

Amino acids and fatty acids in patients with beta thalassemia major

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Summary. *Background:* Oxidative damage and increasing of lipid peroxidation are caused by chronic iron overload in patients with beta thalassemia major. Fatty acids are important structural elements for palmitoylation of membrane proteins which constitute a great part of natural membranes. Oxidative damages caused by reactive oxygen derives in thalassemic erythrocytes can be determined with lipid peroxidation, protein oxidation, and antioxidant system elements. The aim of study was to evaluate the relationship between amino acid and fatty acid levels with iron overload and antioxidant enzymes in beta thalassemia major. *Methods:* A total 40 patients with beta thalassemia major with regular blood transfusion and chelating agents were included in the study. The levels of serum amino acid, fatty acid, ferritin, antioxidant enzymes and malondialdehyde were measured. *Results:* Only C16- palmitoyl level was found significantly low in patients, other fatty acids and amino acids were in normal range. There were lower malondialdehyde and ferritin levels in patients with low C-16 palmitoyl level ($p < 0.05$). *Conclusions:* The high levels of ferritin and malondialdehyde in the patients with low C16-palmitoyl levels might be caused by this fatty acid's preventative effect on oxidative stress. (www.actabiomedica.it)

Key words: amino acids, antioxidants, fatty acids, ferritin, thalassemia major

Introduction

Many recent studies about oxidative stress and antioxidant system in patients with beta thalassemia major (β -TM) show decreasing in antioxidant levels and increasing in the lipid peroxidation of erythrocyte membranes. The oxidative stress causes are the unpaired excess alpha chains, non-Hb iron and low levels of intracellular hemoglobin (1). Since the cross-reactivity between free radicals and molecules that including unsaturated fat and sulphate, proteins that have amino acids such as phenylalanine, tyrosine, tryptophan, histidine, and methionine are easily affected by free radicals (2). Fatty acids are important structural elements for palmitoylation of membrane proteins

which constitute a great part of natural membranes (3). It has been argued that fatty acids are metabolic energy sources, play an important role in cell homeostasis, affects immune system, and that some of them show antimicrobial and anticancer activity (4). The aim of study was to evaluate the relationship between amino acid and fatty acid levels with iron overload and oxidative stress in β -TM.

Methods

A total 40 patients with β -TM aged 7–30 years were included in this study. Twenty-two of 40 had splenectomy. Blood samples were taken as late as pos-

sible, at least 3-4 weeks after the last transfusion. The patients did not take any medications for at least 1 week prior to blood sample collections except iron chelating agents. All patients have been treated with regular blood transfusion (15 cc/kg per month) and chelating agents (30 mg/kg/day deferasirox). The blood samples of the patients were taken for the levels of serum ferritin, amino acids, fatty acids, malondialdehyde (MDA), catalase (CAT), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD). As amino acids; valine, leucine/isoleucine, methionine, phenylalanine, tyrosine, aspartate, glutamate, argininosuccinate, ornithine, citrulline, glycine, alanine, arginine, phenylalanine/tyrosine and as free fatty acids; free carnitine, C2-acetyl, C3-propionyl, C4-butyryl, C5:1-tiglyl, C5-isovaleryl, C4-OH-3-OH-butyryl, C6-hexanoyl, C5-OH-3-OH-isovaleryl, C8-octanoyl, C10-decanoyl, C5-DC-glutaryl, C12-dodecanoyl, methylglutaryl, C14:1, C14-myristoyl, C14-OH-3-OH-myristoyl, C16:1-palmitoleyl, C16-palmitoyl, C18:1-OH-3-OH-oleoyl, C16-OH-3-OH-palmitoyl, C18:1-oleoyl, C18-stearoyl, C16:1-OH-3-OH-palmitoleyl levels were measured by using electrospray tandem mass spectrometry method (5). Ferritin levels were studied at the same day and the samples for the antioxidant study stored at -80°C and analyzed by spectrophotometric method. The SOD level was measured by the method described by Williams al (6). Measurement of GSH-Px activity was based on the method of Paglia and Valentine (7). The activity of catalase enzyme was measured by the Aebi method (8). MDA levels were measured by the double heating method of Draper and Hadley (9).

Serum AST and ALT estimation was done by semi auto analyser. Serum level of AST >40 IU/L and ALT >38 IU/L were considered abnormal (10).

Exclusion criteria were samples with recorded hemolysis, and lypemia.

Statistical Analysis: SPSS 15.0 packaged software was used for the statistical evaluations for the statistical analysis, Mann Whitney U test was used to compare the data and Pearson correlation coefficient was computed to show the correlation between variables. This study was carried out with the permission of Ethics Committee of Medical School of Suleyman Demirel University and consents of the patients' relatives within The Helsinki Rules.

Results

The study involved 40 patients (62.5% female), with the mean age of 18.58±5.7 years. 10 patients were older than 20 years, 22 patients were between 15-19 years old, and 8 patients were younger than 14 years.

Mean serum ferritin was 4533±2116 (577-10741) ng/mL. Mean AST was 84.90±9.92 IU/L and mean ALT was 108.32±15.97 IU/L which were higher than normal value. Serum AST and ALT levels were found to be above three times the normal range in 27.5% and 20% of patients, respectively. 13 (32.5%) of the patients' serum ferritin levels were between 2000 and 4000 ng/mL, 12 (30%) of the patients' serum ferritin levels between 4000 and 6000 ng/mL and 10 (25%) of the children had even higher serum ferritin levels (>6000 ng/mL) in spite of the chelation. Only 5 (12.5%) patients maintained serum ferritin levels <2000 ng/mL. Serum liver enzymes at various levels of serum ferritin levels were as shown in Table 1. The statistically significant difference in AST and ALT was observed once the serum ferritin crossed level of 4000 ng/mL ($p < 0.05$).

As liver enzymes were analyzed at different serum ferritin levels, simultaneously rising as the serum ferritin was increasing. A steep rise in liver enzyme was noticed after the level of 2000 ng/mL as shown in Figure 1.

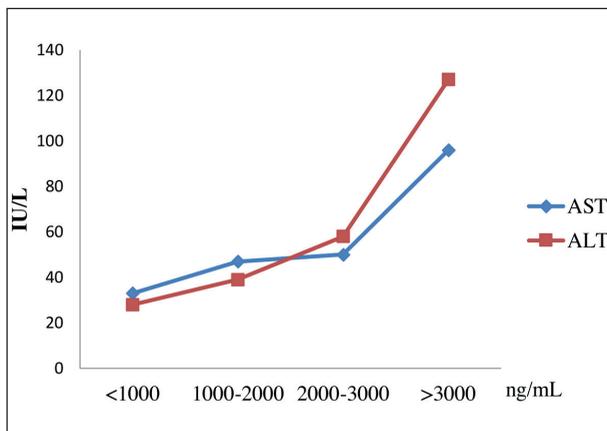
As fatty acids, free carnitine, C2-acetyl, C3-propionyl, C16-palmitoyl were found as low in 1 (2.5%), 3 (7.5%), 8 (20%) patients; respectively, C18:1-oleoyl was found as high in 2 patients (5%). Other fatty acid levels were found in normal range. The mean value of MDA of 14 patients with low C-16 palmitoyl levels was 155.47±16 nmol/gr, while the mean value of MDA of 26 patients with normal C-16 palmitoyl levels was 129.38±11 nmol/gr. This MDA difference between low and normal C-16 palmitoyl levels is statistically significant ($p < 0.05$). While the mean value of ferritin levels of 14 patients with low levels of C-16 palmitoyl was 5595.85±517 ng/dL, the mean value of ferritin levels of 26 patients with normal C-16 palmitoyl levels was 3993.0±390 ng/dL. This difference in ferritin levels is statistically significant ($p < 0.05$) (Table 2).

The relationship between ferritin and MDA with antioxidant enzyme levels was evaluated. Although SOD level increases and MDA, GSH-Px, catalase levels decrease with ferritin increases, there was no sig-

Table 1. Liver enzymes at different serum ferritin levels

Ferritin (ng/mL)	n	AST (IU/L) (mean \pm SD)	P	ALT (IU/L) (mean \pm SD)	P
<2000	5	44.40 \pm 27.01	>0.05	36.80 \pm 20.14	>0.05
2000-4000	13	58.38 \pm 30.06	>0.05	65.15 \pm 35.42	>0.05
4000-6000	12	92.08 \pm 50.83	0.02	118.33 \pm 98.89	0.02
>6000	10	131.00 \pm 89.57	0.02	188.20 \pm 132.87	0.01

n: Number of cases; SD: Standard deviation

**Figure 1.** Trend of liver enzymes with rising serum ferritin

nificant correlation between ferritin and antioxidants (Table 2).

Correlation between ferritin and amino acid levels were evaluated. It was found only weak negative

correlation between ferritin and glutamate. There was no significant correlation between ferritin and other amino acid levels. We also evaluated the correlation between ferritin and fatty acid levels. As a consequence, we found a weak negative correlation among ferritin, carnitine, C6-hexanoyl, C8, C12-dodecanoyl, and C14.

Discussion

Liver is the earliest organ affected by iron overload in thalassemia children and serum AST and ALT are raised due to peroxidative injury and direct toxic effect of iron on liver cells. In present study the serum ferritin concentration was very high in β -thalassemic children in spite of chelation therapy. Like other chronic diseases requiring long-life treatment, adherence to treatment is a major concern for β -TM patients. Poor adherence

Table 2. Comparison of C16-palmitoyl values with MDA, level of antioxidant enzymes and ferritin

C16-palmitoyl	n	MDA $\bar{X} \pm S_{\bar{x}}$	CAT $\bar{X} \pm S_{\bar{x}}$	GSH-Px $\bar{X} \pm S_{\bar{x}}$	SOD $\bar{X} \pm S_{\bar{x}}$	Ferritin $\bar{X} \pm S_{\bar{x}}$
Normal	26	129.38 \pm 11	396.82 \pm 61	59.75 \pm 2	1956.73 \pm 87	3993.00 \pm 390
Low	14	155.47 \pm 16	322.69 \pm 65	52.24 \pm 2	1866.22 \pm 106	5595.85 \pm 517
p		0.02	0.70	0.05	0.61	0.01

*Mann Whitney U

n: Number of cases, \bar{X} : Arithmetic mean, $S_{\bar{x}}$: Standard error mean

MDA: Malondialdehyde, CAT: Catalase, GSH-Px: Glutathione Peroxidase, SOD: Superoxide Dismutase

Table 3. Correlations between ferritin, MDA, CAT, GSH-Px, SOD

	MDA (r;p)	CAT (r;p)	GSH-Px (r;p)	SOD (r;p)
Ferritin	-0.012;0.943	-0.106;0.515	-0.014;0.933	0.274;0.092
MDA		0.036;0.830	0.147;0.380	-0.060;0.726
CAT			-0.006;0.971	0.203;0.216
GSH-Px				0.062;0.707

r: Pearson's correlation coefficient

MDA: Malondialdehyde, CAT: Catalase, GSH-Px: Glutathione Peroxidase, SOD: Superoxide Dismutase

remains a prevalent and persistent problem in these patients, with the reported rates ranging from 30 to 80 percent (11,12). It has been revealed that poor adherence to therapeutic regimen is associated with poor clinical outcomes including deranged liver functions. The study showed that AST and ALT were raised significantly ($p < 0.05$) and continue to rise as ferritin crosses 2000 ng/ml. These findings are in agreement with other previous studies who reported that serum ferritin increases liver enzymes also increases (13-16).

Fatty acids are important structural elements of natural membranes and constitute a great part of these membranes. Oxidative damages caused by reactive oxygen derivatives in thalassemic erythrocytes can be determined with lipid peroxidation, protein oxidation, and antioxidant immune system elements. Increasing of lipid peroxidation occurs with increasing of MDA (3,4,17,18). The MDA levels of patients have low fatty acid levels were evaluated and found only correlation between C16-palmitoyl. The mean value of MDA levels of the patients with low C16-palmitoyl levels was higher than that of the patients with normal C16-palmitoyl levels ($p < 0.05$). This decreasing in C16-palmitoyl level may demonstrate tissue damage caused by free radicals. There has been no published study about free fatty acid levels of patients with thalassemia major.

There are various studies about antioxidant enzyme levels (18-22). As compensatory reply to lipid peroxidation, some studies show increase in antioxidant enzyme (SOD, GSH-Px, CAT) levels while some other studies show decrease in these enzyme levels (23,24). This result can be explained by the increasing of oxidative stress (25) or insufficient chelation treatment causes iron overload (26). In this study,

erythrocyte GSH-Px, SOD and CAT enzyme activities of patients with low and normal serum fatty acid and amino acid levels were compared and there was no statistically significant difference.

Increased oxidative degradation caused by iron overload is important in pathogenesis of thalassemia. Increment of lipid peroxidation in chronic iron overload was shown in experimental animals and organs of patients with thalassemia. Livrea et al. have detected the positive correlation between serum ferritin level and MDA level in 42 patients with β -TM and have 1.866 \pm 996 ng/mL mean level of ferritin (27). In a similar study, Naithani et al. have detected serum ferritin level as 3.709 \pm 1.625 ng/mL in 50 patients with β -TM and observed negative correlation between serum ferritin level and GSH-Px level (25). There was no significant correlation between serum ferritin level and antioxidant enzyme levels (CAT, SOD, GSH-Px) with MDA was detected in the patients with β -TM who had 4.554 \pm 2.095 ng/mL of serum ferritin levels. Our findings give rise to thought that there might be other factors play role in the increment of oxidative stress and peroxidative tissue damage by free radical production than iron overload.

Vander Jagt DJ et al. reported a decrement in concentration of plasma amino acid and increment in urinary amino acid loss and they pointed out that this might contribute to the decrease in growth rate in children with sickle cell anemia (28). In a similar study was showed a significant decrease in isoleucine, phenylalanine, tyrosine, taurine, glutamine levels in children with thalassemia major comparing to the control group (29). In our study, as essential amino acids, methionine level was found as low in 2 patients and as

non-essential amino acids, glycine level was found as low in 4 patients; other amino acid levels were found in normal range. No significant relationship between MDA, antioxidant enzymes and ferritin levels of the patients with low normal levels of methionine and glycine was found.

Palmitoylation is important especially in stabilization of cell membranes. The most important fatty acids for palmitoylation are 16 carbon saturated fatty acids (4). S-palmitoylation involves the attachment of a 16-carbon long fatty acid chain to the cysteine residues of proteins (30). In our study, C16-palmitoyl levels of 14 patients were found as low. Ferritin and MDA levels of the patients with low C16-palmitoyl levels were higher than those of the patients with normal C16-palmitoyl levels. These findings can be associated with free radical production in which iron overload plays role and that results in peroxidative tissue damage. In addition, high levels of ferritin and MDA in the patients with low C16-palmitoyl levels might be because of this fatty acid's preventative effect on oxidative stress.

In conclusion, oxidative damage and increase in lipid peroxidation are caused by chronic iron overload in patients with β -TM. The most significant indicator of this damage is the increase in MDA levels. Membrane lipids and proteins show hypersensitivity to peroxidative damage caused by iron. In our study, the most significant finding was the decrease in C16-palmitoyl level whereas no significant result about other amino acid and fatty acid levels was found. In patients with low C16-palmitoyl levels, high ferritin and malondialdehyde levels may be due to the inhibitory effect of this fatty acid on oxidative stress.

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Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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