The Benefits of Vitamin C and Vitamin E in Children with β-Thalassemia with High Oxidative Stress

Thasinas Dissayabutra MD*,
Piyaratana Tosukhowong PhD*, Panya Seksan MD**

*Department of Biochemistry, Faculty of Medicine, Chulalongkorn University
**Hematology Unit, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University

The present study aimed to determine the benefits of vitamin C and vitamin E as antioxidant supplements in β-Thalassemia children who are at risk of iron overload due to multiple blood transfusion and high oxidative stress. Antioxidant status, oxidative products, plasma free hemoglobin, total hemoglobin and bilirubin were discussed. Twenty children who had laboratory confirmation of major β-Thalassemia at least 6 months with history of packed red cell transfusion without iron chelation were recruited. The informed consent for blood sample collection and antioxidant medication was performed. Most patients (85%) had hyperferritinemia and all of them had high oxidative stress. All of them had low vitamin C and vitamin E level at recruitment. Three months after vitamin C and vitamin E supplementation, plasma vitamin C, vitamin E and glutathione were significantly increased, while total bilirubin was slightly decreased without significance. Other parameters included total antioxidant status (TAS), plasma and erythrocyte malondialdehyde (MDA), hemoglobin and plasma free hemoglobin had no differences during the study period.

Conclusion: *β-Thalassemia major children who had multiple blood transfusion are at risk in iron overload and high oxidative stress. From the present study, no significant improvement in raising hemoglobin and concerning low dose vitamin C is not contraindication in β-Thalassemia patients. Therefore, vitamin C plus vitamin E supplementation have benefits more than vitamin E alone in promoting antioxidant status and may enhance liver function as total bilirubin tends to decrease.*

Keywords: *Vitamin C, Vitamin E, Oxidative stress, Thalassemia, Antioxidant*

J Med Assoc Thai 2005; 88(Suppl 4): S317-21
Full text. e-Journal: *http://www.medassocthai.org/journal*

Millions of people especially Southeast Asia natives are affected by β-Thalassemia, a disease caused by excessive extravascular hemolysis due to abnormal hemoglobin synthesis. Patients who have severe hemolysis called β-Thalassemia major have to receive multiple blood transfusion, and if without iron chelating treatment, iron overload occurs. When body iron excesses, increased free iron induces free radical oxygen species (ROS) as in Fenton's reaction

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + .OH + OH^-$$

Hydrogen peroxide is one of free radicals and can be eliminated by glutathione. In the presence of free plasma iron, hydrogen peroxide is oxidized into hydroxyl ion which are high potency radicals and hazardous to biochemical substance. It oxidizes protein and lipid at cell membrane[1,2], causes cellular integrity disequilibrium, leakage and cell death. This oxidative stress phenomenon explains why β-Thalassemia major patients usually have progressive anemia from intravascular hemolysis, abnormal liver function test, diabetes mellitus and restrictive pulmonary disease as its complications[1-5].

Excessive free radical is not the only reason in the etiology of Thalassemia consequences. Chakraborty and Meral found that deficiency of antioxidants in these patients had also existed.[6-7] Vitamin E or tocopherol, one of the most potent natural body antioxidants, was found to be deficient. Tesoriere found that MDA was normalized after vitamin E treatment for 6 months[8] and Suthutvoravut found improvement in glutathione peroxidase activity after vitamin E supplement for 2 months[9]. Many reports shown the similar outcome that supplementation of vitamin E can improve

Correspondence to : Dissayabutra T, Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.

biochemical indicators but no clinical improvement or hemoglobin rising was found[10-14]. Only Das[15] reported weight gain after vitamin E supplement for four weeks.

Vitamin C or ascorbic acid was also studied and found to be lowered in these patients but no study of supplement was done due to its potential to produce free radicals, called pro-oxidant. The effect of excess intake of vitamin C was found as a report case of hemochromatosis patient who took 1 gram of vitamin C per day for one year against medical advice expired due to cardiac failure. The hypothesis is that vitamin C reduces ferric ion (Fe^{3+}) which deposits in tissue to ferrous ion (Fe^{2+}) which can be redistributed into the bloodstream and accumulates at myocyte, causes myocardium dysfunction and eventually death. However, vitamin C has a particular role in vitamin E recycling and some reports have found vitamin C deficiency in Thalassemia patient[16]. The dietary recommendation limits vitamin C ingestion in iron overload patient to 500 milligram per day unless there is vitamin C deficiency coexists[17]. Therefore, the authors considered low dose vitamin C (100 milligram per day) supplementation could have benefits in protecting against oxidative stress induced complications.

Material and Method

Patients

The present study was perspective study of twenty β-Thalassemia major children of the pediatric hematology unit, King Chulalongkorn Memorial Hospital. The patients, aged between 5 and 15 years, were diagnosed with β-Thalassemia by hemoglobin typing. Duration of treatment was 2 and 12 years, each of them received 6 to 100 doses of blood transfusion in the past and no iron chelating treatment was given. Seven of them had splenectomy. Patients eligible for the study were consent by their parents for blood collection and medication. The study was approved by the Chulalongkorn Hospital ethic committee. The patient's data included demographic information, history of treatment, blood transfusion, ferritin level within one year, concurrent illness and current medication. The exclusion criterias were acute or chronic infection, other hematologic disease comorbidity, chronic renal failure, malnutrition, antioxidant or herbal medicine taking, and patients who were suspected to acquire vitamin C and vitamin E allergy. All of the patients were asked to collect blood sample at the time of recruitement, at 1 month and at 3 months after antioxidant supplementation. The indicators were vitamin C, vitamin E, total antioxidant status (TAS), glutathione (GSH), plasma and erythrocyte malondialdehyde (P-MDA, R-MDA), plasma free hemoglobin (P-Hb), total hemoglobin (Hb) and total bilirubin. Supplementation of 100 gram per day of vitamin C, 400 milligram of vitamin E in patient weighed less than 20 kilograms and 600 milligram of vitamin E in patient weighed at least 20 kilograms were given. Data were recorded in the case record form.

Statistical Analysis

Data were analyzed using descriptive and inferential statistics. The differences in the continuous variables were compared by using the paired-T test (SPSS for Window version 11.0, Chicago, USA). A p-value of $<0.05$was considered statistical significant.

Results

Twenty children whose aged ranged from 5 to15 years old; 9 boys and 11 girls were diagnosed with 18 β–Thalassemia/Hb E and 2 homozygous β_0-Thalassemia. They were treated at King Chulalongkorn Memorial Hospital for 2-12 years (average 5.5 years) and received 6-100 packed red cells transfusions (average 45.5 transfusions.) Eighteen of them had measured ferritin level within 1 year before recruitment and 17 of them have hyperferritinemia which indicated iron overload condition (range 0.5-8712 ng/dL; mean 2442 ng/dL; normal 7-140 ng/dL.)

For the antioxidant aspect, the authors measured serum vitamin C, vitamin E, glutathione and total antioxidant status (TAS), all of them were deficiency. The oxidative stress aspect, the authors measured the oxidative products; plasma malondialdehyde (P-MDA) and erythrocyte malondialdehyde (R-MDA), both of them were exceeded. In addition, the authors measured free plasma hemoglobin (P-Hb), red cell hemoglobin (Hb) and total bilirubin (TB) as the destruction of target tissues. The results were demonstrated in Table 1.

After the period of treatment, vitamin C and vitamin E increased significantly but could not be normalized (normal ranges are 6-14 milligram per liter and 11.6-23.2 micromole, $P = 0.02$ and <0.001 respectively.) Glutathione was also increased after treatment with both antioxidants ($P = 0.011$), but TAS was not changed. Oxidative products, Hb and P-Hb had no changed. TB was lowered but no statistical significance at the 3 months after treatment ($P = 0.086$)

Discussion

The present study indicates that those

Table 1. The values of vitamin C, vitamin E, glutathione, TAS, P-MDA, R-MDA, P-Hb, TB at the 0, 1 and 3 months (* $p < 0.05$ compared with baseline and ** $p < 0.01$ compared with baseline)

	At 0 month	At 1 month	At 3 months	Unit
Vitamin C	1.269 ± 1.274	1.952 ± 1.341*	2.595 ± 1.965*	mg/L
Vitamin E	9.686 ± 3.380	11.151 ± 2.784	11.488 ± 3.377**	mM
Glutathione	7.760 ± 1.161	8.560 ± 0.978*	8.751 ± 1.448*	M/gHb
TAS	1.938 ± 0.365	2.062 ± 0.205	1.995 ± 0.257	mM
P-MDA	5.380 ± 3.318	5.056 ± 2.269	4.804 ± 1.678	mM
R-MDA	14.280 ± 8.479	10.457 ± 4.635	12.831 ± 6.176	mM
P-Hb	21.691 ± 8.465	18.759 ± 8.074	26.984 ± 15.027	mg/dL
Hb	7.385 ± 1.208	7.355 ± 0.907	7.380 ± 0.900	g/dL
TB	2.562 ± 0.933	2.425 ± 0.805	2.178 ± 0.828	mg/dL

β–Thalassemia children have oxidative stress and antioxidant deficiency even without iron overload status. Supplementation of vitamin C and vitamin E can increase plasma antioxidant levels but they were still lowered than normal population. This was due to critically excessive oxidative stress. The best strategy in treatment these patients should be iron chelation with antioxidant therapy. Consideration of antioxidants should be included types, varieties, doses and duration.

Glutathione is an important antioxidant in red blood cell membrane[18] and necessary in vitamin C recycle pathway. All of the β–Thalassemia patients in the present study had ascorbic acid deficiency, the ascorbic acid recycle was activated and glutathione depletion was its consequence. After supplementation of vitamin C and vitamin E, the authors found glutathione level was increased. Therefore, vitamin C supplementation in these patients was proved to have benefit. The authors suggested that low dose vitamin C may not be contraindication in β–Thalassemia patients who are at risk of vitamin C deficiency. However, the present study was done in a short period therefore we lacked the long term evidence for any complication. Patients who receive vitamin C supplement should be closely observed and monitored by electrocardiogram periodically to detect myocardial dysfunction.

Theoretically, vitamin E and glutathione are the red cell protective antioxidants[19], increasing level both of them should decrease intravascular hemolysis. The present study found that plasma free hemoglobin was not decreased, the authors assumed that this was due to plasma free iron, the main cause of oxidative stress, was not treated and there are other antioxidants involved in red blood cell protection. However, Jamshidi[20] studied in bilirubin after treating patients with vitamin E alone for 7 months and found no change. The authors observed that total bilirubin (TB) in the present study for 3 months was insignificantly decreased. This may be the evidence that combination of vitamin C and vitamin E supplementation has benefits beyond vitamin E supplement alone.

Vitamin C deficiency was common in the present patients in the present study. Low dose vitamin C supplement (100 milligram per day) was given and the authors did not find an increase of oxidative products. On the contrary, the authors found some evidences that supplementation of vitamin C plus vitamin E had more benefit than supplementation of vitamin E alone.

In conclusion, oxidative stress and antioxidant deficiency were commonly found in β–Thalassemia major children. Supplement of vitamin E and low dose vitamin C have the benefit of increasing antioxidant status more than supplementation of vitamin E alone.

Acknowledgements

The present study was supported by a grant from the Vejdusit foundation, Bangkok Hospital. 2003. The authors wish to thank all of the pediatric hematologic unit staffs, Srisakul Jatuporn MD, Mr Sanan Lamath, Mr Jamras Mani, Miss Yuwadi Waiyapongpijitra who are staff of the Department of Biochemistry for their assistance with the study.

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ประโยชน์ของวิตามินซีและวิตามินอีในผู้ป่วยเด็กเบต้าธาลัสซีเมียที่มีภาวะอนุมูลอิสระเกิน

ฐสิณัส ดิษยบุตร, ปิยะรัตน์ โตสุโขวงศ์, ปัญญา เสกสรรค์

วัตถุประสงค์: *เพื่อศึกษาประโยชน์ของวิตามินซีและวิตามินอีในด้านการเป็นสารต้านอนุมูลอิสระเพื่อใช้รักษาผู้ป่วยเด็กโรคเบต้าธาลัสซีเมียที่เสี่ยงต่อภาวะเหล็กเกินจากการได้รับการถ่ายเลือดหลายครั้งและมีภาวะอนุมูลอิสระเกินในร่างกาย*

วิธีการศึกษา: *เป็นการศึกษาไปข้างหน้า การศึกษากระทำในผู้ป่วยเด็กที่ได้รับการวินิจฉัยว่าเป็นเบต้าธาลัสซีเมียโดยการทำฮีโมโกลบิน อิเลคโทรโฟเรซิสจำนวน 20 คนที่มารับการตรวจรักษาที่แผนกโลหิตวิทยาเด็ก โรงพยาบาลจุฬาลงกรณ์ โดยมีประวัติได้รับเลือดและไม่ได้รับยาขับเหล็กมาก่อน ผู้เข้าร่วมโครงการวิจัยจะถูกเก็บตัวอย่างเลือดและได้รับประทานวิตามินซีและวิตามินอีเป็นเวลาสามเดือน*

ผลการศึกษา: *ผู้ป่วยร้อยละ 85 มีระดับเฟอร์ริตินในเลือดสูงก่อนเข้าร่วมโครงการและผู้ป่วยทุกคนมีระดับสารอนุมูลอิสระเกิน โดยมีระดับวิตามินซีและวิตามินอีต่ำทุกคน หลังได้รับยานานสามเดือน ระดับวิตามินซี วิตามินอีและกลูทาไธโอนเพิ่มขึ้นอย่างมีนัยสำคัญ ค่าบิลิรูบินทั้งหมดลดลงอย่างไม่มีนัยสำคัญ ขณะที่ค่าระดับสารต้านอนุมูลอิสระทั้งหมด มาลอนไดอัลดีไฮด์ในพลาสมาและในเซลล์เม็ดเลือดแดง ฮีโมโกลบินอิสระในพลาสมาและฮีโมโกลบินทั้งหมดไม่มีการเปลี่ยนแปลงในระยะเวลาที่ศึกษา*

สรุป: *ผู้ป่วยเด็กเบต้าธาลัสซีเมียที่ได้รับเลือดเป็นประจำมีความเสี่ยงต่อการเกิดภาวะเหล็กเกินและสารอนุมูลอิสระเกิน การให้วิตามินซีร่วมกับวิตามินอีมีประโยชน์ในการเพิ่มสารต้านอนุมูลอิสระและอาจช่วยลดระดับบิลิรูบินได้ดีกว่าการให้วิตามินอีเพียงอย่างเดียว และการให้วิตามินซีขนาดต่ำไม่เป็นข้อห้ามในผู้ป่วยโรคธาลัสซีเมีย*