Differentially expressed plasma proteins of β-thalassemia/hemoglobin E patients in response to curcuminoids/vitamin E antioxidant cocktails

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ABSTRACT

Objective: Iron overload and oxidative stress are the major causes of serious complications and mortality in thalassemic patients. Our previous work supports the synergistic effects of antioxidant cocktails (curcuminoids or vitamin E, N-acetylcysteine, and deferiprone) in treatment of β-thalassemia/Hb E patients. This further 2-DE-based proteomic study aimed to identify the plasma proteins that expressed differentially in response to antioxidant cocktails.

Methods: Frozen plasma samples of ten normal subjects and ten β-thalassemia/Hb E patients at three-time points (baseline, month 6, and month 12) were reduced the dynamic range of proteome using ProteoMiner kit and separated proteins by two-dimensional gel electrophoresis. Differentially expressed proteins were identified using tandem mass spectrometry. Several plasma proteins were validated by ELISA and Western blot analysis.

Results: Thirteen and 11 proteins were identified with altered expression levels in the curcuminoids- and vitamin E cocktail groups, respectively. The associations between vitronectin (VTN) expression and total bilirubin levels, as well as between serum paraoxonase/arylesterase 1 (PON1) expression and blood reactive oxygen species were observed. Validation results were consistent with proteomics results.

Discussion and conclusions: These plasma proteins may provide better understanding of the mechanisms underlying the therapeutic effects of antioxidant cocktails in thalassemic patients.

Introduction

β-thalassemia/Hb E has the phenotypic diversity and high prevalence in Southeast Asia. It is responsible for up to 50% severe cases of β-thalassemia worldwide [1]. In β-thalassemia, primary and secondary iron overload induce oxidative stress leading to several pathological consequences such as anemia, hypercoagulability, hepatosplenomegaly, tissue injuries, and organ dysfunctions [2]. Therefore, antioxidants are required to alleviate or prevent such oxidative damages.

Previous studies revealed the therapeutic effects of antioxidants in thalassemic patients including curcuminoids [3], N-acetylcysteine [4], and vitamin E [5]. In recent years, there is an increasing interest in using a combination of antioxidants to treat many diseases. However, only a few clinical studies were performed in thalassemia [6–8]. Our previous study showed that one-year treatment with two antioxidant cocktails consisting of hydrophobic- (curcuminoids/ vitamin E), hydrophilic antioxidant (N-acetylcysteine), and oral iron chelator (deferiprone) could improve anemia, decrease oxidative stress, and reverse hypercoagulability in β-thalassemia/Hb E patients [7]. Although the beneficial effects of these antioxidant cocktails in thalassemic patients have been demonstrated, the underlying mechanisms of such effects still remain to be elucidated. Notably, proteomic approach has been widely used to explore biomolecular alteration associated with therapeutic effects of herbal extract in various diseases [9–11].

Interestingly, two-dimensional fluorescence difference gel electrophoresis had been performed in albumin- and IgG-depleted plasma of β-thalassemia/Hb E patients treated with curcuminoids for 12 months [12]. All proteins with differential expression in response to the treatment were coagulation proteins which are related to hypercoagulable state in these patients. However, the limitation of the aforementioned technique is insufficient removal of high-abundance proteins, hindering the detection of low-abundance plasma proteins. Therefore, in the present study, we apply low-abundance protein enrichment approach in order to reduce high-abundance proteins.
proteins in plasma samples before proceeding with proteomic techniques to determine plasma proteome changes as well as proteins that are associated with improved clinico-biochemical conditions in thalassemic patients after antioxidant cocktails treatments.

Materials and methods

Plasma specimens

This study was approved by the Siriraj Institutional Review Board of Mahidol University, Thailand. Three time points (baseline, month 6, and month 12) of plasma from each 10 non-splenectomized, non-transfusion dependent β-thalassemia/HbE patients treated with curcuminoids- (M/F = 5/5, age 35 ± 3 Y/O) or vitamin E antioxidant cocktail (M/F = 4/6, age 34 ± 3 Y/O) were obtained from our previous project [7]. Plasma were derived from the patients who completed the treatment for 12 months, did not receive transfusion throughout the treatment, had improvements in pathological parameters, and signed informed consent form (Table 1). Plasma from 10 normal subjects was collected with the inclusion criteria as follows: (i) normal Hb typing (A2A); (ii) sex- and age-matched patients; (iii) no history of severe diseases; (iv) no alcohol consumption or smoking; and (v) no pregnancy or breastfeeding. All plasma specimens were stored at −80°C until used.

Sample preparation and two-dimensional gel electrophoresis

The ProteoMiner™ Protein Enrichment Kit (Bio-Rad Laboratories, Hercules, CA, USA) was used to reduce broad concentration range of plasma proteins. Depleted plasma was removed ionic contaminants using a 2-D Clean-Up Kit (GE Healthcare Biosciences Corp., Piscataway, NJ, USA). The pellet was resuspended in lysis buffer and measured the concentration by the Bradford Total Protein Assay (Bio-Rad Laboratories). Each 75 micrograms of plasma proteins were loaded overnight on 7 cm pH3-10 nonlinear IPG strips (GE Healthcare Biosciences) followed by the first-dimensional separation using the Ettan IPGphor 3 Isoelectric Focusing Unit (GE Healthcare Biosciences Corp.) and the second-dimensional separation on 12.5% acrylamide gel, 12 mA/gel for 3 h. Gels were stained with SYPRO® Ruby Protein Gel Stain (Invitrogen Corp., Carlsbad, CA, USA) and scanned using a Typhoon FLA 7000 (GE Healthcare). After scanning, gels were stained with Coomassie® Brilliant Blue R 250 dye (Bio-Rad Laboratories) for mass spectrometric analysis.

Spot quantitation was performed with the ImageMaster 2D Platinum 7.0 software Package (GE Healthcare). The intensity of each spot was evaluated as the relative intensity volume (%vol) normalized with the total intensities of all spots on the same gel.

In-gel digestion and LC-MS/MS analysis

Protein spots with significant difference between groups (p < .05) were excised, destained, reduced, and alkylated before incubating at 37°C overnight with a digestion buffer containing 10 ng/µl trypsin. After stopping the reaction with 2% trifluoroacetic acid, the peptides were purified using the ZipTip® Pipette Tips (C18 resin, P10; Millipore Corp., Bedford, MA, USA).

Mass spectrometric analysis was performed using nanoflow liquid chromatography coupled with electrospray ionization/ion trap tandem-mass spectrometry (Bruker Corp., Coventry, UK). Peak lists with a range from 50 to 3000 m/z were searched using the Mascot searching tool (www.matrixscience.com) against the Swiss-Prot human database. Research parameters were set as described in Weeraphan et al. [12].

Enzyme-linked immunosorbent assay (ELISA)

A total of 70 plasma samples consisting of 10 normal, 30 curcuminoids cocktail-treated group (10 each at baseline, month 6, and month 12), and 30 vitamin E cocktail-treated group (10 each at baseline, month 6, and month 12) were analyzed using the commercial ELISA kits for vitronectin and serum paraoxonase/
arylesterase 1 (Cloud-Clone Corp., Houston, TX, USA) following the manufacturer's supplied protocols.

**Western blot analysis**

Ten micrograms total protein of depleted plasma from healthy controls (n = 10) and patients at baseline, month 6, and month 12 (n = 10 for each group) were resolved in 12.5% SDS-PAGE gels under the constant current 10 mA/gel for 3 h. Proteins were transferred to PDVF membranes under the constant voltage (100 V for 1 h and 11 min) and the membranes were then blocked with 5% bovine serum albumin (BSA) in tris-buffered saline/Tween® 20 (TBS/T) at room temperature for one hour. Primary antibodies used were mouse monoclonal anti-apoliprotein E (1:1000 dilution, Abcam, Cambridge, MA, USA), rabbit polyclonal anti-prothrombin (1:1000 dilution, Abcam), and mouse monoclonal anti-apoA1 (1:10,000 dilution, Abcam). Blocked membranes were incubated with the primary antibodies at 4°C overnight, washed with TBS/T, and then incubated with respective horseradish peroxidase (HRP)-conjugated secondary antibodies (1:2000 dilution, Dako, Denmark) at room temperature for one hour. Detection was performed using WesternBright ECL (Advansta, California, USA) and ImageQuantTM LAS 4000 digital imaging system (GE Healthcare).

**Statistical analysis**

The unpaired Student’s t-test was used to analyze the differences of data between normal and patient groups, whereas the differences between time points of treatment were performed with the paired t-test using SPSS 18.0. Data represented in this study were mean ± standard error of the mean (SEM) and the significance was reported at p-value < .05.

**Results**

There were 1230 ± 28, 1092 ± 28, 1143 ± 27, and 1107 ± 30 spots detected on 2-DE images of the groups of normal volunteers, patients at baseline, month 6, and month 12 of curcuminoids cocktail treatment, respectively. Sixteen spots corresponding 13 proteins presented quantitative differences between time points of the treatment (Table 2). Furthermore, 14 differentially expressed spots from patients’ plasma at baseline (1064 ± 37 spots), month 6 (1209 ± 21), and month 12 (1144 ± 34) of vitamin E cocktail treatment were identified as 11 proteins (Table 3).

In this study, we observed the significant correlations between vitronectin (VTN) spot intensities and total bilirubin obtained from our previous study [7] (r = −0.517, p = .004 in curcuminoids group; r = −0.384, p = .036 in vitamin E group), as well as between serum paraoxonase/arylesterase 1 (PON1) and blood reactive oxygen species (r = −0.434, p = .016 in curcuminoids group) (Figures 1 and 2).

In agreement with the 2-DE results, the levels of VTN in patients’ plasma analyzed by ELISA assays were significantly decreased compared to normal (214 ± 7 and 223 ± 7 µg/ml for baseline curcuminoids and vitamin E groups, respectively, 269 ± 7 µg/ml for normal; p < .001), similar to that observed in PON1 (21.0 ± 0.2 µg/ml for

**Table 2. Plasma proteins differentially expressed in β-thalassemia/Hb E patients treated with curcuminoids antioxidant cocktail.**

| Protein                          | MW (kDa)/pl | MS/MS score | Peptide matches | % Coverage | % Volume protein intensity |
|----------------------------------|-------------|-------------|-----------------|------------|--------------------------|
| **Adhesive protein**             |             |             |                 |            |                          |
| Vitronecin                       | 55.07/5.55  | 184         | 3               | 8          | Normal: 1.007 ± 0.110; Baseline: 0.686 ± 0.046; Month 6: 0.913 ± 0.055; Month 12: 0.758 ± 0.071 |
| Vitronecin fragment 1             | 55.07/5.55  | 101         | 2               | 5          | Normal: 0.375 ± 0.044; Baseline: 0.223 ± 0.016; Month 6: 0.243 ± 0.019; Month 12: 0.280 ± 0.028 |
| **Antioxidants**                 |             |             |                 |            |                          |
| Paraoxonase/arylesterase 1        | 39.88/5.08  | 82          | 3               | 7          | Normal: 0.234 ± 0.030; Baseline: 0.153 ± 0.006; Month 6: 0.161 ± 0.010; Month 12: 0.213 ± 0.023 |
| Protein AMBP                      | 39.89/5.95  | 85          | 1               | 3          | Normal: 0.027 ± 0.004; Baseline: 0.012 ± 0.001; Month 6: 0.012 ± 0.001; Month 12: 0.016 ± 0.002 |
| **Cell motility, actin-binding proteins** |        |             |                 |            |                          |
| Myosin-1 fragment 1               | 223.98/5.59 | 76          | 2               | 1          | Normal: 0.203 ± 0.013; Baseline: 0.159 ± 0.013; Month 6: 0.212 ± 0.019; Month 12: 0.216 ± 0.023 |
| Tropomysin alpha-3 chain          | 28.91/4.72  | 103         | 4               | 12         | Normal: 0.062 ± 0.014; Baseline: 0.008 ± 0.001; Month 6: 0.017 ± 0.003; Month 12: 0.024 ± 0.006 |
| **Lipoproteins**                 |             |             |                 |            |                          |
| Apolipoprotein E isoform 1        | 36.25/5.65  | 149         | 10              | 25         | Normal: 0.148 ± 0.030; Baseline: 0.284 ± 0.013; Month 6: 0.233 ± 0.011; Month 12: 0.207 ± 0.020 |
| Apolipoprotein E isoform 2        | 36.25/5.65  | 177         | 11              | 28         | Normal: 0.255 ± 0.041; Baseline: 0.519 ± 0.019; Month 6: 0.442 ± 0.022; Month 12: 0.394 ± 0.041 |
| Apolipoprotein A-I fragment 1      | 30.76/5.36  | 85          | 1               | 4          | Normal: 0.023 ± 0.008; Baseline: 0.006 ± 0.001; Month 6: 0.012 ± 0.003; Month 12: 0.009 ± 0.003 |
| Apolipoprotein C-II               | 8.91/4.66   | 172         | 4               | 73         | Normal: 0.142 ± 0.025; Baseline: 0.214 ± 0.020; Month 6: 0.166 ± 0.016; Month 12: 0.155 ± 0.013 |
| **Immune system proteins**        |             |             |                 |            |                          |
| Complement factor H fragment 1    | 143.68/6.21 | 318         | 11              | 11         | Normal: 0.069 ± 0.007; Baseline: 0.049 ± 0.004; Month 6: 0.066 ± 0.007; Month 12: 0.062 ± 0.006 |
| C4b-binding protein alpha chain fragment 1 | 69.04/7.15 | 83          | 2               | 4          | Normal: 0.024 ± 0.003; Baseline: 0.014 ± 0.001; Month 6: 0.023 ± 0.003; Month 12: 0.026 ± 0.002 |
| **Coagulation proteins**          |             |             |                 |            |                          |
| Prothrombin fragment 1            | 71.48/5.64  | 148         | 5               | 9          | Normal: 0.220 ± 0.032; Baseline: 0.079 ± 0.007; Month 6: 0.086 ± 0.009; Month 12: 0.102 ± 0.013 |
| Fibrinogen gamma chain fragment 1  | 52.11/5.37  | 173         | 3               | 7          | Normal: 0.056 ± 0.022; Baseline: 0.007 ± 0.001; Month 6: 0.011 ± 0.002; Month 12: 0.013 ± 0.002 |
| **Carrier protein**               |             |             |                 |            |                          |
| Transthyretin isoform 1            | 15.99/5.52  | 81          | 2               | 23         | Normal: 0.031 ± 0.005; Baseline: 0.092 ± 0.006; Month 6: 0.121 ± 0.011; Month 12: 0.069 ± 0.005 |
| Transthyretin isoform 2            | 15.99/5.52  | 131         | 2               | 23         | Normal: 0.380 ± 0.023; Baseline: 0.772 ± 0.026; Month 6: 0.685 ± 0.034; Month 12: 0.594 ± 0.035 |

Note: Values were mean ± SEM. * Compared to normal, p < .05; † Compared to baseline, p < .05; ‡ Compared to normal, p < .001; § Compared to baseline, p < .001.
baseline curcuminoids group, 3.5 ± 0.3 µg/ml for normal; p < .001 (Figures 1 and 2). The antioxidant cocktails could enhance their levels in patients’ plasma. During the curcuminoids cocktail treatment, VTN levels increased from 214 ± 7 (baseline) to 232 ± 9 (month 6, p < .05) and 275 ± 15 µg/ml (month 12, p < .001). In the group of vitamin E cocktail, VTN increased from 223 ± 7 (baseline) to 267 ± 12 (month 6, p < .05) and 279 ± 16 µg/ml (month 12, p < .05). PON1 in plasma of patients treated with curcuminoids cocktail rose from 2.1 ± 0.2 µg/ml (baseline) to 2.7 ± 0.1 and 2.6 ± 0.1 µg/ml (month 6 and month 12, respectively, p < .001).

By using Western blot analysis, prothrombin, apoE, and apoA-I were detected as bands at 70, 34, and 30 kDa, respectively (Figure 3).

Table 3. Plasma proteins differentially expressed in β-thalassemia/Hb E patients treated with vitamin E antioxidant cocktail.

| Protein                              | MW (kDa)/ pI | MS/MS score | Peptide matches | % Coverage | Normal | Baseline | Month 6 | Month 12 |
|--------------------------------------|--------------|-------------|-----------------|------------|--------|----------|--------|----------|
| **Cell motility: actin-binding proteins** |              |             |                 |            |        |          |        |          |
| Gelsolin                             | 86.04/5.90   | 74          | 1               | 1          | 0.019 ± 0.004 | 0.007 ± 0.001a | 0.007 ± 0.002 | 0.010 ± 0.001b  |
| **Immune system proteins**           |              |             |                 |            |        |          |        |          |
| Complement C3 fragment 1             | 188.6/6.02   | 308         | 6               | 18         | 0.003 ± 0.001 | 0.036 ± 0.002c | 0.033 ± 0.003 | 0.021 ± 0.003d  |
| Complement C3 fragment 2             | 188.6/6.02   | 127         | 5               | 2          | 0.058 ± 0.009 | 0.391 ± 0.017c | 0.320 ± 0.016a | 0.268 ± 0.010c  |
| Complement factor H                  | 143.68/6.21  | 318         | 11              | 11         | 0.069 ± 0.007 | 0.046 ± 0.004a | 0.057 ± 0.005a | 0.063 ± 0.008b  |
| **Inflammatory response protein**    |              |             |                 |            |        |          |        |          |
| CD5 antigen-like                     | 39.60/5.28   | 133         | 2               | 7          | 0.262 ± 0.035 | 0.564 ± 0.053c | 0.495 ± 0.045b | 0.485 ± 0.046b  |
| **Lipoproteins**                     |              |             |                 |            |        |          |        |          |
| Apolipoprotein A-IV                  | 45.37/5.28   | 57          | 1               | 3          | 0.079 ± 0.009 | 0.048 ± 0.006a | 0.061 ± 0.009 | 0.093 ± 0.010d  |
| Apolipoprotein E isoform 2           | 36.25/5.65   | 177         | 11              | 28         | 0.255 ± 0.041 | 0.523 ± 0.038c | 0.477 ± 0.036 | 0.395 ± 0.030d  |
| Apolipoprotein A-I fragment 1        | 30.76/5.56   | 85          | 1               | 4          | 0.023 ± 0.008 | 0.005 ± 0.001a | 0.007 ± 0.001a | 0.009 ± 0.002d  |
| **Coagulation proteins**             |              |             |                 |            |        |          |        |          |
| Fibrinogen gamma chain fragment 1    | 52.11/5.37   | 191         | 4               | 12         | 0.046 ± 0.004 | 0.019 ± 0.003c | 0.024 ± 0.005 | 0.033 ± 0.006b  |
| Fibrinogen gamma chain fragment 2    | 52.11/5.37   | 61          | 1               | 3          | 0.020 ± 0.003 | 0.008 ± 0.001a | 0.010 ± 0.001 | 0.012 ± 0.001d  |
| **Antioxidants**                     |              |             |                 |            |        |          |        |          |
| Glutathione peroxidase 3             | 25.76/8.26   | 119         | 2               | 11         | 0.150 ± 0.009 | 0.176 ± 0.006a | 0.149 ± 0.008b | 0.155 ± 0.006b  |
| Haptoglobin-related protein fragment 1| 39.52/6.63   | 143         | 2               | 3          | 0.015 ± 0.004 | 0.004 ± 0.001a | 0.005 ± 0.001b | 0.004 ± 0.001  |
| **Adhesive protein**                 |              |             |                 |            |        |          |        |          |
| Vitronectin fragment 1               | 55.07/5.55   | 101         | 2               | 5          | 0.375 ± 0.044 | 0.226 ± 0.029a | 0.291 ± 0.033b | 0.261 ± 0.031b  |
| Vitronectin fragment 2               | 55.07/5.55   | 35          | 1               | 2          | 0.018 ± 0.003 | 0.010 ± 0.001a | 0.017 ± 0.002b | 0.018 ± 0.003b  |

Note: Values were mean ± SEM. a Compared to normal, p < .05; b Compared to baseline, p < .05; c Compared to normal, p < .001; d Compared to baseline, p < .001.

![Figure 1](image-url). (A) Correlations between vitronectin intensities and total bilirubin levels; (B) The ELISA results of vitronectin in normal subjects and patients treated with curcuminoids and vitamin E antioxidant cocktails. *p < .001 compared to normal; **p < .05 compared to normal; *p < .001 compared to baseline; b p < .05 compared to baseline.
apoE were significantly up-regulated after treating with curcuminoids cocktail for 12 months, whereas there were no significant changes in the vitamin E cocktail group. Both antioxidant cocktails significantly reduced ApoA-I expressions which were consistent with proteomic results.

Discussion

In our previous study [7], both curcuminoids- and vitamin E cocktails significantly decreased iron-induced oxidative stress, increased Hb concentrations, and reduced hypercoagulability in β-thalassemia/Hb E patients. ProteoMiner™ Protein Enrichment Kit removed 96.6% of total plasma protein from patients (data not shown), similar to previous studies [13,14]. The proteomic results revealed 13 and 11 plasma proteins with quantitative changes in response to curcuminoids- and vitamin E cocktails, respectively (Tables 2 and 3).

Iron-induced oxidative membrane damage including phosphatidylserine (PS) exposure and band 3 clustering is responsible to the complement-mediated hemolysis and hypercoagulability in thalassemia [15–17]. The significantly increased amounts of immunoglobulins, complement C3, and the membrane attack

![Figure 2](image2.png)

**Figure 2.** (A) Correlations between paraoxonase/arylesterase 1 intensities and blood reactive oxygen species; (B) The ELISA results of paraoxonase/arylesterase 1 in normal subjects and patients treated with curcuminoids antioxidant cocktail. * p < .001 compared to normal; ** p < .05 compared to normal; † p < .05 compared to baseline.

![Figure 3](image3.png)

**Figure 3.** (A) The bands of Western blots corresponding to prothrombin, apolipoprotein E, and apolipoprotein A-1 of normal subjects (N), thalassemic patients at baseline, month 6 (M6), and month 12 (M12) after curcuminoids- and vitamin E antioxidant cocktails treatments. The graphs (B) and (C) represent the quantitation of the Western blots for these proteins as percentage of the mean value for normal subjects in curcuminoids- and vitamin E antioxidant cocktails groups, respectively. Values were mean ± SEM. * p < .05 and ** p < .01 compared to baseline.
complex C5b-9 on the circulating RBC membrane from β-thalassemia/Hb E patients were reported [18]. Oxidative stress has been found to upregulate complement C3, a central protein of the complement system, and antioxidant treatment could suppress its expression [19]. This supports our result that the complement C3 levels were decreased throughout the vitamin E cocktail treatment.

Besides alleviation of oxidative stress, antioxidant cocktails may partly improve anemia by the inhibitory effects of curcumin [20] and glutathione [21] on the complement system with unknown mechanism. The increased levels of various inhibitors of complement pathway (e.g. vitronectin, complement factor H, and C4b-binding protein) were also indicated (Tables 2 and 3). Vitronectin inhibits complement-mediated hemolysis at the common terminal pathway by blocking the late complement complex (C5b-9) formation [22]. The moderate correlations between vitronectin and total bilirubin levels, a marker of extravascular and intravascular hemolysis [23], were found in both groups (Figure 1). Complement factor H has been reported to inhibit the alternative pathway by preventing the formation of C3 and C5 convertases [24], while the alpha chain of C4b-binding protein inhibits the classical and lectin pathways [25].

The proteomic results showed the enhanced levels of coagulation proteins, fibrinogen gamma chain and prothrombin, following the cocktail treatments due to decreased consumption of such coagulation factors as a result of reverse hypercoagulability (Table 1), supported by a previous study [12].

The abnormality of plasma lipid profile in thalassemic patients including decreased levels of total cholesterol, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), together with increased levels of triglycerides have been reported [26–28]. Our results showed the altered levels of apolipoprotein A-I, A-IV, C-II, and E in response to the treatments. Apolipoprotein E (ApoE) plays important role in regulation of circulating lipoprotein levels, involving in the cholesterol- and triglyceride-rich lipoproteins transport and clearance [29,30]. High apoE levels in our thalassemic plasma were confirmed by Western blot analysis as well as reported in blood microparticles from β-thalassemia/Hb E patients [30]. The levels were decreased toward normal values during the treatments. Apolipoprotein A-I (apoA-I), the major component of HDL, contributes to antioxidant effect of HDL-C by removing oxidized phospholipids and lipid hydroperoxides from LDL, thus preventing LDL oxidation and reducing atherogenesis risk [31]. Curcumin and vitamin E exert anti-atherosclerosis through several mechanisms including increases of plasma HDL-C and apo A-I expressions [32,33]. ApoA-I levels were significantly reduced in plasma of β-thalassemia/Hb E patients and treatments with the antioxidant cocktails increased plasma apoA-I levels, as confirmed by Western blot analysis.

Serum paraoxonase/arylesterase 1 (PON1) is an antioxidant enzyme carried on HDL that also hydrolyzes lipid peroxidation products in LDL [34]. Decreased activities of PON1 associated with increased oxidative stress, decreased Hb levels, and increased atherosclerosis risk have been reported in thalassemic patients [34–37]. Curcuminoids cocktail enhances PON1 intensities in correlation with decreased ROS levels, which was consistent with the ELISA results (Figure 2), supporting by a previous in vivo study [38].

Glutathione peroxidase 3 (GPx3) also detoxifies hydrogen peroxide and lipid peroxides in the circulation and oxidized LDL. Oxidative stress and hypoxia are strong inducers of GPx3 expression [39,40], therefore the plasma GPx3 levels were decreased after vitamin E cocktail treatment, as well as observed in thalassemic patients chelated with deferiprone [41], or with vitamin E alone [42]. Therefore, antioxidant cocktails may provide anti-atherosclerosis benefit to thalassemic patients through increasing the expressions of apoA-I, PON-1, and GPx3. However, several proteins were also reported with unknown relation to thalassemia. They were alpha-1-microglobulin/bikunin precursor (AMBP), CDS antigen-like protein, haptenoglobin-related protein, myosin-1, gelsolin, tropomyosin alpha-3 chain, and transthyretin.

In conclusion, plasma proteomic analysis has identified several proteins which may be associated with the mechanisms underlying the pleiotropic effects of antioxidant cocktails. Further studies are required to investigate their pathophysiological roles that could improve our understanding and may lead to novel mechanisms or targets for treatment of thalassemia.

Disclosure statement

No potential conflict of interest was reported by the authors.

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