THESIS TITLE: Effects of Vitamin E on the Responsiveness of Platelets in Splenectomized β-thalassemia/Hemoglobin E

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## ABSTACT

Thalassemia is a congenital hemolytic anaemia. Beta-thalassemia/ Haemoglobin E ( β-thal/HbE) contritutes to the major portion of thalassemic population living patients in Thailand that possess clinical problems of varying degrees and frequently need of medical attention. Hypoxemia has commonly been found in the splenectomized thalassemic patients (Sp-thal) and are considered a serious clinical syndrome, resulting from pulmonary arterial thrombosis. Hyperfunction of the platelets was suspected as the major cause of this clinical problem and its associated consequences.

This study has defined the abnormalities of platelet functions in 6 splenectomized β-thal/Hb E (Sp-β-thal/HbE ) patients who were splenectonized for more than 5 years. The aggregatability of platelets in the whole blood (WB) and platelet rich plasma (PRP) were performed in a chronolog aggregometer (using impedance and optical aggregometry). One male and 5 female patients, aged 21-38 years old who experienced varying degrees of iron overloading were recruited into this study. They

were told not to take aspirin or other nonsteroid antiinflammatory drugs that were known to affect platelet functions, at least two weeks before the studies. Six normal healthy volunteers in the comparable age groups were served as controls. Blood sample (25 ml) were taken via the scalp vein directly dropped into the clean siliconized glass tube, containing 2.5 ml of 3.2 % sodium citrate. Impedance aggregation of the WB were performed within one hour while the optical aggregation of platelets in the PRP were performed within three hours after the blood sampling.

The responsiveness of platelets to both mechanical and chemical stimuli were studied using a continuous stirring magnetic bar (speed at 1000 rpm) and the addition of either ADP (0.2-4.0 μM) or collagen (0.039-2.50 μg/ml). Thiobarbituric acid reactive substances (TBARs) appeared in the plasma was detected using fluorometric method and used as a marker for lipid peroxidation. Levels of serum vitamin E were monitored using HPLC. A complete blood count (CBC) were performrmed by an H1-technicon cell analyzer.

This study demonstrated that platelets in the WB of all six Sp-β-thal/HbE were hyperreactive to both mechanical and chemical stimuli. Spontaneous aggregation of the platelets in the WB responding to stirring magnitic bar was found in all cases of the Sp-β-thal/HbE but not a single case occured in the normal. Platelets of the Sp-β-thal/HbE were more sensitive to ADP and collagen. The minimum effective concentrations (MEC) of ADP that were needed to aggregate platelets were five-fold less in the Sp-β-thal/HbE (0.2 μM) than that was need in normal volunteers (1.0 μM) and over 30-fold less of collagen (0.08 μg/ml in Sp-β-thal/HbE while more than 1.25 μg/ml in normal) to initiate platelet aggregation in the WB. The same picture applied to platelets in the PRP: the MEC of ADP that induces irreversible platelet aggregation were 0.5, 1.0 μM (Sp-β-thal/ HbE) and 2.0 μM (normal volunteers) and with collagen were 0.16 μg/ml (Sp-β-thal/HbE ) and 0.625 μg/ml (normal volunteers). Levels of vitamin E in these patients were confirmed low 0.25 mg/dL (Sp-β-thal/HbE ) against 0.5 mg/dL (normal volunteers). On the contary, marker of lipid peroxidation was higher in Sp-β-thal/HbE (0.42 nm/ml ) and low (0.14 nm/ml) in normal volunteers.

Daily doses of vitamin E (525 IU or 2x525 IU) were given daily to the Sp-β-thal/HbE patients for up to three months. The effect of vitamin E on the reactivity of platelets, TBARs (marker of lipid peroxidation) and levels of vitamin E in the plasma were respectively followed. Vitamin E inhibited platelet responses to ADP and collagen in both the WB and PRP after daily supplementation of vitamin E 525 IU for one month. A significant suppression were also noted with ADP 0.5 μM in the WB ($p < 0.05$) and ADP 0.5 or 1.0 μM in the PRP model ($p < 0.05$). Vitamin E also inhibited ATP release in response to 2.0 μM ADP. TBARs, marker of lipid peroxidation in the plasma was significantly lowered, from 0.42 nm/ml (before vitamin E) to 0.17 nm/ml (after two months of vitamin E supplementation). Three months after the cessation of vitamin E supplementation, all these parameters with no exception of any returned to their pretreatment values.

These data strongly suggested that the Sp-β-thal/HbE were under severe oxidative stress, as vitamin E (the chain breaking antioxidant) was depleted, resulting in elevation of lipid hydroperoxides in the plasma and thus the hyperreactiivity of platelets. It is likely that these could be the consequence of iron overloading in thalassemia patients as a good correlation between the levels of serum ferritin and plasma lipid hydroperoxides was established. Daily supplementation with mega-dose of vitamin E seems to work effectively in inhibiting the oxidative stress in Sp-β-thal/HbE as the hyperreactive platelets were modulated and degrees of lipid peroxidation lowered.

Studies of the conventional reactive-oxygen scavenger enzymes, i.e., erythrocyte glutathionne peroxidase ( r-GSH-Px), erythrocyte glutathione reductase (r-GSSG-Rd), erythrocyte catalase (r-CA), and plasma glutathione peroxidase (p-GSH-Px) were also performed in these studies. Significant elevation of r-GSH-Px and r-GSSG-Rd activities were recorded while the activity of p-GSH-Px was suppressed in the Sp-β-thal/HbE patients. No significant difference was noted with the r-CA activity. Supplementation of vitamin E (1050 IU) for six weeks seem to show suppessive effect on the activity of r-GSH-Px. The activities of r-GSSG-Rd, and p-GSH-Px on the other hand were increased after supplementation of vitamin E. It is not known if the effect of vitamin E acts to suppress the

oxidative stress or directly interfere with these oxidative scavenging enzymes activities in the assay tubes.

It is concluded that platelets of Sp-β-thal/HbE are hyperreactive to both mechanical and chemical stimuli. Iron overloading in the thalassemic blood could be an underling cause of the excessive oxidative stress as demonstrated by a good correlation of serum ferritin, plasma TBARs, increased activities of the scavenging enzymes and the severely depleted of vitamin E. Supplementation of vitamin E restored the abnormal parameters back towards the direction of healthy volunteers, beside the subjective benefit expressed by the thalassemia subjects. It thus suggested that clinical trials of vitamin E supplementation in the Sp-β-thal/HbE should be considered.