Study antioxidant activity in Thalassemia Iraqi patients
دراسة الفعالية التأكسدية عند مرضى الثلاسيميا العراقيين

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Abstract

The aims of this study was to estimate the total antioxidant activity level in serum among the Thalassemia Iraq patients. We estimated total free radical scavenging activity in the120 serum of Thalassemic patients their age range from 2-27years. The DPPH activity of serum ranged from 0.1-3.11µmol/ml, the mean serum level of total antioxidants in Thalassemia patients was significantly lower than the control group, and no relation to age or sex. We concluded that DPPH is useful for evaluating the total antioxidant capacity.

Key wards:DPPH- Free radicals- Total antioxidant activity, Thalassemia

الملخص

تهدف الدراسة إلى قياس الفعالية التأكسدية الكلية عند مرضى الثلاسيميا في بغداد بواسطة استخدام مركب (DPPH)، أجريت هذه الدراسة على 120عينة من مصل المرضى المصابين بالثلاسيميا تتراوح أعمارهم بين 2-27 سنة بالإضافة إلى 25 عينة من أشخاص أصحاء كمجموعة سيطرة، اظهرت النتائج انخفاضا ملحوظا في مستوى مضادات الأكسدة عند المرضى مقارنة مع مجموعة السيطرة ولا توجد علاقة واضحة للعمر أو الجنس مع مستوى مضادات الأكسدة. لذالك تعتبر طريقة القياس بواسطة (DPPH) هي طريقة سهلة لقياس الفعالية الكلية التأكسدية.

الكلمات المفتاحية: قياس الفعالية التأكسدية، الثلاسيميا، DPPH

Introduction

Free radicals are highly reactive compounds inherently unstable, since they contain "extra" energy [1]. They are created in the body during normal metabolic functions or introduced from the environment, to reduce their energy load, free radicals react with certain chemicals in the body to produce reactive oxygen species (ROS), it interfere with the cell's ability to function normally [2]. Antioxidants work in several ways such as reduction the energy of the free radical, stopping the free radical from forming in the first place, or to interrupt an oxidizing chain reaction to minimize the damage caused by free radicals. The generation of reactive oxygen species (ROS) is a steady-state cellular event in respiring cells [2, 3]. Their production can be grossly amplified in response to a variety of path physiological conditions such as inflammation, immunologic disorders, hypoxia, hyperoxia, and metabolism of drug or alcohol, exposure to UV or therapeutic radiation, and deficiency in antioxidant vitamins [1,4]. Uncontrolled production of ROS often leads to damage of cellular macromolecules (DNA, protein, and lipids) and other small antioxidant molecules.

A number of major cellular defense mechanisms exist to neutralize and combat the damaging effects of these reactive substances. The enzymatic system such as (superoxide dismutase, catalase, and glutathione peroxidase), functionsby direct or sequential removal of ROS. Metal binding proteins targeted to bind iron and copper ions, ensure that these Fenton metals are cryptic. Non enzymatic defense consists of scavenging molecules such as (GSH, ubiquinols, uric acid) that are endogenously produced or derived from the diet (vitamins C and E, lipoidacid, selenium, riboflavin, zinc, and the carotenoids). These antioxidant nutrients occupy distinct cellular compartments and among them; there are active re-cycling [5]. Thalassemia is a hereditary disorder with higher potential for oxidative damage due to chronic redox imbalance in red cells that often results in clinical manifestation of hemolys in patients with this disorder. The release of hemoglobin during hemolysis and the subsequent therapeutic transfusion lead to systemic iron overloading that further potentiates the generation of ROS. The body produces several antioxidant enzymes, including superoxide dismutase (SOD), catalase, and glutathione peroxidase, which neutralize many types of free radicals [6]. A simple method that has been developed to determine the antioxidant activity of foods utilizes the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical [7]. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. The color turns from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes paired with a hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H [8].

Aims of this study to estimate the total antioxidant activity level in serum among the Thalassemia in Iraqi patients.

Materials and Methods

120 EDTA blood samples of major –Thalassemic patients were collected during January to April 2010 from Ibn Al-Baladi pediatrics hospital, and 25 healthy individual as control group from Baghdad under the Genetic Engineering and Biotechnology Institute human ethic improvement (No. 10095 date 25 Apr 2010).

Total ant oxidation activity assay:-

1. Serum samples will be determined for oxidative status by using Total antioxidant activity assay.
2. Working radical 2, 2-diphenyl-1-picrylhyydracyl (DPPH) was freshly prepared in 5mM before daily using for 2 hour and was kept in the dark at room temperature.
3. Then 175ul of serum volume of each samples were pipette to micro plate well.
4. Five tubes was use as standard control 10.25, 41.02, 656.25, 164.06, 2.625 and 10.500uM of Gallic acid.
5. Then 25ul of working radical 2, 2-diphenyl-1-picrylhyydracyl (DPPH) was pipetting all micro plate well.
6. The reaction mixture was kept in the dark at room temperature for 30 minute and then the 518 nm absorption was measured.
7. The antioxidant activity was calculated by compare with standard curve of Gallic acid.
8. Hemoglobin concentration measured by taken blood samples (0.02 ml) from the carotid artery and added to 5 ml of Drabkin reagent containing :(0.048 g / l KCN, 0.18 g / l K Fe CN, 0.136 g / l KHPO and 0.1 g/ l of detergent, the samples were mixed and incubated at room temperature for 5 min and measuredspectrophotometrically at 540 nm [9].

Statistics assay:

Statistically we use the t-test values to estimate the significant difference at $P < 0.01$ between the β-thalassemia patients and control groups. These results reflect the point which indicate that the

Results:

The hemoglobin and the hematocrit values were found to be lower in the patients with thalassemia when compared with control. These results were found to be statistically significant Table (1). The serum level of total antioxidants in thalassemic patients ranged from 0.1-3.11mmol/L with a mean of 2.1+0.67 mmol/L which was significantly lower than that of the control group =7.986, P<0.001

Table (1).

Table (1): Complete blood count results with total antioxidant of the patients and the healthy control.

	Hb(g/dl) *	Htc(%)	MCV(fl)	MCH(pg)	Total antioxidant
Beta-thalassemia major patients	9.3± 1.8	26.2±4.3	82.9±3.71	27.8±1.9	2.1±0.67
Healthy control	13.1±0.49	38.3±2.2	80.89±3.1	27±0.99	4.03±0.67
P value	<0.001	<0.05	>0.05	>0.05	<0.001**

***Hb, hemoglobin; Htc, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscularhemoglobin.**
***Values were given as mean ± standard deviation.**
****Highly significant.**

Discussion

Free radicals attack various biological molecules, membranes and tissues to induce free radical-mediated chain oxidations [10]. It has been found that aqueousperoxyl radicals include the oxidation of phospholipids liposomal membranes, for example, the aqueous peroxyl radicals attack membranes and induce the oxidation of lipids and proteins to cause possible hemolysis [11]. Oxidative damage especially due to iron overload and depletion of antioxidant status play an important role in pathogenesis of thalassemias. Increased oxidative damage in thalassemias may be due to the depletion of lipid soluble antioxidants [12]. Beta-thalassemia patients severe from iron overload lead *in vivo* lipid peroxidation and increase in the antioxidant enzyme levels of superoxide dismutase(SOD) and glutathione peroxidase(GPX) [12,15]. Iron is the major generator of free radicals. In moderate quantities, free radicals are necessary for cell survival; but in large quantities they are cytotoxic [12]. There is balance physical separation between iron and the cell membrane , which is maintained by chelating of free iron via its carrier proteins . This separation is required in order to prevent the ions from catalyzing lipid and protein peroxidation via their participation in the Fenton reaction [13].Under various pathological conditions associated with iron overload, including thalassemia, there is evidence of an increase in low molecular weight iron in serum [9,14]. This promotes peroxidative damage to cell and organelle membranes in organs that accumulate excess iron including liver pituitary gland, pancreas and heart [13]. The chelation therapy given to these patients was suggested to play an inhibitory effect on the production of oxygen radicals due to lipid peroxidation and the Fenton reaction [15,16]. We concluded that DPPH is useful for evaluating the total antioxidant capacity.

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