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Evaluation of Hepatic Enzymes in major β-thalassemic Patients using Deferasirox

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Abstract

β-thalassemia major is a genetic disease that causes sever defect in normal hemoglobin synthesis. The patients with β-thalassemia major need periodic blood transfusions that can result in accumulation of body iron, so treatment with iron chelating agent is required. Complications of this iron overload affecting many vital organs, including the liver. The aim of this work was to evaluate liver enzymes in β -thalassemia major patients with deferasirox versus without it. Two groups of β-thalassemia major patients were involved in this study named group A; 40 β-thalassemia patients of blood transfusion dependent without deferasirox, group B; 40 β-thalassemia patients of blood transfusion dependent on deferasirox. In addition to group C, 40 normal subjects as a control group. Samples of serum were obtained from all participants to be tested for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase and ferritin. The biochemical data of the patients on blood transfusion without deferasirox showed significant increases in the mean serum levels of aminotransferases and ferritin in comparison with control. Whereas the patients on blood transfusion with deferasirox exhibit significant increases in the means serum levels of alkaline phosphatase activity and ferritin in comparison with control. Iron overload may cause liver injury, shown by significant increases of; ALT and AST activities and elevated ferritin level in serum of transfusion dependent patients of β-thalassemia major. Administration of deferasirox for β-thalassemia major patients causes elevation of serum ALP activity and ferritin level.

Key words: **β-thalassemia, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, ferritin.**

فحوصات وظيفة الكبد لمرضى الثلاسيميا الكبرى نوع بيتا من الذين يستخدمون عقار الديفيراسيروكس

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الخلاصة

يتميز مرض الثلاسيميا الكبرى نوع بيتا بخلل في تصنيع الهيموغلوبين أ. إن الأفراد المصابين يعتمدون على عمليات نقل الدم المتكررة مما يؤدي الى زيادة الحديد في الجسم مما يستوجب استعمال دواء لطرح الحديد الزائد. ان المضاعفات التي تتعلق بالحديد الزائد والآثار الجانبية للعلاج يؤثران سلبا على العديد من الأعضاء في الجسم ومنها الكبد. تهدف هذه الدراسة لتقييم وظائف الكبد لدى مرضى الثلاسيميا نوع بيتا ممن يستخدمون عقار الديفيراسيروكس ومقارنتهم مع مرضى الثلاسيميا ممن لا يستخدمون هذا العقار. تشمل الدراسة مجموعتان من مرضى الثلاسيميا نوع بيتا. المجموعة (أ) تتكون من ٤٠ مريض ممن يعتمدون على النقل الدوري للدم، والمجموعة (ب) تتكون من ٤٠ مريض ممن يعتمدون على النقل الدوري للدم ويتناولون عقار الديفيراسيروكس، بالإضافة إلى مجموعة الضبط (ج) و تتكون من ٤٠ طفل من الأصحاء. تم جمع عينات مصل الدم لغرض قياس مستوى فعالية إنزيم ناقل أمين الالانين، وناقل أمين الاسبارتيت، والفوسفاتيز القاعدي ومستوى الحديدين في مصل الدم. اظهرت نتائج الفحوصات الكيموحيوية للمرضى الذين لا يستعملون عقار الديفيراسيروكس زيادة معنوية في معدلات فعاليات إنزيمات نواقل الأمين وكذلك الفرتين في مصل الدم مقارنة بمجموعة الضبط. بينما المرضى المعتمدين على النقل الدوري للدم ويستعملون عقار الديفيراسيروكس اظهروا زيادة معنوية بمعدل مستوى فعالية الفوسفاتيز القاعدي ومستوى الحديدين في مصل الدم مقارنة بمجموعة الضبط. يستنتج من نتائج هذه الدراسة وجود اضطرابات ظاهرية في وظائف الكبد وذلك بسبب زيادة مستويات فعالية إنزيم ناقل أمين الالانين و إنزيم ناقل أمين الاسبارتيت وكذلك زيادة الفرتين في مصل الدم لدى مرضى الثلاسيميا الكبرى نوع بيتا المعتمدين على النقل الدوري للدم. من جهة اخرى فقد ادى استعمال عقار الديفيراسيروكس لمرضى الثلاسيميا الكبرى نوع بيتا المعتمدين على النقل الدوري للدم الى زيادة مستوى فعالية إنزيم الفوسفاتيز القاعدي والى زيادة فرتين مصل الدم.

الكلمات المفتاحية: **بيتا ثلاسيميا، إنزيم ناقل أمين الالانين، إنزيم ناقل أمين الاسبارتيت، إنزيم الفوسفاتيز القاعدي، الفرتين.**

Introduction

Thalassemias are genetic disorders of hemoglobin synthesis where the production of normal hemoglobin is partly or completely suppressed. This suppression may be due to a defective synthesis of any globin chains. Many forms thalassemia have been discovered and called by the defected globin chain

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The common thalassemia of clinical interest are α- and β-thalassemia[1]. Normally hemoglobin (Hb) is made up of two α-globin and two β-globin chains ($alpha_2$ $beta_2$). β-thalassemia forms are hereditary diseases of blood in which reduced or absent β-globin chain production of hemoglobin (Hb) tetramer (β^+) or (β^0) respectively, resulting in diminutive normal Hb in red blood cells (RBC), diminutive production of RBC and then anemia[2]

The formation of normal α-globin chains in β-thalassemia patients continues as normal, that result in accumulation of unmatched α-globin in the erythroid precursors. This increased free α-globin chains cannot synthesis tetramers of Hb, instead precipitate in the bone marrow forming inclusion bodies that causes intramedullary destruction of erythroid precursors resulting in ineffective erythropoiesis characterized in all forms of β-thalassemias[3]. β-thalassemia major patients require repeated blood transfusion for normal life, patients on blood transfusion always have iron overload as a result to the periodic blood transfusion and ineffective erythropoiesis[4]. The adverse effects of iron overload may be seen on certain vital organs like the liver, endocrine glands, heart, kidneys and lungs[5]. Iron over load can cause increased cellular accumulation of labile part of iron in certain parenchymal tissues, including the above-mentioned organs causing iron toxicity. Iron toxicity can cause production of reactive oxygen species (ROS), such as free radicals where, it was proved that labile iron is the key mediator of the toxicity[6]. Deferasirox is the most recent drug used as an oral chelator that moves iron from stores by binding to the ferric atom (Fe^{+3}) of iron[7]. The main metabolic pathway for this chelating agent is through glucuronidation and biliary excretion[7].

The hepatic cells are the major tissues of iron storage so when a case of iron overload is present, it is regarded as the most important target for the therapy with deferasirox[8]. The well-known complications that appear in thalassemic patients on deferasirox drug are the transient elevation of serum liver transaminases activities and serum creatinine[9].

Materials and Methods

Patients and control selection:

Eighty β-thalassemic major patients all were dependent on blood transfusion with age ranged from 6-60 months who were presented to Ibn-Alatheer teaching hospital/ department of thalassemia in Mosul City/ Iraq were participated in this study since 1st of October 2011 to the 30th of March 2012. The enrolled participants were diagnosed as patients with β-thalassemia major according to hemoglobin variants using hemoglobin electrophoresis. Those Patients were classified into two groups:

Group A

consisted of 40 patients depend on blood transfusion that were not received deferasirox as chelating therapy. Their ages range from 6-60 months (with a mean of 21.12 months).

Group B

consisted of 40 patients depend on blood transfusion that were treated with 10-30 mg/kg body weight of the chelating agent deferasirox daily. Their ages range from 21-60 months (with a mean of 40.68 months).

Group C

Apparently healthy 40 normal subjects, with unknown diseases and not received any chronic therapy participate in this study. Their ages range from 6-60 months (with a mean of 25.2 months).

A written informed consent of the study details was obtained from each of the participant's parents. The ethical approved on the study design and investigations was obtained from the local Mosul Medical College ethical committee.

About 5 ml of venous blood was obtained from each child of the three studied groups in plain tubes, left 15 minutes at room temperature to form clot, followed by centrifugation to separate serum, that were then feezed deeply at -20 C°.

The biochemical measurements were carried at the laboratory of Ibn-Alatheer teaching hospital in Mosul / Iraq.Serum Aminotransferases (AST and ALT) activities were determined calorimetrically according to (Wooton and Freeman, 1982) by using a kit obtained from Biomeriux Company (France).

Serum Alkaline phosphatase (ALP) activity was determined by colorimetric reaction method, using a kit method supplied by (Biolabo, France).

Serum ferritin was determined by an enzyme liked assay method[10], by using a kit obtained from Biomerieux (France).

Statistical analysis

The mean, standard deviation (SD), unpaired t-test, fisher Freeman Halton test, ANOVA test were used as standard statistical analysis methods for analyzing the data of this work[11]. The results were considered statistically significant when $P \leq 0.05$[12].

Results and Discussion

The comparison of serum ALT activity in transfusion dependent β-thalassemia patients (Group A) with controls (Group C) showed a significant increase in the mean of serum ALT activity in Group A. While the comparison of transfusion dependent β-thalassemia patients using deferasirox (Group B) with Group C showed no significant increase in the mean of serum ALT activity (Tables 1 and 2 respectively). On comparing serum AST activity in Group A with Group C, there was a significant increase in Group A, while in Group B, there was no a significant difference in the mean of serum AST activity in

comparison with that of Group C (Tables 1 and 2 respectively).

Table1. Differences in Serum ALT, AST, ALP and Ferritin between Group A and C.

parameters	***Mean ± SD***		
	Group A	***Group C***	
ALT activity (IU/l)	54.12±39.28	17.00±9.83	**P-value* <0.001
AST activity (IU/l)	57.82±31.71	19.700±13.41	**P-value* <0.001
ALP activity (IU/l)	134.39±27.65	114.23±21.64	*P-value* > 0.05 (NS)
Ferritin (ng/ml)	1763.35±1285.44	43.6 ±18.85	**P-value* <0.001

* Significant difference between groups exists at p≤0.05.
NS: not significant.
ANOVA (One away Analysis of variance) test was used to compare the results of various parameters between thalassemic patients with the controls.

Table2. Differences in Serum ALT, AST, ALP and Ferritin between Group B and C.

parameters	***Mean ± SD***		
	Group B	***Group C***	
ALT activity (IU/l)	28.65±19.88	17±9.83	*P-value* > 0.05 (NS)
AST activity (IU/l)	20.65±12.6	19.7±13.41	*P-value* > 0.05 (NS)
ALP activity (IU/l)	148.27±62.04	114.23±21.64	**P-value* <0.001
Ferritin(ng/ml)	2622.92±843.89	43.6 ±18.85	**P-value* <0.001

* Significant difference between groups exists at p≤0.05.
NS: not significant.
ANOVA (One away Analysis of variance) test was used to compare the results of various parameters between thalassemic patients with the controls.

The mean of serum ALP activity showed no significant increase in group A in comparison with group C, while in group B, there was a significant increase in the mean of serum ALP activity (Tables 1 and 2 respectively).

The mean of serum ferritin showed a significant increase in both groups of patients (Groups A and B) from that of Group C (Tables 1and 2 respectively).

The results of this work showed significant differences in the serum of ALT and AST activities between transfusion dependent β-thalassemia patients (Group A) and controls (Group C) (Table1). Iron-induced oxidative stress is known to be one of the most important factors causing liver cell injury in thalassemic patients (Patpan et al.)[13], leading to increased liver enzyme activities[14]. The results of this work are in accordance with that of another study (Patpan et al.)[13] where, it was found that significant elevations in serum; ferritin, AST, ALT activities in β-thalassemia major (βTM) patients on blood transfusion group when compared with their controls. The results of this study also agree with other investigators (Sonali et al.; Setoodeh et al) [15,16]. However, periodic blood transfusions is necessary as life-saving and improving the quality of life for the patients with βTM, even it causes excessive iron overload, that was regarded as an important clinical complication of the treatment [17]. In the present study, the results of Group B showed no significant increase in the mean of serum ALT and AST activities in comparison to Group C (Table2). This can be attributed to the fact that deferasirox therapy reduces hepatocellular inflammation and improves liver functions that may be linked to the reduction in liver iron concentration and serum ferritin[18].

In the present study, the results concerning the mean serum ALT and AST activities that compared according to different age ranges for each of group A and group B patients, no significant changes were observed (Table3 and 4 respectively). This may indicate that age did not adversely affect the liver functions. Soliman et al. (2014)[19] reported that the variations in ALT and AST activities in βTM patients who are under a regular treatment with deferoxamine were not correlated with the age of the patients.

Table3. Effect of Age on Serum ALT, AST, ALP and Ferritin in Group A.

Parameters	***Mean ± SD***		***P-value***
	< 2 years(n=20)	***≥ 2 years(n=20)***	
ALT activity (IU/l)	54.80±40.04	53.45±39.54	*P-value* > 0.05 (NS)
AST activity (IU/l)	56.2±28.86	59.45±35.01	*P-value* > 0.05 (NS)
ALP activity (IU/l)	114.8±16.03	153.99±22.46	**P-value* <0.001
Ferritin (ng/ml)	1212.1±427.73	2314.6±1602.31	**P-value* <0.001

* Significant difference between groups exists at p≤0.05.
NS: not significant.

ANOVA (One away Analysis of variance) test was used to compare the results of various parameters between thalassemic patients with the controls.

Table4. Effect of Age on Serum ALT, AST, ALP and Ferritin in Group B.

parameters	**Mean ± SD**		**P-value**
	<3years (n=22)	***≥ 3years (n=18)***	
ALT activity (IU/l)	29.72±20.66	27.33±19.41	*P-value* > 0.05 (NS)
AST activity (IU/l)	24.13±14.4	16.38±8.58	*P-value* > 0.05 (NS)
ALP activity (IU/l)	136.44±40.59	162.74±79.95	**P-value* <0.001
Ferritin (ng/ml)	2450.09±839.09	2834.16±823.47	**P-value* <0.05

* Significant difference between groups exists at p≤0.05.
NS: not significant**.**

In the present study, the mean serum ALT activity showed a significant increase according to increased ferritin ranges for group A only (Table5 and 6 respectively). This result means that ALT activity reflecting liver cell injuries due to toxic iron overload.

Table5. Effect of Ferritin levels on Serum ALT, AST and ALP in Group A.

parameters	**Mean ± SD**			**P-value**
	<1000 (n=9)	**1000-2000 (n=22)**	**>2000 (n=9)**	
ALT activity (IU/l)	33±17.05	56.09±37.93	70.44±51.42	*P-value <0.05
AST activity (IU/l)	49.33±18.1	54.68±30.57	74±41.45	P-value > 0.05 (NS)
ALP activity (IU/l)	116.58±25	131.84±19.76	158.45±32	*P-value <0.05

* Significant difference between groups exists at p≤0.05.
NS: not significant**.**

Table6. Effect of Ferritin levels on Serum ALT, AST and ALP in Group B.

parameters	**Mean ± SD**		**P-value**
	≤ 2500 (n=20)	**>2500 (n=20)**	
ALT activity (IU/l)	24.45 ±16.37	32.85 ±22.5	P-value > 0.05 (NS)
AST activity (IU/l)	18 ±11.81	23.3 ±13.11	P-value > 0.05 (NS)
ALP activity (IU/l)	152.15 ±41.67	144.4 ±78.31	P-value > 0.05 (NS)

* Significant difference between groups exists at p≤0.05.
NS: not significant**.**

The data of this work showed that the mean level of serum ALP activity in Group A is not significantly higher than in group C (Table1) and this is in accordance with the results of Younus and Bashi, (2012)[20] who reported that the alkaline phosphatase activity was not significantly increased in the patients with βTM when compared with that of their control group. In the present study ALP activity is significantly higher in group B in comparison to Group C (Table2). These results may be accounted to be due to that serum ALP activity may originate from the liver, bone, intestine and placenta, in addition to the elevation of serum activity that result from hepatobiliary and non-hepatic (bone disease and childhood growth) causes of elevated serum ALP activity[21]. Osteoporosis may result due to chronic request for blood cell production by over stimulation of the hematopoietic system that causes an increase in the number of osteoclasts and osteoblasts, leading to accelerated bone turnover and increase serum ALP activity[22]. Bone demineralization which commonly occurs in β-thalassemia patients also causes an elevation in the serum ALP activity. Moreover, the results of the present study were in agreement with those of Baldini et al. (2010)[23] where they studied the level of serum alkaline phosphatase activity in adult Caucasian β-thalassemic who were on deferasirox therapy. The result of the present study showed a significant increase of serum ALP activity according to age ranges for both Groups A and B (Table3 and 4 respectively) and for different serum ferritin ranges for group A only (Table5 and 6 respectively). These results may be accounted due to iron overload itself, as it is known that it cause increase liver enzyme activities[14]. In addition, the toxicity of iron on the bone lead to the bone demineralization and so increased serum ALP activity.

The results of the present study showed a significant increase in the mean level of serum ferritin (major physiological role is to store iron) in patient's groups in comparison with the control group (Table1 and 2 respectively) (iron overload is usually manifested by a high serum ferritin levels(5)). A significant increase in the mean level of serum ferritin also was seen in patient's subgroups according to age (Table3 and 4 respectively). There are many mechanisms to absorb or store or to transfer iron, but no mechanism to excrete iron outside the body. So it is important to find a way to get rid of excess iron that are accumulated due to periodic blood transfusion in βTM patients where it is found that every unit of blood contains about 200-250 mg of iron[24]. Another source of iron may exist in some βTM patients as more iron is absorbed from the diet as a response to ineffective erythropoiesis [25]. The results of this study showed that the mean of serum ferritin levels in patients of group B was significantly higher than that of group A despite using deferasirox (Table7).

Table7.Differences in Serum ALT, AST, ALP and Ferritin between Group A and B.

parameters	Mean ± SD		
	Group A	Group B	
ALT activity (IU⁄l)	54.1250±39.28	28.65±19.88	*P-value <0.05
AST activity (IU⁄l)	57.82±31.71	20.65±12.6	*P-value <0.001
ALP activity (IU⁄l)	134.39±27.65	148.27±62.04	*P-value <0.001
Ferritin (ng⁄ml)	1763.35±1285.14	2622.92±843.89	*P-value <0.01

* Significant difference between groups exists at p≤0.05.
NS: not significant.
ANOVA (One away Analysis of variance) test was used to compare the results of various parameters between thalassemic patients with the controls

This may be due to the short duration of administration of deferasirox which does not exceed 6 months or may be due to those patients in group B were older than that of group A (mean of age 21.12 and 40.68 months respectively. The results of the present work are in accordance with that of other investigators [26,27] where they reported, evidence of iron overload manifested as elevated serum ferritin levels in all patients with βTM whether were using or not using chelating agents. They proved that the serum ferritin level is in correlation with age.

Conclusion

Iron overload may cause liver injury. This is reflected by significant elevation of serum; ALT and AST activities and elevated serum ferritin level in transfusion dependent β-thalassemia major patients. On the other hand, administration of deferasirox in transfusion dependent β-thalassemia major patients causes no significant elevation in serum AST and ALT, but significant elevation of serum ALP activity that might be caused by bone demineralization or may be originated from the liver, bone, intestine or other tissues or due to

chronic blood transfusion which accelerated bone turnover and increase serum ALP activity.

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