Thesis Title Antioxidant Status and Tissue Damage in β-thalassemia Hemoglobin E

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ABSTRACT

β-Thalassemia / hemoglobin E (β-thal / Hb E) is claimed to be susceptible to the oxidative stress since the red cells contains isolated globin chains and large quantities of iron, which may serve as an initiator of oxidative stress. The antioxidant status and tissue damage were used as indicators of oxidative stress in 10 normal subjects (2 males and 8 females); aged 21 - 38 years, and 10 patients of β-thal / Hb E (2 males and 8 females); age 22 - 41 years, that further divided into two subgroups: 6 non - splenectomized and 4 splenectomized. All subjects were not given blood transfusion within 3 months prior study. The results showed that the total GSH-Px and Se-GSH-Px activities were significantly increased in β-thal / Hb E patients ($P < 0.001$) whereas non-Se-GSH-Px activity was not statistically significant ($P > 0.05$). In addition, plasma selenium level of β-thal / Hb E was significantly lower than that of non-thalassemic subjects ($P < 0.005$), but red blood cell selenium content of β-thal / Hb E patients was significantly higher than that of non-thalassemic subjects ($P < 0.05$). However, reduced glutathione (GSH), essential substance for protective mechanisms against oxidative damage, in red cell of patients with β-thal / Hb E was significantly lower than that of normal subjects. Oxidised glutathione

(GSSG) of β-thal / Hb E was not significantly different but had an upward trend to increase. The ratio of GSH / GSSG was decreased significantly in β-thal / Hb E ($P < 0.001$). There was also a negative correlation between Se-GSH-Px activity and hemoglobin level ($r=-0.59, P<0.001$).A reactive antioxidant enzyme activity may thus protect the increasing of reactive oxygen species in the case of β-thal / Hb E. Furthermore, higher levels of serum iron, transferrin iron saturation, serum ferritin and lower level of total iron binding capacity (TIBC) were also found in β-thal / Hb E patients. There were also a positive correlation between total GSH-Px activity and serum iron ($r=0.71, P<0.0001$), total GSH-Px activity and serum ferritin ($r=0.66, P<0.0001$), non-Se-GSH-Px activity and serum iron ($r= 0.76, P<0.005$). From these results, we concluded that β-thal / Hb E patients were subjective to iron overload and expected to produce reactive oxygen species which serve as an initiator of oxidative stress to damage cells and tissues. The levels of plasma antioxidants such as vitamin E, A and β - carotene were significantly decreased in β-thal / Hb E ($P < 0.001$) as compared to the non-thalassemic control. The level of plasma vitamin E and serum ferritin, β-carotene and serum iron had a negative correlation ($r= -0.66, P<0.001$ and $r= -0.70, P<0.001$, respectively) and also found a negative correlation between vitamin E or A and hemoglobin level ($r= -0.57, P<0.05$ and $r= -0.59, P<0.05$, respectively). These results indicated that the cells or tissues of β-thal / Hb E were prone to be oxidised by reactive oxygen species owing to the shortage of natural antioxidants. The higher level of red cell MDA including a positive correlation with serum iron ($r=0.63, P<0.001$) and a negative correlation with β-carotene ($r= -0.58, P<0.0001$), were found in β-thal / Hb E as well. These suggested that the red cell membranes of thalassemic patients might be subjective to oxidative damage corresponding to the higher level of plasma uric acid. Plasma uric acid may be increased by turnover rate of purine metabolism from short survival of cells and / or

by ischemia reperfusion from anemia and abnormality of red cell morphology. Creatine kinase (CK) and lactate dehydrogenase (LDH) as indices of tissue membrane damage were also determined in this study. The CK activity in plasma of β-thal / Hb E was not significantly different from normal control but total LDH activity of patients with β-thal / Hb E was significantly higher ($P< 0.001$). Extremely significant increases of LDH_1 and LDH_2 ($P< 0.001$, $P< 0.005$, respectively) in β-thal / Hb E were also found but no significant difference were investigated in LDH_3, LDH_4 and LDH_5 of patients with β-thal / Hb E. These LDH isoenzymes results indicated that red blood cells but not other tissues were destroyed or damaged by reactive oxygen species. This may be due to the high protective role of scavenging enzymes activities, or high adaptation of the defense mechanism in other tissues to prevent themselves from the oxidative damage.