. A higher RBC count and sTfR levels in non-deletional alpha-thalassemia is consistent with a previous study that showed that sTfR levels had a significantly positive correlation with RBC count [31]. This result suggested that alpha-thalassemia syndromes have a relatively higher red cell count compared to Hb levels due to increased erythropoietic activity. Additionally, the level of sTfR was higher among patients with non-deletional alpha-thalassemia who underwent splenectomy than in non-splenectomized patients. This is consistent with the previous finding that splenectomy in Hb H-CS disease improved anemia but did not reduce the sTfR levels [26]. In patients with beta-thalassemia who underwent splenectomy, the level of sTfR is significantly higher than in non-splenectomized patients. This result might be helpful in that splenectomy could improve only sTfR levels in patients with beta-thalassemia. The sTfR levels seem to be higher among patients with non-deletional alpha-thalassemia who were NTDT compared to those patients with TDT, but the liver iron concentration was slightly higher in the patients who received regular

blood transfusions than those patients who received an occasional blood transfusion. In contrast, the sTfR levels in patients with beta-thalassemia were not different in either transfusion status. This result is consistent with a previous study that showed that sTfR is a good marker of expanded erythropoiesis, reflecting the amount of iron absorption in the explored NTDT patients [32]. The previous study showed that the sTfR is a marker of erythropoiesis and had a strong correlation with hepcidin level. Elevation of sTfR is associated with hepcidin reduction which promotes iron absorption in the anemic state [33]. This result, therefore, demonstrated that the high levels of sTfR in patients with non-deletional alpha-thalassemia who were just occasionally transfused represented inadequate blood transfusion in this group of patients. The sTfR therefore might be less specific for ineffective erythropoiesis and therefore might be considered as a common biomarker of erythropoietic activity [22, 33].

The limitations of this study were as follows: (1) The number of patients with deletional α-thalassemia was too small to demonstrate statistically significant findings as compared to the other genotype groups. (2) None of the patients with deletional α-thalassemia had undergone splenectomy. Therefore, the effect of splenectomy in this genotype group could not be explored. This study, however, clearly demonstrates the differences in the level of ineffective erythropoietic biomarkers among patients with beta-thalassemia and alpha-thalassemia with a variety of thalassemia genotypes.

In conclusion, the levels of GDF-15 and sTfR were different across the genotype groups. Patients with beta-thalassemia and non-deletional alpha-thalassemia had the highest degree of ineffective erythropoiesis. The level of PS-exposed RBCs was significantly high among splenectomized patients with beta-thalassemia. Patients with non-deletional alpha-thalassemia who underwent splenectomy showed improvement of anemia and degree of ineffective erythropoiesis. Splenectomy can be considered in patients with non-deletional alpha-thalassemia but not in patients with beta-thalassemia. These findings highlight the need for different therapeutic approaches between beta-thalassemia and alpha-thalassemia.

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Disclosure of conflict of interest

None.

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