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EVALUATION OF CONVENTIONAL RENAL FUNCTION TESTS IN PATIENTS WITH β-THALASSEMIA MAJOR USING DEFERASIROX

FATIMAH HAITHAM FATHI[1], YUSRA AHMED HUSSEIN ALHIALLY[2] AND AHMED YAHYA DALLAL BASHI[3*]

[1]Department of Pharmaceutical Science, College of Pharmacy
[2]Department of Science, College of Nursing
[3]Department of Biochemistry, College of Medicine, University of Mosul, Mosul, Iraq

β-thalassemia major is characterised by grossly defective synthesis of haemoglobin A along with increased production of haemoglobin F, impaired red blood cells production and increased haemolysis of the defective red blood cells. Affected individuals are dependent on repeated blood transfusions and iron chelating therapy. Patients on blood transfusion may develop complications related to iron overload and adverse effects of chelating agent which has harmful effects on many organs in the body including the kidneys. The present study was aimed to investigate renal functions in paediatric patients with transfusion dependent β-thalassemia major using deferasirox. This study consisted of two groups of β-thalassemia patients; group A, 25 transfusion dependent β-thalassemia patients, age range 0.5–5.0 years; group B, 25 transfusion dependent β-thalassemia patients receiving deferasirox as chelating agent, age range 1.75–5.0 years. In addition, 25 subjects (age range 0.5–5.0 years) served as control group C. Blood samples were collected and serum was tested for creatinine, urea and ferritin. The results showed a significant increase in the mean of serum creatinine, and a significant decrease in the mean creatinine clearance in group B when compared with the control group (p≤0.05). In addition, there was a significant increase in the mean serum ferritin in group A and B when compared with the control group (p≤0.001). On the other hand, there were no significant changes in the mean serum urea between groups A and B when compared with the control group. In conclusion the results of the present study indicated no deterioration in renal functions tests (estimated using serum creatinine, urea and creatinine clearance) in β-thalassemia patients in both groups A and B. Nevertheless, subclinical alteration in renal functions could be expected in those patients, so that measurement of other early marker of glomerular and tubular dysfunction is recommended.

Keywords: β-thalassemia, Creatinine, Urea, Creatinine clearance, Ferritin

INTRODUCTION

Thalassemia refers to a disease characterised by decreased or absent production of one or more globin chains (α or β) (Silberstein 2008). Impaired globin chain synthesis causes impaired production of haemoglobin and eventually results in a hypochromic microcytic anaemia because of defective haemoglobinisation of the red blood cells (Silberstein 2008). According to the type of globin chain affected, thalassemia can be classified into different categories; the most common of these is β-thalassemia, which is characterised by impaired β-globin chain synthesis (Alsamarrai *et al.* 2008). β-thalassemia major is an autosomal

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recessive disease that leads to a severe haemolytic anaemia in early infancy (Ghone *et al.* 2008). Depletion or impaired synthesis of β-globin chain can result in an imbalanced production of β-globin chains towards higher production of α-chain, which converts haemoglobin from a normal oxygen transporting function into toxic inclusion bodies, causing peripheral erythrocyte haemolysis (Kassab-Chekir *et al.* 2003; Joshi *et al.* 1983). Patients with β-thalassemia major usually suffer from iron overload as a consequence of recurrent transfusion and ineffective erythropoiesis (Swaminathan *et al.* 2007; Das *et al.* 2004). Glutathione decrease is another reason for destabilisation of haemoglobin (Naithani *et al.* 2006; Scott and Eaton 1995). Iron overload may have adverse effects on several organs including the heart, liver, endocrine glands, lungs and kidneys (Malik, Syed and Ahmed 2009; Lahiry, Al-Attar and Hegele 2008; Olivieri 1999).

Deferasirox (Exjade®, Novarti, Switzerland, ICL670) is an oral chelator with high iron-binding potency and selectivity (Nick *et al.* 2003). It is chelated to the iron from the reticulo-endothelial (RE) cells as well as various parenchymal organs and the chelated iron is cleared by the liver and excreted through the bile (Hershko *et al.* 2001). The most prevalent complications that arise from thalassemia and deferasirox are transient increase of serum creatinine and liver transaminases (Eshghi *et al.* 2011). Several investigations about renal involvement in adult patients with β-thalassemia major have been reported but there is a dearth of paediatric data (Aldudak *et al.* 2000; Koliakos *et al.* 2000) and the main underlying cause of tubular dysfunction in these patients remains unknown (Sumboonnanonda *et al.* 2003). However, renal damage can be attributed to chronic anaemia and iron overload (Aldudak *et al.* 2000). This prospective study was aimed to investigate renal involvement in paediatric patients with transfusion dependent β-thalassemia major and in those taking deferasirox, using conventional and early markers of glomerular dysfunctions.

METHODS

Patients

Fifty patients all having transfusion dependent β-thalassemia of age range from 0.5–5.0 years attending the Thalassemia Centre in Ibn-Alatheer Paediatric Teaching Hospital in Nineveh province, Iraq, were enrolled in this study beginning 1 October 2011 to 30 March 2012. All patients were diagnosed as having β-thalassemia major depending on haemoglobin variants tested by haemoglobin electrophoresis. All patients chosen did not have any history of previous or recent infection. None of the individuals enrolled in this study had previous history of procedures or ultrasound for kidney. None of the patients chosen was receiving treatments other than deferasirox (Exjade® 30 mg/kg/day) as an iron chelating therapy when indicated.

The patients were divided into two groups: group A, 25 transfusion dependent β-thalassemia patients not using deferasirox iron chelator with age range 0.5–5.0 years old; group B, 25 transfusion dependent β-thalassemia patients all receiving deferasirox iron chelator with age range 1.75–5.0 years old. Control group (group C) consisted of 25 apparently healthy non-thalassemic individuals with age range from 0.5–5.0 years old.

Approval of the study was obtained from The Scientific and Human Ethical Committee, Department of Biochemistry, College of Medicine, Mosul University, Iraq.

Specimens and Methods

Venous blood samples, about 5 mL were collected from each individual of the studied groups in plain tubes. Plain tubes samples were then placed aside for 15–30 min to enable blood coagulation to occur. Blood were then centrifuged for 10 min at 4000 rpm in order to obtain serum samples, separated and collected in aliquots, and finally deep frozen at –20°C.

All biochemical analyses were performed at the laboratory of Ibn-Sena Teaching Hospital in Nineveh province, Iraq. The selection of reagents used in this study was based on accuracy, reliability and availability, and were purchased as kits.

Serum creatinine was measured by Jaffe reaction method that depends on using alkaline picrate (Bartels and Bohmer 1972), using a Reflotron creatinine kit (Roche Diagnostics GmbH, Mannheim, Germany). Serum urea was measured by enzymatic method (Fawcett and Scott 1960), using a Reflotron urea kit supplied by Roche Diagnostics GmbH (Mannheim, Germany). Creatinine clearance was calculated according to Shull *et al*. (1978):

Creatinine clearance (mL/ min/ 1.73m^2) = [(0.035 × age) + (0.236 × 100)]/ serum creatinine.

Serum ferritin was measured by an enzyme-linked assay method (Koivunen and Krogsrud 2006), using a Vidas® Ferritin Assay kit supplied by Biomerieux (Chemin de L'orme, Marcy L'etoile, France).

Statistical Analysis

Standard statistical methods for the analysis of data in this study were used to determine the mean, standard deviation (SD), unpaired t-test, fisher Freeman Halton test and ANOVA test (Motulsky 2003). The statistical results were considered significant at $p \leq 0.05$ (McDonald 2009).

RESULTS

For comparison purpose, the results of the present study were classified according to different age ranges [group A (≤2years and >2 years) and group B (≤3 years and >3 years)] and according to serum ferritin levels [group A (≤1500 ng/mL and >1500 ng/mL) and group B (≤2500 ng/mL and >2500 ng/mL)].

The results showed no significant differences in the mean serum levels of creatinine and urea, and mean creatinine clearance in group A when compared with group C. Whereas, a significant difference ($p \leq 0.001$) was seen in the mean serum level of ferritin between group A and group C (Table 1).

The mean serum levels of creatinine in group B showed a significant increase ($p \leq 0.05$) compared to group C. In contrast, the mean creatinine clearance in group B was significantly decreased ($p \leq 0.05$) when compared to group C. Serum ferritin was also significantly increased ($p \leq 0.001$) compared to group C (Table 2).

Within group A, significant increases in the mean serum level of creatinine and of ferritin were observed when the results were compared between different age groups. The decrease in creatinine clearance was also significantly different ($p \leq 0.05$) (Table 3).

Within group B, significant increases in the mean serum level of creatinine and mean creatinine clearance ($p \leq 0.05$) and mean serum level of ferritin ($p \leq 0.01$) were observed between the two age groups (Table 4).

In group A (Table 5), a significant increase in the mean serum level of creatinine and a significant decrease in the mean of creatinine clearance ($p \leq 0.05$) were observed among patients with different ranges of serum ferritin. In group B (Table 6), no significant changes in the mean serum level of creatinine and urea, and mean creatinine clearance ($p > 0.05$) were observed among patients with different ranges of serum ferritin.

Table 1: Differences in the mean values of serum creatinine, urea and creatinine clearance between group A and group C.

Parameters	Mean±SD		*p* value
	Group A (n=25)	Group C (n=25)	
Serum creatinine (µmol/L)	35.04±5.98	38.62±5.22	NS
Serum urea (mmol/L)	4.36±1.29	4.17±1.31	NS
Creatinine clearance (mL/min)	77.96±11.74	75.4±7.48	NS
Serum ferritin (ng/mL)	1759.92±1312.11	49.03±19.44	≤0.001

Notes: Unpaired Student's t-test, *p*<0.05
NS – not significant

Table 2: Differences in the mean values of serum creatinine, urea and creatinine clearance between group B and group C.

Parameters	Mean±SD		*p* value
	Group B (n=25)	Group C (n=25)	
Serum creatinine (µmol/L)	44.56±8.37	38.62±5.22	≤0.05
Serum urea (mmol/L)	4.08±1.59	4.17±1.31	NS
Creatinine clearance (mL/min)	73.79±10.86	75.4±7.48	≤0.05
Serum ferritin (ng/mL)	2948.8±736.93	49.03±19.44	≤0.001

Notes: Unpaired Student's t-test, *p*<0.05
NS – not significant

Table 3: Effect of age on the renal function parameters and serum ferritin levels in group A.

Parameters	Mean±SD		*p* value
	≤2 years (n=13)	>2 years (n=12)	
Serum creatinine (µmol/L)	32±5.7	38.33±4.47	≤0.05
Serum urea (mmol/L)	4.34±1.29	4.39±1.35	NS
Creatinine clearance (mL/min)	80.77±14.03	74.9±8.16	≤0.05
Serum ferritin (ng/mL)	1240.53±527.27	2381.18±611.86	≤0.05

Notes: Unpaired Student's t-test, *p*<0.05
NS – not significant

Table 4: Effect of age on the renal function parameters and serum ferritin levels in group B.

Parameters	Mean±SD		*p* value
	≤3 years (n=12)	>3 years (n=13)	
Serum creatinine (μmol/L)	43.91±2.23	45.15±11.61	≤0.05
Serum urea (mmol/L)	4.12±1.46	4.17±1.7	NS
Creatinine clearance (mL/min)	69.32±6.92	79.46±12.27	≤0.05
Serum ferritin (ng/mL)	2845.25±1436.68	3044.38±51.83	≤0.01

Notes: Unpaired Student's t-test, *p*<0.05
NS – not significant

Table 5: Effect of serum ferritin levels on the renal function parameters in group A.

Parameters	Mean±SD		*p* value
	≤1500 ng/mL (n=13)	≥1500 ng/mL (n=12)	
Serum creatinine (μmol/L)	32.76±6.74	37.5±3.98	≤0.05
Serum urea (mmol/L)	4.2±1.17	4.53±1.45	NS
Creatinine clearance (mL/min)	82.02±14.7	74.35±7.06	≤0.05

Notes: Unpaired Student's t-test, *p*<0.05
NS – not significant

Table 6: Effect of serum ferritin levels on the renal function parameters in group B.

Parameters	Mean±SD		*p* value
	≤2500 ng/mL (n=7)	>2500 ng/mL (n=18)	
Serum creatinine (μmol/L)	43.14±0.89	45.11±9.88	NS
Serum urea (mmol/L)	4.92±1.13	3.75±1.61	NS
Creatinine clearance (mL/min)	72.47±8.13	75.42±12.21	NS

Notes: Unpaired Student's t-test, *p*<0.05
NS – not significant

DISCUSSION

The results of this study showed a significant increase in the mean serum creatinine level in group B when compared to the control group, which may be related to over-chelation effect of deferasirox especially in patients receiving less frequent of blood transfusion with high dose of deferasirox (Cappellini 2008). Our findings are in agreement with that of Eshghi *et al*. (2011), who also found raised serum creatinine in 24.1% of their patients. Serum creatinine was significantly higher in the group that received higher doses of deferasirox and they concluded that the serum creatinine increases with increased dose of deferasirox.

We found a significant decrease in creatinine clearance in group B when compared to the control group. Deferasirox has characteristics such as marked iron-

chelating ability and prolonged plasma clearance that could contribute to renal toxicity (Kontoghiorghes 2007). In addition, Cappellini *et al.* (2011) have reported that creatinine clearance decreased during the first 6 months of deferasirox treatment, which then remained stable for the remainder of the study. Cappellini *et al.* (2006) found a decrease in renal functions in up to one third of patients treated with deferasirox; accordingly kidney toxicity may be a major issue in the management of patients receiving deferasirox (Papadopoulos *et al.* 2010).

A progressively significant increase in serum creatinine with increased age was found in patients from groups A and B. In addition, the mean level of serum creatinine significantly increased according to different ranges of elevated serum ferritin among group A. This can be explained by that the treatment of β-thalassemia patients with blood transfusion and iron chelating therapy provide the chance for normal growth with increasing body mass index (Safaei-asl, Maleknejad and Heidarzadeh 2009). These results may also indicate that renal disorders are not rare in patients with β-thalassemia major and that they may increase in term of frequency with age and increased duration of transfusion (Mohkam *et al.* 2008). Helin, Axenram and Grubb (1998), found a positive correlation between age and serum creatinine in patients with β-thalassemia major.

The decrease in creatinine clearance with age of patients in group A observed in this study would suggest that renal dysfunction in these patients could be the result of chronic anaemia and iron overload (Koliakos *et al.* 2000). On the other hand, creatinine clearance significantly increased with age among patients in group B. This can be attributed to the increased body mass index of the patients (Safaei-asl, Maleknejad and Heidarzadeh 2009). Papadopoulos *et al.* (2010) found a significant positive correlation of creatinine clearance with age of β-thalassemia major patients.

Among patients in group A, we found that the group with higher serum ferritin had significantly lower creatinine clearance compared to those with lower serum ferritin. The underlying mechanism for renal dysfunctions in patients with β-thalassemia major is not clear. They seem to be multifactorial, attributed mainly to include long-standing anaemia, chronic hypoxia and iron overload (Koliakos *et al.* 2000).

The highly significant levels of serum ferritin seen in groups A and B in comparison to the control group, and the significant increase in the level of serum ferritin seen between subgroups of patients according to age, may be accounted for the repeated blood transfusion and lack for the mechanism of excreting excess iron overload on the human body. It is therefore inevitable that patients who undergo regular transfusion therapy to treat chronic anaemia, such as those with β-thalassemia will develop iron overload, since every unit of blood contains approximately 200 mg of iron (Porter 2001). Iron overload from transfusions may be exacerbated in some patients due to increased absorption of iron from the diet in response to ineffective erythropoiesis (Taher and Cappellini 2009). Iron overload can be manifested by a high serum ferritin levels (Ikram, Hassan and Younas 2004).

The mean serum urea was within normal range in both groups A and B, and no significant difference was seen compared to that of group C. It appears that no significant deterioration in glomerular function regarding the filtration of urea in β-thalassemia patients. These results are in agreement with that of Shfik *et al.* (2011).

Our data confirm the incidence of glomerular dysfunction in transfusion dependent β-thalassemia major paediatric patients which could be attributed to oxidative stress and deferasirox therapy. It is recommended that patients treated with deferasirox are monitored regularly for iron status and renal functions.

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