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ORIGINAL ARTICLE

Increased endothelial activation in α-thalassemia disease

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Abstract

One complication of thalassemia is thromboembolism (TE), which is caused by an abnormal red blood cell surface, as well as endothelial and platelet activation. These findings are commonly observed in severe β-thalassemia. However, limited information on α-thalassemia exists. This study enrolled subjects with deletional and non-deletional α-thalassemia and normal controls (NC). Plasma and serum of subjects were tested for endothelial activation markers including thrombomodulin (TM), vascular cell adhesion molecule-1 (VCAM-1), and von Willebrand factor antigen as well as platelet activation markers including thromboxane B2 and platelet factor 4. A total of 179 subjects were enrolled: 29 in the deletional group (mean age 13.3 ± 4.4 years), 31 in the non-deletional group (mean age 12.9 ± 4.8 years), and 119 in the NC group (mean age 13.6 ± 3.0 years). Twenty nine percent of subjects in the non-deletional group received regular red blood cell transfusion and iron chelator administration. Serum ferritin level was higher in the non-deletional group than that in the deletional group. Multivariate analysis demonstrated that VCAM-1 and TM levels were increased significantly in α-thalassemia compared with NC group (816.8 ± 131.0 vs 593.9 ± 49.0 ng/ml, and 4.9 ± 0.7 vs 4.0 ± 0.4 ng/ml, $P < 0.001$ respectively). VCAM-1 and TM levels in the non-deletional group were significantly higher than that in the deletional group. The present study demonstrated endothelial activation in children with α-thalassemia disease, especially those in the non-deletional group, which might be one risk factor for TE in α-thalassemia disease.

Keywords α-Thalassemia · Children · Endothelial activation · Thromboembolism

Abbreviations

BMI	Body mass index
β-TG	β-Thromboglobulin
CBC	Complete blood count
CECs	Circulating endothelial cells
CS	Hb constant spring
DNA	Deoxyribonucleic acid
ELAM	E-selectin
F1.2	Prothrombin fragment 1 + 2
Hb	Hemoglobin
HPLC	High-performance liquid chromatography
ICAM-1	Intercellular adhesion molecule-1
MCH	Mean corpuscular hemoglobin
MCV	Mean corpuscular volume
NC	Normal controls
NTDT	Non-transfusion dependent thalassemia
PF4	Platelet factor 4
PS	Hb Paksé
RBC	Red blood cell
RDW	Red cell distribution width
TAT	Thrombin-antithrombin complex
TBX2	Thromboxane B2
TDT	Transfusion-dependent thalassemia
TE	Thromboembolism
TM	Thrombomodulin
VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelial growth factor
VWF:Ag	Von Willebrand factor antigen

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Introduction

Alpha (α)-thalassemia disease, an autosomal recessive inherited hemolytic anemia, is caused by abnormal α-globin chain synthesis from deletional and/or non-deletional

mutations of the α-globin genes [1–3]. It is prevalent in Mediterranean countries, Central Asia, Southeast Asia, the North Coast of Africa, and North America [4–6].

Four α-globin genes are located in chromosome 16. The most severe form of α-thalassemia disease is hemoglobin (Hb) Bart's hydrops fetalis, in which all 4 α-globin genes are deleted. Therefore, patients usually die from severe anemia after birth. A less severe form is HbH disease (termed deletional type α-thalassemia disease), in which 3 α-globin genes are deleted. Common types of α-deletions include Southeast Asian ($--^{SEA}$), Thai ($--^{THAI}$), 3.7 kb of deoxyribonucleic acid(DNA) ($-\alpha^{3.7}$), and 4.2 kb of DNA ($-\alpha^{4.2}$). Almost all deletional types or HbH patients have non-transfusion-dependent thalassemia (NTDT). However, patients with a non-deletional type such as Hb constant spring (CS, $-\alpha^{CS}\alpha$) or Hb Paksé (PS, $-\alpha^{PS}\alpha$) usually have more severe symptoms and require red blood cell (RBC) transfusion [7–9]. Non-deletional α-thalassemia gives rise to a more severe reduction in α-globin chain synthesis compared with the deletional type because mutations usually affect the α2 gene, which normally accounts for 2–3 times more α-globin mRNA and α-globin chain synthesis than the α1 gene [8, 10–12].

Thromboembolism (TE) is one of the complications of thalassemia. The incidence rates in β-thalassemia major and β-thalassemia intermedia were reported as 0.9%–4.0% and 3.9%–29.0%, respectively [13]. Even though clinical manifestations of α-thalassemia disease are not as severe as β-thalassemia, TE was reported in α-thalassemia patients, especially adult post-splenectomized patients with venous TE, pulmonary embolism, or portal vein thrombosis [14–18].

The mechanisms of hypercoagulability in α-thalassemia disease have been studied since 1995, mostly in adult patients. Previous studies demonstrated the increased susceptibility of RBC to monocyte phagocytosis because of reduced sialic acid content and the irregular distribution of sialic acid on α-thalassemia RBC surfaces compared with β-thalassemic RBC [19]. Evidence for endothelial activation was indicated by increased thrombomodulin (TM) [20], intercellular adhesion molecule-1 (ICAM-1), E-selectin (ELAM-1), vascular cell adhesion molecule-1 (VCAM-1) [21, 22], circulating endothelial cells (CECs), and vascular endothelial growth factor (VEGF), which are released from activated vascular endothelial cells. Furthermore, protein C and protein S levels in all types of thalassemia patients were significantly decreased compared with normal controls (NCs) [23]. Levels of β-thromboglobulin (β-TG), a marker of platelet activation, were significantly higher in post-splenectomized HbH compared with non-splenectomized HbH patients [24]. However, in children, the coagulation markers; D-dimer, thrombin-antithrombin complex (TAT), and prothrombin fragment 1 + 2 (F1.2) were not significantly increased compared with NC [25]. The studies of endothelial and platelet activation especially in children with α-thalassemia disease are limited. Therefore, the current study investigated endothelial and platelet activation in children with α-thalassemia disease.

Materials and methods

Study population

Subjects aged 1–20 years who were diagnosed with α-thalassemia disease at the Hematology Clinic of the Department of Pediatrics, Ramathibodi Hospital, Faculty of Medicine, Mahidol University were enrolled into the study from 1 March 2016 to 28 February 2017. Subjects aged 1–20 years, who had normal Hb typing and no medical conditions, were enrolled as the NC group. Ethical approval for the study protocol was obtained from the Ethical Clearance Committee on Human Rights Related to Research to Research Involving Human Subjects, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Thailand (ID 12-59-09).

Inclusion criteria

Subjects diagnosed with α-thalassemia disease from hemoglobin typing by high-performance liquid chromatography (HPLC) and DNA analysis by multiplex PCR for α-thalassemia were enrolled. Then, subjects were divided into non-deletional α-thalassemia and deletional α-thalassemia disease groups based on the result of the DNA study. Informed consent was obtained from all subjects for being included in the study.

Exclusion criteria

Subjects who received blood components over than RBC 1 month and antiplatelet medication (e.g., aspirin) 1 week prior to blood collection were excluded. In addition, subjects who had an infection within 1 week or other underlying medical conditions were not enrolled in this study.

Data collection

In the α-thalassemia group, age, gender, body mass index (BMI) Z-score, result of hemoglobin typing and DNA study, liver and splenic size, history and frequency of RBC transfusion, iron chelation, complete blood count (CBC), and ferritin level were recorded. In the NC group, age, gender, BMI Z-score, hemoglobin typing, CBC, and ferritin level were recorded.

BMI Z-score provides a relative measure of adiposity adjusted for age and gender. For children below 5 years old, BMI Z-score was calculated according to WHO Anthro for personal computers, version 3.2.2, 2011: Software for assessing

growth and development of the world's children. Geneva: WHO, 2010 (http://www.who.int/childgrowth/software/en/) and children 5 to 19 years old, BMI Z-score was calculated by WHO AnthroPlus for personal computers Manual: Software for assessing growth of the world's children and adolescents. Geneva: WHO, 2009 (http://www.who.int/growthref/tools/en/).

Laboratory assays

After receiving informed consent, a sample of venous blood was drawn from each individual by the double-syringe technique and divided into two tubes supplemented with 3.2% sodium citrate (9:1) and a plain tube for clotted blood. Specimens with sodium citrate and clotted blood were centrifuged within 1 h after collection at 1500×*g* for 10 min at room temperature. Plasma taken from the sodium citrate-anticoagulated blood sample was kept at − 80 °C until testing for von Willebrand factor antigen (VWF:Ag) by ELISA (Dako, Glostrup, Denmark). Serum taken from clotted blood was kept at − 80 °C until testing for vascular cell adhesion molecule-1 (VCAM-1) using VCAM-1/CD106 Quantikine ELISA Kits (Bio-Techno/R&D Systems, Minneapolis, MN, USA); TM using Thrombomodulin (CD141) Human ELISA Kits (Abcam, UK); Thromboxane B2 (TBX2) using Thromboxane B2 Parameter Assay Kits (Bio-Techno/R&D Systems); and platelet factor 4 (PF4) levels using Human CXCL/PF4 Quantikine ELISA Kits (Bio-Techno/R&D Systems, USA). ELISA results were obtained using a plate reader (Biotek Instruments, Winooski, USA).

Statistical analysis

PASW 18.0 statistical software (SPSS, Chicago, IL, USA) was used for statistical analyses. Descriptive statistics were expressed as the mean ± SD, median (interquartile range or range), and percent. Differences between groups were assessed using the independent *t* test, chi-square test, and Mann-Whitney *U* test. Multivariate analysis was used to adjust the parameters that had been reported or statistically significantly shown to affect the results of the present study. A statistically significant difference was defined when $P < 0.05$.

Results

Demographic data

A total of 179 subjects were enrolled in this study, of which 60 subjects had α-thalassemia disease and 119 were NC. There was no statistical difference in age, gender, and BMI Z-score between α-thalassemia subjects and NC. The 60 subjects, age ranged from 5 to 20 years, were separated into 2 groups: 31 non-deletional α-thalassemia disease group and 29 deletional α-thalassemia disease group**.** In the non-deletional α-thalassemia group, 23 subjects (74.2%) had HbH/CS disease (−−/α^{CS}α, β/β), 5 subjects (16.1%) had AE Bart's disease with HbCS (−−/α^{CS}α, β^{E}/β), and 3 subjects (9.7%) had HbH/PS disease (−−/α^{PS}α, β/β). In the deletional α-thalassemia group, 27 subjects (93.1%) had HbH disease (−−/−α, β/β), 1 subject (3.5%) had AE Bart's disease (−−/−α, β^{E}/β), and 1 subject (3.5%) had EF Bart's disease (−−/−α, β^{E}/β^{E}).

Nine out of 31 subjects in the non-deletional α-thalassemia group (29.0%) received regular RBC transfusion: 6 (19.4%) subjects received transfusion every 4 weeks and 1 subject each (3.2%) received transfusion every 3 weeks and every 8 weeks. Eight subjects in the transfusion subgroup were diagnosed with HbH/CS disease and 1 was diagnosed with AE Bart's disease with HbCS. Six subjects had received deferiprone as an iron chelator. The median (range) times of patients receiving regular RBC transfusion and iron chelator were 4.5 (2–8) years and 3.1 (1–6) years, respectively. The demographic data of all subjects is shown in Table 1.

None of the α-thalassemia subjects had a history of thromboembolic events or splenectomy. Liver and splenic size, history of regular RBC transfusion, and iron chelator use were significantly higher in the non-deletional α-thalassemia group compared with the deletional α-thalassemia group (Table 1).

Hematologic parameters and ferritin level

The α-thalassemia group had a significantly lower Hb, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) levels, but higher red cell distribution width (RDW), and ferritin level compared with the NC group ($P < 0.05$). In the subgroup analysis, the non-deletional group had significantly lower RBC counts, hemoglobin, and platelet count levels, but higher ferritin level compared with the deletional group ($P < 0.05$) (Table 1).

Endothelial activation parameters

The multivariate analysis, adjusted endothelial activation parameters with age, gender, BMI Z-score, Hb, and ferritin levels, demonstrated that VCAM-1 level in the α-thalassemia group was significantly higher when compared with the NC group (816.8 ± 131.0 vs 593.9 ± 49.0 ng/ml, $P < 0.001$). VCAM-1 level in the non-deletional group was significantly higher when compared with the deletional group (886.0 ± 140.4 vs 742.8 ± 63.9 ng/ml, $P < 0.001$). TM level was significantly higher in the α-thalassemia group compared with

Table 1 Demographic data of α-thalassemia patients and the normal control (NC) group

Parameter	α-Thalassemia patients		NC
	Non-deletional group $n = 31$	Deletional group $n = 29$	$n = 119$
Genotype (*N*)			
—— $^{SEA}/-\alpha^{3.7}$		21	
—— $^{THAI}/-\alpha^{3.7}$		2	
—— $^{SEA}/-\alpha^{4.2}$		1	
—— $^{SEA}/-\alpha^{3.7}$, β^E/β		1	
—— $^{SEA}/-\alpha^{3.7}$, β^E/β^E		1	
—— ^{SEA}/HBA1 c.264C > G		1	
—— ^{SEA}/HBA2 c1 A del		1	
—— ^{SEA}/unknown mutation		1	
—— $^{SEA}/\alpha^{CS}\alpha$	23		
—— $^{SEA}/\alpha^{PS}\alpha$	3		
—— $^{SEA}/\alpha^{CS}\alpha$, β^E/β	5		
Sex, female/male	13:18	14:15	62:57
Age, years	12.9 ± 4.8	13.3 ± 4.4	13.6 ± 3.0
BMI Z-score	− 0.4[2] (− 1.4,0.3)	− 0.9 (− 1.6,0.8)	− 0.4 (− 1.2,0.8)[b]
Liver (cm) below RCM	1 (0, 3)*	0 (0,0)	ND
Spleen (cm) below LCM	2 (0, 5)*	0 (0, 0)	ND
Regular RBC transfusion, *n*/%	9.0/29%*	0.0	ND
Chelator, *n*/%	6.0/19%*	0.0	ND
Laboratory tests			
RBC (× 10^6/μL)	4.5 ± 0.7*	5.9 ± 0.8	5.0 ± 0.5
Hb (g/dL)	8.5 ± 1.1*	9.8 ± 0.9	13.7 ± 1.3**
MCV (fL)	67.7 ± 8.2*	56.6 ± 5.7	83.6 ± 6.7**
MCH (pg)	19.0 ± 3.2*	16.7 ± 1.8	27.5 ± 2.6**
RDW, %	24.2 ± 4.6	22.4 ± 3.1	13.0 ± 1.2**
Platelet (× 10^3/μL)	291.0 (238.0, 349.0)*	351.0 (298.0, 411.0)	294.0 (252.0, 337.0)
Ferritin (ng/mL)	209.2 (103.0, 353.6)*	64.5 (41.1, 103.1)	56.6 (41.1–77.0)**[a]

$-\alpha^{3.7}$; g.34164_37967 del 3804, $-\alpha^{4.2}$; g.31291_35545 del 4255, α^{CS}; HBA_2 c.427 T > C, α^{PS}; HBA_2 c.429A > T, $-^{SEA}$; g.26264_45564 del 19,301, $-\beta^E$; HBB:c.79G > C, $-^{THAI}$; g. 10664_44164 del 33,501

Hb hemoglobin, *LCM* left costal margin, *MCH* mean corpuscular hemoglobin, *MCV* mean corpuscular volume, *ND* no data, *RBC* red blood cell, *RCM* right costal margin, *RDW* red cell distribution width

***Statistical significance between deletional α-thalassemia and non-deletional α-thalassemia group; Statistical significance between α-thalassemia diseases and NC

[a] $n = 60$

[b] $n = 98$

the level in the NC group (4.9 ± 0.7 vs 4.0 ± 0.4 ng/ml, $P < 0.001$). TM level in the non-deletional group was significantly higher when compared with the level in the deletional group (5.1 ± 0.7 vs 4.8 ± 0.6 ng/ml, $P < 0.001$). VWF:Ag levels were in the normal range in all groups; the significantly higher level was demonstrated in NC when compared to the α-thalassemia groups (115.6 ± 9.6 vs 96.5 ± 9.9%, $P = 0.004$). VWF Ag level was also significantly lower in non-deletional when compared to the deletional groups (93.0 ± 8.8 vs 100.3 ± 9.7%, $P < 0.001$) (Table 2; Fig. 1).

Platelet activation parameters

The multivariate analysis, adjusted platelet activation parameters with age, gender, BMI Z-score, and Hb levels, demonstrated that PF4 and TBX2 levels in the α-thalassemia group were not significantly higher than the levels in the NC group. There was no difference in TBX2 level in the non-deletional group compared with the level in the deletional group. The PF4 level in the non-deletional group was significantly lower when compared with the level in the deletional group

(6980.5 ± 992.3 vs 7797.1 ± 887.2 ng/ml, $P < 0.001$) (Table 2; Figs. 1).

Subgroup analysis of transfusion and non-transfusion-dependent α-thalassemia

Subjects with α-thalassemia disease were divided into NTDT and transfusion-dependent thalassemia (TDT) group according to a history of regular RBC transfusion [26]. The multivariate analysis, adjusted endothelial activation parameters with age, gender, BMI Z-score, Hb, and ferritin levels, demonstrated that VCAM-1 and TM levels were significantly higher in the TDT group compared with the NTDT group (1039.6 ± 164.1 vs 775.5 ± 73.8 ng/ml, $P < 0.001$ and 5.5 ± 0.5 vs 4.9 ± 0.7 ng/ml, $P = 0.015$ respectively). The PF4 and TBX2 levels were significantly lower in the TDT group compared with the levels in the NTDT group (6702.1 ± 735.7 vs 7465.2 ± 1010.9 ng/ml, $P = 0.035$ and 2.9 ± 1.2 vs 4.4 ± 1.8 ng/ml, $P = 0.02$ respectively) (Fig. 2).

Discussion

Thromboembolic events have been reported in α-thalassemia patients especially in splenectomized patients [14–18]. Mechanisms related to the hypercoagulable state in α-thalassemia include firstly endothelial activation shown by increased CECs [23], endothelial markers such as ELAM-1 in adults with HbH disease and HbH/CS disease [22], TM levels in adults with HbH disease and HbH/CS disease [20], and ICAM-1 levels in adolescent HbH disease with or without splenectomy [24]. The second was platelet activation indicated by increased β-TG levels [24]. The third was coagulation stimulation demonstrated by increased F1.2 levels [24]. However, coagulation stimulation, including D-dimer, F1.2, and TAT levels, was not significantly increased in a study that enrolled patients with a lower age compared with previous study (median 13 years vs 16 years old) [24, 25]. There have been limited studies of endothelial and platelet activations especially in children with α-thalassemia disease; therefore, the present study enrolled children diagnosed with α-thalassemia disease with mean age of 13.1 ± 4.6 years. We divided the subjects into two groups, non-deletional and deletional α-thalassemia disease, because more severe symptoms are associated with non-deletional α-thalassemia disease [1, 4, 8–11, 19, 27–30]. Patients in the non-deletional α-thalassemia group received more RBC transfusions. In addition, 29% of patients in the non-deletional α-thalassemia group received regular RBC transfusion every 3–8 weeks to maintain Hb level of at least 8 g/dL. The mean ± SD of Hb level in the non-deletional group was lower when compared with the level in the deletional group. Ferritin level was higher in the non-deletional group compared with the level in the deletional group due to higher number of subjects received RBC transfusion.

Evidence for endothelial activation was demonstrated in the present study by increased VCAM-1 and TM levels in α-thalassemia disease compared with the levels in the NC group. Patients in the non-deletional group had significantly higher VCAM-1 level compared with the level in the deletional group. In addition, patients who received regular RBC transfusion in the non-deletional α-thalassemia group had significantly higher VCAM-1 and TM levels when compared with those who received occasional RBC transfusion. This result indicated that severe symptoms of α-thalassemia patients were related to increased endothelial activation. The present study demonstrated the increased endothelial activation in α-thalassemia disease, which was similar to the result in previous studies of children using ICAM-1 as a marker and in adults using CECs, TM, and ELAM-1 as markers of endothelial activation [20, 22–24].

For platelet activation, the present study selected TBX2 and PF4 as markers of platelet activation. TBX2 is converted from thromboxane A2, which increases during platelet activation while PF4 is stored in α granules and released into the bloodstream after platelet activation. Both parameters were not significantly increased in the non-deletional and deletional groups when compared with the levels in the NC group. The difference in our results compared with the previous study that reported increased β-TG levels in α-thalassemia disease [24] might be explained by the younger age group, as higher age group is associated with more platelet activation than the lower age group [31]. The other parameters that might affect the results were the lower severity of symptoms and ferritin levels in the present study. The more severe symptoms were observed in the previous study which enrolled 11 splenectomized α-thalassemia patients and 20 non-splenectomized α-thalassemia patients, while the present study had no patients with splenectomy and a higher baseline Hb level compared with those in the previous study [24]. Another important aspect might be the treatment with regular RBC transfusion in the present study, which accounted for 29% of patients in the non-deletional group. The regular RBC transfusion in thalassemia was able to reduce platelet activation in severe thalassemia disease [32]. This evidence was demonstrated by our analysis of patients stratified into TDT and NTDT groups. TBX2 and PF4 levels in the TDT group were significantly lower when compared with the NTDT group. Normally, multivitamin and folic acid is given to thalassemia patients. At our institute, vitamin E (10 U/kg) is an option to all thalassemia patients for the antioxidant effect. The present study showed that 93.3% of the patients desired to

Table 2 Multivariate analysis compared laboratory results of α-thalassemia subgroup and normal control (NC) group

Parameter	Non-deletional group $n = 31$	Adjusted non-deletional group[a] $n = 31$	Deletional group $n = 29$	Adjusted deletional group[a] $n = 29$	α-Thalassemia group $n = 60$	Adjusted α-thalassemia group[a] $n = 60$	NC	Adjusted NC[a]	*P* value[b]	*P* value[c]	*P* value[d]	*P* value[e]	*P* value[f]
VCAM-1 (ng/ml)	920.9 ± 202.0	886.0 ± 140.4	699.1 ± 126.5	742.8 ± 63.9	813.7 ± 202.1	816.8 ± 131.0	602.0 ± 172.5	593.9 ± 49.0	< 0.001[a]	< 0.001[a]	< 0.001[a]	< 0.001[a]	< 0.001[a]
VWF:Ag (%)	88.2 ± 28.6	93.0 ± 8.8	103.2 ± 32.3	100.3 ± 9.7	95.5 ± 31.1	96.5 ± 9.9	114.8 ± 48.4	115.6 ± 9.6	< 0.001[a]	< 0.001[a]	< 0.001[a]	0.004[a]	< 0.001[a]
TM (ng/ml)	5.1 ± 1.6	5.1 ± 0.7	4.7 ± 1.2	4.8 ± 0.6	4.9 ± 1.4	4.9 ± 0.7	4.0 ± 1.2	4.0 ± 0.4	< 0.001[a]	< 0.001[a]	< 0.001[a]	0.063[a]	< 0.001[a]
TBX2 (ng/ml)	3.5 ± 4.5	4.0 ± 1.8	4.8 ± 6.3	4.3 ± 1.8	4.1 ± 5.4	4.1 ± 1.8	6.5 ± 4.5	3.9 ± 1.9	0.809[a]	0.366[a]	0.483[a]	0.533[a]	0.652[a]
PF4 (ng/ml)	6767.2 ± 1673.7	6932.5 ± 834.5	8025.0 ± 2065.8	7797.7 ± 995.9	7375.2 ± 1962.5	7350.7 ± 1007.4	9947.6 ± 3086.5	10,055.3 ± 10,483.5	< 0.001[a]	< 0.001[a]	< 0.001[a]	< 0.001[a]	< 0.001[a]

Number of normal controls for VCAM−1 = 60, VWF:Ag = 60, TM = 60, TBX2 = 20 and PF4 = 39. Mean age (year): VCAM-1, VWF:Ag, TM =13.8 ± 1.1, TBX2 = 12.3 ± 2.5 and PF4 = 14.0 ± 4.7

NA not available, *PF4* Platelet factor 4, *TBX2* thromboxane B2, *TM* thrombomodulin, *VCAM-1* vascular cell adhesion molecule-1, *VWFAg* von Willebrand factor antigen

[a] The values are adjusted with age, sex, BMI Z-score, Hb and ferritin

[b] Comparison of non-deletional group and NC

[c] Comparison of deletional group and NC

[d] Comparison of α-thalassemia group and NC

[e] Comparison of the non-deletional and deletional groups

[f] Comparison of non-deletional, deletional group and NC

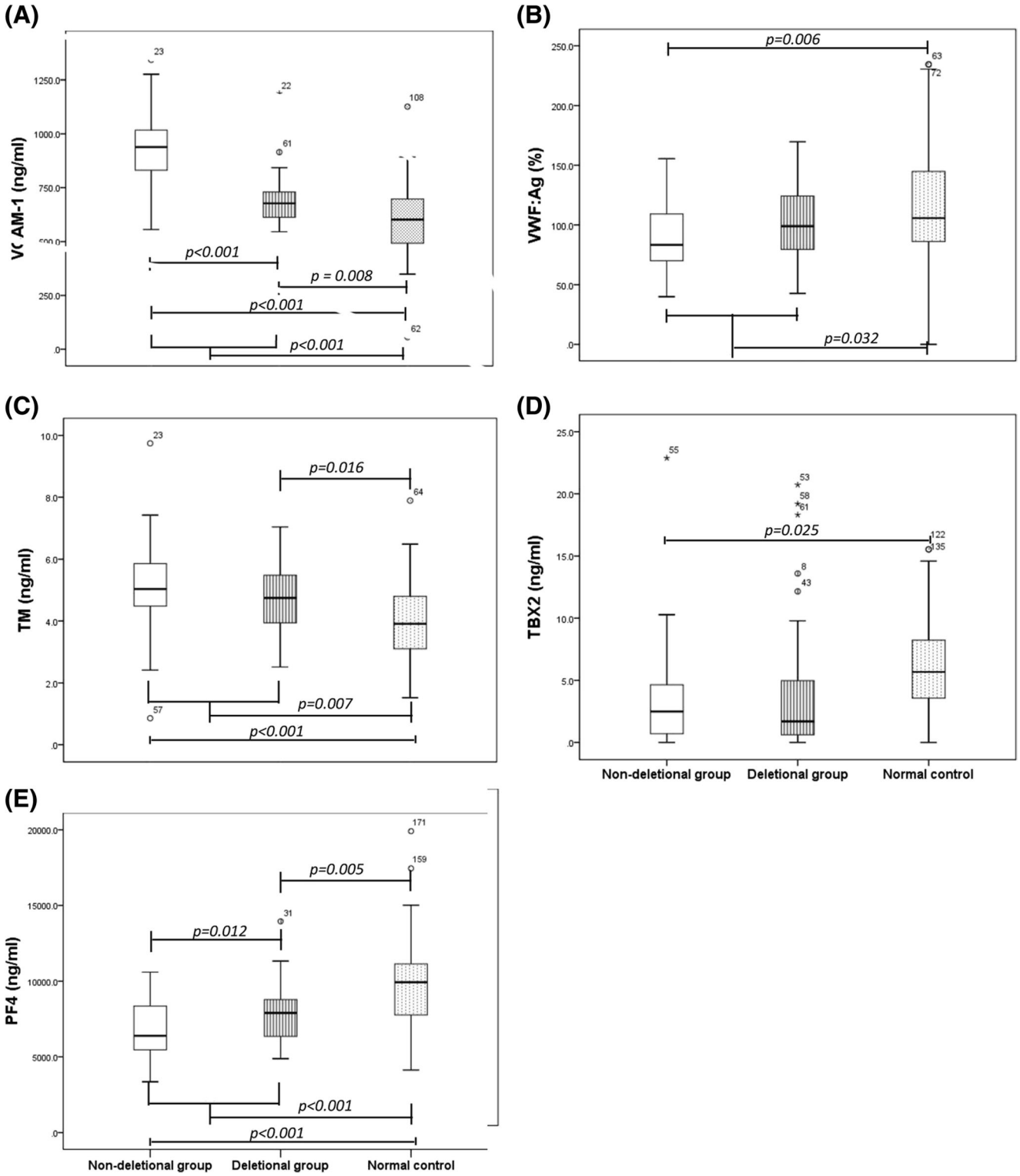

Fig. 1 Multivariate analysis comparison of laboratory results between subjects with α-thalassemia disease (non-deletional and deletional group) and normal controls, vascular cell adhesion molecule-1 (VCAM-1) (**a**), von Willebrand factor antigen (VWF:Ag) (**b**), thrombomodulin (TM) (**c**), thromboxane B2 (TBX2) (**d**), and platelet factor 4
(PF4) (**e**). Subjects in non-deletional α-thalassemia ($n = 31$), deletional α-thalassemia ($n = 29$), and normal control groups. Number of normal controls for VCAM-1 = 60, VWF: Ag = 60, TM = 60, TBX2 = 20, and PF4 = 39.

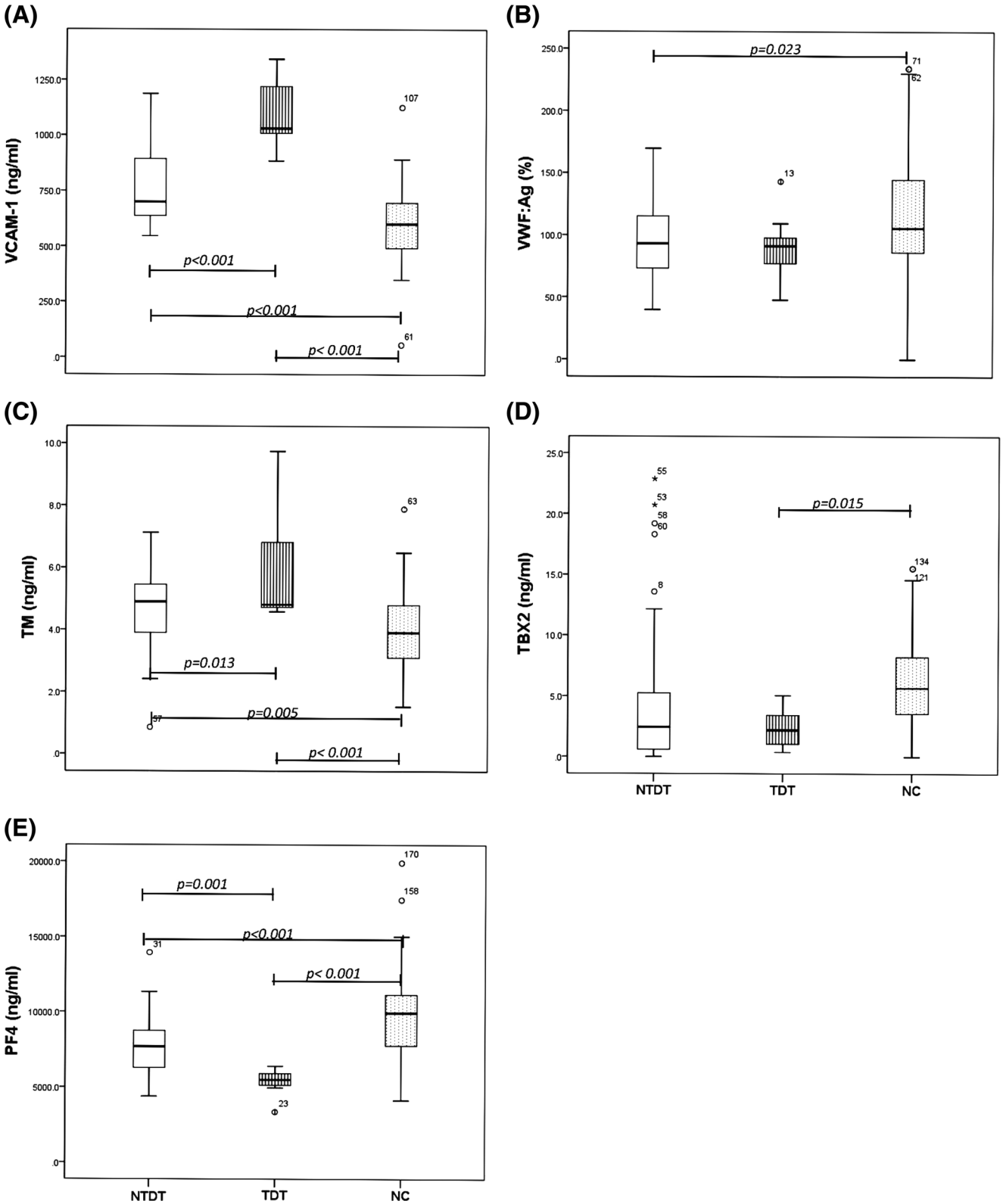

Fig. 2 Multivariate analysis comparison of laboratory results between subjects with HbH [non-transfusion dependent thalassemia (NTDT, $n = 51$) and transfusion-dependent thalassemia (TDT, $n = 9$)] and normal controls, vascular cell adhesion molecule-1 (VCAM-1) (**a**), von Willebrand factor antigen (VWF:Ag) (**b**), thrombomodulin (TM) (**c**), thromboxane B2 (TBX2) (**d**), and platelet factor 4 (PF4) (**e**). Number of normal controls for VCAM-1 = 60, VWF:Ag = 60, TM = 60, TBX2 = 20, and PF4 = 39

take vitamin E supplement. As a result, vitamin E intake may cause lower levels of TBX2 and PF4 than usual; therefore, the difference in platelet activation in thalassemia when compared to NC might not be observed. Vitamin E or α-tocopherol was demonstrated to decrease platelet aggregation-induced release of [^{14}C] 5 hydroxytryptamine from platelets as shown in the previous study [33]. Further study is required to verify this hypothesis.

In conclusion, the present study demonstrated endothelial activation in children with α-thalassemia, especially non-deletional α-thalassemia disease. Endothelial activation may be one of the risk factors for the development of TE in α-thalassemia patients. Although regular RBC transfusion was able to reduce platelet activation, further study and long-term follow-up is required to demonstrate the association of endothelial activation and TE in this population.

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Compliance with ethical standards Ethical approval for the study protocol was obtained from the Ethical Clearance Committee on Human Rights Related to Research to Research Involving Human Subjects, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Thailand (ID 12-59-09).

Conflict of interest The authors declare that they have no conflicts of interest.

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