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Original Article

Vitamin E effect on osmotic fragility in β thalassemia major

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Abstract

Background Blood transfusion remains the main therapy for anemia in β thalassemia major patients. However, frequent transfusions can cause oxidative stress in response to iron overload. Vitamin E is considered to be the best lipid-soluble exogenous antioxidant in humans. It can protect phospholipid membrane from peroxidation. Erythrocyte osmotic fragility is a useful test to assess for the improvement of red blood cells in thalassemia patients after vitamin E supplementation.

Objective To investigate the effect of vitamin E for improving erythrocyte osmotic fragility in β- thalassemia major and for decreasing the need for frequent transfusions.

Methods This was a double blind placebo controlled randomized clinical trial on children aged 2-14 years with thalassemia major who received frequent blood transfusions. Fifty subjects were divided into 2 groups: group I with vitamin E supplementation and group II with placebo, as a control group, for a period of 1 month. Pre- and post-treatment data on erythrocyte osmotic fragility and hemoglobin level were analyzed with non-paired T-test.

Results Improved erythrocyte osmotic fragility was found: in group I, pre-treatment 31.59 (SD 6.342)% to post-treatment 38.08 (SD 7.165)%, compared to the control group pre-treatment 34.40 (SD 6.985)% to post-treatment 29.26 (SD 9.011)% (P=0.0001). Comparison of the mean delta Hb level in group I was 0.94 (SD 0.605) gr% and that of group II was - 0.23 (SD 1.199) gr% (P= 0.0001).

Conclusion Vitamin E supplementation improves erythrocyte fragility and Hb level in β-thalassemia major pediatric patients. **[Paediatr Indones. 2014;54:280-3.]**.

Keywords: *β thalassemia major, vitamin E, erythrocyte fragillity*

Thalassemia is a recessive, autosomal, hereditary and congenital hemolytic anemia. It is caused by defect of globin chain synthesis. The most frequent type found in Indonesia is β-thalassemia. β-thalassemia arises as a consequence of decreased or absent synthesis of the β-globin chain.[1]

Oxidative stress in β-thalassemia can be caused by excess α-globin chains which form an unstable monomer, producing free oxygen radical. Moreover, iron overload due to chronic blood transfusion can generate a peroxidation in β-thalassemia major patients, increasing superoxide dismutase and erythrocyte glutathione peroxidase activity. Both the accumulation of excess α-chain and iron overload result in increased red blood cell destruction and decreased antioxidant levels. Abnormal hemoglobin level in thalassemia patients can induce free radical production, which can oxidize components of membrane of erythrocyte.[2-4]

Antioxidants can prevent lipid membrane peroxidation due to free radical oxidation. Vitamin E is

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considered to be the best free radical chain-breaking antioxidant. It may protect against oxidative stress.[1-3,5] Vitamin E supplementation can increase plasma α-tocopherol levels and reduce plasma oxidant levels in β-thalassemia.[6]

The erythrocyte fragility test was the first laboratory screen for thalassemia.[1,7] The test involves a cross-reaction between the defective erythrocyte membrane and malonyldialdehyde (MDA). Malonyldialdehyde is a lipid peroxidation product. The erythrocyte membranes of thalassemic patients are more resistant to osmotic pressure than normal erythrocytes. Hence, erythrocyte fragility in thalassemic patients is lower than normal.[7] This fragility test is not used as much anymore.

We investigated the effect of vitamin E supplementation on erythrocyte osmotic fragility in β-thalassemia major pediatric patients. We used the fragility test to assess the improvement of erythrocyte membranes after vitamin E supplementation, with the hope of decreasing the blood transfusion frequency needed in thalassemia patients.

Methods

This study was a double-blind, placebo controlled randomized clinical trial. Subjects were β-thalassemia major patients hospitalized in the Thalassemic Ward at Dr. Kariadi Hospital and fulfilled the following inclusion criteria: received frequent blood transfusions (10-20 times transfusion), aged 2-14 years, had no other hematologic disorders, and did not consume other antioxidant or herbal supplementation. Exclusion criteria were allergy to vitamin E, or could not continue the study.

Subjects were selected with consecutive sampling and were randomized using block allocation. The patients were randomized into 2 groups: group I (treatment group) received 200 IU vitamin E supplementation twice a day for 1 month; group II (control group) received a placebo for 1 month. This study was approved by the Ethics Committee of Medical Research, Diponegoro University.

Erythrocyte fragility was determined by one tube osmotic fragility test using a *Coleman Junior II model 6120* spectrophotometer at 440 nm wavelength. All parameters were expressed as mean and standard deviation (SD). Statistically significant differences between values were analyzed using non-paired, sample T-test. The criterion for significance was $P<0.05$.

Results

Fifty-five patients met the inclusion criteria, but 5 patients did not continue the study because they did not return for regular clinic visits.

Distribution of subjects' characteristics were similar between the treatment and control groups (**Table 1**). Erythrocyte fragility was similar in the two groups before supplementation. We observed significant improvement in erythrocyte fragility in group I after vitamin E supplementation ($P=0.0001$) (**Table 2**.)

There was a significant difference in erythrocyte osmotic fragility delta (Δ fragility) between the two groups. Mean erythrocyte osmotic fragility delta increased in group I (6.49) whereas mean erythrocyte

Table 1. Subjects' characteristics

Characteristics	Group	
	I (n = 25)	II (n = 25)
Mean age (SD), years	9.70 (3.02)	8.13 (3.32)
Birth weight (SD), kg	22.60 (6.76)	20.59 (6.38)
Height (SD), cm	120.8 (1.9)	112.1 (15.1)
Gender		
Male, n	12	14
Female, n	13	11
Nutritional status, n		
Malnourished	6	3
Moderately malnourished	9	14
Normal	10	8
Clinical manifestations, n		
Cooley facies	16	17
Anemia	23	22
Icterus	6	7
Hepatomegaly	6	7
Splenomegaly	17	17
Splenectomy	2	1

Table 2. The distribution of mean erythrocyte osmotic fragility in each group

Group	Fragility (SD), %		P value
	Pre-	Post-	
I	31.59 (6.34)	38.08 (7.17)	0.0001
II	34.40 (6.99)	29.26 (9.01)	0.018
P value	0.121	0.0001	

osmotic fragility delta in group II decreased (-5.14) (**Table 3**).

The delta Hb level was significantly different between the two groups (P=0.0001). The mean delta Hb in group I (0.94) was positive, whereas it was negative in group II (-0.23) (**Table 4**).

Table 3. Comparison of erythrocyte osmotic fragility delta (Δ fragility) between group I and II

Group	Δ fragility (SD)	P value
I	6.49 (6.68)	0.0001
II	-5.14 (10.14)	

Table 4. The difference of mean delta Hb level between group I and II

Group	Mean delta Hb level (SD)	P value
I	0.94 (0.61)	0.0001
II	-0.23 (1.20)	

$ independent T-test

Discussion

Our study is consistent with a study which found that daily supplementation of 200 IU vitamin E for 3 months significantly reduced superoxide dismutase activity and increased total antioxidant capacity in Hb E carriers.[6] The hemolysis rate of erythrocytes in thalassemia major patients approached that of normal cells after 1 month of 200 IU vitamin E supplementation. Further investigation is needed to assess whether it is caused by effect of vitamin E deficiency on erythrocytes or lower MDA resulted in erythrocyte membrane.[6]

Another study reported on the effect of vitamin E on erythrocytes, hemoglobin (Hb) derivatives and the rate of alkaline denaturation of Hb in homozygous β-thalassemic patients. This study showed that antioxidant vitamin supplementation reduced the hemolysis of red blood cells and improved the hemoglobin levels in thalassemic patients.[8] Similarly, we found that Hb level improved in the vitamin E supplementation group.

A study investigated the oxidant-antioxidant status in β-thalassemia major patients receiving routine transfusions. This study showed that peroxidation products such as erythrocyte superoxide dismutase (ESOD) and plasma malonyldialdehyde (MDA) levels were higher in thalassemia major patients, whereas serum vitamin E levels were lower in patients with thalassemia major than in healthy children. Increased oxidative damage in thalassemia patients may be due to the depletion of lipid soluble antioxidants such as vitamin E.[9] Similarly, we found an improvement in oxidative status in β-mayor thalassemia after vitamin E supplementation as shown by improved erythrocyte osmotic fragility. Malonyldialdehyde (MDA) and SOD are lipid peroxidation products. Reduced cross-link between MDA and spectrin from erythrocyte membranes can improve oxidative status. In this study, the improvement of oxidative status was shown by increasing erythrocyte osmotic fragility.[5]

Although there was improvement in erythrocyte osmotic fragility in the treatment group, it did not achieve normal levels. Perhaps the duration of vitamin E supplementation was too short or the dose of vitamin E needed to be increased. Another study gave 200 IU vitamin E supplementation for 3 months and found increased total antioxidant capacity in Hb E carriers.[6] In addition, Mahjoub *et al*. reported reduced lipid peroxidation on erythrocyte membranes after 550 IU daily of vitamin E supplementation.[10]

In conclusion, in β-thalassemia major patients, erythrocyte osmotic fragility and Hb levels significantly improve in the vitamin E suplementation group compared to the placebo group. We recommend that 200 IU vitamin E daily to be used as a lipophilic antioxidant in red blood cells for pediatric thalassemic patients.

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