Total Antioxidant Capacity As Indicative of Oxidative Stress on β-Thalassemia Patients

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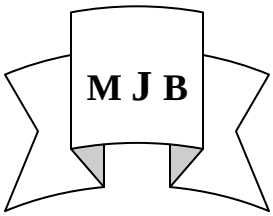

Abstract

Total antioxidant capacity (TAC), glutathione peroxidase(Gpx) and glutathione S transferase(GST) for patients with β- thalassemia major (n=20) and β-thalassemia minor (n=20) compared with apparent healthy control subjects (n=18) were study. The aim of this study is to consider mechanisms of free radical formation in the body, the consequences of free radical induced tissue damage, and the function of antioxidant defence systems in thalassemia major and minor patients. The indicative parameter of conjugated diene hydroperoxide with the total antioxidant capacity and the enzymatic antioxidant system activity (glutathione peroxidase(Gpx) and glutathione S-transferase(GST)), were evaluated. This is a new study to describe total antioxidant capacity in the thalassemia patients.

Keywords: total antioxidant capacity (TAC), glutathione peroxidase(Gpx), glutathione S transferase(GST), fetal hemoglobin (HbF), Adult hemoglobin(HbA_2),

الخلاصه

يتضمن البحث دراسة سعة مضادات الأكسدة الكلي ،وانزيمي الكلوتاثيون بيروكسيديزGpx والكلوتاثايون S ترانزفيرز(GST) لمرضى البيتا ثلاسيميا (٢٠ شخص مصاب بالثلاسيميا العظمى و٢٠ شخص مصاب بالثلاسيميا الصغرى)، تم سحب العينات من مستشفى الولادة والطفل في بابل وتم تشخيص المرض من قبل الكادر الطبي المتخصص فيها، مقارنة بمجموعة الأصحاء (١٨ شخص) وكان الهدف من البحث هو دراسة علاقة الجذور الحرة المتولدة بتأثير المرض مع دفاع الجسم ضدها من خلال قياس سعة مضادات الأكسدة الكلي ومضادات الأكسدة الأنزيمية. وتعتبر قياس مضادات الأكسدة الكلي هي دراسة جديدة لمرضى فقر الدم البحري.

Introduction

The term thalassemia major and minor are used to indicated the severity of the clinical disorder. Thalassemia major is characterized by severe anemia, hypochromic microcytic red blood cells(RBCs), signs of accelerated hemolysis and regeneration, marked enlargement to the liver and spleen. Thalassemia minor is a common, mild condition that is characterized by the hypochromia, mild microcytosis of RBCs and usually mild elevation in the RBC count(1,2).

The hemoglobin levels of β-Thalassemia major may be as lower 3-4 g/dl, and a low mean cellular hemoglobin(MCH), low mean cell volume(MCV), mild morphological alterations of their RBCs, an increased levels of adult hemoglobin(HbA_2), the level of fetal hemoglobin HbF is always higher than normal in the range of 10 to 90% and a low β/α globin chain ratio on biosynthesis. Most patients with β-Thalassemia minor have two fold elevated levels of hemoglobin A_2 (HbA_2), the average 5.1% approximately twice the normal level (1.5-3.5%), the HbA_2/ HbA_1 ratio is 1:20 instead of the normal 1:40, and the HbF (1 to 3%) of total hemoglobin occur during the early year of live(1,2).

Free radical production occurs continuously in all cells as part of normal cellular function. However, excess free radical production originating from endogenous or exogenous sources might play a role in many diseases(3).Antioxidants prevent free radical induced tissue damage by preventing the formation of radicals, scavenging them, or by promoting their decomposition(4).

Free radicals are eliminated from the body by their interaction with antioxidants. Two classes of antioxidants are known: 1) the low molecular weight compounds such as vitamin C,E,A, glutathione, and 2) the protein albumin, glutathione peroxidase, superoxide dismutase. Total antioxidant capacity(TAC) parameters summarizes overall activity of antioxidants and antioxidant enzymes(5). The physiological role of antioxidants, as this definition suggests, is to prevent damage to cellular components arising as a consequence of chemical reactions involving free radicals. In recent years, a substantial body of evidence has developed supporting a key role for free radicals in many fundamental cellular reactions and suggesting that oxidative stress might be important in the pathophysiology of common diseases. Because radicals have the capacity to react in an indiscriminate manner leading to damage to almost any cellular component, an extensive range of antioxidant defences, both endogenous and exogenous, are present to protect cellular components from free radical induced damage. (6,7)

Glutathione peroxidases catalyze the oxidation of glutathione at the expense of a hydroperoxide, which might be hydrogen peroxide or another species such as a lipid hydroperoxide (8).

$$ROOH + 2GSH \rightarrow GSSG + H_2O + ROH$$

Other peroxides, including lipid hydroperoxides, can also act as substrates for these enzymes, which might therefore play a role in repairing damage resulting from lipid peroxidation. Glutathione peroxidases require selenium at the active site, and deficiency might occur in the presence of severe selenium deficiency (9). Several glutathione peroxidase enzymes are encoded by discrete genes (10). The plasma form of glutathione peroxidase is believed to be synthesised mainly in the kidney. Within cells, the highest concentrations are found in liver although glutathione peroxidase is widely distributed in almost all tissues. The predominant subcellular distribution is in the cytosol and mitochondria, suggesting that glutathione peroxidase is the main scavenger of hydrogen peroxide in these subcellular compartments. The activity of the enzyme is dependent on the constant availability of reduced glutathione (11). The ratio of reduced to oxidised glutathione is usually kept very high as a result of the activity of the enzyme glutathione reductase (12).

$$GSSG + NADPH + H^+ \rightarrow 2GSH + NADP^+$$

The glutathione transferase is effective as important catalysts the bio transformation of xenbiotics, including drugs (13), and catalysts the formation of thioether bond by conjugated ionic compound with reduce glutathione (GSH) to form products which can excreted by the bile as GSH conjugated and then cleavage glycine or glutamine and acetylation the free amino acids of cysteinyl to give the end products, mercapturic acid (14).

The aim of this study is to consider mechanisms of free radical formation in the body, the consequences of free radical induced tissue damage, and the function of antioxidant defence systems in thalassemia major and minor patients.

Materials and methods

- **Patients:** Patients affected by β-thalassemia major and minor **(** 20 persons for each type**),** aged 10-35 years, controls healthy individuals, aged 10-35 years were recruited. In this study patients were under un regular chelating therapy with deferoxamine and did not involve intake of ascorbate. Blood from thalassemia patients and controls was collected during the may

and September 2007, after clotting, serum was separated by centrifugation and divided in several aliquots. The analytical determinations described below were either performed immediately, or serum was stored at 20 º C and used within 72 hours.

Biochemical analysis.

-Determination of Total Antioxidant Capacity (TAC):
The assay is based on the reduction of molybdenum(VI) to molybdenum(V)by the formation of a green phosphomolybdenum complex at acid PH(15). The total antioxidant capacity was expressed as ascorbic acid equivalent (AAE) at λ max 695nm.

-Determination the activity of serum glutathione peroxidase (GPX):
The activity were measure by the method described by Rolruck *et al.*(16), briefly, reaction mixture contained 0.2ml of tris-HCl buffer (0.4M) PH=7, 0.1ml of sodium azide (10mM), 0.2ml of the serum (of patients and controls) and 0.1ml of tertiary butyl hydroperoxide, the contents were incubate at 37C° for 10 minutes. The reaction was arrest by 0.4ml of 10% trichloro acetic acid (TCA) and centrifuged. The supernatant was assay for glutathione content by using Ellmans reagent.

-Determination the activity of serum glutathione S transferase (GST): The principle to determine their activity was using the reaction between 1-choro-2,4-dinitrobenzene and glutathione in presence of GST, which described by Habig *etal.*(17) , the enzyme activity were expressed as (U/L).

Statistical analysis.
All results are expressed as mean ± SD(standard deviation), comparison between thalassemia patients and controls was performed by the Student's t- test. Pearson's correlations were used to determine relationship between parameters studied taken $P \leq 0.05$ as the lowest limited of significant.

Results
The changes of the serum antioxidant value were investigated, compared with controls, this value was shown in Table 1 and Fig. 1. The antioxidants species in serum contribute to a different extent to the total antioxidant capacity due to their relative concentration and number of radicals scavenged per mole of compound (18). compared with controls the TAC levels were decrease by (43%) and (30%) for thalassemia patients major and minor respectively. The decreased of TAC value appears to the result of the marked decrease of vitamin C.
Results are expressed as mean ± SD. Number of patients and controls volunteers, * value were significant for thalassemia major compared with controls, ** for thalassemia minor compared with controls, *** value were no significant for thalassemia major and minor.
Table 1, and Fig 2, 3 show Gpx and GST activity in serum of patients major and minor compared with controls, the Gpx activity were significantly lower than (9%) and (4%) in serum of thalassemia major and minor respectively versus apparent healthy controls. While the GST activity were significantly higher than (25%) and (18%) for thalassemia major and minor respectively compared with controls subjects. Not significant result shown between thalassemia major and minor subjects.
The levels of Gpx activity were a positive correlate with GST activity for the thalassemia major and minor patients(r= 0.05, P= 0.00),(r=0.1, P=0.00) respectively.
Serum levels of TAC were a positive correlated with Gpx activity for the thalassemia major patients(r= 0.08, P=0.00), and a inversely correlated for the thalassemia minor patients (r= -0.2, P=0.00), while the correlate between GST activity and TAC was a positive for the thalassemia major patients(r=0.2, P=0.00), and a inversely correlated for the

thalassemia minor patients(r=-0.25, P=0.00).

Discussion

The depletion of total antioxidant capacity(TAC) induced by oxidative stress is eliminated by release and stock organ antioxidant, mainly from liver and adipose tissue and the induction or activation of antioxidant enzymes(19). β-thalassemia major and minor are characterized by an overproduction of free radicals, i.e. when the antioxidant defense of an organism is overwhelmed or are established when a deficit of defenses of the organism against oxidation occurs. The primary defense against oxidative stress in extracellular fluids results from a number of low molecular weight antioxidant molecules either water – (ex. ascorbic acid) or lipid-soluble (ex.Vitamin E). These antioxidants can also be generated during normal metabolism (ex. uric acid, bilirubin, albumin, thiols) or introduced in the body by the consumption of dietary products rich in antioxidants (olive oil, fruits and vegetables, tea, wine, etc)(20). At a later phase of oxidative stress, The TAC falls due to depletion of antioxidant, low molecular weight antioxidants penetrate specific locations in the cell where oxidative stress may occur and protect against free radicals (21).

The sum of endogenous and food-derived antioxidants represents the total antioxidant activity of the extracellular fluid. In addition, the levels of these antioxidants are suitable not only as a protection against oxidation, but could also reflect their consumption during acute oxidative stress states. The cooperation among different antioxidants provides a greater protection against attack by reactive oxygen or nitrogen radicals, than any single compound alone(22).

Thus, the overall antioxidant capacity may give more relevant biological information compared to that obtained by the measurement of individual parameters, as it considers the cumulative effect of all antioxidants present in serum and body fluids(22).

Glutathione(GSH) and its related enzymes are well implicated in the circumvention of cellular oxidative stress and maintenance of intracellular thiol redox status(23). Gpx activity is a key component of the glutathione homeostasis. The decreasing of Gpx activity may indicate a protective role of Gpx induced oxidative stress(24) and could be illustrated directly by the low GSH content which necessitate low Gpx activity and due to produce increased oxidative stress, the depletion of Gpx may be beyond to it has broader protective of H_2O_2 production and other hydroperoxide(25). Moreover, the elevated levels of $O_2^{\cdot -}$ in serum of these patients produce the depression of Gpx activity (26). The higher levels of GST activity in serum of thalassemia patients may be due to increase synthesis of this enzyme under oxidative stress to protect the body from toxic compound (27), which is caused damage for high tissue iron levels. This suggest that in thalassemia patients the observed increased of GST activity was due to the iron chelating therapy.

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