Vitamin E Supplement Improves Erythrocyte Membrane Fluidity of Thalassemia: An ESR Spin Labeling Study

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Background: *β-thalassemia/Hemoglobin E (β-thal/Hb E) is prevalent in Thailand. The imbalance of globin chains in red blood cells is the primary cause of this anemic disease. The excess α-globin in β-thal/Hb E causes typical damage(s) to membrane of erythroblasts and erythrocytes. By using three paramagnetic labeled compounds (5-, 12-, and 16-spin labeled stearic acids, SLS), the changes of the molecular motion in the lipid bilayer of thalassemic RBCs that have structural modification can be detected.*

Objective: *to investigate erythrocyte membrane fluidity and the effect of vitamin E treatment in β-thalassemia/Hemoglobin E patients by using spin labeling techniques.*

Material and Method: *The erythrocyte membrane fluidity was investigated in nine splenectomized and five non-splenectomized β-thalassemia/hemoglobin E (β-thal/Hb E) patients using EPR spin labeling techniques. To determine the effect of vitamin E on erythrocyte membrane fluidity, only the splenectomized patients were enrolled. Patients were divided into two groups. The first group received 350 mg vitamin E daily for a period of 1 month (n = 5) and the second group received placebo for an equal period (n = 4). Three paramagnetic fatty acid, 5-, 12-, and 16-doxyl stearic acids, (5-, 12- and 16-DS) were used to label phospholipids layer near both the surface (5-DS) and the deeper hydrophobic region of membrane (12-and 16-DS). Lipid peroxidation (TBARs) was measured using a colorimetric method. Vitamin E was measured with high performance liquid chromatography (HPLC).*

Results: *Significantly higher values of erythrocyte membrane fluidity were revealed with 12-, 16-DS in splenectomized patients, as compared with non-splenectomized patients and normal subjects. In β-thal/Hb E patients, fluidity values, both outer hyperfine splitting ($2T_{//}$) and order parameter (S) of 12-DS showed inverse correlation with serum TBARs. There was no significant difference between the fluidity values measured with 5-DS. After vitamin E supplementation, the erythrocyte membrane fluidity was decreased in almost all patients. In contrast to the vitamin E supplementation group, increased erythrocyte membrane fluidity was demonstrated in the placebo group. Vitamin E supplementation also had effect on other clinical parameters such as increased plasma vitamin E, decreased serum TBARs and no change in hemoglobin.*

Conclusion: *The present results suggested the abnormal motion of lipid in the deeper phospholipids region of membrane. In addition, vitamin E supplementation may have a role in the prevention of erythrocyte membrane damage of these patients.*

Keywords: *Thalassemia, Membrane fluidity, Vitamin E, Spin labeling*

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β-Thalassemia is the most common hemoglobinopathy in Thailand and South East Asia. The imbalance of globin chains, particularly excess of α-chain is the primary cause of membrane damage of erythroblasts and erythrocytes. Ineffective erythropoiesis and increased peripheral hemolysis are also associated consequences[1,2]. The clinical features include anemia, ineffective erythropoiesis and chronic iron overload. Level of serum iron is elevated as transferrin becomes fully saturated and there is the appearance of non-transferrin bound iron[3]. The latter circulating pool of iron determines the toxicity of iron overload or the varying degree of oxidative stress in thalassemia. Therefore, oxidative damage has been implicated as an important mechanism that is

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likely to account for the pathology of β-thalassemic erythrocytes[4,5].

Alpha-tocopherol (Vitamin E), an antioxidant that participates in the termination of free radical chain reactions propagated by the polyunsaturated fatty acid of the membrane phospholipids, were low in both serum and red blood cells (RBCs) membranes of thalassemic patients[6]. Unchern et al[7] demonstrated that supplementation of vitamin E for 3 months is capable of reducing oxidative stress and improving platelet function in the patients. Recently, a study by Pfeifer et al[8] has also demonstrated an increase in GSH with decrease in lipid peroxidation in RBCs without improvement of anemia in β-thalassemia after 3 months of the supplementation. However, the effect of vitamin E on integrity and quality of RBCs membrane has not been evaluated.

The aim of the present study was to demonstrate the changes of the molecular motion in the lipid bilayer of thalassemic RBCs, the so-called "membrane fluidity"[9-14], in relation to oxidative stress by using EPR spin labeling technique with different paramagnetic probes (5-, 12-, and 16-doxyl stearic acids, DS). These spin labels embed in the biological membrane and exhibit their freedom of anisotropic motion in conformity with the position of the nitroxide ring on the alkyl fatty acid chain[9,12]. The spin labeling technique has been established as a valuable tool to obtain conformational and dynamic data concerning the physical state of the biological membrane[9-14]. Furthermore, the effect of vitamin E on erythrocyte membrane fluidity in thalassemia patients after one-month vitamin E administration is also demonstrated.

Material and Method

Subjects

Fourteen β-thalassemia/Hb E (9 splenectomized and 5 non-splenectomized) patients and ten normal healthy subjects were enrolled. They were asked not to take any medication, except for the daily folic acid supplementation for thalassemic patients, for at least two weeks before joining the studies. No patients received a blood transfusion for at least three months prior to the present study.

To determine the effect of vitamin E, the splenectomized β-thalassemia/Hb E (β-thal/Hb E) patients were divided into two groups. The first group received 350 mg vitamin E daily for a period of one month (n = 5) and the second group received placebo for an equal period (n = 4). Complete blood count (CBC) was measured before and after one month of supplement by an H-1 Technicon analyzer. The present study protocol was approved by the Committee of Ethical Practice and the Ethical, and Scientific Review Subcommittee of Mahidol University. All subjects were informed about the scope and objective of the present study before giving their informed consent.

Material

All chemicals were obtained commercially and used without further purification. Three spin labeling compounds (5-doxyl-stearic acid, 12-doxyl-stearic acid, and 16-doxyl stearic acid), 2-thiobarbituric acid and butylated hydroxytoluene were obtained from Sigma Chemical Co. (St. Louis, USA). Oral preparation of vitamin E and its placebo used in the present study are the generous gift of the Pharma Nord (Denmark). Each capsule of the vitamin preparation contains dl-α-Tocopherol 350 mg (525 IU), vegetable oil 175 mg, gelatin 149 mg, glycerol 54 mg and 22 mg of purified water. Vitamin E is excluded in the placebo preparation.

Method

Preparation of human erythrocytes

Venous blood was obtained from normal and thalassemic patients, using heparin as anticoagulant (50 unit/ml whole blood). Whole blood was centrifuged at 2,000 g for 10 minutes. Red blood cells were washed three times in five volumes of phosphate buffered saline (0.1 M PBS, pH 7.4), care being taken to remove the buffy coat. The washed erythrocytes were then adjusted to 50% hematocrit by PBS.

Spin labeling and electron spin resonance (ESR) measurement[15]

Three doxyl stearic acid spin labels (DS): 5-, 12- and 16-DS were used as labeling compounds. These are stearic acid analogues and each has a nitroxyl ring at the fifth, twelfth and sixteenth carbon position counted from the carboxyl group of the acyl-chain, respectively.

The incorporation of these spin labels into the erythrocyte membrane bilayer was done by the following procedure. A thin film of the label compounds was prepared by placing 50 ml of 1, 2-dichloroethane containing 0.1 mg/ml of spin label compounds in a small test tube and evaporating the 1, 2-dichloroethane with a stream of nitrogen. The 0.5 ml of 50% hematocrit in PBS buffer was added to the spin label tube. Tube was gently shaken at room temperature for 15 minutes. Final concentrations of spin labels were 2.36×10^{-5} M. The labeled erythrocytes

were transferred to a capillary tube and ESR spectra were obtained at room temperature on JEOL X-band spectrometer, Model JES-RE1X (JEOL Ltd., Tokyo, Japan).

Calculation of membrane fluidity

The representative spectrum of DS spin labels embedded in the erythrocyte membrane is shown in Fig. 1. The observed values of the outer ($2T_{//}$) and inner ($2T_{\perp}$) hyperfine splitting (in Gauss) were used to calculate the order parameter (S) in 5- and 12-DS according to the formula (1) of Gaffney[10].

Formula (1) $$S = \frac{T_{//}-T_{\perp}+(-c)}{T_{//}+2T_{\perp}+(+2c)} \times 1.723$$

where $c = 1.4\,G - 0.053\,(T_{//}-T_{\perp})$

However, in the case of 16-DS, the outer splitting value could not be measured since the low-field peak was not resolved. Therefore, order parameter was determined from the observed inner splitting value and the calculated outer splitting value using the relation:

$$T_{//} = 3a - 2T_{\perp};$$

where a is the isotropic hyperfine splitting and 3a was taken to be 44.5 G[16].

Another parameter, peak height ratio (h_o/h_{-1}), where h_o is the mid-field height and h_{-1} is the high-field height (Fig. 1), was used to aid comparison of membrane fluidity since it has higher sensitivities than the outer hyperfine splitting and order parameter used in the measurement with 16-DS. Although peak height ratios were derived for the analysis of isotropic motion, they could be used as parameters of 16-DS anisotropic mobility for the purpose of comparison[11,14].

Greater values of membrane fluidity, *i.e.*, greater freedom of motion of spin labels in the double membrane, are associated with smaller values of outer hyperfine splitting, order parameter and peak height ratio.

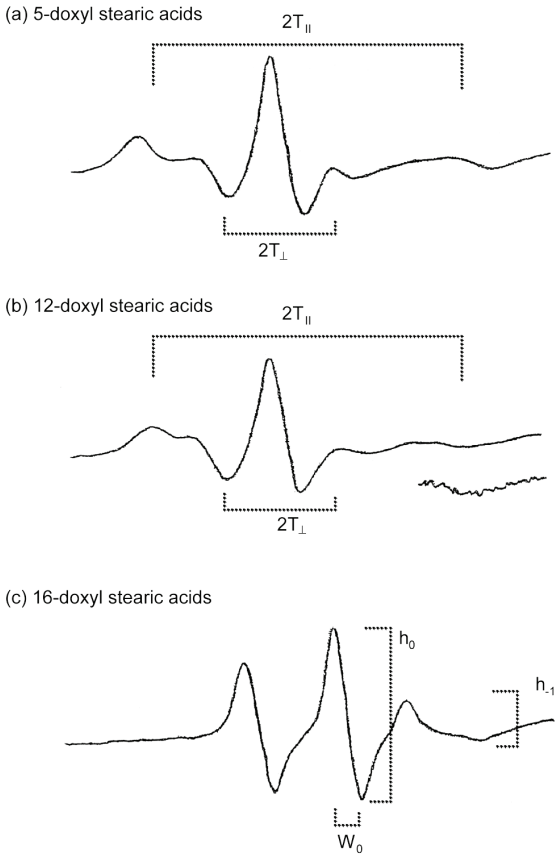

Fig. 1 The representative electron spin resonance spectra of 5-DS (a), 12-DS (b) and 16-DS (c) embedded in the erythrocyte membrane

Determination of lipid peroxidation

Lipid peroxidation (TBARs) level was measured fluorometrically using a spectrofluorometer (Perkin-Elmer LS55 luminescence spectrometer) with excitation and emission wavelengths at 515 and 553 nm, respectively. 1, 1, 3, 3-Tetraethoxypropane was used as the standard[17].

High performance liquid chromatography (HPLC) determination of vitamin E

The modification of HPLC method as described by Seta et al[18] and Zaspel et al[19] was used. Plasma (100 μl) samples were sequentially extracted with methanol and hexane. After drying the hexane layer under N_2 and re-dissolving with isopropanol, the extract was injected into the HPLC system, which consisted of a Waters 600E Multisolvent Delivery system and a Waters 717 plus autosampler module (Waters, Milford, MA). Vitamin E level was determined with a Jusco FP 210 fluorescence detector (excitation wavelength, 295 nm and emission wavelength 370 nm). The software program, Millennium 32™ (Waters, Milford, MA), was used for data analysis. The separation was carried out on a Hypersil BDS C18 column (4.6 mm x 250 mm, 5 μ) and a guard column (Javalin, 10 x 4 mm; Thermo Electron Co., Cheshire, UK) with isocratic acetonitride/isopropanol (75:25, v/v) as mobile phase. The flow rate was 1.2 ml/min. The temperature of the column was controlled at 50°C.

Statistical analysis

All values are expressed as mean ± SD, frequency and percentage. The statistical analysis was performed using Stat View™ for Windows version 5. Mann-Whitney-U Test was applied in comparing data from unpaired two groups. Fishers' Exact test was used for comparing counting number. The Wilcoxon Sign Rank Test was also used for assessing the data from paired two groups. The correlation between two parameters was assessed by Spearman's rank correlation Test. The p-value of less than 0.05 is considered to be statistically significant.

Results

Demographics, hematological parameters, lipid peroxidation (TBARs) and Vitamin E level in normal and thalassemic patients

Twenty-four patients were enrolled in the present study, out of which 10 patients were categorized as normal, nine as splenectomized thalassemia and five as non-splenectomized thalassemia. The average age of normal, non-splenectomized thalassemia and splenectomized thalassemia were 26.0 ± 5.5, 31.6 ± 15.2, and 28.5 ± 6.2 years, respectively. There were no statistical differences with regard to the age, sex distribution between normal and thalassemia groups.

The hematological data showed that patients with β-thal/Hb E were those with anemia, with microcytic and hypochromic RBCs (Table 1). The presence of microcytic RBC was the result of the ineffective erythropoiesis and rapid destruction of the RBC. The increase in numbers of circulating nucleated cells, especially in those with splenectomized thalassemia, suggested that either more immature RBC or the appearance of more fragmented cells in the absence of spleen contributes to the image picked up by the H-1 cell analyzer.

The levels of plasma vitamin E and lipid peroxidation in the different groups are shown in Table 1. In agreement with previous studies[7], plasma vitamin E levels in both non-splenectomized and splenectomized β-thal/Hb E subjects were markedly low when compared with normal subjects. The marked reduction in vitamin E level, which is the chain-breaking antioxidant, presumably indicated an excessive oxidative stress in β-thal/Hb E. This corresponded to the extent of lipid peroxidation in the patients.

Detection of erythrocytic membrane fluidity by ESR

Higher membrane fluidity of splenectomized β-thal/Hb E was observed with 12-DS and 16-DS (Table 2). The values of outer hyperfine splitting ($2T_{//}$) and order parameter (S) of 12-DS and peak height ratio of 16-DS were significantly lower in splenectomized β-thal/Hb E erythrocytes. There was no significant difference between the fluidity values when measured

Table 1. Demographics, hematological parameters, lipid peroxidation and vitamin E levels of normal subjects and β-thalassemia/Hb E patients

Parameter	Normal subjects (n = 10)	β-thalassemia/Hb E patients	
		Non-splenectomized (n = 5)	Splenectomized (n = 9)
Age (years)	26.0 ± 5.5	31.6 ± 15.2	28.5 ± 6.2
Sex			
Male	4 (40.0%)	1 (20.0%)	5 (55.5%)
Female	6 (60.0%)	4 (80.0%)	4 (44.5%)
CBC			
WBC (109/l)	5.7 ± 1.1	6.5 ± 2.4	56.0 ± 23.6***
RBC (1012/l)	4.4 ± 0.5	3.8 ± 0.5	2.7 ± 0.4***
Hb (g/dl)	12.2 ± 1.2	6.9 ± 1.0**	6.1 ± 1.0***
Hct (%)	36.6 ± 3.7	22.1 ± 2.5**	20.4 ± 3.2***
MCV (fl)	83.0 ± 3.9	59.0 ± 4.0**	74.8 ± 6.3**
Plt (109/l)	207 ± 40	252 ± 51	622 ± 162**
TBARs (nmole/ml)	0.16 ± 0.09	0.09 ± 0.05	0.33 ± 0.22*
Vitamin E (mg/ml)	8.9 ± 1.8	1.0 ± 0.8**	0.6 ± 0.3**

Values are expressed as the mean ± SD, *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$ compared with normal
Hematological data were derived from Technicon H-1 analyzer (WBC = white blood cell; RBC = red blood cell; Hb = hemoglobin; Hct = hematocrit; MCV = mean corpuscular volume; Plt = platelet)

with 5-DS, which represents the fluidity of rather shallow sites of the erythrocyte membrane.

In addition, significant negative correlations between fluidity parameter of 12-DS and serum TBARs were detected. The r values for $2T_{//}$ and S were -0.711 ($p = 0.010$) and -0.685 ($p = 0.013$), respectively.

Effect of vitamin E on erythrocyte membrane fluidity

The markedly increased plasma vitamin E with decreased serum TBARs was observed in patients receiving vitamin E supplementation (Table 3). However, there was no significant difference in CBC data before and after 1 month of placebo and vitamin E supplementation (data no shown).

To study the fluidity changes after in vitamin E supplementation, the membrane fluidity value using the peak height ratio (h_o/h_{-1}) of 16-DS was compared in splenectomized β-thalassemia/Hb E patients both before starting and one month after vitamin E supplementation.

Fig 2a shows the peak height ratio of 16-DS from those five individuals splenectomized β-thal/Hb E before the start of the regimen and after one month. The erythrocyte membrane fluidity was decreased in all patients, except for patient number 03, whose peak height ratio slightly decreased after 1 month. The mean and SD of peak height ratio before and after vitamin E supplementation were 4.876 ± 0.137 and 5.191 ± 0.404, respectively ($p = 0.138$).

Fig 2b shows the peak height ratio of 16-DS from those four individuals with splenectomized β-thalassemia/Hb E who receiving the placebo. In contrast to the vitamin E supplementation group, erythrocyte membrane fluidity was further slightly increased in all patients, except for patient number 07, whose peak height ratio increased after one month. The mean of peak height ratio was slightly decreased in this group of patients after one month receiving placebo. The mean and SD of peak height ratio in 16-DS before and after treatment with placebo were 5.153 ± 0.177 and 5.007 ± 0.204, respectively ($p = 0.465$).

Discussion

Oxidative damage of red blood cell membrane is an important pathology of β-thalassemia, which leads to hyperhemolysis and short red blood cells survival. The degraded products of unbound α-globin subunits such as hemin, heme, and free iron may bind to the membrane and catalyze free radical reaction. The enhanced production of ROS was demonstrated

Table 2. Outer hyperfine splitting ($2T_{//}$) and order parameter (S) of three different spin labels in the erythrocytic membrane

Parameter	DS	Normal subjects (n = 10)	β-thalassemia/Hb E patients	
			Non-splenectomized (n = 5)	Splenectomized (n = 9)
$2T_{//}$ (G)	5-DS	55.82 ± 0.49	55.95 ± 0.42	55.39 ± 0.43
	12-DS	47.88 ± 0.72	47.88 ± 0.84	46.44 ± 0.54**
	16-DS	44.56 ± 0.89	44.36 ± 0.91	44.45 ± 0.53
Order parameter	5-DS	0.699 ± 0.013	0.702 ± 0.016	0.694 ± 0.013
	12-DS	0.530 ± 0.020	0.545 ± 0.017	0.516 ± 0.014*
	16-DS	0.387 ± 0.027	0.381 ± 0.028	0.384 ± 0.016
Peak height ratio (h_o/h_{-1})	16-DS	5.25 ± 0.26	5.14 ± 0.16	4.99 ± 0.21*

Values are expressed as the mean ± SD, ** $p < 0.01$ and * $p < 0.05$ compared with normal

Table 3. Plasma vitamin E and serum TBARs of splenectomized β-thal/Hb E before and after vitamin E supplementation

	Plasma vitamin E (μg/ml)		Serum TBARs (nmol/ml)	
	Before	After 1 month	Before	After 1 month
Placebo group (n = 4)	0.66 ± 0.05	1.75 ± 1.18	0.35 ± 0.14	0.38 ± 0.08
Vitamin E group (n = 5)	0.59 ± 0.41	5.19 ± 1.37*	0.31 ± 0.28	0.19 ± 0.09

Values are expressed as the mean ± SD, * $p < 0.05$ compared with normal

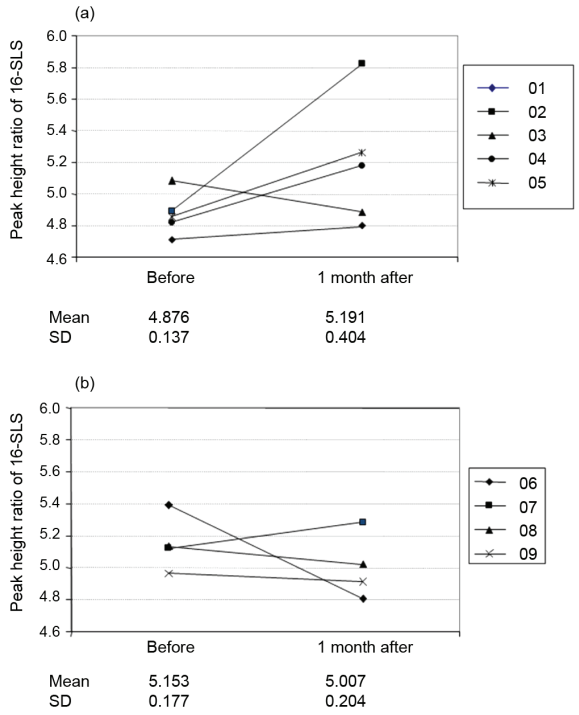

Fig. 2 Peak height ratios of 16-DS before starting vitamin E and one month after supplementation in individual splenectomized β-thalassemia/Hb E patients (a) and peak height ratios of 16-DS in individual splenectomized β-thalassemia/Hb E patients receiving placebo (b)

in β-thalassemia RBCs[20]. Recently, the correlations between imbalance globin chain synthesis, reactive oxygen species and red blood cell properties have been demonstrated in thalassemic mice[21]. Characteristics of the damages such as reduction in the membrane content of phosphatidylethanolamine[6], externalization of phosphatidylserine[22] have been reported in the β-thalassemic erythrocytes. The perturbations of membranous lipids in thalassemic RBCs were reflected in the enhanced uptake of Merocyanine-540 dye, indicating changes in the membrane phospholipids asymmetry[23]. All evidence suggested that lipid peroxidation could play an important role.

Electron spin resonance (EPR) spectroscopy of spine-labeled compounds involves the use of molecular probe to explore a physical environment on the molecular scale. Considerable knowledge of structure of biological membranes has been acquired using nitroxide spin labels[24]. In the present study, fatty acid spin labels (5-, 12- and 16-DS) were used to investigate the erythrocyte membrane fluidity in these patients. These parameters represented the nature of change that occurs in the lipid compartment of their erythrocytes membrane. The erythrocyte membrane fluidity is a dynamic physical property of the membrane (three-dimensional flexibility of the membrane hydrocarbon interior). It is not equivalent to the deformability which is a specific functional characteristic of erythrocyte membranes and which permits erythrocytes to undergo rapid and often extreme changes in shape as they circulate *in vivo*, particularly in the spleen[9-14].

With EPR spin labeling, level of erythrocyte membrane fluidity was significantly increased in the splenectomized β-thalassemia/Hb E patients compared to those in non-splenectomized thalassemic patients and normal subjects. Changes in these ESR parameters were detected only by the more lipophilic labeled stearic acids (12- and 16-DS) which were located nearby the unsaturated bonds of fatty acid chains in phospholipids molecules. Consistent with the evidence found in lipoproteins separated from thalassemic plasma, a more hydrophobic region of membrane was suggested to be a major target site of oxidative damage[25]. Udyaningsih-Freisleben et al[26] have demonstrated a decreased membrane fluidity in the lipophilic region of isolated erythrocyte membrane from transfusion-dependent thalassemic patients. This conflict with the present finding may indicate that variable severity of the patient's conditions could determine the stage and progression of the membrane damage.

The increased peroxidative damage to tissue with the depletion of endogeneous antioxidant was supported by an increase of plasma lipid peroxidation products (TBARs) and a marked decrease of plasma antioxidant, such as vitamin E. Even though, membrane fluidity was not marked significant difference, the authors found that the increasing of the membrane fluidity of the β-thalassemia/Hb E erythrocyte was inversely correlated with the level of oxidative stress markers (TBARs). Furthermore, the level of erythrocyte membrane fluidity in splenectomized β-thalassemia/Hb E can be reduced by the supplementation of vitamin E, which is a lipid soluble, chain-breaking antioxidant. Thus, vitamin E could prevent oxidatively modified lipids in the erythrocyte membrane.

In the present study, the authors could not demonstrate the benefit of vitamin E on red blood cell survival because of a short period of the supplementation. However, the authors would strongly recommend the supplement of vitamin E or other lipid

soluble antioxidants in β-thalassemia in addition to iron chelation therapy. Improvement of membrane integrity may not only increase erythrocyte half-life but also improve the function of other cells and cellular components.

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Potential conflicts of interest

None.

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การให้วิตามินอีทดแทนทำให้การเปลี่ยนแปลงของเยื่อหุ้มเม็ดเลือดแดงธาลัสซีเมียดีขึ้น

วีระศักดิ์ สุทธิพรพลางกูร, นพวรรณ ภู่มาลา มอราเลส, สุภีนันท์ อัญเชิญ, ยุพิน สังวรินทะ, อุดม จันทรารักษ์ศรี, สุทัศน์ ฟู่เจริญ

ภูมิหลัง: *โรคบีต้าธาลัสซีเมีย/ฮีโมโกลบินอีพบได้บ่อยในประเทศไทย ความไม่สมดุลของโกลบินในเม็ดเลือดแดงเป็นสาเหตุแรกที่ทำให้เกิดภาวะซีด โดยแอลฟาโกลบินที่เกินเป็นสาเหตุที่ทำให้เกิดการทำลายเยื่อหุ้มเม็ดเลือดแดง การใช้สารเคมีที่มีการติดสลากด้วยสารเหนี่ยวนำแม่เหล็กทำให้สามารถตรวจจับการเปลี่ยนแปลงของเยื่อหุ้มเม็ดเลือดแดงธาลัสซีเมียได้*

วัตถุประสงค์: *การตรวจจับการเปลี่ยนแปลงของเยื่อหุ้มเม็ดเลือดแดงธาลัสซีเมีย และผลของการให้วิตามินอีทดแทนที่เกิดขึ้นในโรคธาลัสซีเมีย โดยเทคนิคการใช้สารเคมีที่มีการติดสลากด้วยสารเหนี่ยวนำแม่เหล็ก การเปลี่ยนแปลงของเยื่อหุ้มเม็ดเลือดแดงทำในผู้ป่วยธาลัสซีเมียที่ตัดม้าม 9 คน และผู้ป่วยธาลัสซีเมียที่ไม่ตัดม้าม 5 คน ส่วนการให้วิตามินอีทดแทนทำในผู้ป่วยธาลัสซีเมียที่ตัดม้ามแล้วจำนวน 9 คน โดยแบ่งเป็น 2 กลุ่ม คือ กลุ่มที่ได้รับวิตามินอีทดแทนจำนวน 5 คน และกลุ่มที่ได้รับยาหลอกจำนวน 4 คน สารเคมีที่มีการติดสลากด้วยสารเหนี่ยวนำแม่เหล็ก 3 ชนิด ถูกนำมาใช้โดยการเปลี่ยนแปลงของเยื่อหุ้มเม็ดเลือดแดงในชั้นตื้นใช้ 5-ด็อกซี่สเตียรริกแอซิด ส่วนในชั้นลึกใช้ 12-ด็อกซี่สเตียรริกแอซิด กับ 16-ด็อกซี่สเตียรริกแอซิด*

ผลการศึกษา: *การวิจัยพบว่าเยื่อหุ้มเม็ดเลือดแดงในผู้ป่วยธาลัสซีเมียที่ตัดม้ามแล้วมีการเปลี่ยนแปลงมากกว่าเมื่อเปรียบเทียบกับผู้ป่วยธาลัสซีเมียที่ไม่ตัดม้าม และกลุ่มปกติเมื่อใช้ 12-ด็อกซี่สเตียรริกแอซิด และ 16-ด็อกซี่สเตียรริกแอซิด แต่ไม่พบการเปลี่ยนแปลงนี้เมื่อใช้ 5-ด็อกซี่สเตียรริกแอซิด การให้วิตามินอีทดแทนในผู้ป่วยธาลัสซีเมียทำให้การเปลี่ยนแปลงที่เกิดขึ้นที่เยื่อหุ้มเม็ดเลือดแดงดังกล่าวข้างต้นลดลง*

สรุป: *คณะผู้วิจัยจึงเสนอว่าการเปลี่ยนแปลงที่เกิดขึ้นที่เยื่อหุ้มเม็ดเลือดแดงอยู่ในชั้นลึก และการให้วิตามินอีทดแทนมีบทบาทในการป้องกันการทำลายเยื่อหุ้มเม็ดเลือดแดงในผู้ป่วยธาลัสซีเมีย*