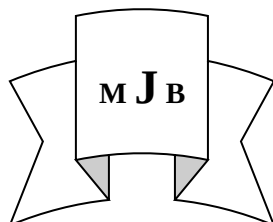


The Effect of Deferasirox on the Oxidative Stress and Inflammation in Iron Overloaded Beta-Thalassemic Patients

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Abstract

Iron overload represents a serious complication in transfusion dependent β -thalassemic patients. Once the body's storage capacity is exceeded, free iron catalyzes the formation of highly reactive oxygen species, which leads to membranes, DNA and protein damage. This process leads to cellular death, tissue necrosis and inflammatory reaction in multiple organs particularly liver, heart, and endocrine glands. Indeed organs failure due to chronic iron overload represent the major cause of death in patients with β -thalassemia major who receive blood transfusions regularly. Iron chelation represents life-saving therapy that lead to a significant improvement in the survival of transfusion dependent β -thalassemic patients. Deferasirox is an orally administered once daily iron chelator that mobilize tissue iron by forming soluble stable complexes with iron that are then excreted in feces.

This study aimed to evaluate the effect of deferasirox treatment on the oxidative stress and inflammation in iron overloaded β -thalassemic patients.

Forty one β -thalassemia major patients were enrolled in this study they were diagnosed by specialist pediatric physicians depending on the clinical features, family history and confirmed by laboratory analysis of blood film, Hb-electrophoresis and serum ferritin. They were free of infections. All patients were on monthly blood transfusion and regular daily oral chelation therapy with deferasirox (30-40mg/kg/day). The patients were monitored monthly for six months by measuring [serum C-reactive protein (CRP), serum malondialdehyde (MDA) and serum glutathione (GSH)]. Base line study for all the above investigations was done.

The results showed: There was a significant decrease ($P < 0.01$) in the means of serum CRP and serum MDA, while there was a significant increase ($P < 0.01$) in the serum level of GSH after six months of DFX treatment.

It was concluded that: Deferasirox can play important role in controlling the oxidative stress and inflammation which are secondary to iron overload. It is noticed from the significant decrease in the oxidative parameter (MDA), the inflammatory parameter (CRP) and from the significant increase in the antioxidant (GSH).

الخلاصة

زياده الحديد تمثل واحده من المضاعفات الخطيرة لدى مرضى الثلاسيميا الكبرى نوع بيتا المعتمدين على نقل الدم. عند تجاوز قدرة الجسم على تخزين الحديد يحفز الحديد الحر تكوين أنواع الاوكسجين العاليه الفعاليه، الأمر الذي يؤدي إلى تلف الاغشيه و الحمض النووي والبروتين. هذه العملية تؤدي إلى الموت الخلوي ونخر الأنسجة ورد فعل التهابي في اجهزه متعدده خاصة الكبد والقلب والغدد الصماء . في الواقع فشل الاعضاء بسبب الحديد الزائد المزمن يمثل السبب الرئيسي للوفاة لمرضى الثلاسيميا الكبرى نوع بيتا والذين يتلقون نقل الدم بشكل منتظم. عملية إزالة الحديد يمثل العلاج المنقذ للحياة والذي يؤدي إلى تحسن كبير في بقاء مرضى الثلاسيميا المعتمدين على نقل الدم على قيد الحياة. عقار (الديفرازيروكس) الذي يعطى عن طريق الفم مرة واحدة يومياً، يزيل الحديد من الأنسجة من خلال تشكيل معقد قابل للذوبان مع الحديد ليتم افرازه فيما بعد عن طريق البراز. هدفت هذه الدراسة إلى تقييم تأثير العلاج بعقار (الديفرازيروكس) على جهد الاكسدة والالتهاب الناتج عنه لمرضى الثلاسيميا نوع بيتا والذين يعانون من تراكم الحديد.

في هذه الدراسة تم ادخال ٤١ من مرضى الثلاسيميا الكبرى الذين تم تشخيصهم من قبل أطباء متخصصين بطب الاطفال اعتمادا على المظاهر السريرية والتاريخ العائلي وأكدت التشخيص التحاليل المختبرية لصوره الدم ، والترحيل الكهربائي لخضاب الدم وتركيز الفيريتين في المصل. كان جميع المرضى المختارين غير مصابين بأي التهاب . جميع المرضى كانوا معتمدين على نقل الدم الشهري وعلى العلاج اليومي المنتظم لازالة الحديد بعقار (الديفرازيروكس) عن طريق الفم بجرعه (٣٠-٤٠ ملغم/ كم/ يوم). تم مراقبة المرضى شهريا لمدة ستة أشهر من خلال قياس [بروتين سي الفعال (CRP) ، مالوندايلديهاد (MDA) ، الكلوتاثيون (GSH) في المصل] ولقد تم اجراء دراسه خط البدايه لكل الاختبارات اعلاه.

أظهرت النتائج : وجود انخفاض معنوي في تركيز بروتين سي الفعال (CRP) وتركيز المالوندايلديهاد (MDA) في المصل. بينما كانت هناك زيادة معنويه في مستوى الكلوتاثيون (GSH) في المصل بعد ستة أشهر من العلاج بواسطه الديفرازيروكس.

يستنتج من ذلك ان :

الديفرازيروكس يمكن أن يلعب دورا هاما في السيطرة على الاكسدة والالتهابات الناتجه عنها في مرضى الثلاسيميا نوع بيتا والمتسببه من الحديد الزائد . وهذا يلاحظ من الانخفاض الكبير في كل من ثابت الاكسده المالوندايلديهاد (MDA) و ثابت الالتهاب بروتين سي الفعال (CRP) ومن خلال الزيادة كبيرة في مضاد الاكسده الكلوتاثيون (GSH).

Introduction

Thalassemia refers to a heterogeneous group of genetic blood diseases characterized by decreased synthesis of one of the two types of polypeptide chains (α or β) that form the normal adult human hemoglobin molecule HbA($\alpha_2 \beta_2$) resulting in defect in hemoglobin synthesis of red blood cell (RBC) and anemia [1]. Thalassemia is among the most common genetic disorder worldwide, occurring more frequently in the Mediterranean, Indian subcontinent, Africa and south East Asia [2]. Thalassemias are classified according to which chain of the hemoglobin molecule is affected into α thalassemia and β thalassemia . In β thalassemia there is a defect in the production of β globin usually results from disabling point mutations in the β gene on chromosome 11 causing no (β^0) or reduced (β^+) β chain production [3]. β thalassemia are the most important types of thalassemia because they are so common and usually produce severe anemia in their homozygous and compound heterozygous states [4]. Beta thalassemia major (severe homozygous or mixed heterozygous β -thalassemia) also called (Cooley anemia) characterized by severe illness with long-term, transfusion-dependent

anemia and entails a risk of iron overload and multiorgan involvement [5]. Iron overload represents a serious complication in transfusion dependent β -thalassemic patients. Free iron catalyzes the formation of highly reactive oxygen species, which leads to membranes, DNA and protein damage, this process leads to cellular death, tissue necrosis and inflammatory reaction in multiple organs particularly liver, heart, and endocrine glands. Indeed organs failure due to chronic iron overload represent the major cause of death in patients with β -thalassemia major who receive blood transfusions regularly [6]. Iron chelation represents life-saving therapy that lead to a significant improvement in the survival of transfusion dependent β -thalassemic patients. Deferasirox (DFX) is an orally administered once daily iron chelator that mobilize tissue iron by forming soluble stable complexes with iron that are then excreted in feces [7]. It is a tridentate iron chelators requiring two molecules of drug to form a stable complex with iron [8]. DFX active molecule is highly lipophilic with molecular weight (373 Daltons), it can readily enters most of the cells and efficiently reaches the major intracellular sites of iron accumulation

[9]. Due to its lipophilicity and its relatively small size DFX is well absorbed through the gastrointestinal tract [10]. Its half-life ($t_{1/2}$) is between 8 and 16 hours, allowing a once-daily administration. After the oral administration (dissolved in water or orange or apple juice) it is rapidly absorbed and its plasma level is in the therapeutic range for 18 to 24 hours [11]. Thus, after a dose, the chelating effect lasts all day. DFX is highly (99%) bound to serum albumin and has a volume of distribution of approximately 14 L in adults. Glucuronidation is the main metabolic pathway for DFX, with subsequent biliary excretion [10]. DFX therapy should be started when a patient has evidence of chronic iron overload, such as the transfusion of approximately 100 ml/kg of packed red blood cells (approximately 20 units for a 40 kg patient) and a serum ferritin consistently greater than 1000 $\mu\text{g/L}$. The recommended starting daily dose is 20 mg per kg body weight. Serum ferritin should be monitored monthly and the dose adjusted if necessary every 3-6 months based on serum ferritin trends and attainment of clinical goals. Dose adjustments should be made in increments of 5 or 10mg/kg. If the serum ferritin consistently falls below 500 $\mu\text{g/L}$, temporary interruption of therapy should be considered [10]. In patients not adequately controlled on daily doses of 30 mg/kg, daily doses of up to 40 mg/kg may be considered. Doses above 40 mg/kg are not recommended because there is only limited experience with doses above this level [12]. The most frequent adverse reactions reported during treatment with DFX in adult and pediatric patients are Gastrointestinal disturbances in about 26% of patients [13], Mild, non-progressive increases in serum creatinine, mostly within the

normal range, occurred in about 36% of patients [10] and increase in serum transaminases: elevations in serum glutamate pyruvate transaminase (SGPT) have occurred in few patients [14].

Patients , Materials and Methods

The study was conducted in the thalassemia and other inherited hemolytic anemia center at Babylon Maternity and Children Hospital in Hilla city from October/2011 to April /2012. A total of 41 β -thalassemia major patients (22 males and 19 females) were enrolled in this study. Their ages ranged from 2 to 15 years. They were diagnosed by specialist pediatric physicians depending on the clinical features, family history and confirmed by laboratory analysis of blood film, Hb-electrophoresis and serum ferritin. All patients were on monthly blood transfusion and regular daily oral chelation therapy with deferasirox (30-40mg/kg/day).

The study design was as follow: the patients were monitored monthly for six months by measuring serum (C-reactive protein, MDA and GSH) and base line study for all the above investigation was done.

The patients are subdivided into two groups according to their serum ferritin levels which can predict the patients chances to develop cardiac disease, serum ferritin levels equal or above 2500 $\mu\text{g/L}$ are associated with high patient risk in having cardiac disease while serum ferritin below 2500 $\mu\text{g/L}$ are associated with improved cardiac disease-free survival [15]. Our two groups were as follow: the high risk group, those patients had serum ferritin above 2500($\mu\text{g/L}$), they were (30 patients) and the low risk group, those patients had serum ferritin below 2500($\mu\text{g/L}$) and they were (11 patients). It is worthy to mention that those patients were not suffering from

fever and infections, particularly the infections by: human immune deficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV).

Serum C-reactive protein (CRP) was measured by minineph instrument using commercially available kit from Binding Site Group Ltd company, UK. Serum malondialdehyde (MDA) was measured by colorimetric method using kit from North West Company, USA. Serum glutathione (GSH) was measured by colorimetric method using kit from North West Company, USA.

Statistical analysis

The data were analyzed by using SPSS program version 17. Repeated measurement ANOVA test and LSD were used to determine the level of significance in the change of all the

measured parameters during the treatment period and also to determine the significant level of the difference between the two groups (high and low risk group). The changes and differences were considered significant when the probability (P) was less than 0.05 ($P < 0.05$) and highly significant when the probability (P) was less than 0.01 ($P < 0.01$) [16].

Results

The means of serum CRP concentration showed a highly significant decrease from the base line after six months treatment with deferasirox (30-40mg/kg/day) in both treated groups ($P < 0.01$). While there was no significant difference in the effect of deferasirox on the means of serum CRP concentration between the high and low risk groups ($P > 0.05$), as shown below in figure (1).

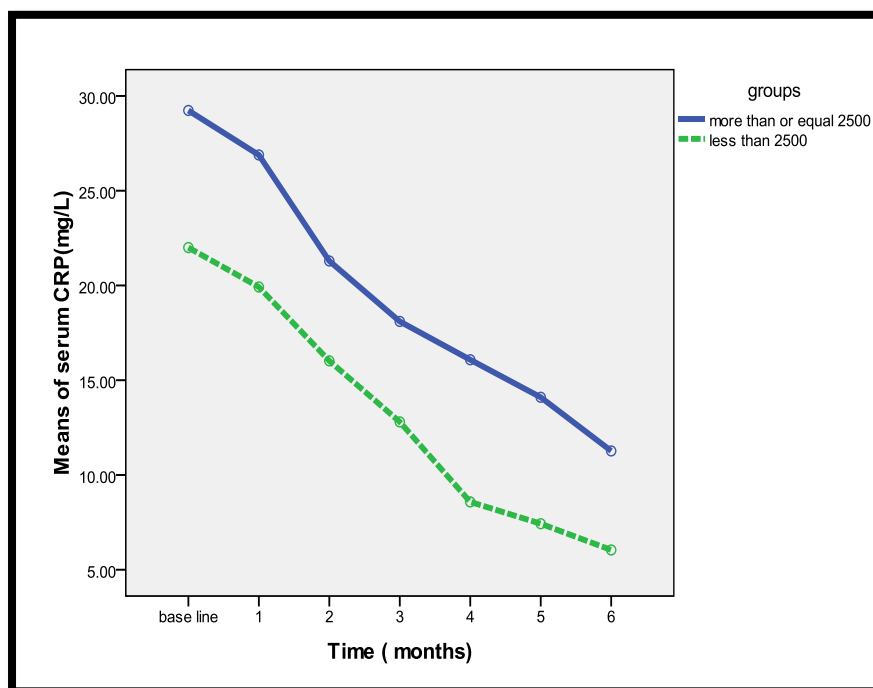


Figure 1 The effect of deferasirox treatment on serum CRP

The means of serum MDA concentration showed a highly significant decrease from the base line after six months treatment with deferasirox (30-40mg/kg/day) in both

treated groups ($P < 0.01$). While there was no significant difference in the effect of deferasirox on the means of serum MDA concentration between the

high and low risk groups ($P > 0.05$), as shown below in figure (2).

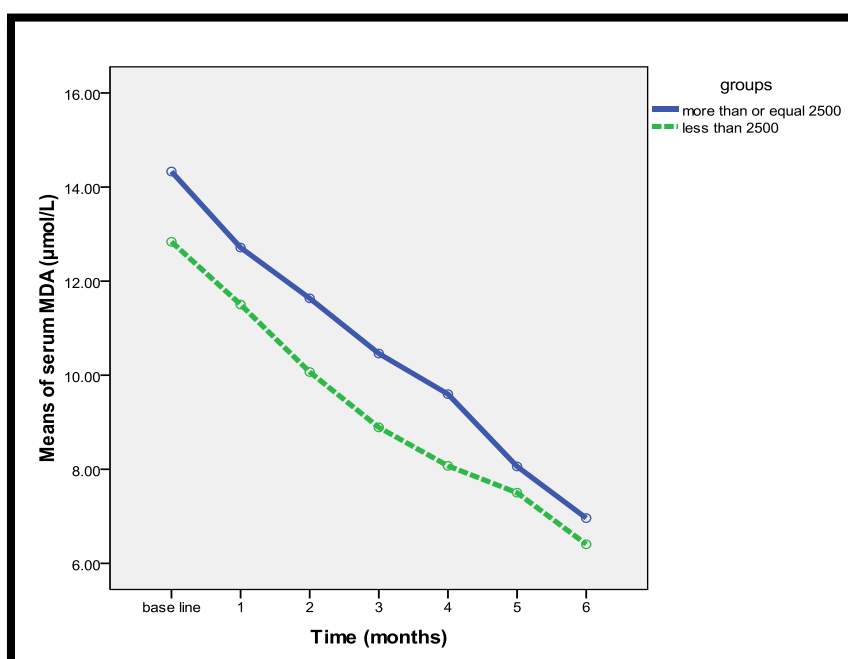


Figure 2: The effect of deferasirox treatment on serum MDA.

The means of serum GSH concentration showed a highly significant increase from the base line after six months treatment with deferasirox (30-40mg/kg/day) in both treated groups ($P < 0.01$). However

there was no significant difference in the effect of deferasirox on the means of serum GSH concentration between the high risk and the low risk group ($P > 0.05$), as shown below in figure (3).

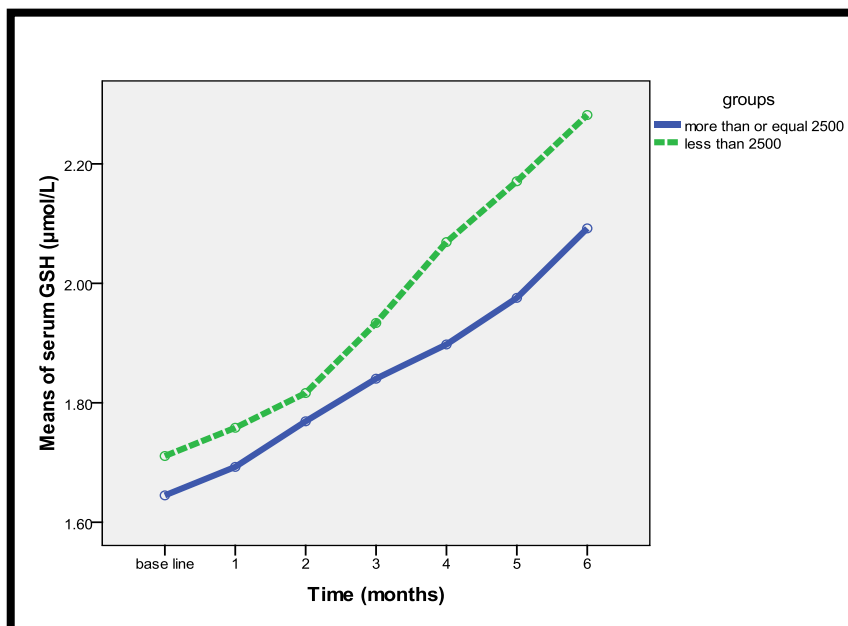


Figure 3 The effect of deferasirox treatment on serum GSH

Discussion

We measured serum MDA concentration as a marker of tissue injury and oxidative stress because MDA is a product and a well-recognized biomarker of lipid peroxidation [17]. At baseline we found elevated levels of serum MDA in thalassaemic patients that enrolled in this study; it may result from several mechanisms:

First: plasma MDA could be increased in thalassemia patients because the increasing of circulating erythroid precursors and peripheral blood erythrocytes that have a high density of unpaired α -hemoglobin chains. The excess α -chains in thalassemic red blood cells are unstable and prone to denaturation and oxidation. The α -chains in thalassemic red blood cells can autoxidise, release heme that disintegrate releasing toxic free iron which in turn generate reactive oxygen species (ROS) by Fenton and Haber-Weiss reactions. ROS increase lipid peroxidation and thus enhance MDA levels [18].

Second: plasma MDA may be increased in iron overloaded thalassemia patients because the excess of free iron lead to the formation of highly toxic ROS via Fenton and Haber-Weiss reactions. ROS peroxidate tissue lipid. Lipid peroxidation products such as MDA leaks into the plasma during the peroxidation process so the plasma level of MDA will be elevated [17].

With DFX treatment we found the means of serum MDA significantly declined from the baseline in both of the treated patients groups. This finding is agreed with [19] in which they confirmed that malondialdehyde levels could be controlled by DFX. This can be explained as follow: DFX chelate the excess of iron, reducing the circulating and intracellular free iron [9, 20] that lead to decrease the

formation of ROS and lipid peroxidation. Since MDA is one of the lipid peroxidation products, its serum level will be markedly decreased in response to DFX treatment [19].

The serum level of GSH was too low at the base line. Suggesting major consumption due to oxidative stress that result from iron overload [21]. Since GSH is the major thiol antioxidant and redox buffer of the cell [22], its level will be low in case of oxidative damage.

After six months treatment with deferasirox we found a significant increase in the mean of serum GSH from the baseline in both of the treated patients groups. These results are agree with [23] where they confirmed that DFX iron chelation therapy can act as an antioxidant by decreasing intra- and extra-cellular toxic iron species, reducing oxidative stress and decrease GSH consumption.

We found an elevated serum level of the inflammatory marker CRP at the base line. That is because in transfusion dependent thalassemic patient when iron overload exceed the storage capacity of the cell, free iron start to deposit in the organs and serve as precursor for various chemical reactions to produce ROS that in turn cause peroxidative damage to cellular components, mainly in cellular membranes lead to cell damage and tissue necrosis. This enhance the release of interleukin-6 (IL-6) from monocyte and macrophage during the inflammatory response to tissue damage [19]. CRP release from hepatocytes will be increased in response to IL-6 and serum CRP concentrations will closely follow the course of the acute-phase response to inflammation or tissue necrosis [24].

In our study we found a significant decline in the mean of serum CRP from the baseline after six months treatment with defersirox in

both of the treated patients groups, these results are agree with [19] in which they confirmed that DFX treatment can significantly decrease CRP.

This is can be explained as follow: Chelation the excess of iron by DFX decrease the free iron, diminishing the iron-induced oxidative tissue injury and the possible stress to circulating monocytes and macrophages of the reticuloendothelial system. Reduced oxidative stress has been shown to lower monocyte and macrophage IL-6 release (25, 26) thus the serum CRP level will be lowered.

Conclusions

The oxidative stress and inflammation in β -thalassemic pateints resulted from iron overload can be effectively controlled by deferasirox chelation therapy. It is noticed from the significant decrease in the oxidative parameter (MDA), the inflammatory parameter (CRP) and from the significant increase in the antioxidant (GSH).

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