Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-nahrain.edu.iq

Role of Vitamin E, L-Carnitine and Melatonin in Management of β-Thalassemia Major

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Abstract

Background β-thalassemia major is an inherited disease resulting from decrease or total lack of β globin chains. Patients with this disease need repeated blood transfusion for survival. This may cause oxidative stress and tissue injury due to iron overload and depletion of antioxidant enzymes.

Objective Evaluation the role of vitamin E, L- carnitine and melatonin supplementation in management of β-thalassemia major patients.

Methods Forty five patients with β-thalassemia major were allocated to three groups A, B and C treated with vitamin E, L-carnitine and melatonin respectively. Serum malondialdehyde, serum reduced glutathione, serum ferritin, Hb, PCV, MCV, MCH, and MCHC levels and RBCs count were measured before and after treatment.

Results A significant decrease was observed in serum malondialdehyde and ferritin level after therapy in all treated groups; whereas, no significant ($P > 0.05$) changes in glutathione level after treatment in all groups. Hb level and RBC count increased significantly in group A (vitamin E), whereas, PCV, MCV, MCH and MCHC levels did not change significantly in all treated groups.

Conclusion Vitamin E, L- carnitine and melatonin have beneficial effects of in reducing lipid peroxidation and iron overload in patients with β-thalassemia major. These antioxidants may increase the life span of RBCs, which manifested by significant increase in Hb level in vitamin E treated group and significant decrease in serum ferritin level in all treated groups.

Keywords Beta-thalassemia, malondialdehyde, glutathione, ferritin, hematological parameters, vitamin E, L-carnitine, melatonin.

List of Abbreviation: HbF = fetal hemoglobin, SOD = superoxide dismutase, HOCl = hypochlorous acid, MDA = malondialdehyde, GSH = glutathione, TBA = thiobarbituric acid, RBC = red blood cell count, Hb = hemoglobin, PCV = packed cell volume, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration.

Introduction

Beta thalassemia is a hereditary anemia resulting from defects in the production of β-globin chains. Depending on clinical severity, three forms are distinguished, namely, thalassemia major, thalassemia intermedia and thalassemia minor [(1)]. β-Thalassemia major, is a clinically severe disorder that results in the transfusion dependent state which creates a state of iron overload [(2,3)]. Once reticulo-endothelial stores saturate, iron deposition increases in parenchymal tissues such as endocrine glands, hepatocytes, and myocardium [(4)]. The toxicity of iron is attributed to its ability to catalyze free-radical reactions that have life-threatening consequences. Iron could catalyze the oxidative breakdown of most biomolecules such as lipids, sugars, amino acids, DNA etc. [(1)].
Iron overload induces extra ferritin protein synthesis but the protein is overfilled with the extra iron that damages ferritin, with conversion

to toxic hemosiderin [5]. Vitamin E, is an important lipid-soluble antioxidant in humans, has been used as a potential agent to help protection against oxidative stress in thalassemia patients [6]. Due to increased consumption in homozygous β-thlassemia, low plasma levels of α-tocopherol, may induce lipid peroxidation within the red blood cells and consequently hemolysis [7]. Poor physical fitness is a common problem among thalassemic patients. L- carnitine plays an essential role in fatty acid beta-oxidation. In a clinical study, it was found that L-carnitine seems to be a safe and effective adjunctive therapeutic approach in thalassemic patients, and improves their cardiac performance and physical fitness [8]. In the treatment of thalassemia, newer approaches have been tried as alternative to standard therapy. Butyrate analogues such as L-carnitine have been found to increase fetal hemoglobin (HbF) synthesis and hence used in treatment of β- thalassemia. It also protects erythrocytes from oxidative stress, stabilizes the cell membrane, increasing the life span of red blood cells and is found to inhibit apoptosis in different diseases and that, thalassemic children had significant DNA double-strand breaks in their leukocytes and can be ameliorated by L-carnitine supplementation [1]. In a study conducted by Merchant *et al*, L-carnitine levels were found to be lower in thalassemics as compared to age matched controls [9]. Melatonin is a potent scavenger especially for hydroxyl and peroxyl radicals, inhibits apoptosis in normal cells, influences activities and cellular mRNA levels of antioxidant enzymes including superoxide dismutase (SOD), glutathione peroxidase, and glutathione reductase [10]. Recent preliminary results have shown that melatonin can prevent hypochlorous acid (HOCl) mediated heme destruction in hemoglobin and highlights a new mean by which melatonin can exhibit its protective effect [11].

The aim of this study was to evaluate the effects of vitamin E, L- Carnitine and melatonin supplementation on the levels of oxidative stress (malondialdehyde and glutathione), ferritin and hematological parameters in β-thalassemia major patients.

Methods

Patients Selection and drugs treatment:

This study was carried out between June 2011 and May 2012 on 45 patients with β-thalassemia major in Thalassemia Center in Al-Kut Hospital after giving the informed consent of the patients and agreement of the hospital, the patient age ranges were 10-34 years (mean ± SD = 19.12 ± 6.29), all the selected patients s were under regular blood transfusion and regular chelation therapy with deferoxamine (Desferal®, and they did not take any other antioxidants preparations) at the time of study.

The patients were allocated to three groups and were treated as follow:

Group A: 15 Patients (9 females and 6 males), were treated orally with 200 mg/day vitamin E (United pharmaceuticals, Jordan), 100 mg at morning and 100 mg at night for three months, in addition to other drugs prescribed according to center drug policy.

Group B: 15 Patients (8 females and 7 males), were treated orally with 30 mg/kg/day **L-carnitine (**Ultimate nutrition, USA), taken daily in two divided doses at morning and night for three months, in addition to other drugs prescribed according to center drug policy.

Group C: 15 Patients (9 females and 6 males) were treated orally with 3 mg/day **melatonin** soft gels (Vitane pharmaceuticals Inc., USA) taken at night at bedtime for two months with a break for one week between the two months, in addition to other drugs prescribed according to center drug policy. (**Melatonin** was administered to patients with ages of eighteen years old and above, since the safety of melatonin in subjects below eighteen years old has not been established.

Sample collection and preparation:

Five milliliters of blood were obtained from each patients by venipuncture, taken on routine visit to the thalassemia center within the first hour of visit, and before starting blood transfusion,

which considered (baseline or before treatment), and then at every visit to check the changes in the liver enzymes (to ensure the safety of administered drug), and at the end of treatment period (after treatment).

The period of treatment was three months, except for melatonin, which administered for two months separated by one week free interval.

All blood samples were collected in plain tubes; erythrocytes were separated by centrifugation at 3000 rpm for 10 minutes, the serum obtained was used for biochemical analysis.

Assay methods

1- Measurement of serum malondialdehyde (MDA) level: MDA measurement is based on the reaction of thiobarbituric acid (TBA) (BDH chemicals, Ltd. Poole, England) with MDA forming a pink colored MDA-TBA adduct that its light absorbance measured at 532 nm [(12)].

2- Measurement of serum glutathione (GSH) level: Reduced glutathione was determined based on the reaction of GSH with DTNB (5,5-Dithiobis (2- nitrobenzoic acid)) (BDH chemicals, Ltd. Poole, England) at pH 8 to produce a colored complex which absorb light at 412 nm and it is directlyproportional to GSH concentration [(13)].

3- Measurement of serum Ferritin: Serum ferritin level was measured by using minividas kit (Biomerieux SA, France).

4- Measurement of hematological parameters: RBC (Red Blood Cell count), Hb (Hemoglobin concentration), Hematocrit or PCV (packed cell volume), MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Hemoglobin) and MCHC (Mean Corpuscular Hemoglobin Concentration) were measured in whole blood on the day of collection using Micros (60), blood automated Coulter counter **(**Horiba ABX, France**).**

Statistical analysis

Numerical data are presented as mean ± SD. The paired Student t-test was used to compare data obtained from each group before and after treatment, P-value less than 0.05 considered significant.

Results

Out of the 45 total numbers of patients involved in the study, 39 patients completed the study. While six patients were excluded, one patient due to marked elevation of liver enzymes (hepatitis C), two patients due to poor compliance, two patients due to gastrointestinal side effects (abdominal pain) and one patient due to sedative effect of melatonin.

In all treated groups, post treatment serum MDA level was significantly decreased as compared to pretreatment (3.9 ± 2.9 µM/L versus 1.74 ± 0.83 µM/L in group A; 3.44 ± 1.7 µM/L versus 2.07 ± 0.93 µM/L in group B; and 3.46 ± 1.3µM/L versus 2.33 ± 1.2 µM/L in group C).

Similarly, post treatment serum ferritin level was significantly decreased in all treated groups (6029.2 ± 3250 ng ml versus 4831 ± 3014 ng/ml in group A; 5435.9 ± 2905 ng/ml versus 4102.8 ± 1645 ng/mL in group B; and 5435.9 ± 2905 ng/mL versus 4391.6 ± 2517 ng/ mL in group C).

On the contrary, post treatment Hb level and RBC count were increased significantly in group A only (7.9 ± 1.5 g/dL versus 8.5 ± 1.2 g/dL; 3 ± 0.6 $x10^{12}$/L versus 3.3±0.45 $x10^{12}$/L, respectively). Concerning post treatment GSH level, PCV, MCV, MCH and MCHC, no significant change was observed versus pretreatment in all treated groups (Tables 1 through 3).

Discussion

Malondialdehyde (MDA), a terminal compound of lipid peroxidation, is used widely as an index of oxidative status. Increased plasma MDA levels have been observed in patients affected by β-thalassemia major [(14)].

Results of present study showed that vitamin E significantly ($P < 0.05$) decrease serum MDA level and these results are in agreement with results obtained by Palasuwan *et al* [(6)]. Vitamin E can transfer its phenolic hydrogen to a peroxyl free radical of a peroxidized PUFA, thereby breaking the radical chain reaction and preventing the peroxidation of PUFA in cellular and subcellular membrane phospholipids [(15)]. Although no clinical studies were found about the effect of L-carnitine and melatonin on MDA

and some parameters that investigated in this study in β-thalassemia major.

Table 1. Effect of vitamin E on serum MDA, GSH, ferritin, hemoglobin (Hb), PCV, RBCs, MCV, MCH and MCHC levels in β- thalassemia major patients

Parameters	Effect of Vitamin E	
	Pretreatment	Post treatment
Malondialdehyde (µM/L)	3.9 ± 2.9	1.74 ± 0.83*
GSH(µM/L)	1.77 ± 0.49	1.51 ± 0.15
Serum Ferritin (ng/mL)	6029.2 ± 3250	4831 ± 3014**
Hemoglobin (g/dL)	7.9 ± 1.5	8.5 ± 1.2*
PCV (L/L)	24.6 ± 5.4	26.5 ± 3.6*
RBC ($x10^{12}$/L)	3.0 ± 0.6	3.3 ± 0.45
MCV (fL)	79.3 ± 5.1	78.1 ± 3.6
MCH (pg/cell)	26.46 ± 2.53	25.85 ± 3.34
MCHC (g/dL)	32.33 ± 2.002	32.3 ± 2.38

* = $P < 0.05$, ** = $P < 0.001$

Table 2. Effect of L-carnitine on serum MDA, GSH, ferritin, hemoglobin (Hb), PCV, RBCs, MCV, MCH and MCHC levels in β- thalassemia major patients

Parameters	Effect of L-Carnitine	
	Pretreatment	Post treatment
Malondialdehyde (µM/L)	3.44 ± 1.7	2.07 ± 0.93*
GSH(µM/L)	1.54 ± 0.23	1.57 ± 0.29
Serum Ferritin (ng/mL)	6259.7 ± 2269	4102.8 ± 1645**
Hemoglobin (g/dL)	8.7 ± 0.87	8.8 ± 1.5
PCV (L/L)	27.8 ± 2.7	28.5 ± 4.9
RBC ($x10^{12}$/L)	3.58 ± 0.39	3.65 ± 0.68
MCV (fL)	78 ± 5.8	78.4 ± 7.9
MCH (pg/cell)	24.4 ± 2	24.1 ± 2.6
MCHC (g/dL)	31.12 ± 1.177	31.09 ± 0.8964

* = $P < 0.05$, ** = $P < 0.001$

Table 3. Effect of melatonin on serum MDA, GSH, ferritin, hemoglobin (Hb), PCV, RBCs, MCV, MCH and MCHC levels in β- thalassemia major patients

Parameters	Effect of Vitamin E	
	Pretreatment	Post treatment
Malondialdehyde (µM/L)	3.46 ± 1.3	2.33 ± 1.2**
GSH (µM/L)	1.38 ± 0.28	1.35 ± 0.46
Serum Ferritin (ng/mL)	5435.9 ± 2905	4391.6 ± 2517**
Hemoglobin (g/dL)	7.4 ± 0.85	7.7 ± 0.81
PCV (L/L)	24.1 ± 3.3	24.9 ± 2.8
RBC ($x10^{12}$/L)	3.23 ± 0.62	3.35 ± 0.53
MCV (fL)	75.1 ± 3.8	74.3 ± 4.5
MCH (pg/cell)	23.4 ± 2.5	24.2 ± 2.1
MCHC (g/dL)	31.38 ± 1.92	31.25 ± 1.17

** = $P < 0.001$

The results also demonstrated that oral administration of L-carnitine significantly (P < 0.001) decreases serum MDA level, in study done by Ates *et al* to determine the antioxidant properties of the L-carnitine in the treatment of patients with age-related macular degeneration. The MDA level was significantly reduced at the end of the 3-month period (P < 0.001) [16]. The present results also found that administration of melatonin significantly (P < 0.001) decreases serum MDA level. In one study, Herrera *et al* reported that melatonin prevents the oxidative stress changes induced by intravenous administration of iron and erythropoietin in doses commonly used to treat anemia in chronic hemodialysis patients and had no adverse side effects, and that oral dose of melatonin (0.3 mg/kg) prevents the increase in MDA level [17]. In addition to that, it was found that melatonin administration (5 mg daily for 30 days), resulted in a significant reduction in the MDA level in elderly primary essential hypertensive patients [18].

The results of present study have demonstrated a significant decrease in serum ferritin level after treatment with antioxidants in all groups. These results were in agreement with results obtained by Attia *et al* who reported that administration of vitamins E, C and A to homozygous β-thalassemic patients for twelve months results in significant (P < 0.05) decreases in ferritin values [7]. Glutathione is the most abundant erythrocyte thiol and a principal reducing agent for sulphydryl enzymes and hemoglobin, the loss of function of hemoglobin due to autoxidation is restored by the intervention of reduced glutathione [19]. The glutathione level did not show significant (P > 0.05) difference in all treated groups when compared to before treatment level.

Red cells from β-thalassaemia patients generally display a shortened life span with overt haemolysis. In this condition, the red cell membrane is under increased oxidant stress with a threat to membrane integrity. Reduced glutathione (GSH) prevents oxidative damage to red blood cells [16].

Hb level and RBC count showed significant (P < 0.05) increase after treatment with vitamin E only, Attia *et al* found that the value of Hb improve after treatment vitamins (E, C and A) for twelve months in homozygous β-thalassemic patients [7]. In addition to that, Tesoriere *et al* showed that vitamin E significantly (P < 0.05) increased the RBC count in β-thalassemia intermedia patients [20]. While in groups B and C, there was non-significant increase in Hb level, El-Beshlawy *et al* found a significant increase in the blood transfusion intervals after L-carnitine administration and improvement in the cardiac performance and physical fitness in patients with thalassemia major, and however, there was no significant change in hemoglobin concentration [8].

The results also did not show significant (P > 0.05) differences in PCV, MCV, MCH and MCHC levels after treatment in all treated groups. In a study, Palasuwan *et al* reported that PCV and MCV did not change significantly after treatment with vitamin E 200 I.U. daily for 3 months in hemoglobin-E carriers [21]. While in another study, Karimi et al. demonstrated that the combination of L-carnitine with hydroxyurea could be more effective in improving hematologic parameters in patients with β-thalassemia intermedia than hydroxyurea alone [22]. Di Iorio *et al* have demonstrated that L-carnitine administration was effective as adjunctive treatment of anemia associated with chronic kidney disease in many β-thalassemic patients [23]. The effect of L-carnitine supplementation in the present study showed good activity against MDA serum ferritin level, but with little activity in improving the hematological parameters, which may require increasing the dose or prolonging the treatment period as were seen in some clinical studies. The administration of melatonin improved several hematological parameters, but only the Hb value showed significant increase. Tesoriere *et al* investigated the antioxidant activity of melatonin in human erythrocytes, exposed to oxidative stress by cumene hydroperoxide (cumOOH), and found that melatonin was

actively taken up into erythrocytes under oxidative stress, and is consumed in the defense of the cell, delaying Hb denaturation and release of hemin. RBCs are highly exposed to oxygen and can be a site for radical formation, under pathological conditions, which results in their destruction. A protective role of melatonin should be explored in hemolytic diseases [24].

Arushanian *et al* reported that melatonin leads to a significant increase in the hemoglobin level and erythrocyte number [25]. In addition to that, Maulood *et al* found that melatonin could improve hematological complications in bleomycin treated rats [26]. Melatonin also was effective to reverse the deleterious effects of radiation on the blood parameters in rats in study by Ozmerdivenli et *al* [27]. Finally, the thalassemic patients those had completed the present study were well tolerated the antioxidant agents, with few adverse effects, which were mild and limited, no allergic reactions were seen to any of the antioxidant agents.

In conclusion, iron overload in β-thalassemia major, causes tissue injury and organ damage. The poor compliance of patients with iron chelation therapy, made the iron chelaters alone in β-thalassemia major is not sufficient to neutralize the da8mage induced by free radicals. The marked role of antioxidants given in present study in decreasing lipid peroxidation (through significant reduction of MDA), serum ferritin and the improvement in some hematological parameters were encouraging to suggest the addition of these antioxidants to the classical drug regimen of β-thalassemia major.

Acknowledgment

We thank Dr. Muntather Al-Saidee and Dr. Laith Sami Dr. Suhad Faleh for their great support and help.

Author Contribution

Dr. Ahmed M. Taqi Al-Mosawi collected the data and participated the statistical analysis and drafting of the article. Dr. Faruk H. Al-Jawad contributed in the designing of the proposal, analysis and interpretation of the data; Dr. Safaa A. Al-Badri participated in the follow up of patients during period of study and in drafting of the article.

Conflict of Interest

The authors declare no conflict of interest.

Funding

Personal funding

References

1. Ragab SM, Mahfouz RG. L-Carnitine ameliorates the iron mediated DNA degradation in peripheral leukocytes of β- thalassemic children. Egypt J Med Hum Genet. 2010; 11(1): 17-31.
2. Thein SL. Pathophysiology of {beta} thalassemia: a guide to molecular therapies. Hematology (Am Soc Hematol Educ Program). 2005: 31–7.
3. Hoffman R. Hematology: Basic Principles and Practice. 4th ed. Elsevier: Churchill Livingstone; 2005. p. 262-275.
4. Borgna-Pignatti C, Rugolotto S, De Stefano P, et al. Survival and complications in patients with thalassemia major treated with transfusion and desferoxamine. Haematologica. 2004; 89: 1187-93.
5. Theil E. Ferritin iron minerals are chelator targets, antioxidants, and coated, dietary iron. Ann N Y Acad Sci. 2010; 3(4): 197-204.
6. Palasuwan A, Sriprapun M, Dahlan W, et al. Antioxidant protection in hemoglobin E trait subjects after vitamin E supplementation. Southeast Asian J Trop Med Public. 2008; 39(1): 82-.9
7. Attia MM, Sayed AM, Ibrahim FA, et al. Effect of antioxidant vitamins on the oxidant/antioxidant status and liver function in homozygous beta-thalassemia. Romanian J Biophys. 2011; 21(2):93-106.
8. El-Beshlawy A, El-Accaoui R, Abd El-Sattar M, et al. Effect of L-carnitine on the physical fitness of thalassemic patients. Ann Hematol. 2007; 86(1): 31-4.
9. Merchant R, Jain A, Udani A, et al. L-carnitine in beta-thalassemia. Indian Pediatr. 2010; 47(2): 165-7.
10. Sener A, Ozsavici D, Bingol-Ozakpinar O, et al. Oxidized-LDL and Fe3+/ascorbic acid-induced oxidative modifications and phosphatidyl serine exposure in human platelets are reduced by melatonin. Folia Biologica. 2009; 55: 45-52.
11. Galijasevic S, Abdulhamid I, Abu-Soud HM. Melatonin is a potent inhibitor for myeloperoxidase Biochemistry, 2008; 47: 2668-77.
12. Stocks J, Dormandy TL. The auto-oxidation of human red cell lipids induced by hydrogen peroxide. Br J Haematol. 1971; 20: 95-111.

13. Godin DV, Wahaieb SA, Garent ME. Antioxidant enzyme alteration in experimental and clinical diabetes. Mol Cell Biochem. 1988; 84: 223-31.

14. Gunarsih A, Amalia P, Boediman I. Variables associated with malondialdehyde level in thalassemia major patients. Paediatr Indones. 2012; 52(3): 125-31.

15. Fang YZ, Yang S, Wu G. Free radicals, antioxidants, and nutrition. Nutrition. 2002; 18: 872-9.

16. Ates OH, Alp HH, Mumcu U, et al. The Effect of L-carnitine treatment on levels of malondialdehyde and glutathione in patients with age related macular degeneration. Eurasian J Med. 2008; 40: 1-5.

17. Herrera J, Nava M, Romero F, et al. Melatonin prevents oxidative stress resulting from iron and erythropoietin administration. Am J Kidney Dis. 2001; 37(4): 750-7.

18. Kedziora-Kornatowska K, Szewczyk-Golec K, Czuczejko J, et al. Antioxidative effects of melatonin administration in elderly primary essential hypertension patients. J Pineal Res. 2008; 45(3): 312-7.

19. Ponnazhagan S, Sarkar R. Enzymes of the pentose phosphate pathway in glutathione-regulated membrane protection in β-Thalassaemia. Eur J Clin Chem Clin Biochem. 1992; 30: 481-4.

20. Tesoriere L, D'Arpa D, Butera D, et al. Oral supplements of vitamin E improve measures of oxidative stress in plasma and reduce oxidative damage to LDL and erythrocytes in beta-thalassemia intermedia patients. Free Radic Res. 2001; 34(5): 529-40.

21. Palasuwan A, Soogarun S, Wiwanitkit V, et al. Preliminary study of the effect of vitamin E supplementation on the antioxidant status of hemoglobin-E carriers. Southeast Asian J Trop Med Public Health. 2006; 37(3): 184-9.

22. Karimi M, Mohammadi F, Behmanesh F, et al. Effect of combination therapy of hydroxyurea with L-carnitine and magnesium chloride on hematologic parameters and cardiac function of patients with beta-thalassemia intermedia. Eur J Haematol. 2010; 84(1): 52-8.

23. Di Iorio BR, Guastaferro P, Cillo N, et al. Long-term L-Carnitine Administration reduces Erythropoietin Resistance in Chronic Hemodialysis Thalassemia Minor Patients. Drug Target Insights. 2007; 2: 1-7.

24. Tesoriere L, D'Arpa D, Conti S, et al. Melatonin protects human red blood cells from oxidative hemolysis: new insights into the radical-scavenging activity. J Pineal Res. 1999; 27(2): 95-105.

25. Arushanian SA, Beier EB, Mastiagina OA, et al. Melatonin effect on the hematological indices of healthy humans. Eksp Klin Farmako. 2006; 69: 36-8.

26. Maulood IM. Effect of low molecular weight heparin, melatonin and their combination on plasma endothelin -1, serum electrolytes and some hematological variables in bleomycin treated rats. J Duhok Univ. 2009; 12(1): 161-6.

27. Ozmerdivenli R, Karacabey K, Gundogdu C, et al. Protective role of melatonin on blood parameters following irradiation in rat. Afr J Biotechnol. 2011; 10(80): 18564-8.

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Received 10th Mar. 2013: Accepted 27th Jan. 2014