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Determination of Some Oxidative Stress Parameters and Antioxidants in Sample of Iraqi Beta Thalassemia Major Patients

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Abstract
Repeated blood transfusion in beta thalassemia major patients may lead to peroxidative tissue injury by secondary iron overload. In the present study, 100 patients(50 male+50 female) with beta thalassemia major patients with age (5-20) years and 60 healthy control were included during their attendance at Abin Al_Baladi hospital in Baghdad. Malondialdehyde ,Superoxide Dismutase and Vitamin E, were measured by using kits.The results showed A highly significant ($p<0.01$)increase in the levels of Malondialdehyde and Superoxide Dismutase, whereas, significant p(<0.01)decrease in the levels of vitamin-E, This suggest that oxidative stress and reduced antioxidant defense mechanism play an important role in pathogenesis of beta thalassemia major.
Key words: Beta thalassemia major, Oxidative stress, Antioxidants.

تحديد بعض معايير جهد الأكسدة ومضادات الأكسدة في عينة من مرضى بيتا الثلاسيميا الكبرى العراقيين

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الخلاصة
أن تكرار نقل الدم في المرضى بيتا ثلاسيميا الكبرى يؤدي إلى إصابة الأنسجة نتيجه الحديد الزائد الثانوي. في هذه الدراسة،كان 100 عينه من المرضى (50 ذكور + 50 إناث) مع مرضى بيتا ثلاسيميا الكبرى في فئه العمريه (5−20) سنوات و 60 عينه من الاصحاء أثناء حضورهم في مستشفى أبن البلدي في بغداد.ماليندياهايد،ودسميتوز وفيتامين(اي)،وقياسها باستخدام الكتات وأضهرت النتائج زيادة كبيرة جدا في مستويات ماليندهايد ودستميوز(ع<0.01)في حين انخفاض في فيتامين (اي) ذا يشير إلى أن الاكسدة وانخفاض آلية الدفاع المضادة للأكسدة تلعب دورا هاما في التسبب في بيتا ثلاسيميا الكبرى.

Introduction

Beta thalassemia major is the most widespread type of thalassemia as it is common in certain populations., every year more than 10,000 children are born with thalassemia major. It produces severe anemia in its homozygous state [1]. About 190 million people throughout the world have genetic mutations associated with different hemoglobinopathies and more than 90 million of them carry defective genes leading to thalassemia [2, 3].The disease is associated with profound anemia, jaundice,splenomegaly, expanded bone marrow space, siderosis and cardiomegaly. These symptoms

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appear after about 2-4 months of age. Impaired erythropoeisis, hemolysis in the peripheral circulation and deposition of excess iron in the tissues, are some of the causes of clinical manifestations [2].Earlier studies have shown that, in thalassemia there is excess production of reactive oxygen intermediates, such as superoxide anion (O_2–), hydroxyl radical (OH·), singlet oxygen and hydrogen peroxide (H_2O_2) within the erythrocytes, all these events lead to oxidative stress. This oxidative stress and a possible consequential accelerated apoptosis may contribute to shortened life span of erythrocytes.In thalassemic RBCs, non-hemoglobin iron is increased. Free irons or aggregates of ferritin and deposits of hemosiderin are catalysts of lipids and protein peroxidation via the Fenton reaction:

$$Fe_{2+} + H_2O_2 \rightarrow OH\bullet + OH^- + Fe_{3+}$$

Data have been collected suggesting that increased lipid peroxidation takes place in thalassemic RBCs.[4] Malondialdehyde (MDA), a product of lipid peroxidation is generated in excess amounts in supporting the fact that large amount of membrane bound iron is present in thalassemic erythrocytes [5,2]. Trace metals, especially Iron are implicated as causative agents in excessive generation of free radical which are capable of causing oxidative damage to erythrocytes [1]. Hydroxyl radicals (OH) are the most reactive oxygen free radical species capable of direct oxidative damage to macromolecules including DNA, protein, and lipid membranes. The effect of excess results of oxygen free radicals, such as DNA strand breaks and membrane blebbing, match the hallmark feature of apoptosis [6]. Antioxidants are complex and diverse group of molecules that protect key biological sites from oxidative damage. They scavenge free radicals and other reactive oxygen species (ROS) [7]. The body has developed several endogenous antioxidant defense systems classified into two groups such as enzymatic and non -enzymatic. The enzymatic defense system includes different endogenous enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and non -enzymatic defense system included vitamin E, vitamin C and reduced glutathione (GSH)[8] SOD is an important endogenous antioxidant enzyme act as the first line defense system against ROS which scavenges superoxide radicals to H_2O_2[9].The present study was initiated to evaluate the level of some oxidants and antioxidants in beta thalassemia major.

Materlals and methods

This study was carried out in Abin AL-Baladi Hospital, Thalassemia Center. statistically present study included 160 subjects whose age were 5-20 years, 60 subjects were regarded as healthy controls and remaining 100 were patients with beta thalassemia major.They were clinically diagnosed on the basis of severe anemia and haemoglobin electrophoresis.All the patients were examined regularly once or twice a month by clinicians. They were regularly receiving erythrocytes transfusions every month Transfusion characteristics and duration of transfusion were similar in all the patients .eight ml of venous blood was collected from the subjects under aseptic condition. Serum were separated by centrifugation at 3000 rpm for 10 min at room temperature. Analysis of all the biochemical parameters was done manually using kits.

This kit uses enzyme-linked immune sorbent assay (ELISA)based on biotin double antibody sandwich technology to assay MDA,SOD,and Vit E. Blood from thalassemia patients was collected just before the transfusion .The samples were stored at 4°C before analysis.

Results and discussion

The level of MDA in Table-1, was increased highly significant($p<0.001$) in β- thalassemic patients (2.807 ± 0.06 nmol/ml) in comparasion to healthy control (1.009 ± 0.03 nmol/ml), Absence of beta globin chains lead to accumulation of unpaired alpha globin chains. Excess presence of the alpha globin chains is a primary reason for the cellular oxidative damage and also iron overload. As a result of both high plasma iron and high intracellular non hemoglobiniron in beta thalassemia, there is an enhanced generation of ROS[8, 10]. Moreover, repeated blood transfusion causes iron overload which increases free radical production and peroxidative damage of tissues. In such condition, depletion of endogenous antioxidants may be expected. Peroxidative damage of lipids was indicated by the increase in serum MDA levels following oxidation of polyunsaturated fatty acids(PUFAs)[11] Also in Table-1 there was highly significant ($p<0.001$) increase in the level of SOD value (37.48 ± 0.66 µ/ml) in β- thalassemic patients as compared to control (15.73 ± 0.34 µ/ml). SOD scavenges superoxide radicals to form hydrogen peroxide and protects the cell membrane from its damage. Increased Erythrocyte SOD activity may be due to blood transfusion and increase in the proportion of younger erythrocytes, as a compensatory mechanism after increased oxidative stress [9].

The level of Vit.E was decreased highly significant ($p<0.001$) in β- thalassemic patients (8.08 ± 0.14 µmol/l) in comparison to healthy control (15.82 ± 0.20 µmol/l), Vitamin E plays a key role in protecting cells against oxidative damage. The antioxidant role of vitamin E is attributed to its ability in quenching highly reactive lipid peroxide intermediate by donating hydrogen and this prevents extraction of hydrogen from PUFA. This assists in restricting self-perpetuated lipid peroxidation chain reaction [2, 11].

Table 1 - Level of serum MDA, SOD and Vit. E in B-thalassemia major and controls (Mean ± SE)

Group	Mean ± SE		
	Parameters		
	MDA (nmol/ml)	SOD (µ/ml)	Vit. E (µmol/l)
Patients	2.807 ± 0.06	37.48 ± 0.66	8.08 ± 0.14
Control	1.009 ± 0.03	15.73 ± 0.34	15.82 ± 0.20
T-test value	0.161 **	1.774 **	0.475 **
** (P<0.01).			

*p<0.01 - Highly significant.

This study indicates that oxidative stress may be a major cause of hemolysis in beta thalassemia major. The administration of selective antioxidants such as vitamin E might represent a promising way of counteracting the oxidative damage.

References

1. Widad, N.M. and Al-Naama, L. Meaad. **2003**. Trace element in patients with beta thalassemia major. *Haem* , 6(3), pp: 376-83.
2. Das, N., Chowdhury, T.D. and Chattopadhyay, A. Datta. **2004**. Attenuation of oxidation stress - induced changes in thalassemic erythrocytes by Vitamin E. *Pol J Pharmocol*, 56, pp: 85-96.
3. Ambekar, S.S., Phadke, M. A., Balpande, D. N. Mokashi, G. D., Khedkar, V. A., et al. **2001**. The prevalence and Heterogeneity of Beta Thalassemia Mutation in The Western Maharashtra Population, A hospital based study. *IJHG*,1(3), pp: 219-223.
4. Meerang, M., Nair, J., Sirankapracha, P., Thephinlap, C., Srichairatanakool, S. and Fucharoen, S. **2008**. Increased urinary 1, N6-ethenodeoxyadenosine and 3, N4- ethenodeoxycytidine excretion in thalassemia patients: Markers for lipid peroxidation- induced DNA damage. *Free Radic.Biol.Med*. 44(10), pp: 1863-1868.
5. Livrea, M. A.**,** Tesoriere, L.**,** Pintaudi, A. M.**,** Calabrese, A., Maggio, A., Freisleben, H. J., et al**. 1996.** Oxidative stress and Antioxidant status in beta thalassemia major: Iron overload and depletion of lipid - soluble antioxidants. *Blood*, 88, pp: 3608-3614.
6. Ragab, S. M. and Mahfouz, R. G. **2010**. L–Carnitine ameliorates the iron mediated DNA degradation in peripheral leukocytes of β- thalassemic children.*Egypt. J. Med. Hum. Genet*. , 11(1), pp: 17-31.
7. Baumgartener, T. G. **1999.** Vitamins In: Van Way C.W. ed. Nutrition secrets. *Internet Journal of Medicinal Update*.1, pp: 1-17.
8. Ghone, R. A., Kumbar, K. M. and Suryakar, A. N. **2008**. Oxidative stress and disturbance in antioxidant balance in beta thalassemia major. *Ind J Clini Biochem*, 23, pp: 337-340.
9. Landis, G.N. and Tower J. **2005.** Superoxide dismutase evolution and life span regulation. *Mech. Ageing Dev*., 126, pp: 365–379.
10. Kassab-Chekir A**,** Laradi S**,** Ferchichi S**,** Haj Khelil A**,** Feki M, Amri F, et al**. 2003** Oxidant, antioxidant status and metabolic data in patients with beta-thalassemia. *Clin. Chim. Acta.*, 338(1-2), pp: 79-86.
11. Kalpravidh, R. W., Siritanaratkul, N., Insain, P. and Charoensakdi, R. **2010** .Improvement in oxidative stress and antioxidant parameters inβ-thalassemia/Hb E patients treated with curcuminoids. *Clinical Biochemistry*, 43, pp: 424–429.