

ANTIOXIDANT IN PLASMA OF HEMOGLOBIN-E TRAIT

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Abstract. A study of antioxidant levels among Thai subjects with a hemoglobin E trait was undertaken. The objective of this study was to determine whether the antioxidant level would be disturbed in the HbE condition. All 185 volunteer subjects, 171 normal healthy subjects and 14 HbE carriers were recruited. For each case, an antioxidant determination was performed using the Trolox equivalent antioxidant capacity (TEAC) method. According to this study, the average antioxidant level in the healthy group was 3.439 ± 0.220 mM Trolox equivalent, and in HbE trait group was 3.276 ± 0.209 mM Trolox equivalent. There was a significant decrease of the antioxidant level in the HbE trait group ($p = 0.008$).

INTRODUCTION

Thalassemia is the most common chronic hereditary hemolytic anemia due to the imbalance of the globin chain synthesis. The severity of impaired globin chain generates the diversity of thalassemic phenotypes. β -thalassemia arises as a consequence of decreased or absent synthesis of the β -globin chain. As the result of altered β -globin chain biosynthesis, the concentration of the $\alpha_2\beta_2$ -hemoglobin tetramer (HbA) is substantially reduced, or absent. The excess pool of unpaired α -hemoglobin chains leads to erythrocyte damage by oxidative means which might be further exaggerated by the heme (Joshi *et al*, 1983).

β -thalassemia hemoglobin E is one of the most common forms in Thailand, and mostly due to the interaction of β^0 -thalassemia or β^+ -thalassemia with HbE. HbE β -thalassemia shows a remarkable degree of variability of clinical expressions, some of which similar to homozygous β -thalassemia (Swarup *et al*, 1961; Frischer *et al*, 1975). The iron overload is the consequence in the β -thalassemia HbE after repeated blood transfusions. Iron overload can generate the peroxidative status in β -thalassemia major and the increase of superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity (Kassab-Chekir *et al*, 2003). Severe oxidative damage is observed in erythrocytes due to the presence of excess α -globin chains (Pearson *et al*, 1973). Thus, both the accumulation of excess alpha chains and iron overloaded would instigate increased red blood cell destruction, resulting in decreased antioxidants. Given that β -thalassemia HbE is the interaction between β -

thalassemia carrier and HbE trait (AE), in this study we aimed to investigate the level of antioxidants in those HbE carriers.

MATERIALS AND METHODS

Chemicals

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was purchased from Aldrich Chemical. ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt) and potassium persulfate were purchased from Sigma-Aldrich (St Louise, MO, USA). All chemicals were of analytical grade and available locally.

Subjects and clinical samples.

The HbE trait population, or carriers, were diagnosed by hemoglobin electrophoresis analysis and confirmed under the supervision of medical professionals in our laboratory. Fourteen HbE carriers (AE) (mean age of 25.86 ± 13.97 years) were recruited, with informed consent, for this study. A control population of 171 subjects was recruited, with informed consent, from healthy people (mean age of 38.2 ± 17.0 years).

Blood sampling

Venous blood samples were taken after fasting overnight. Blood samples were collected in heparinized tubes (Li Heparin 500 U/10 ml). Within 30 minutes of venipuncture, plasma was separated by centrifugation of blood sample at 950g for 15 minutes. Plasma samples were blown with nitrogen gas and stored at -20°C until further analysis.

Sample analysis

Total antioxidant capacity was measured by using a radical cation decolorization assay (Re *et al*, 1999).

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This assay was based on the inhibition by antioxidants of the absorbance of the free radical cation from ABTS. ABTS was incubated with potassium persulfate to produce the free radical cation (ABTS^{o+}). This had a relatively stable blue-green color, which was measured at 734 nm. Antioxidant compounds will suppress the absorbance of ABTS^{o+} to an extent, on a time scale, dependent on the antioxidant capacity in plasma. This assay was calibrated using Trolox (a water soluble vitamin E analog) as a standard.

Statistical analysis

All parameters were expressed as mean ± SD, and statistically significant differences between values were obtained using the two-tailed Student’s unpaired *t*-test. The criteria for significance was *p*<0.01.

RESULTS

Total antioxidant capacity from HbE carriers (trait) and control subjects are presented in Table 1. The average total antioxidant level in the healthy group was 3.276 ± 0.209 mM Trolox equivalent and, in HbE trait group, was 3.439 ± 0.220 mM Trolox equivalent (Fig 1). There was a significant decrease of the antioxidant level in the HbE trait group (*p* = 0.008).

DISCUSSION

The total antioxidant capacity in our HbE trait subjects showed a significant decrease compared with the control subjects. This result indicated the depletion of the oxidative defense mechanism to neutralize the damaging effects of oxidative species (Cighetti *et al*, 2002; Kassab-Chekir *et al*, 2003). Scott *et al* (1990) demonstrated that the excess α-hemoglobin chains oxidized, released heme, and generated reactive oxygen species in red blood cells. The interaction between these reactive oxygen species and the heme-derived iron (Scott *et al*, 1991) has been shown to result in a “biologic Fenton reaction” (Sadrzadeh *et al*, 1984) that catalyzed the production of the hydroxyl radical

Table 1
Total antioxidant capacity (mean ± SD) from carrier and control subjects.

	Total antioxidant capacity (mM Trolox equivalent)
HbE trait (n=14)	3.276 ± 0.209
Control (n=171)	3.439 ± 0.220

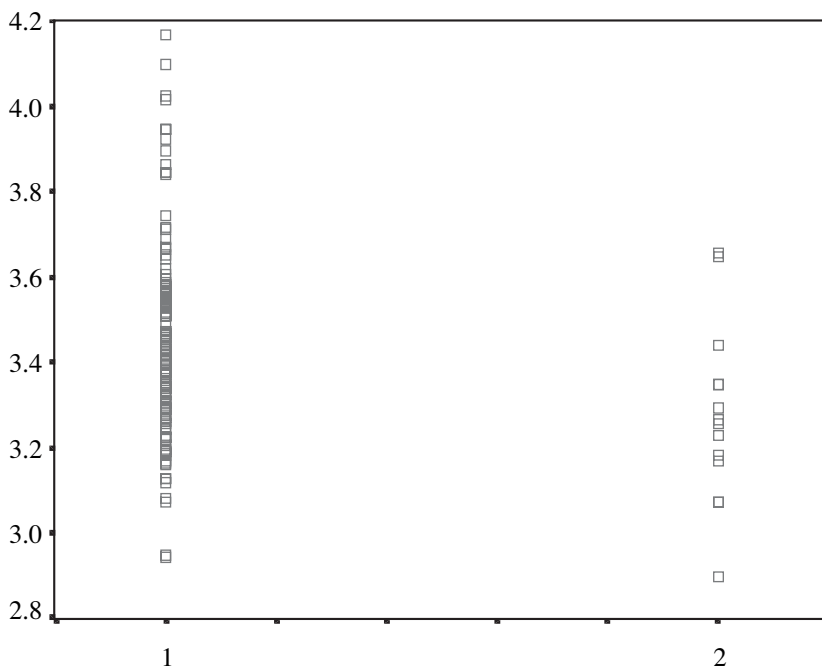


Fig 1- Scattergram showing the distribution of total antioxidant capacity in this study (X axis represents groups: group 1 (controls) and group 2 (HbE carriers); Y axis represents total antioxidant capacity in Trolox equivalent unit).

in thalassemic erythrocytes. HbE trait is quite different from β -thalassemia diseases. In HbE, the defect is at the amino acid residue 26 that causes the change of the amino acid glycine to lysine. The result is as if beta chains were absent at this point, thus showing the thalassemia-like nature of the HbE trait. Hence, the offspring of β -thalassemia carriers and HbE carriers (HbE trait) might suffer from β -thalassemia HbE disease. Interestingly, the depletion of the oxidative defense (lower antioxidant) might be due to an increase in iron accumulation that has not yet overloaded, which contrasts with β -thalassemia diseases (Scott *et al*, 1991). Iron accumulation in these carriers can occur because of the short red cell life span, represented by a reduction in the mean corpuscular volume (MCV), and increased red cell count when compare to the Hb concentration. As a result, β -thalassemia HbE patients can have normal daily life. *In vivo* studies on model β -thalassemia erythrocytes have shown a significant production of intracellular hydrogen peroxide and methemoglobin that was correlated with the autoxidation rate of the α -hemoglobin chain. This phenomenon is implicated in the pathway of cellular oxidant stress in β -thalassemia (Scott *et al*, 1993). If the red cells of those who have HbE genes acting as β -thalassemia erythrocytes, as mentioned above, intracellular hydrogen peroxide and methemoglobin will be another mechanism that enhanced the lowering of antioxidants in the patients. An increased oxidative stress and a decreased antioxidant status enhance peroxidative damage to cell or organelle membranes in organs with excess iron accumulation, such as in the liver, pituitary gland and heart. Thus, HbE carriers should be taken under care to prevent damage from high tissue iron levels. Further research should be undertaken, with more subjects, to find the exact mechanism involved in the lowering of antioxidants.

REFERENCES

- Cighetti G, Duca L, Bortone L, *et al*. Oxidative status and malondialdehyde as a biomarker for beta-thalassemia patients. *Eur J Clin Invest* 2002;32 (suppl 1): 55-60.
- Frischer H, Bowman J. Hemoglobin E, an oxidatively unstable mutation. *J Lab Clin Med* 1975;85:531-9.
- Joshi W, Leb L, Piotrowski J, Fortier N, Snyder LM. Increased sensitivity of isolated alpha subunits of normal human hemoglobin to oxidative damage and crosslinkage with spectrin. *J Lab Clin Med* 1983;102:46-52.
- Kassab-Chekir A, Laradi S, Ferchichi S, *et al*. Oxidant, antioxidant status and metabolic data in patients with beta-thalassemia. *Clin Chem Acta* 2003;338: 79-86.
- Peason HA, O'Brien RT, McIntosh S. Screening for thalassemia trait by electronic measurement of mean corpuscular volume. *New Engl J Med* 1973;288:351-3.
- Re R, Pellegrini N, Proteggente A, Pannala A, *et al*. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Rad Bio Med* 1999;26:1231-7.
- Sadrzadeh SM, Graf HE, Penter SS, Hallaway PE, Eaton JW. Haemoglobin: a biologic Fenton reaction. *J Biol Chem* 1984;259:14354-6.
- Scott MD, Rouyer-Fessard P, Lubin BH, Beuzard Y. Entrapment of purified α -hemoglobin chains in normal erythrocytes: a model for thalassemia. *J Biol Chem* 1990;265:17953-9.
- Scott MD, van den Berg JJM, Repka T, *et al*. Effect of excess α -hemoglobin chains on cellular and membrane oxidation in model β -thalassemic erythrocyte. *Clin Invest* 1993; 91: 1706-12.
- Scott MD, Wagner TC, Lubin BH, Eaton JW. 'Loose' iron: an important element in the pathogenesis of damage within beta thalassemic erythrocytes. *Blood* 1991; 78(suppl 1): 772.
- Swarup S, Chatterjee JB, Ghosh SK. Observations on erythropoiesis in HbE-thalassemia disease, a study with Cr^{51} and Fe^{59} . *Indian J Pathol Bacteriol* 1961;4:1.