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ORIGINAL ARTICLE

Association of Iron Overload with Oxidative Stress, Hepatic Damage and Dyslipidemia in Transfusion-Dependent β-Thalassemia/HbE Patients

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Abstract Blood transfusion can be a life-saving therapy for β-thalassemia major and β-thalassemia/HbE (β-TM) patients with chronic anemia, major caused severe iron overload particularly in β-TM patients received only blood transfusion therapy. We aim to evaluate the association of iron overload with oxidative stress, liver damage, and elevated very low density lipoprotein cholesterol (VLDL-C) in transfusion-dependent β-TM patients. Serum ferritin, malondialdehyde (MDA), liver profiles, triglycerides levels, and VLDL-C were significantly higher while total cholesterol, low-density lipoprotein cholesterol, high density lipoprotein cholesterol and total antioxidant capacity were lower in β-TM than controls. Serum ferritin was significantly correlated with MDA, liver enzymes and lipid profiles ($p < 0.05$). Multiple forward stepwise linear regression analyses of the significant variables showed that in these β-TM patients, independent predictors of iron overload were MDA ($\beta = 0.410$, $r^2 = 0.671$, $p < 0.001$), ALT ($\beta = 0.493$, $r^2 = 0.578$, $p < 0.001$), and VLDL-C ($\beta = 0.253$, $r^2 = 0.711$, $p < 0.001$). In conclusion, iron overload associated with increased oxidative stress, lipid peroxidation, liver damage, decreased TC, LDL-C, HDL-C and over production of VLDL-C, is significantly problem in transfusion-dependent β-TM patients. These appeared the major cause of future morbidity and mortality in β-TM patients.

Keywords β-Thalassemia major · β-Thalassemia/HbE · Iron overload · Oxidative stress · Hepatic damage · Dyslipidemia

Abbreviations

Hb	Hemoglobin
Hct	Hematocrit
TB	Total bilirubin
DB	Direct bilirubin
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
TC	Total cholesterol
HDL-C	High density lipoprotein cholesterol
LDL-C	Low density lipoprotein cholesterol
VLDL-C	Very low density lipoprotein cholesterol
MDA	Malondialdehyde
RC	Red blood cell
/g Hb	Per gram hemoglobin
TAC	Total antioxidant capacity

Introduction

Thalassemia syndrome is considered the most common genetic disorder worldwide. In fact, about 250 million people in the world carry thalassemia genes mainly in the Mediterranean area, the Middle East, the Indian subcontinent, and in

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Southeast Asia and about 500,000 new born per year were severe thalassemia [1]. In Thailand, about 1 % of Thai populations (~600,000 persons) were thalassemia patients [2] and about 40 % of Thai populations were thalassemia trait [3]. β-Thalassemia major (homozygous form), is an autosomal recessive disease causes a severe, microcytic, hemolytic anemia that necessitates frequent transfusions beginning in infancy [4]. The co-expression form of β-mutant thalassemic Hb with HbE is the other form of β-thalassemia, shows a remarkable variability in clinical expression similar to homozygous β-thalassemia [5]. In β-thalassemia major and β-thalassemias/HbE (β-TM) disorder, both hemolysis and anemia are prominent; therefore patients need continuous transfusions. However, due to a large amount of frequent transfusions lead to iron overload and accumulation of iron in heart, liver, pancreas and endocrine organs. The continuous iron accumulation can overwhelm the liver and caused hepatotoxicity [6]. Transfusion of red blood cells, chelation and splenectomy, this triad is still for the traditional treatment of β-TM in our country. Then, complications from iron excess are still numerous and more frequent in these β-TM patients.

It is the decreased or impaired β-globin biosynthesis in β-TM that leads to ineffective erythropoiesis and plays a crucial role in producing oxidative stress [7]. Moreover, the iron within the heme is free to generate H_2O_2 mainly via Fenton reaction. Anemia and excessive reactive oxygen species (ROS) is the hallmark of thalassemia [8]. Iron chelators are the mainstay of thalassemia treatment while antioxidants have the potential to guard against oxidative hemolysis and its clinical manifestation in β-TM patients [9]. Oxidative stress makes an important contribution to numerous pathologies including cardiovascular, cancer and degenerative diseases [9].

Dyslipoproteinemia in thalassemia [10, 11] is a consequence of lipid peroxidation associated with iron overload [12]. The low cholesterol levels were related to depletion of vitamin E and severity of thalassemia [10, 13]. Oxidative modification of thalassemic LDL had been reported [14, 15] which was related to the increased protein and triglyceride (TG) content but decreased cholesterol and vitamin E content [14]. Circulating malondialdehyde (MDA) is an oxidative stress marker and tissue injury via lipid peroxidation. Thus, this research aims to investigate iron overload associated with hepatic damage, increased oxidative stress, elevated TG-rich lipoprotein remnants (very low density lipoprotein cholesterol, VLDL-C) in transfusion-dependent β-TM patients.

Materials and Methods

Subjects

One hundred and eleven β-thalassemia major and β-thalassemia/HbE patient (β-TM patients) volunteers were drawn from the Thalassemia Clinic Unit of Phrae Hospital (26 males and 24 females, ages range 4–16 years) and the Vachira Phuket Hospital, Thailand (26 males and 34 females, aged 5–18 years). Diagnosis was based on the age of onset of anemia, and all hematological parameters determined by hematology analyzer. Peripheral smear and hemoglobin (Hb) typing was determined with high performance liquid chromatography, and confirmed with clinical manifestation. In β-TM patients, 84 were received only blood transfusion-dependent therapy and never received iron chelation treatment there were 7 spleenectomized [4 from Phrae Hospital (3 males, 1 female); 3 from Vachira Phuket Hospital (2 males, 1 female)], and 26 received blood transfusion with infrequent iron chelation therapy [9 from Phrae Hospital (4 males, 5 female); 17 from Vachira Phuket Hospital (9 males, 8 female)]. Infrequent iron chelation therapy was the decision of the physician at each time to request desferrioxamine; chelating agent by subcutaneous 8–12 h nightly infusion. All β-TM received more than 54 ± 25 transfusions (mean ± SD). 60 healthy children volunteers were also recruited from the general population making up the respective hospital catchment areas Phrae, (20 males and 20 females, ages 3–16 years) and Phuket (10 males and 10 females, ages 5–18 years). The criteria for the healthy participants included the absence of any history of blood transfusion, no condition which limited mobility, no life-threatening diseases, and no other diseases. All control and thalassemic participants were negative for blood hepatitis C, hepatitis B, and HIV virus. The parents of all participants signed an informed consent form prior to enrollment and the study was approved by the Ethics Committee of Naresuan University, Phrae Hospital and Vachira Phuket Hospital.

Blood Sampling

Blood samples were obtained from β-TM patients immediately before a transfusion (22–26 days after previous transfusion) and healthy volunteers via venipuncture after obtaining informed consent. Serum was separated by centrifugation and stored for the major biomarkers assay.

Complete Blood Cell Count

Complete blood cell counts were performed in EDTA-whole blood by using Sysmex SF-3000 hematology analyzer system, Sysmex, Japan).

Preparation for Red Blood Cells (RBCs) Suspensions

Red Blood Cells of EDTA treated blood were isolated by centrifugation at 1,500×g for 10 min, washed three times with two volumes of phosphate buffer saline solution (PBS,

pH 7.4) and finally re-suspended using enough buffer to make a target concentration equivalent to a hematocrit of 5 %. The washed RBCs were recentrifuged and then re-suspended them in double distilled water to hemolyse the cells and centrifuged again at 15,000×*g* for 40 min [16]. The resulting hemolysate was used for MDA determination in RBCs (as RC-MDA).

Liver Function Test

Total bilirubin (TB), direct bilirubin (DB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were determined by standard kinetic methods using Olympus AU 640 autoanalyzer (Olympus Diagnostic System, Japan).

Lipid Profiles Assay

Serum total cholesterol (TC), TG, and high density lipoprotein cholesterol (HDL-C) were measured by using an enzymatic procedure with a Hitachi 912 auto-analyzer (Roche Diagnostic, Switzerland). We calculated the low density lipoprotein cholesterol (LDL-C) with Friedewald's formula in specimens with TG levels <400 mg/dL (<4.52 mmol/L). We also estimated VLDL-C concentration according to the formula of Hattori et al. [17] as: VLDL-C = −0.01 (TC − HDL-C) + 0.16 TG.

Ferritin Assay

Ferritin measurement was based on microparticle enzyme immunoassay (MEIA) technology using Abbott reagents with the Axsym system (Abbott Laboratories, IL, USA).

MDA Assay

The method is based on the formation of red (pink) chromophore following the reaction of thiobarbituric acid (TBA) with MDA and the other breakdown products of peroxidized lipids called thiobarbituric acid reactive substance (TBARS). One molecule of MDA reacts with two molecules of TBA to yield a pink pigment with maximum absorption at 532 nm. This was measured by spectrophotometry using 1,1,3,3-tetraethoxypropane (TEP) as standard as described previously [18]. The final results were expressed as μmol of MDA formed per liters of serum. Intra-assay and inter-assay imprecision were 3.24 and 5.78 %, respectively. The normal range of MDA was <3.5 μmol/L.

Total Antioxidant Status

The method is based on formation of the $ABTS^{\cdot+}$ cation [2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] and its scavenging by antioxidant sample constituents (serum) measured by spectrophotometry at 600 nm (decay of green/blue color absorption is inversely associated with antioxidant sample content and the control antioxidant is Trolox, a hydrophilic vitamin E analog) [19].

Statistical Analysis

All data are presented as median and interquartile range (non-normally distributed data), and were tested by using Shapiro–Wilk test. Mann–Whitney *U* test was used to analyze the differences for these non-normally distributed data. Correlation between iron overload with liver profiles (TB, DB, AST, ALT and ALP levels), lipid profiles (TC, TG, HDL-C and LDL-C) and MDA were analyzed with Spearman's rho correlation test. Clinical variables that correlated with iron overload (ferritin) in these β-TM patients were tested as independent variables by using multivariate forward stepwise linear regression analysis. Statistical analysis was done with SPSS (v 17.0 SPSS Inc, Chicago, USA). All p values <0.05 were considered as significant.

Results

All clinical characteristics of 111 β-TM patients [age 10.0 (8.0–13.0 years), the male and female ratio was 51/59] and 60 control subjects [age 12.0 (10.0–14.0 years), the male and female ratio was 30/30] are compared and summarized in Table 1.

Iron overload and increased in these β-TM patients MDA, RC-MDA, ferritin levels were significantly higher in these β-TM patients than control subjects ($p < 0.001$).

Liver damage in these β-TM patients Liver profiles (TB, DB, AST, ALT, ALP) were significantly higher in these β-TM patients than control subjects ($p < 0.001$).

Dyslipidemia in these β-TM patients VLDL-C and TG levels were significantly higher, while TC, HDL-C, LDL-C and TAC levels were lower in these β-TM patients than control subjects ($p < 0.001$).

β-TM patients who received infrequent iron chelation and the splenectomized β-TM patients showed increased oxidative stress, liver damage and reduced TAC results similar to the other β-TM patients as shown in Tables 2, 3. Only serum ferritin concentration was tended to be lower (but not statistically significant) in β-TM patients who received infrequent iron chelation (Table 2).

For Spearman's correlation analysis We found that ferritin was significantly correlated statistically with MDA, AST, ALT, ALP TC, and TG; respectively) and also inversely correlated with HDL-C and TAC and the others variables as demonstrated in Table 4. We used multiple

Table 1 Comparison of the clinical characteristics of all β-TM patients with their controls

Variables	β-TM patients ($n = 111$)	Control subjects ($n = 60$)	p value
Age (years)	10.0 (8.0–13.0)*	12.0 (10.0–14.0)*	0.029
Hb (g/L)	65.0 (54.0–77.0)	130.0 (123.0–139.0)	<0.001
Hct (%)	20.8 (18.1–24.5)	39.4 (36.8–41.6)	<0.001
WBC × 10^9 (cells/L)	10.53 (7.69–30.16)	6.63 (5.83–7.81)	<0.001
Platelet × 10^9 (cells/L)	271.0 (205.0–530.0)	272.0 (218.8–317.8)	0.204
TB (μmol/L)	34.2 (27.3–49.5)	9.41 (3.42–13.68)	<0.001
DB (μmol/L)	6.84 (6.84–10.3)	3.42 (1.71–3.42)	<0.001
AST (U/L)	65.0 (56.0–95.0)	20.0 (15.3–24.8)	<0.001
ALT (U/L)	81.0 (69.0–123.0)	12.0 (10.0–15.8)	<0.001
ALP (U/L)	130.0 (103.0–176.0)	71.0 (56.3–84.0)	<0.001
TC (mmol/L)	2.94 (2.45–3.46)	4.50 (4.19–4.88)	<0.001
TG (mmol/L)	1.66 (1.41–2.06)	0.73 (0.60–1.06)	<0.001
LDL-C (mmol/L)	1.42 (1.16–1.88)	2.46 (2.21–3.04)	<0.001
HDL-C (mmol/L)	0.65 (0.49–0.83)	1.59 (1.37–1.75)	<0.001
VLDL-C (mmol/L)	0.550 (0.447–0.669)	0.241 (0.194–0.351)	<0.001
Ferritin (μg/L)	2,622.3 (1,686.0–5,000.0)	60.4 (48.3–75.8)	<0.001
MDA (μmol/L)	9.0 (6.4–14.8)	2.78 (2.24–3.18)	<0.001
RC-MDA/gHb (μmol/L)	7.55 (6.23–8.94)	3.83 (1.15–3.98)	<0.001
TAC (mmol TroloxEquiv/L)	0.470 (0.000–0.520)	5.55 (4.55–5.77)	<0.001

p value <0.05, significant;
* median (interquartile)

Hb hemoglobin, *Hct* hematocrit, *TB* total bilirubin, *DB* direct bilirubin, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *ALP* alkaline phosphatase, *TC* total cholesterol, *HDL-C* high density lipoprotein cholesterol, *LDL-C* low density lipoprotein cholesterol, *VLDL-C* very low density lipoprotein cholesterol, *MDA* malondialdehyde, *RC* red blood cell, */g Hb* per gram hemoglobin, *TAC* total antioxidant capacity

forward stepwise linear regression analysis to examine effects of variables in the association of these variables with iron overload in β-TM patients. Statistics are listed in Table 5. MDA, ALT and VLDL-C showed strong association with iron overload, which remained highly significant after adjusting for any clinical or laboratory confounding variables [MDA ($\beta = 0.410$, $r^2 = 0.671$, $p < 0.001$) ALT ($\beta = 0.493$, $r^2 = 0.578$, $p < 0.001$), and VLDL-C ($\beta = 0.253$, $r^2 = 0.711$, $p < 0.001$)].

Discussion

In our study, all β-TM patients had iron overload, elevated serum liver profiles (TB, DB, AST, ALT, and ALP), increased oxidative stress, decreased TAC level and decreased TC, LDL-C and HDL-C levels [14] were commonly occurred in β-TM patients. Both increased lipid peroxidation and decreased TAC mean that all circulating antioxidant defenses are overwhelmed and organs, HDL-C and other antioxidant agents are no longer protected and undergo oxidative damage. Iron overload showed significantly correlated with oxidative stress markers (MDA and TAC), liver damage, lipid profile and the other variables as shown in Table 4. Iron accumulation initiates in the reticulo-endothelial system (bone marrow, spleen) and then in the hepatocytes, the heart and the endocrine glands [6, 20]. Normally, liver has a large capacity for storage of excess iron in the form of ferritin and can be mobilized when needed to other areas of the body. Hepatocellular damage due to Fe^{2+}-initiated peroxide damage of the lysosomal membrane lipids. Liver function tests in β-TM patients were 3–4 folds higher compared to controls indicating liver damage. Elevated bilirubin may arise from both hemolytic process and hepatic lesions. Furthermore, the increase ALP may cause from cholestasis syndrome [21]. Liver lipid peroxidation also increases leakage of ferritin, AST, ALT, ALP, and MDA, into the circulation. MDA levels may leak from the liver strongly correlates with liver iron overload [22]. As in our study, chronic transfusion can overwhelm liver with iron accumulation and caused hepatotoxicity from oxidative damage in β-TM patients [6]. MDA levels, liver enzymes and lipid profile were similar to those without or with inadequate chelation or splenectomy, as also noted in previous studies [23, 24]. Only serum ferritin concentrations had a tendency to decrease in inadequate chelation patients. Increased circulating concentrations may be caused from hemolysis, anemia and continuous transfusions in all β-TM patients if they didn't receive regular iron chelation therapy. Regularly transfusion has determined a decrease in extra-medullar erythropoiesis and has also decreased the number of non functional red cells that needed to be destroyed in the splenic tissue. Therefore, splenomegaly tends to develop later. The criteria for splenectomy is a blood consumption greater than 50 % above the mean requirement [more than 200–250 mL/(kg year)] of pure red cells, to maintain a pre-transfusion Hb around 9 g/dL [25]. The response to splenectomy is variable from patient to patient, but it is usually satisfactory, and it is long-lasting [26].

Table 2 Comparison of the clinical characteristics of β-TM patients received blood transfusion therapy only with β-TM received blood transfusion with infrequent iron chelation therapy

Variables	β-TM ($n = 85$) (blood transfusion therapy)	β-TM ($n = 26$) (blood transfusion with infrequent chelation therapy)	p value
Age (years)	11.0 (8.5–13.0)*	8.0 (6.0–12.3)*	0.030
Hb (g/L)	63.0 (55.0–75.0)	71.5 (52.5–85.0)	0.387
Hct (%)	20.4 (18.3–23.6)	22.6 (17.9–25.8)	0.486
WBC $\times 10^9$ (cells/L)	11.31 (8.17–32.18)	10.19 (7.03–17.74)	0.256
Platelet $\times 10^9$ (cells/L)	263.0 (190.5–545.0)	354.0 (237.0–459.8)	0.403
TB (μmol/L)	35.91 (27.36–53.01)	28.22 (23.51–39.33)	0.061
DB (μmol/L)	8.55 (6.84–10.26)	6.84 (6.41–10.26)	0.391
AST (U/L)	67.0 (55.5–97.0)	62.5 (55.5–69.5)	0.283
ALT (U/L)	84.0 (69.0–125.0)	79.0 (72.8–102.8)	0.895
ALP (U/L)	130.0 (106.0–172.5)	129.0 (93.8–179.8)	0.631
TC (mmol/L)	2.97 (2.44–3.46)	2.93 (2.59–3.48)	0.676
TG (mmol/L)	1.65 (1.39–1.95)	1.80 (1.55–2.24)	0.144
LDL-C (mmol/L)	1.45 (1.15–1.89)	1.36 (1.12–1.86)	0.604
HDL-C (mmol/L)	0.62 (0.47–0.83)	0.79 (0.59–0.83)	0.069
VLDL-C (mmol/l)	0.550 (0.454–0.674)	0.534 (0.405–0.669)	0.611
Ferritin (μg/L)	2,624.1 (1,734.0–5,000.0)	2,624.1 (1,734.0–5,000.0)	0.160
MDA (μmol/L)	9.4 (6.39–16.18)	8.70 (6.68–13.40)	0.730
RC-MDA/g Hb (μmol/L)	7.68 (6.27–8.99)	7.33 (6.17–8.84)	0.920
TAC (mmol Trolox Equiv/L)	0.470 (0.000–0.5350)	0.465 (0.000–0.510)	0.775

p value <0.05, significant;
* median (interquartile)

Hb hemoglobin, *Hct* hematocrit, *TB* total bilirubin, *DB* direct bilirubin, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *ALP* alkaline phosphatase, *TC* total cholesterol, *HDL-C* high density lipoprotein cholesterol, *LDL-C* low density lipoprotein cholesterol, *VLDL-C* very low density lipoprotein cholesterol, *MDA* malondialdehyde, *RC* red blood cell, */g Hb* per gram hemoglobin, *TAC* total antioxidant capacity

Table 3 Comparison of the clinical characteristics of the splenectomized β-TM patients with β-TM patients (without splenectomized)

Variables	Splenectomized β-TM ($n = 7$)	β-TM without splenectomized ($n = 103$)	p value
Age (years)	12.0 (8.0–19.0)*	10.0 (7.0–13.0)*	0.298
Hb (g/L)	63.0 (55.0–75.0)	65.0 (54.3–76.8)	0.528
Hct (%)	20.4 (18.3–23.6)	20.7 (18.0–24.4)	0.420
WBC $\times 10^9$ (cells/L)	9.65 (6.56–10.53)	11.64 (7.78–30.39)	0.338
Platelet $\times 10^9$ (cells/L)	224.0 (207.0–297.0)	287.0 (202.0–530.0)	0.536
TB (μmol/L)	37.62 (15.39–51.30)	34.20 (27.36–49.59)	0.875
DB (μmol/L)	8.55 (5.13–10.26)	6.84 (6.84–10.26)	0.941
AST (U/L)	64.0 (54.0–98.0)	65.0 (56.0–94.0)	0.937
ALT (U/L)	84.0 (69.0–131.0)	80.5 (69.0–107.3)	0.981
ALP (U/L)	145.0 (127.0–187.0)	130.0 (102.3–175.5)	0.501
TC (mmol/L)	2.61 (2.24–3.12)	2.97 (2.48–3.46)	0.214
TG (mmol/L)	1.74 (1.51–1.88)	1.66 (1.41–2.09)	0.776
LDL-C (mmol/L)	1.37 (0.93–1.88)	1.42 (1.17–1.89)	0.508
HDL-C (mmol/L)	0.49 (0.39–0.80)	0.66 (0.49–0.83)	0.140
VLDL-C (mmol/l)	0.565 (0.511–0.669)	0.545 (0.444–0.671)	0.489
Ferritin (μg/L)	3,128.0 (658.0–4,909.0)	2,610.2 (1,686.3–5,000.0)	0.923
MDA (μmol/L)	7.68 (5.38–12.40)	9.20 (6.63–15.69)	0.329
RC-MDA/g Hb (μmol/L)	8.12 (7.20–9.46)	7.55 (6.22–8.87)	0.305
TAC (mmol Trolox Equiv/L)	0.510 (0.000–2.41)	0.470 (0.000–0.510)	0.432

P value <0.05, significant;
* median (interquartile)

Hb hemoglobin, *Hct* hematocrit, *TB* total bilirubin, *DB* direct bilirubin, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *ALP* alkaline phosphatase, *TC* total cholesterol, *HDL-C* high density lipoprotein cholesterol, *LDL-C* low density lipoprotein cholesterol, *VLDL-C* very low density lipoprotein cholesterol, *MDA* malondialdehyde, *RC* red blood cell, */g Hb* per gram hemoglobin, *TAC* total antioxidant capacity

Oxidative stress in thalassemia diseases is due to the key redox reactions of hemoglobin that take place in the presence of hydrogen peroxide (H_2O_2) and superoxide anion radicals ($O^{2\cdot-}$). According to the Haber–Weiss reactions, ·OH and molecular oxygen can be formed. The reaction can be catalyzed by Fe^{3+} and is a possible source of ·OH. Besides iron causing oxidative stress and the absence of the β-globin chain, causes self aggregation of the unpaired α-

Table 4 Correlation of variables among ferritin, MDA, ALT, HDL-C and VLDL-C in β-TM patients

Correlation between parameters		Correlation coefficient	
		r	*P* value
Ferritin	MDA	0.471	0.014
	TAC	−0.255	0.007
	AST	0.578	<0.001
	ALT	0.535	<0.001
	ALP	0.420	<0.001
	TC	0.255	0.006
	TG	0.527	<0.001
	HDL-C	−0.466	<0.001
	LDL-C	0.206	0.030
MDA	WBC	0.255	0.007
	AST	0.347	<0.001
	ALT	0.226	0.017
	ALP	0.220	0.020
	RC-MDA/g Hb	0.426	<0.001
	TC	0.264	0.005
	TG	0.250	0.008
	HDL-C	−0.292	0.002
	LDL-C	0.290	0.002
ALT	Hb	−0.411	<0.001
	Hct	−0.439	<0.001
	AST	0.841	<0.001
	TG	0.542	<0.001
	HDL-C	−0.576	<0.001
HDL-C	Hb	0.559	<0.001
	Hct	0.556	<0.001
	DB	−0.288	0.002
	AST	−0.553	<0.001
	TC	0.229	0.015
	TG	−0.514	<0.001
VLDL-C	Hb	−0.256	0.007
	Hct	−0.317	0.001
	AST	0.504	<0.001
	ALT	0.544	<0.001
	ALP	0.226	0.017
	Ferritin	0.523	<0.001
	MDA	0.241	0.011
	HDL-C	−0.522	<0.001

Hb hemoglobin, *Hct* hematocrit, *TB* total bilirubin, *DB* direct bilirubin, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *ALP* alkaline phosphatase, *TC* total cholesterol, *HDL-C* high density lipoprotein cholesterol, *LDL-C* low density lipoprotein cholesterol, *VLDL-C* very low density lipoprotein cholesterol, *MDA* malondialdehyde, *TAC* total antioxidant capacity, *RC* red blood cell, */g Hb* per gram hemoglobin

chains, this also cause oxidative membrane damage destroying the immature erythroblasts within the bone marrow [21]. MDA in thalassemic erythrocytes was substantially greater compared to controls ($p < 0.001$), which accords with previous work [27]. This increased MDA reaffirms the assumption that it reacts with membrane polyunsaturated fatty acid leading to hemolysis [14, 22]. A similarly membrane labiality may contribute to cell death in other tissues. Furthermore, the greatly diminished antioxidant viability further exacerbates fragility of cell membranes. Elevated MDA demonstrated the LDL-C from β-TM patients are more prone to form peroxides. Thus, increased relative risk of CVD and organ damage (particular in liver) could be linked to the greater oxidative stress and oxidation in LDL-C even in patients with lower in TC and LDL-C concentrations. These may be the better markers for prediction of the oxidative stress and CVD events in the future.

Table 5 Multiple forward stepwise linear regression analyses of the significant variables showed that ALT, MDA and VLDL-C were independent predictors of iron overload in β-TM patients

Variables	β	R^2	Adjusted R^2	*p* value
MDA	0.321	0.674	0.671	<0.001
ALT	0.400	0.578	0.576	<0.001
VLDL-C	0.253	0.711	0.706	<0.001

MDA malondialdehyde, *ALT* alanine aminotransferase, *VLDL-C* very low density lipoprotein cholesterol

Lipid profiles in our study revealed significantly lower TC, LDL-C, HDL-C and TAC levels but higher VLDL-C, TG and MDA in β-TM patients. Hypocholesterolemia has been documented in various hemolytic disorders including β-TM patients [28]. The mechanisms of hypocholesterolemia in β-TM patients were hypothesized as: (i) plasma dilution due to anemia, (ii) increased cholesterol requirement associated with erythroid hyperplasia, (iii) macrophage system activation with release of cytokines, (iv) increased cholesterol uptake by reticulo-endothelial system, and (v) liver damage secondary to iron overload [28]. These low levels of TC and LDL-C in help protect β-TM patients against CVD caused by oxidative stress [29]. Moreover, TAC and HDL-C were found significantly lower and may play the protective role against oxidative stress and LDL-C oxidation [29]. Decreased HDL-C levels may cause from oxidatively modified from the increasing oxidative stress. The changing in β-thalassemic HDL-C are also related to low cholesterol concentration. Many β-TM patients with low levels of HDL-C also have hypertriglyceridemia levels, and elevated concentrations of highly atherogenic triglyceride-rich lipoprotein remnants (derived from chylomicrons and VLDL-C). Iron overload caused an increased oxidative stress appeared to be a deleterious factor leading to insulin resistance, β-cell dysfunction, impaired glucose tolerance, and ultimately, T2D in β-TM patients as in our recent study [30]. Insulin resistance cause

increased VLDL production. Increased VLDL production and impaired VLDL lipolysis link a high triglycerides level with a low HDL-C concentration. This constellation of metabolically linked factors is most like the condition of metabolic syndrome [31]. β-TM patients are primarily due to liver disease, heart disease, diabetes, infection and malignancy as the major future caused morbidity and mortality [32].

Most β-TM patients in our country have not regularly received iron chelating agent, perhaps because of socio-economic disparities in our country. Thus, β-TM patients regularly received only blood transfusion therapy and some received inadequate iron chelating agent, particularly in the rural area as in our present study. Therefore, iron overload and increased oxidative stress commonly occurred in these β-TM patients.

Conclusion

Iron overload commonly occurred in transfusion-dependent β-TM patients, the major cause of increased oxidative stress, liver damage, and over-production of VLDL-C. Given plethora serious complications diseases associated with iron overload and increased oxidative stress in β-TM patients.

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Conflict of interest No conflict of interest.

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