



Improvement in oxidative stress and antioxidant parameters in β -thalassemia/Hb E patients treated with curcuminoids

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ABSTRACT

Objectives: To evaluate the hematological profile, oxidative stress, and antioxidant parameters in β -thalassemia/Hb E patients treated with curcuminoids for 12 months.

Design and methods: Twenty-one β -thalassemia/Hb E patients were given 2 capsules of 250 mg each of curcuminoids (a total of 500 mg) daily for 12 months. Blood was collected every 2 months during treatment and 3 months after withdrawal and was determined for complete blood count, malonyldialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), reduced glutathione (GSH) in red blood cells (RBC), and non-transferrin bound iron (NTBI) in serum.

Results: The increased oxidative stress in β -thalassemia/Hb E patients was shown by higher levels of MDA, SOD, GSH-Px in RBC, serum NTBI, and lower level of RBC GSH. Curcuminoids administration resulted in improvement of all the measured parameters as long as they were administered. After 3 months withdrawal of treatment, all parameters returned close to baseline levels.

Conclusion: Curcuminoids may be used to ameliorate oxidative damage in patients with β -thalassemia/Hb E disease.

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Introduction

Thalassemia is a common hereditary disorder worldwide caused by quantitative defect of α - and/or non- α -globins leading to the imbalance ratio of 2 types of paired globins that make up hemoglobin [1]. The excess of α or β chains of hemoglobin (Hb) A, is relatively unstable, and eventually disintegrates and precipitates in the red blood cells (RBC) [2]. As a result, heme is separated from globin and free radicals are generated mainly via Fenton reaction following peripheral hemolysis, premature apoptosis, and then anemia which is the hallmark of thalassemia [3]. Intracellular iron is finally released into the circulation [3]. Severe and chronic anemia plays an important role for pathogenesis of complications such as splenomegaly, jaundice, gallstone, defective development, and iron overload.

Another major factor contributing to oxidative stress in thalassemia is due to the excess iron obtained in each blood transfusion and

irrespectively from increased iron absorption [3,4]. When transferrin is fully saturated, non-transferrin bound iron (NTBI) and labile iron pool (LIP) are found and unregulatedly accumulate in organs including heart, liver, and multiple endocrine glands. These toxic iron species also catalyze the formation of oxygen free radicals resulting in deleterious effects to their deposited organs [5].

Hemoglobin E, the most common hemoglobin variant, is endemic in Southeast Asia with the frequency of approximately 50% in the triangle of Thailand, Laos, and Cambodia [6]. Although homozygous Hb E (Hb E/Hb E) provides mild symptoms, heterozygous compound of Hb E and β -thalassemia allele produces profound degree of anemia and markedly increased oxidative stress with a poorly defined mechanism. This contributes β -thalassemia/Hb E to the most common form of severe thalassemia requiring lifelong care and proper treatment.

The present study was carried out to examine whether parameters of oxidative stress are also found in β -thalassemia/Hb E patients and whether they can be ameliorated following treatment with the extract derived from dried rhizomes of curcumin (*Curcuma longa* Linn) also known as turmeric, a natural herb being used as food additive or traditional medicine for centuries. Curcumin extract is non-toxic to animals or humans, even at high doses and the National Cancer

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Institute evaluated curcumin as 'generally recognized as safe' (GRAS) [7]. Curcuminoids, a group of phenolic compounds of curcumin extract, are well known with potential antioxidant, anti-inflammatory, anticancer [7–13] and iron-chelator properties [14]. A variety of functional groups of curcuminoids is related to their biological properties. Although the mechanism of curcuminoids for scavenging free oxygen radicals and chelating NTBI is not well understood, it has been proposed that the β -diketone group and the hydroxyl/methoxy groups on phenyl rings are participating in their antioxidant activity and iron chelating property [15,16].

Our previous studies showed the efficacy of curcuminoids to reduce significantly oxidative stress parameters on RBC membrane in the β -thalassemia/Hb E patients treated with curcumin extract for 3 months [17]. Moreover, the patients felt that their quality of life was improved by having more appetite, and more energy. In this study, we prolonged the treatment period to 12 months analyzing various hematological and biochemical parameters during and after treatment.

Methods

Patients

Twenty-one β -thalassemia/Hb E patients (7 males and 14 females) were recruited from the Division of Hematology, Department of Medicine, Faculty of Medicine Siriraj Hospital, Thailand. All patients were compound heterozygous for β^0 -thalassemia and Hb E, non-blood transfused, and only 4 of these were splenectomized. Their age and Hb concentrations were between 15 and 45 years and 60–80 g/L, respectively. All subjects including the guardian of patients under the age of 18 years signed informed consent and the protocol used in this study was approved by the Siriraj Ethics Committee, Mahidol University, Thailand. The control group consisted of 26 healthy volunteers (9 males and 17 females) at the age between 20 and 45 years old, who had normal hemoglobin typing (Hb A and A₂) and Hb concentrations were in the range of 120–150 g/L.

Treatment and blood collection

At the beginning of the study, 29 mL of peripheral blood was collected once from normal subjects and twice from β -thalassemia/Hb E patients at 2 week interval as baseline. Two milliliters of those was used to analyze complete blood counts and serum from 7 mL clotted blood was used to measure liver and renal function tests, lipid profiles, uric acid, ferritin, and NTBI. The remaining blood in EDTA-coated tubes was centrifuged at 1000×g for 10 min at 4 °C. The packed red cells were washed three times with cold phosphate buffered saline (PBS), pH 7.4. Cells were diluted to 50% hematocrit with the same buffer and analyzed oxidative stress and antioxidant parameters in red blood cells.

All patients received 2 capsules (250 mg each) of curcuminoids daily for 12 months. Curcuminoids capsules, kindly donated from The Government Pharmaceutical Organization, Bangkok, Thailand, are comprised of curcumin, demethoxycurcumin, and bisdemethoxycurcumin in the ratio of 1:0.3:0.1. Blood samples were collected every 2 months during treatment and 3 months after withdrawal.

Hematological parameters

Complete blood counts of all normal and β -thalassemia/Hb E patients at different time points were measured using an automated cell counter Sysmex NE-1500.

Malonyldialdehyde (MDA)

The formation of MDA, a product of lipid peroxidation, was measured in RBC according to the method of Stocks and Dormard

[18] by exposure to hydrogen peroxide solution and reacting with thiobarbituric acid (TBA) to form MDA-TBA complex in acidic and boiling temperature. After cooling, the complex was measured by spectrophotometer at 532 and 600 nm.

Superoxide dismutase (SOD)

SOD activity was determined in RBC based on the ability to inhibit the reaction of nitroblue tetrazolium (NBT) by superoxide anions generated by the reaction of photoreduced riboflavin and oxygen according to Winterbourn [19]. The activity of SOD was expressed as units per gram of Hb, where one unit of SOD activity is defined as the amount of enzyme that inhibits the rate of NBT reduction by 50%.

Glutathione peroxidase (GSH-Px)

The GSH-Px activity was determined in RBC by NADPH oxidation in a coupled reaction system containing t-butyl hydroperoxide and oxidized glutathione by the method of Beutler [20]. The solution was measured at 340 nm for 2 min and the decreased absorbance is directly proportional to the GSH-Px concentration.

Reduced glutathione (GSH)

Reduced glutathione in red blood cells was measured by the reduction of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) to give a stable yellow color [21]. The difference of the absorbance at 412 nm between before and after adding DTNB reagent was used to calculate the concentration of GSH.

Methemoglobin (MHb)

Methemoglobin was measured according to method of Evelyn and Molloy [22]. The value was reported as the percentage of the MHb in total hemoglobin.

Non-transferrin bound iron (NTBI)

The level of NTBI was assayed by the method of Singh and colleagues [23]. Nitrotriacetic acid (NTA) was added in serum for chelating ferric NTBI. The complex of Fe(III)-[NTA]₂ was separated by special membrane and measured by reverse-phase HPLC. An iron chelator (CP22) was added to capture ferric iron from Fe(III)-[NTA]₂. The absorbance of the complex of Fe(III)-[NTA]₂ and Fe(III)-[CP22]₃ was measured at 450 nm using on-line LDC detector.

Biochemical parameters

The parameters of liver function, renal function, lipid profiles, level of uric acid, and serum ferritin analyzed in this study were measured by using an automated analyzer Integra 700 (Roche, Switzerland).

Statistical analysis

The statistical analysis was performed by SPSS 10.0 and data were analyzed using the one-way ANOVA and paired *t*-test to compare the effects of curcuminoids treatment and withdrawal at different time points. Values were considered statistically significant at *p*<0.05.

Results

Hematological parameters of normal and β -thalassemia/Hb E patients treated with curcuminoids for 12 months were measured (Table 1). The hematological parameters of normal group were within reference range whereas β -thalassemia/Hb E patients showed significant difference from normal subjects. Curcuminoids treatment

Table 1
Hematological profile of normal and β -thalassemia/Hb E subjects.

Parameters	Normal (n = 26)	β -thalassemia/Hb E (n = 21)			
		Baseline (before treatment)	Curcuminoids treatment		Withdrawal (month 15)
			Month 6	Month 12	
Hb (g/L)	138 \pm 2	69 \pm 3 ^a	68 \pm 3	68 \pm 3	65 \pm 3
Hct (proportion of 1.0)	0.410 \pm 0.006	0.221 \pm 0.007 ^a	0.219 \pm 0.007	0.220 \pm 0.007	0.214 \pm 0.008
WBC ($\times 10^9$ /L)	5.8 \pm 0.3	22.5 \pm 6.0	26.8 \pm 8.4	22.1 \pm 6.4	26.3 \pm 8.7
RBC ($\times 10^{12}$ /L)	4.6 \pm 0.1	3.5 \pm 0.1 ^a	3.5 \pm 0.1	3.5 \pm 0.1	3.4 \pm 0.1
MCV (fl)	89.1 \pm 0.7	61.8 \pm 1.6 ^a	62.4 \pm 2.0	63.3 \pm 1.9	63.2 \pm 1.9
MCH (pg)	29.8 \pm 0.3	19.3 \pm 0.5 ^a	19.3 \pm 0.6	19.3 \pm 0.5	19.0 \pm 0.5
MCHC (g/L)	335 \pm 2	313 \pm 5	309 \pm 5 ^b	308 \pm 5 ^b	297 \pm 9
Platelet ($\times 10^9$ /L)	228 \pm 14	314 \pm 55	327 \pm 58	302 \pm 51	316 \pm 54
Reticulocyte (proportion of 1.0)	0.014 \pm 0.001	0.036 \pm 0.005 ^c	0.041 \pm 0.005	0.049 \pm 0.014	0.045 \pm 0.007

Values were means \pm SEM.

Abbreviations: Hb, hemoglobin concentration; Hct, hematocrit; WBC, number of white blood cells; RBC, number of red blood cells; MCV, mean cell volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

^a Compared to normal subjects, $p < 0.001$.

^b Compared to baseline (before treatment), $p < 0.05$.

^c Compared to normal subjects, $p < 0.05$.

for 12 months had no apparent effect on hematological parameter. Supplementation of curcuminoids (500 mg daily) to β -thalassemia/Hb E patients for 12 months did not have any effect on liver and kidney function tests and lipid profiles (Table 2).

Biochemical parameters evaluating oxidative stress and antioxidant in normal healthy subjects and β -thalassemia/Hb E patients receiving curcuminoids were shown in Table 3. All parameters analyzed in normal subjects were significantly different from patients ($p < 0.001$). The percentage of MHb was significantly decreased ($p < 0.05$) after administration of curcuminoids for 12 months, while there were no changes in Hb levels. The levels of H₂O₂-induced RBC MDA were significantly higher in the patients than in normal subjects ($p < 0.001$) and reduced significantly following treatment up to 6 months and remained the same for the rest of the year. After withdrawal for 3 months, MDA levels in RBC increased but were still below the level detected before treatment (Fig. 1A).

Curcuminoids treatment for 12 months significantly decreased the activities of SOD and GSH-Px in RBC. SOD activity was decreased after

treatment for 8 months and slightly increased until the end of treatment. After curcuminoids withdrawal for 3 months, SOD reached levels closed to levels detected before treatment (Fig. 1B). Similarly, the RBC GSH-Px activity was gradually reduced during 12 months of curcuminoids treatment and returned to baseline after the withdrawal of curcuminoids (Fig. 1C). The level of reduced glutathione in RBCs' β -thalassemia/Hb E patients increased significantly throughout the period of treatment and slightly decreased after stopping treatment (Fig. 1D). Serum NTBI levels were significantly reduced after treatment for 6 months, and gradually increased after 12 months while ferritin levels in serum did not change (Table 3).

Discussion

Oxidative damage in β -thalassemia/Hb E patients resulted from the accumulation of unpaired α -globins, increased intracellular non-heme iron content, and reduced concentration of normal Hb. High oxidative stress in thalassemia patients is one of the most important

Table 2
Biochemical parameters on the blood of β -thalassemia/Hb E patients (n = 21) treated with curcuminoids for 12 months.

Parameters	Reference range	Baseline (before treatment)	Curcuminoids treatment (month 6)	p^a	Curcuminoids treatment (month 12)	p^b
<i>Liver function</i>						
Aspartate aminotransferase (U/L)	0–37	54.1 \pm 6.4	59.8 \pm 4.9	0.457	52.5 \pm 4.8	0.827
Alanine aminotransferase (U/L)	0–40	46.8 \pm 6.1	41.7 \pm 4.7	0.472	43.8 \pm 4.1	0.671
Alkaline phosphatase (U/L)	39–117	94.8 \pm 8.7	99.9 \pm 6.6	0.641	94.3 \pm 8.2	0.965
Gamma-glutamyltransferase (U/L)	7–50	124 \pm 63	44.6 \pm 4.9	0.130	38.1 \pm 4.5	0.102
Total bilirubin (μ mol/L)	5–20	61.9 \pm 5.1	65.8 \pm 3.6	0.482	63.8 \pm 2.8	0.735
Direct bilirubin (μ mol/L)	0–9	71.8 \pm 45.5	8.5 \pm 0.5	0.095	7.9 \pm 0.3	0.091
Albumin (g/L)	35–55	46.1 \pm 1.6	47.6 \pm 0.6	0.344	46.7 \pm 0.9	0.711
Total protein (g/L)	66–87	76.6 \pm 2.2	78.6 \pm 1.0	0.360	77.7 \pm 1.1	0.611
Globulin (g/L)	15–35	31.8 \pm 1.2	31.0 \pm 1.1	0.653	32.8 \pm 1.2	0.523
<i>Renal function</i>						
Urea (mmol/L)	3–7	4.89 \pm 0.2	4.4 \pm 0.1	0.032 ^c	4.5 \pm 0.12	0.049 ^c
Creatinine (μ mol/L)	44–132	53.9 \pm 3.5	47.7 \pm 3.5	0.108	49.5 \pm 2.7	0.268
<i>Lipid profile</i>						
Cholesterol (mmol/L)	2.6–5.2	2.5 \pm 0.1	2.5 \pm 0.07	0.971	2.5 \pm 0.09	0.866
Triglyceride (mmol/L)	0.8–1.8	0.4 \pm 0.04	0.5 \pm 0.03	0.882	0.5 \pm 0.03	0.693
HDL-Cholesterol (mmol/L)	0.9–2.6	0.7 \pm 0.04	0.7 \pm 0.03	0.715	0.8 \pm 0.03	0.165
LDL-Cholesterol (mmol/L)	1.8–4.1	1.04 \pm 0.09	1.03 \pm 0.07	0.911	1.1 \pm 0.07	0.572
<i>Other</i>						
Uric acid (μ mol/L)	143–416	375 \pm 27	328 \pm 20	0.128	350 \pm 17	0.416

Values were means \pm SEM.

^a Value at month 6 compared to at baseline.

^b Value at month 12 compared to at baseline.

^c Compared to baseline (before treatment), $p < 0.05$.

Table 3

Effects of curcuminoids on oxidative stress and antioxidant parameters in the blood of β -thalassemia/Hb E patients during treatment (month 1–12) and withdrawal for 3 months (month 15).

Parameters	Normal (n = 26)	β -thalassemia/Hb E (n = 21)			Withdrawal (month 15)
		Baseline (before treatment)	Curcuminoids treatment		
			Month 6	Month 12	
Hb (g/L)	138 \pm 2	69 \pm 3 ^a	68 \pm 3	68 \pm 3	65 \pm 3
Methemoglobin (proportion of total Hb)	0.09 \pm 0.01	0.13 \pm 0.01 ^a	0.13 \pm 0.01	0.11 \pm 0.01 ^d	0.13 \pm 0.04
RBC MDA (nmol/g Hb)	566 \pm 22	1596 \pm 45 ^a	1101 \pm 46 ^b	1134 \pm 31 ^b	1338 \pm 44 ^{b,c}
RBC SOD (U/g Hb)	2868 \pm 46	4784 \pm 153 ^a	4028 \pm 125 ^b	4108 \pm 116 ^b	4580 \pm 122 ^c
RBC GSH-Px (U/g Hb)	28.3 \pm 0.4	47.0 \pm 1.4 ^a	37.8 \pm 1.0 ^b	36.7 \pm 1.6 ^b	47.2 \pm 1.3 ^c
RBC GSH (mmol/L)	1.75 \pm 0.06	1.62 \pm 0.07 ^a	1.76 \pm 0.08 ^d	1.78 \pm 0.08 ^d	1.71 \pm 0.09 ^d
Serum ferritin (pmol/L)	211 \pm 9	2417 \pm 260 ^a	2480 \pm 238	2132 \pm 253	2686 \pm 279
Serum NTBI (μ mol/L)	-0.7 \pm 0.2	4.6 \pm 1.0 ^a	2.2 \pm 0.7 ^d	3.9 \pm 0.9	4.8 \pm 0.7

Values were means \pm SEM.

Abbreviations: Hb, hemoglobin concentration; MDA, malondialdehyde; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; GSH, glutathione; NTBI, non-transferrin bound iron.

^a Compared to normal subjects, $p < 0.001$.

^b Compared to baseline (before treatment), $p < 0.001$.

^c Compared to month 12 of treatment, $p < 0.001$.

^d Compared to baseline (before treatment), $p < 0.05$.

factors causing cell injury and organ dysfunction [24–27]. The thallemic RBC showed high oxidative stress as indicated by increasing level of MDA content, following oxidation of polyunsaturated fatty acids (PUFAs). The RBC MDA levels in β -thalassemia/Hb E patients were significantly higher ($p < 0.001$) than in normal controls, comparable to previous studies [2,28–35]. An earlier study showed

that non-heme iron contributed to lipid peroxidation of red cell membrane of thallemia patients, resulting in increased MDA concentrations in RBC [36]. In addition, plasma NTBI may play an important role in production of free radicals leading to the increase of free MDA in serum [37]. Normal subjects do not have NTBI detected in plasma/serum [32,38–40], whereas NTBI in range of 1–12 μ mol/L is

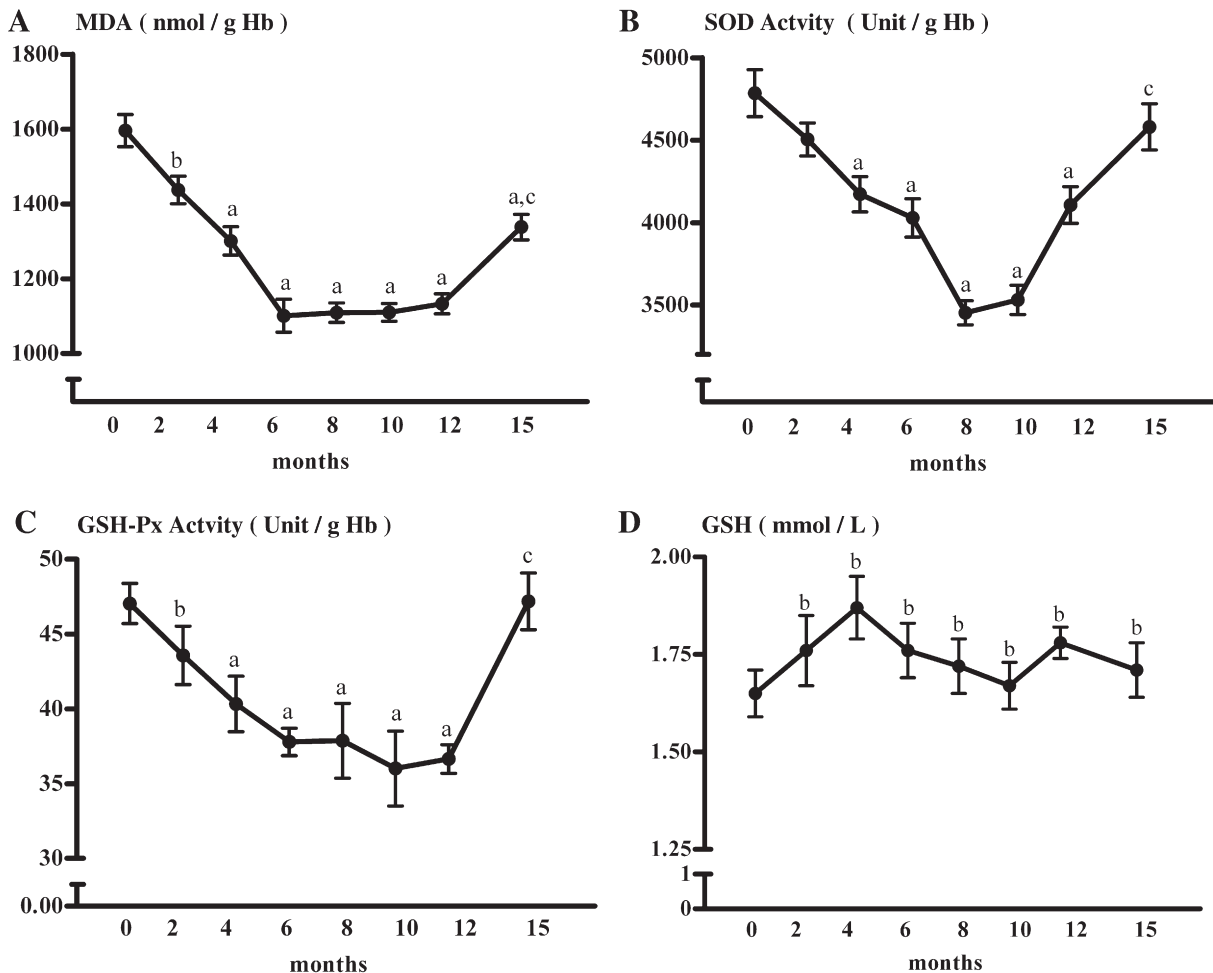


Fig. 1. Levels of (A) malonyldialdehyde (MDA), (B) superoxide dismutase (SOD) activity, (C) glutathione peroxidase (GSH-Px) activity, and (D) reduced glutathione (GSH) (mean \pm SEM) in RBCs of β -thalassemia/Hb E patients receiving curcuminoids for 12 months and following withdrawal for 3 months. ^aCompared with baseline values before treatment, $p < 0.001$. ^bCompared with baseline values before treatment, $p < 0.05$. ^cCompared with levels after 12 month treatment, $p < 0.001$.

present in iron-overloaded patients [41]. A significant 5-fold elevation of serum NTBI was observed in β -thalassemia/Hb E patients when compared to normal controls ($p < 0.001$) and the total (free + bound) serum MDA was found to be positively correlated with serum NTBI levels [28].

The antioxidant status in β -thalassemia/Hb E patients was investigated by direct measurement of cytoprotective enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) inside RBC. The activities of both enzymes in the patients were significantly increased in response of elevated RBC oxidative stress. The upregulation of SOD activity protects the thalassemic RBC by scavenging superoxide radicals and producing more hydrogen peroxide (H_2O_2), which is removed by GSH-Px. The levels of RBC reduced glutathione (GSH), a potent endogenous antioxidant and scavenger of free radicals, in β -thalassemia/Hb E patients were significantly decreased concomitant with the increase in GSH-Px activities, because GSH and its oxidized form (GSSG) are a major redox couple in GSH-Px antioxidative system. These results are similar to previous studies in patients with β -thalassemia major [42,43], β -thalassemia/Hb E [25], and hemoglobin H disease [44].

Since it was shown that the increase in oxidative stress in thalassemia can be ameliorated by antioxidants [29,45–47], we explored whether treatment of β -thalassemia/Hb E patients with curcuminoids for 12 months will yield similar or better results. Curcuminoids at the dose of 500 mg daily for 12 months did not have any toxic and side effects in the patients as evaluated by hematological profiles, liver and renal function tests, lipid profiles, and level of uric acid. The safety of curcuminoids was encouraged by clinical studies in patients with pre-malignant lesions consuming curcumin at the dose of 8 g daily for up to 3 months [48] or even at the single dose of 12 g in healthy volunteers [49].

The levels of serum NTBI were significantly reduced during the first 6 months of treatment and slightly increased afterward, whereas the levels of serum ferritin were nonsignificantly decreased (Table 3). Curcumin is a bidentate chelator of Fe^{3+} where the formation of Fe^{3+} -curcumin complex occurred via the β -diketonate group [50] with a constant of 10^{22} in the cell-free system [51]. Since iron chelators remove NTBI transiently and incompletely [16], it is possible that this is the reason why serum NTBI or MDA levels in RBC decreased during the first 6 months, but remained unchanged or gradually increased up to 12 months. Besides iron-binding, curcumin modulated proteins involved in cellular iron metabolism [14]. In response to curcumin, both transferrin receptor 1 and iron regulatory proteins (IRPs) increased in cultured liver cells. Interestingly, conflict between increased mRNA and decreased protein levels of ferritin was also reported indicating another mode of action of curcumin. This may explain the slight decrease of serum ferritin levels in our patients.

Ratio of cellular GSH/GSSG indicating intracellular redox status plays an important role in redox-dependent signaling pathway. Compared β -thalassemia/Hb E patients with normal subjects [25], the ratios of GSH/GSSG were markedly decreased whereas ROS levels were highly elevated, and it was also found that activities of glutamate-cysteine ligase, the enzyme involved in the rate-limiting step of glutathione synthesis, increased approximately 2-fold in patients. Decreased GSH/GSSG ratio results in upregulation of several enzymes or proteins involved in redox system including SOD, GSH-Px, glutamate-cysteine ligase, thioredoxin reductase, and metallothionein [52]. Another mode of action, curcumin is able to raise GSH/GSSG ratio by increasing cellular GSH content via stimulating Nrf2 expression [53,54] followed by raising Nrf2 nuclear translocation [55] and finally, increasing the expression of glutamate-cysteine ligase [56]. The antioxidant activity of curcuminoids resulted in a significant decrease in the antioxidant enzymes SOD and GSH-Px ($p < 0.001$) concomitant with an increase in GSH levels in RBC and a decrease in the percentage of MHB throughout the period of

treatment. Besides increasing cellular GSH content, curcumin is able to donate H atom from the phenolic groups [57] directly to superoxide anion and hydroxyl radical [58], lowering ROS level.

There were no changes in Hb levels although in in vitro study, curcuminoids showed the protective effect of RBC from free radical-induced hemolysis in a concentration-dependent manner [59]. However, this effect in vivo may be uncertain because of extremely low bioavailability as a consequence of poor solubility of curcumin in an aqueous condition and rapid metabolism in liver and intestine [60]. After single dose of 2 g curcumin for 1 h, undetectable or very low level ($0.006 \pm 0.005 \mu\text{g/mL}$) was found in human serum [61]. Several clinical studies confirming the poor bioavailability of curcumin administered at various dosages were also reported in Anand et al. [60].

The iron-chelating ability of any ligands can be compared using pFe^{3+} defined as $-\log [Fe^{3+}]$ at pH 7.4, 10 μM ligand, and 1 μM Fe^{3+} . Curcumin, the major constituent of curcuminoids used in this study, is a moderate iron chelator ($pFe^{3+} = 16.6$) [14] compared to common clinical iron chelator such as deferoxamine (DFO, $pFe^{3+} = 20$) [62] and deferoxamine (DFP, $pFe^{3+} = 26$) [50]. Supporting the comparison, at equivalent concentrations in vitro study DFO, DFP and curcumin decreased plasma NTBI with the order of $DFP > DFO > \text{curcumin}$; curcumin and deferoxamine, however, act in synergy to increase the rate of NTBI removal [16]. Moreover, the antioxidant capacity of curcuminoids can be increased if given together with vitamin E [63]. Therefore, synergistic effect of curcuminoids with other antioxidants/iron-chelators should be further studied in vivo. This may be a promising strategy for alleviating pathology associated to oxidative stress in patients with various forms of thalassemia, decreasing adverse effects of iron chelators, or eventually may result in an increase in Hb levels.

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