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Effects Of Deferasirox Therapy On High Sensitivity C-Reactive Protein and Oxidative Stress Markers In Iron Overloaded, Beta-Thalassemic Patients

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ABSTRACT

Objective: The study aimed to investigate the effects of iron chelator deferasirox on markers of oxidative-stress and inflammation in Iron Overloaded blood transfused Beta-Thalassemic Patients by measuring malondialdehyde (MDA), total antioxidant capacity (TAC), antioxidant marker glutathione(GSH) and highly-sensitivity C-reactive protein (hs-CRP) in these patients in comparison with control subjects.

Methods: A total of 105 patients with transfusion dependent β-thalassemia major were participated in this study. They were diagnosed by specialized pediatric physicians at Thalassemic Center in Ibn Al-Atheer Teaching Hospital in Mosul city, Iraq, during the period between October 2019 and March 2020. They were divided into two treatment groups. The first group was β- thalassemic patients treated with monthly regular blood transfusion alone and the second group was β- thalassemic patients treated with daily iron chelation therapy with oral deferasirox (DFX) (30-40mg/kg/day) in addition to regular blood transfusion. Sixty-five healthy, not thalassemic subjects, were also participated in this study as a control group. High sensitive C-reactive protein (hs-CRP), marker of inflammation, serum malondialdehyde (MDA) , marker of oxidative stress , serum glutathione (GSH)and total antioxidant capacity (TAC) , markers of antioxidant capacity were measured for both patients and controls.

Results: It has been found that β- thalassemic patients on DFX therapy had a significant decrease ($P < 0.01$) in means hs-CRP and MDA levels, and a significant increase ($P < 0.01$) in TAC and GSH levels as compared with β- thalassemic patients on regular blood transfusion alone and without DFX therapy . In addition , there is highly significant positive correlation between S.ferritin and hs-CRP ($r=0.723$, $P < 0.01$), and between S.ferritin and MDA ($r= 0.675$, $P < 0.01$), while there is a highly significant negative correlation between ferritin and TAC ($r=-0.422$, $P < 0.01$), and between ferritin and GSH ($r=- -0.354$, $P < 0.01$).

Conclusion: Iron chelation therapy with DFX was effective in decreasing MDA and hs-CRP and increasing the antioxidant markers (GSH) and TAC in iron overloaded blood transfused beta thalassemic patients. Thus, DFX therapy can play important role in controlling oxidative stress and inflammation in these patients.

Keywords: Beta thalassemia, oxidative stress, inflammation,TAC, hs-CRP, GSH , malondialdehyde, deferasirox.

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INTRODUCTION

Thalassemias are considered as the most common blood genetic disorders in the world and are attributable to imbalance in the production of the hemoglobin molecule due to either insufficient production of the α- or β-globin chains. Patients with β-thalassemia (β-TM) almost need frequent blood transfusion to survive.[1,2] Failure of organs such as the liver, kidney, and heart due to chronic iron overload is a major cause of death in patients with β-thalassemic major who are regularly receiving blood transfusions.[3,4]

Several iron chelators have been developed to mobilize tissue iron through the formation of complexes that are excreted in the faeces and/or urine.

Deferasirox is a powerful iron chelator ,water soluble antioxidant. It is used as a supplement to withstand oxidative stress in patients with β-TM. [5,6] The oxidative damage observed by the measurement of MDA in β-TM patients receiving Deferasirox remains unclear. Some researchers concluded that small doses of Deferasirox have no harm to β-TM patients, while others restrict its supplementation with chelating drugs only. [7,8,9] The oral iron chelator, deferasirox, is given to patient with beta thalassemia to manage the oxidative stress initiated by the iron overload produced by frequent blood transfusion and defective hematopoiesis. It also enhances the release of iron from its stores to chelating agents. [10, 11] Several studies on the role of Deferasirox in increasing the absorption of iron from GIT suggested the harmfulness of this role in increasing the iron overload and hence the deterioration of the oxidative stress on tissues.[12,13,14]

In the current study, the impact of Deferasirox supplementation on the iron overload and oxidative stress was evaluated in β-thalassemia major patients on regular blood transfusion for survival.

PATIENTS, MATERIALS AND METHODS

A total of 105 patients with β- thalassemia were participated in this study. They were selected from the Thalassemic Center in Ibn Al-Atheer Teaching Hospital in Mosul city, Iraq . The protocol for the study was approved by the Regional Research Ethics Committees of the College of Pharmacy and the Mosul Health Administration.Patients in this study were diagnosed with specialized pediatric physicians based on clinical features, family history, and laboratory analysis of blood film, Hb-electrophoresis, and serum ferritin level.

The patients were subdivided into two groups. Forty β-thalassemic patients (Group A) (21male and 19 female) were receiving blood transfusion only (without iron chelator) with age range (0.6-6) years old on a monthly regular blood transfusion alone and Sixty five β-

thalassemic patients (Group B) were treated with iron chelation therapy with oral deferasirox (DFX) (30-40mg/kg/day) in addition to a monthly regular blood transfusion . Another Sixty five (Group C) apparently healthy subjects, not thalassemic(29 male and 36 female) with age range (0.8-16) years old were also participitated in this study as a control group. High sensitive C-reactive protein (hs-CRP), marker of inflammation, serum malondialdehyde (MDA) , marker of oxidative stress and serum glutathione (GSH)] , marker of antioxidant capacity were measured for both patient and control groups.

Blood samples were taken from each patient and control subjects and tested for serum ferritin, serum MDA, TAC , GSH and hs-CRP. Serum ferritin was measured using the MINI-VIDUS ELISA method, MDA was measured using the Buege and Aust method, where MDA reacts with thiobarbituric acid (TBA) to produce a red colored product. [15,16]

Total antioxidant capacity (TAC) was measured by peroxidase / H2O2 / ABTS colorimetric assay using commercial kits from Randox Laboratories, Belfast, United Kingdom. The antioxidant enzyme glutathione (GSH) was measured in the serum by colorimetric method using kit from North West Company, USA. Highly sensitive CRP was measured using the BioCheck-CRP ELISA kit (BioCheck, Inc., Foster City, California , USA. [17,18] Statistically, the data were expressed as mean ± standard deviation (SD). Statistical comparisons were made using the Students t-test between patients and controls, and a one-way analysis of variance (ANOVA). The Dunnett's Test was used to compare groups of patients.[19] Linear regression analysis and Pearson correlation coefficients (r) were performed to determine the relationship between the parameters studied. Statistical analyses was done by using the Statistical Package for Social Sciences (SPSS) for Windows (Version 26, Chicago, Illinois, USA). A P- value of <0.05 was considered to be statistically significant.

RESULTS

The serum levels of MDA, TAC , GSH and hs-CRP for healthy subjects and patients with β-TM patients were shown in Tables 1,2 and 3 respectively. The serum levels of MDA and hs-CRP were found to be significantly higher (P ≤0.001) and TAC and GSH were significantly lower in β-thalassemia major patients in comparison to healthy controls (Table 1,2, and 3).

By comparing the serum levels of MDA, TAC, GSH and hs-CRP between the three groups of β-TM patients, a highly significant differences (P ≤0.01) were reported in serum MDA levels between group A, with blood transfusion therapy alone, and group B, who received DFX and on regular blood transfusion therapy for survival, whereas the serum levels of TAC were found to be significantly higher (P ≤0.01) and hs-CRP were significantly lower (P ≤0.01) in group B who received DFX with blood transfusion therapy in comparison with group A who received blood transfusion alone (Table 4).The difference in mean of serum levels of MDA, GSH, TAC and hs CRP among the study sampled groups were shown in Figures 1,2,3 and 4 respectively.

Table (1): Concentrations of malondialdehyde (MDA), total antioxidant capacity (TAC), GSH and highly-sensitivity C-reactive protein (hs-CRP) in patients with β-thalassemia before DFX therapy (group A) [n= 40].

Parameters	Mean	SD	Minimum	Maximum
S. ferritin (ng/ml)	1048.9	628.2	214.0	3210.0
hs-CRP(mg/l)	1.17	0.92	0.19	4.97
MDA (nmol/ml)	8.34	2.32	3.70	13.70
TAC (mmol/l)	1.55	0.22	1.09	2.08
GSH (µg/ml)	3.46	1.37	1.56	5.97

Table (2): Concentrations of malondialdehyde (MDA), total antioxidant capacity (TAC), GSH and highly-sensitivity C-reactive protein (hs-CRP) in patients with β-thalassemia after DFX therapy (group B) [n= 65].

Parameters	Mean	SD	Minimum	Maximum
S. ferritin (ng/ml)	3306	1684	860	8046
hs-CRP(mg/l)	1.30	0.94	0.14	4.30
MDA (nmol/ml)	10.05	3.16	3.70	16.15

TAC (mmol/l)	1.53	0.26	1.01	1.932
GSH (µg/ml)	2.37	0.84	1.560	5.43

Table (3): Concentrations of malondialdehyde (MDA), total antioxidant capacity (TAC), GSH and highly-sensitivity C-reactive protein (hs-CRP) in healthy controls (group C) [n= 65].

Parameters	Mean	SD	Minimum	Maximum
S. ferritin (ng/ml)	33.62	27.32	7.00	120.00
hs-CRP(mg/l)	0.40	0.30	0.06	1.60
MDA (nmol/ml)	2.26	0.78	0.50	3.70
TAC (mmol/l)	1.94	0.17	1.52	2.53
GSH (µg/ml)	4.89	0.77	3.53	6.48

Table (4): Comparison of Concentrations of malondialdehyde (MDA), total antioxidant capacity (TAC), GSH and highly-sensitivity C-reactive protein (hs-CRP) among the three groups

Parameters	Group A Mean ± SD	Group B Mean ± SD	Group C Mean ± SD	P-value*
S. ferritin(ng/ml)	1048.9 ± 628.2^{B}	3306 ± 1684^{A}	33.62 ± 27.32^{C}	**0.000**
hs-CRP (mg/l)	1.17 ± 0.92^{A}	1.30 ± 0.94^{A}	0.40 ± 0.30^{B}	**0.000**
MDA (nmol/ml)	8.34 ± 2.32^{B}	10.05 ± 3.16^{A}	2.26 ± 0.78^{C}	**0.000**
TAC (mmol/l)	1.55 ± 0.22^{B}	1.53 ± 0.26^{B}	1.94 ± 0.17^{A}	**0.000**
GSH (µg/ml)	3.46 ± 1.37^{B}	2.37 ± 0.84^{C}	4.89 ± 0.77^{A}	**0.000**

* One-way ANOVA-test with Tukey's Multiple (Pair wise) comparisons was used.

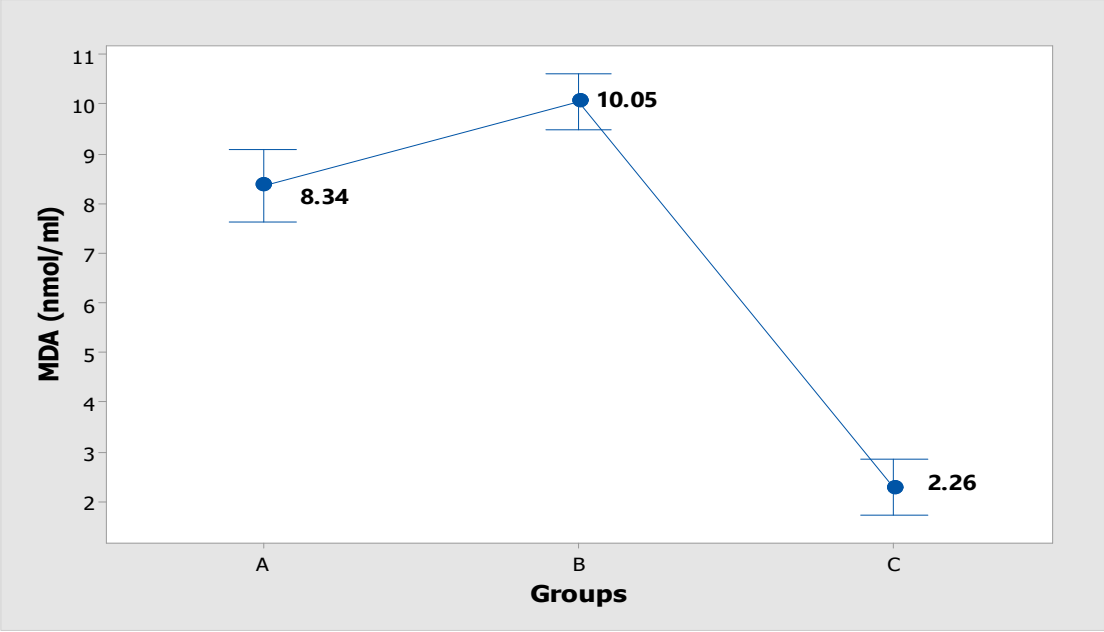

Figure (1): The difference in mean MDA among the study sampled groups.

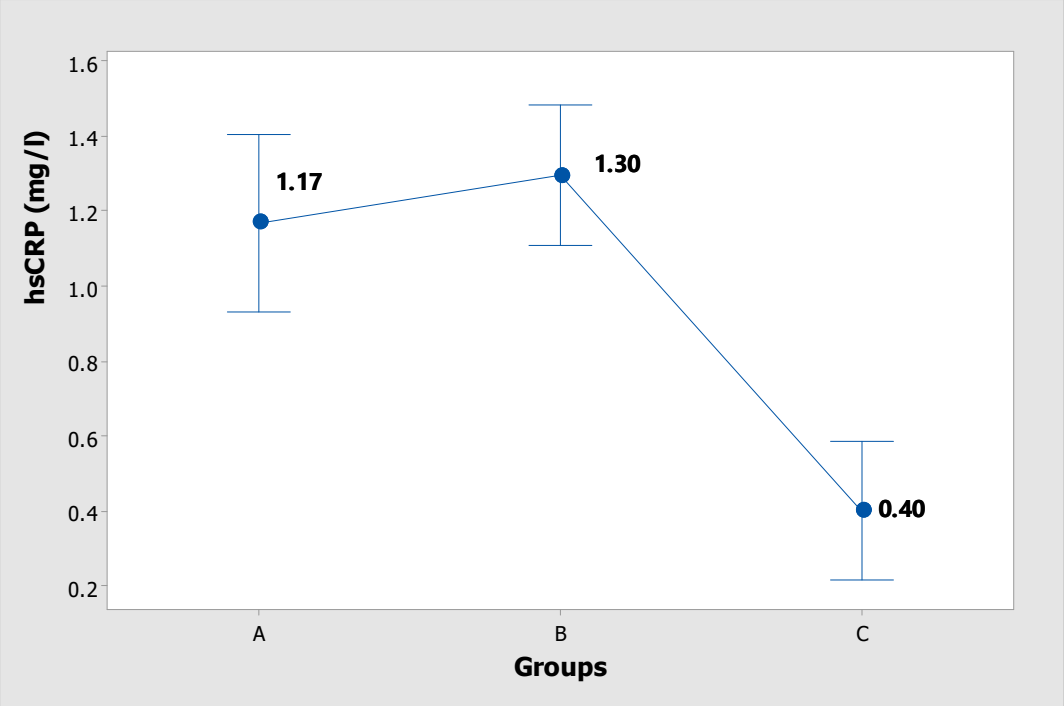

Figure (2): The difference in mean hs-CRP among the study sampled groups.

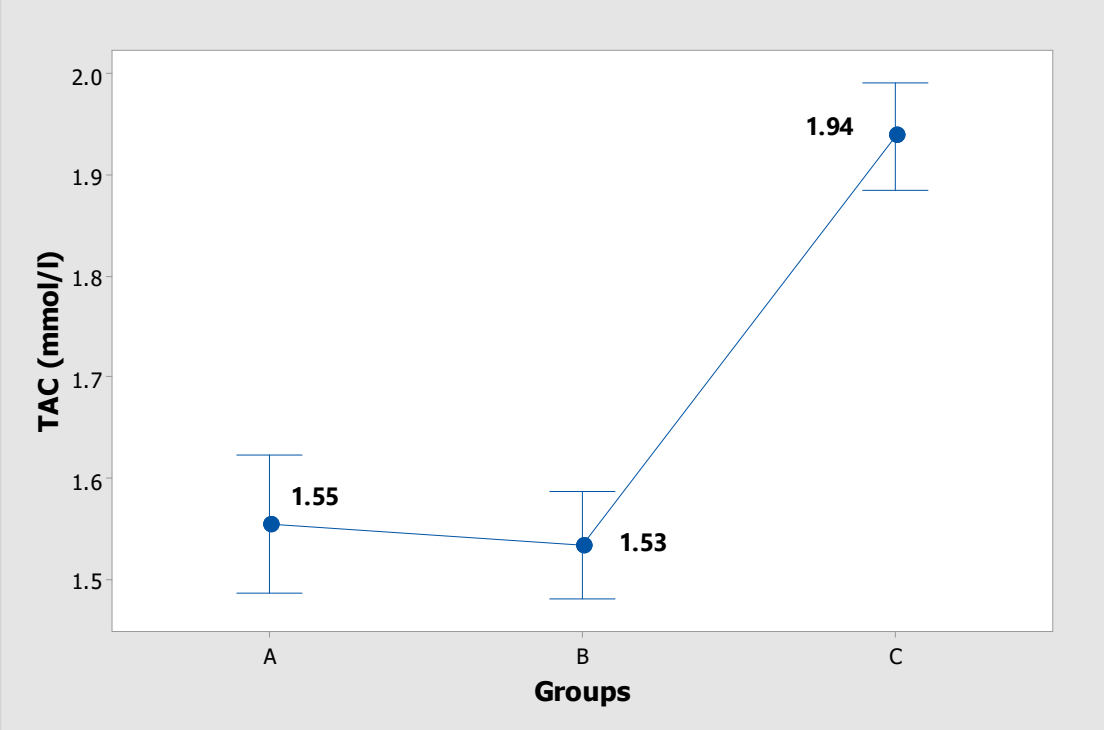

Figure (3): The difference in mean TAC among the study sampled groups.

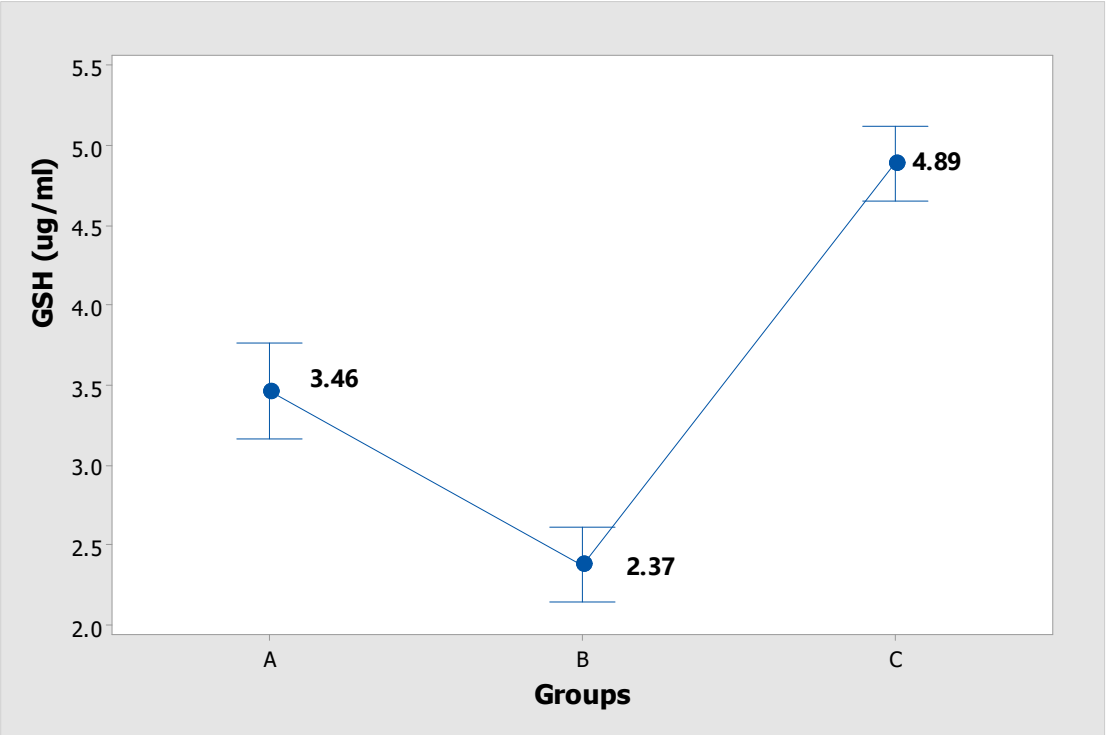

Figure (4): The difference in mean GSH among the study sampled groups.

Besides these, the correlation between serum MDA , serum antioxidant enzymes GSH, Hs-CRP and TAC were also determined in healthy control subjects as well as thalassemic subjects and also between different parameters in different groups. Table (5) , exhibits the correlation matrix between the studied parameters in group A, there is statistically highly significant positive correlation between S.ferritin and hs-CRP (r=0.629, p=0.000), MDA (r=0.502, p=0.001), TAC (r=0.502, p=0.001) as well as there is significant positive correlation between hs-CRP and MDA (r=0.442, p=0.004), hs-CRP and TAC (r=0.442, p=0.004).

Table (5): Correlation matrix between different parameters in group A, (n= 40).

Items	Correlcoef*	Age	WT	Ferritin	hs-CRP	MDA	TAC	GSH
Ferritin	r	0.137	0.103	---	---	---	---	---
	P	0.399	0.526	---	---	---	---	---
hs-CRP	r	0.238	0.253	**0.629**	---	---	---	---
	P	0.140	0.116	**0.000**	---	---	---	---
MDA	r	0.287	0.064	**0.502**	**0.442**	---	---	---
	P	0.073	0.694	**0.001**	**0.004**	---	---	---
TAC	r	0.287	0.064	**0.502**	**0.442**	0.287	---	---
	P	0.073	0.694	**0.001**	**0.004**	0.073	---	---
GSH	r	0.177	-0.013	-0.238	-0.162	-0.107	-0.303	---
	P	0.274	0.938	0.139	0.318	0.510	0.058	---

* Pearson correlation method (r) was used.

Table (6), Exhibits the correlation matrix between the studied parameters in group B, There is highly significant positive correlation between S.ferritin and hs-CRP (r=0.723, p=0.000), between ferritin and MDA (r= 0.675, p=0.000), and there is highly significant negative correlation between ferritin and TAC (r=-0.422, p=0.000), and between ferritin and GSH (r=- -0.354, p=0.004). There is highly significant positive correlation between hs-CRP and MDA (r=0.518, p=0.000), and significant negative correlation between hs-CRP and TAC (r= -0.413, p=0.001), and between hs-CRP and GSH (r=-0.374, p=0.002). in addition there is significant negative correlation between MDA and GSH (r=-0.348, p=0.005).

Table (6): Correlation matrix between different parameters in group B, (n= 65).

Items	Correlcoef*	Age	WT	Ferritin	hs-CRP	MDA	TAC	GSH	Period
Ferritin	r	0.056	-0.156	---	---	---	---	---	---
	P	0.658	0.213	---	---	---	---	---	---

hs-CRP	r	0.075	-0.086	**0.723**	---	---	---	---	---
	P	0.555	0.494	**0.000**	---	---	---	---	---
MDA	r	**0.278**	0.039	**0.675**	**0.518**	---	---	---	---
	P	**0.025**	0.758	**0.000**	**0.000**	---	---	---	---
TAC	r	0.222	**0.298**	**-0.422**	**-0.413**	-0.153	---	---	---
	P	0.076	**0.016**	**0.000**	**0.001**	0.223	---	---	---
GSH	r	-0.063	0.013	**-0.354**	**-0.374**	**-0.348**	0.215	---	---
	P	0.619	0.920	**0.004**	**0.002**	**0.005**	0.085	---	---

* Pearson correlation method (r) was used.

Table (6): Exhibits the correlation matrix between the studied parameters in group B, there is statistically significant positive correlation between ferritin and MDA (r= 0.412, p= 0.001).

Table (7): Correlation matrix between different parameters in group C, (n= 65).

Items	Correlcoef*	Age	WT	Ferritin	hs-CRP	MDA	TAC	GSH
Ferritin	r	0.108	0.116	---	---	---	---	---
	P	0.392	0.359	---	---	---	---	---
hs-CRP	r	0.150	0.133	-0.003	---	---	---	---
	P	0.232	0.291	0.983	---	---	---	---
MDA	r	-0.001	-0.017	**0.412**	0.149	---	---	---
	P	0.991	0.891	**0.001**	0.235	---	---	---
TAC	r	-0.163	-0.212	-0.045	-0.081	-0.065	---	---
	P	0.195	0.090	0.721	0.521	0.607	---	---
GSH	r	0.001	0.012	-0.047	-0.204	0.025	0.068	---
	P	0.993	0.925	0.712	0.103	0.841	0.593	---

* Pearson correlation method (r) was used.

DISCUSSION

Beta thalassemia are the most common hereditary hemoglobin disorders that affect more than 90 million people throughout the world.[1,2,3] Several studies , had been reported the increased oxidative stress in β-TM, and in-creased oxidative damage BT has been related mostly to the generation of free radicals by an excess of denatured beta-globin chains, intracellular iron overload, and low concentration of normal hemoglobin level.

The results of the present study showed significant increase in the mean of MDA and significant decrease in the mean of TAC and GSH in patients groups when compared with control group. Tables(1,2,and 3) respectively.

The present study showed significant increase in the mean of MDA in group B thalassemic patients on deferasirox when compared with group A patients on blood transfusion only and highly significant decrease in mean of GSH in group B than group A whereas there is non-significant difference in mean of TAC between group A and group B. This can be explained that as group B patients are highly iron overloaded than group A, as well as there is positive correlation between MDA and ferritin as shown in tables (3.20), (3.21) and (3.22) {(r=0.502, p=0.001), (r= 0.675, p=0.000) and (r=0.412, p=0.001) respectively} thus increasing iron load will cause subsequent increase of free radicals in turn will cause oxidative tissue damage and lipid peroxidation which reflected by increasing MDA level.

Treatment with DFX found that the means of MDA levels in the serum was significantly decreased from the baseline in both of the treated patients groups. This finding is agreed with the Daniel study, which confirmed that the serum MDA levels could be controlled by DFX.[19] The explanation for that is DFX chelate the excess of iron, reducing the circulating and intracellular free iron that lead to decrease the formation of ROS and lipid peroxidation. Since MDA is one of the products for lipid peroxidation, its serum level will be significantly reduced in response to DFX treatment [17,18].

The serum level of antioxidant marker glutatione (GSH) was decrease at the base line. Suggesting major consumption due to oxidative stress that result from iron overload .[20]

Since GSH is the major antioxidant thiol and redox buffer in the cell, its level will be low in case of oxidative damage. .[21]

Treatment with deferasirox results in a significant increase in the mean of serum GSH for both the treated patients groups. These results agree with other studies where they found that DFX therapy can act as an antioxidant by decreasing intra- and extra-cellular toxic iron species, reducing oxidative stress and decrease GSH consumption.[22,23,24]

Our study found that there is elevated serum level of the inflammatory marker CRP for patient with β-TM. This is because in transfusion dependent beta thalassemic patient when iron overload exceed the storage capacity of the cell , the free iron start to deposit in the organs and by this way 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 (20,21). 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[22,23].

High sensitive-CRP is an acute phase protein, which is synthesized in response to tissue damage. Its production is stimulated mainly by interleukin-6 (IL-6).(22) The results of the present study showed highly significant increase in the mean of hs-CRP in patients groups when compared with healthy control, where as there is non-significant difference between patients groups (A and B), as shown in table (1,2 and 3 respectively).

Hs-CRP is an acute phase protein, which produced with respect to tissue damage and stimulated by interleukin-6 (IL-6). Elevated hs-CRP is important marker for inflammation and diagnostic factor to avoid tissue injury. Hs-CRP was used for determination if significant inflammation happens between participants [24].

The important elevation of hs-CRP is a major inflammatory marker (22,23) and little is known about the role of acute phase proteins in hemoglobinopathies. The inflammatory marker status in our patients was done by direct measurement of this protein. Interestingly, the levels of the C-reactive protein biomarker was less increased in patients in comparison to controls, but are not statistically significant with healthy subjects in response to elevated ferritin and PAB values. Similar to our study, some studies reported the reduction of the hs-CRP levels in patients compared to healthy subjects. Similar studies also reported that hs-CRP concentration in healthy volunteers is lower than thalassemia patients.(17,18) In our study we found a significant decline in the mean of serum CRP from the baseline after six months treatment with deferasirox in both of the treated patients groups, our results were in agreement with other studies which confirmed that DFX treatment can significantly decrease CRP level.[20]

The explaination for the reason is that chelation of the excess iron by DFX decrease the free iron, diminishing the iron-induced oxidative tissue injury and the possible stress to circulatin monocytes and macrophages of the reticuloendothelial system. Reduced oxidative stress has been shown to lower monocyte and macrophage IL-6 release thus the serum CRP level will be lowered. [24, 25]

The results of the present study showed a strong positive correlation between hs-CRP and MDA (r=0.442, p=0.004) which is indicator of oxidative stress but there is no correlation between hs-CRP and antioxidant parameters (TAC and GSH) in group A as shown in Table (5). Whereas, in group B the results showed strong positive correlation between hs-CRP and MDA (r=0.518, p=0.000) and significant negative correlation between hs-CRP and TAC (r=-0.413, p=0.001) and hs-CRP and GSH (r=-0.374, p=0.002) as shown in Table (6),whereas, in healthy control group C, the results showed no correlation between hs-CRP and oxidative stress and antioxidant parameters as shown in Table (7). In thalassemic patients especially in group B who receiving deferasirox exhibited positive correlation between oxidative injury and inflammation, and inverse correlation between antioxidant parameters and inflammation.

The results of the present study showed no correlation between MDA with TAC and GSH in group A as shown in table (5). Whereas in group B, the results showed no correlation between MDA and TAC, and significant negative correlation with GSH (r= -0.348, p=0.005) as shown in Table (6). Whereas in healthy control group C showed no correlation between MDA with TAC and GSH as shown in Table (7). The results of the present study are agreed with study involved 50 thalassemic patients and 50 healthy control their results showed significant negative correlation between MDA and GSH in thalassemic patients and no correlation between MDA and GSH in healthy control.[24,25]

CONCLUSIONS

Inflammation and oxidative stress in β-thalassemic patients resulted from iron overload can be effectively controlled by oral iron chelator, deferasirox . Significant decreases in oxidative parameter (MDA), inflammatory parameter (CRP) and significant increases in antioxidant (GSH) levels were observed in this study.

Our findings indicate that, compared to healthy individuals, there are significantly higher levels of oxidative stress indicators in patients with beta thalassemia who need treatment with deferasirox to prevent endothelial dysfunction and damage to other tissues and organs. In addition, measurement of hs-CRP may be a useful marker for inflammation and a useful diagnostic factor to prevent inflammation.

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