Evaluation of conventional renal function tests in β-thalassemia major patients in Nineveh province

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Abstract

β-thalassemia is an inherited anemia, caused by impaired β-globin chain synthesis. The disease leads to serious health problems unless treated by regular blood transfusion and iron chelating therapy. However, complications from continuous therapy may arise due to iron overload and toxicity of iron chelating therapy, it may affects many organs in the body including the renal system. The present study was directed to study the changes in conventional renal function tests that may occur in β-thalassemia patients. The present study consisted of two groups of β-thalassemia patients; group A, 40 transfusion dependent β-thalassemia patients receiving only regular blood transfusion, age ranged 1-4 years; Group B, 40 transfusion dependent β- thalassemia patients receiving regular blood transfusion and deferroxamine chelation therapy, age ranged 4-20 years old. In addition to a control group C, 40 subjects, age ranged 1-20 years. Blood samples were collected and serum was separated for measurement of creatinine, urea, and ferritin. Urine samples were also collected for measurement of microalbuminuria. There was a significant decrease in serum creatinine, and a significant increase in serum urea in group B when compared with their control ($P \leq 0.01$). In addition, there was a significant increase in serum ferritin in groups A and B when compared with their controls ($P \leq 0.01$). On the other hand, there were no significant changes in microalbuminuria between groups A and B when compared with their controls. The results of the present study showed no deterioration in renal functions in β-thalassemia patients in both groups A and B regarding serum creatinine and urea in addition to microalbuminuria, although, subclinical alteration in renal functions could be expected in those patients, so that measurement of other early markers of renal dysfunction is occasionally recommended.

تقييم وظائف الكلية التقليدية لدى مرضى الثلاسيميا نوع بيتا الكبرى في محافظة نينوى

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الملخص

مرض الثلاسيميا نوع بيتا هو من أمراض فقر الدم الوراثية ، والذي سببه اضطراب في تصنيع سلسلة الكلوبين نوع بيتا للهيموكلوبين الاعتيادي . يؤدي المرض إلى مشاكل صحية عديدة ما لم يعالج بالاعتماد على نقل الدم بشكل منتظم بالإضافة إلى العلاج المساعد على طرح الحديد الزائد من الجسم . إن المضاعفات الناتجة عن هذا المرض وعن علاجه قد تودي إلى اعتلال في وظائف عدد من أعضاء الجسم بسبب زيادة نسبة الحديد أو نتيجة الآثار الجانبية لعلاج إزالة

الحديد الزائد من الجسم. إن الدراسة الحالية قد وجهت لتقييم مدى التغييرات في فحوصات وظائف الكلية الاعتيادية لد ى مرضى الثلاسيميا نوع بيتا وتأثرها بعلاج المرض . وتتضمن الدراسة مجموعتان من مرضى الثلاسيميا نوع بيتا . المجموعة (أ) تتكون من 40 مريض وبمدى أعمار 1-4 سنوات، والمجموعة (ب) تتكون من 40 مريض وبمدى أعمار 4-20 سنة، بالإضافة إلى مجموعة الأشخاص الأصحاء (مجموعة الضبط) وهي المجموعة (ج) وتتكون من 40 شخص تتراوح أعمارهم من 1-20 سنة. تم جمع عينا ت الدم ومن ثم فصل مصل الدم لغرض قياس مستوى الكرياتنين واليوريا والحديدين في مصل الدم. كذلك، تم جمع عينات البول للمرضى لغرض إجراء فحوصات معدل الزلال البولي. أظهرت الدراسة قلة معنوية في معدل مستوى الكرياتنين في مصل الدم بالإضافة إلى زيادة معنوية في معدل مستوى اليوريا لدى المجموعة (ب) من مرضى الثلاسيميا بمقارنتها مع مجموعة الضبط (المجموعة (ج) الخاصة بها). كما أظهرت عدم وجود اختلافات معنوية في معدل الزلال البولي. تبين نتائج الدراسة الحالية عدم وجود اضطرابات ظاهرية في وظائف الكلية لدى مرضى الثلاسيميا نوع بيتا بالاعتماد على مستويات الكرياتنين واليوريا في مصل الدم بالإضافة إلى معدل الزلال البولي. مع ذلك فانه من المتوقع وحسب النتائج ، وجود تغييرات طفيفة وغير ظاهرة سريريا في وظائف الكلية لدى هؤلاء المرضى ، وبذلك ينصح بمتابعة وظائف الكلية لدى مرضى الثلاسيميا عن طريق إجراء فحوصات اشمل وأدق لوظائف الكلية.

Introduction

Thalassemia, is an important health problem especially in endemic areas like the Mediterranean and the Middle east countries. It is an autosomal inherited genetic disorder in hemoglobin synthesis that result in a deficient or limited production of the globin chain subunits of hemoglobin. 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 characterized by impaired beta globin chain synthesis [1, 2, 3]. β-thalassemia major, was described in 1925 by Cooley and Lee, is a life threatening anemia which is characterized by ineffective erythropoiesis, bone marrow expansion, and increase destruction of defective red blood cells (RBCs)[4, 5]. The resultant anemia and other complications can be corrected with repeated regular blood transfusion program [6]; unfortunately, such blood transfusion program will exert its own problems concerning iron overload [7]. The iron overload may have adverse effects on several organs including heart, liver, endocrine glands, lungs, and kidneys [4, 5, 8]. Deferroxamine, an iron chelating agent given by subcutaneous infusion is the standard iron chelating agent used since the late 1960s [6, 9, 10]. Many researchers studied the different complications that arise from the disease and its treatment; however, there are only a few studies on the effects of thalassemia and deferroxamine on renal functions [7, 11]. Renal damage can be attributed to chronic anemia, iron over load and deferroxamine therapy [12]. The aim of present study is to evaluate the possible changes of conventional renal function tests in transfusion dependent β-thalassemia patients and in those taking deferroxamine as iron chelator.

Patients & methods

A total of 80 patients of age ranged 1-20 years old, all are transfusion dependent β-thalassemia patients attending the thalassemia center in Ibn-Alatheer teaching hospital were enrolled in this study, since the 4th of December 2010 to the 1st of June 2011. The patients were diagnosed as β-thalassemia major depending on hemoglobin (Hb) variant test by Hb electrophoresis. The patients were divided into two groups:

1- Group A: 40 transfusion dependent β-thalassemia patients not using deferroxamine iron chelator with age range 1-4 years old.
2- Group B: 40 Transfusion dependent β-thalassemia patients all receiving deferroxamine iron chelator with age range 4-20 years old.

- ***Control (group C):*** consist of 40 non-thalassemic individuals all are apparently healthy with age range 1-20 years old for comparison. This group was divided into two subgroups according to the age range and for convenience in comparison:
    - Group C1: involve 10 subjects with age range 1-4 years old.
    - Group C2: involve 30 subjects with age range 4-20 years old.

Methods

- Venous blood samples: about 5 ml were collected from each individual of the studied groups in plain tubes. The tubes are placed in a water bath at 37°C for 15 minutes for blood clotting to occur. Serum samples were obtained by centrifugation of blood at 4000 rpm for 10 minutes. The serum was divided and placed in 1 ml eppendroff tubes then freezed at -20°C.
- Urine samples: a fresh morning urine sample was collected from each individual, then centrifuged at 3000 rpm for about 10 minutes and the supernatant is placed in 10 ml plain tube. Urine samples were frozen at -20°C to be tested for microalbuminuria.

All the biochemical analysis was performed at the laboratory of higher studies in the department of biochemistry, College of medicine, University Of Mosul, Mosul, Iraq. The selection of reagents used in this study was based on accuracy, reliability, availability, and were purchased as kits. Serum creatinine was measured by Jaffe reaction method [(13)], using a kit supplied by Randox laboratories (UK), by means of UV-VIS spectrophotometer (PD-303 UV, Japan). Serum urea was measured by enzymatic method [(14)], using a kit supplied by Biomerieux (France). Serum ferritin was measured by an enzyme linked assay method [(15)] using a kit supplied by Biomerieux (France), measured automatically with Minividas, Biomerieux (France). Urine microalbuminuria was measured by Micral test, according to Sacks and Bruns[(16)], using a strips supplied by Roche company (Germany). The 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, ANOVA test, in addition to Pearson correlation [(17)]. The statistical results were considered significant at $P \leq 0.05$ [(18)].

(*) significant difference exists at level 0.05 degree of significance.

(**) significant difference exists at level 0.01 degree of significance.

Results

For comparative purpose, the results of the present study were classified as follows:

- According to different age ranges :
    - Group A (≤ 2 years old) and (> 2 years old).
    - Group B (< 10 years old and ≥ 10 years old).
- According to serum ferritin levels:
    - Group A (≤ 2000ng/ml, from 2000-3500ng/ml, and > 3500ng/ml).
    - Group B (< 4000ng/ml, from 4000-6000ng/ml, and > 6000ng/ml).

The results of the present study showed no significant differences in the mean serum levels of urea and creatinine in group A when compared with group C1. Whereas, a significant difference was seen in the mean serum level of ferritin between group A and Group C1 (table 1).

The mean serum level of creatinine in group B showed a significant reduction (P≤0.01) compared to group C2. In contrast, the mean serum urea in group B was significantly increased (P≤0.01) when compared with group C2. Serum ferritin was also significantly increased (P≤0.01) compared to group C2 (table 2). A significant increase (P≤0.05) in the mean serum level of creatinine, plus a significant increase (P≤0.01) in the mean serum ferritin were observed when the results were compared between different age ranges of group B patients (table 4). A significant increase in the mean serum creatinine was observed in between subgroups B (group B) according to different ranges of serum ferritin (table 6). The results of the present study showed no significant difference in the level of urinary microalbumin when a comparison was done between group A and group C1 and between group B and group C2.

Table (1):- differences between group A and control subjects (group C1).

Parameters	***Mean ± SD***		***P-value***
	Group A N=40	***Group C1 N=10***	
Creatinine µmol/l	44.54±8.95	49.14±9.94	NS
Urea mmol/l	4.24±1.3	4.14±1.4	NS
Ferritin ng/ml	2669.47±1667.7	60.19±53.8	0.01**

Table (2):- differences between group B and control subjects (group C2).

Parameters	***Mean ± SD***		***P-value***
	Group B N=40	***Group C2 N=30***	
Creatinine µmol/l	47.29±15.16	68.59±13.48	0.01**
Urea mmol/l	5.42±1.56	4.54±1	0.01**
Ferritin ng/ml	5101.71±2394.3	43.49±47	0.01**

Table (3):- Show the differences between different age ranges of group A in regard to serum levels of creatinine, urea, and ferritin.

Parameters	***Mean ± SD***		***P-value***
	≤2 years old N= 24	***>2years old N=16***	
Creatinine µmol/l	42.78 ± 8.79	47.29 ± 8.76	NS
Urea mmol/l	4.22 ± 1.36	4.28 ± 1.24	NS
Ferritin ng/ml	2169.26±1218.36	3123.48±1411.9	0.05*

Table (4):- Show the differences between different age ranges of group B in regard to serum levels of creatinine, urea, and ferritin.

Parameters	*Mean ± SD*		*P-value*
	< 10 years old N=20	*≥ 10 years old N=20*	
Creatinine µmol/l	41.97 ± 12.64	52.88 ± 15.84	0.05*
Urea mmol/l	5.14 ± 1.41	5.71 ± 1.68	NS
Ferritin ng/ml	3644.52±1341	6558.91±2343.26	0.01**

Table (5):- comparison of creatinine and urea in regard to different ranges of serum ferritin in group A patients.

Parameters	*Mean ± SD*			*P-value*
	≤ 2000 N=15	*2000-3500 N=15*	*≥3500 N=10*	
Creatinine µmol/l	41.7 ± 5.4	46.27 ± 12.4	46.38 ±6.6	NS
Urea mmol/l	3.74 ± 1.18	4.58 ± 1.52	4.54 ± 0.9	NS
Ferritin ng/ml	1243 ± 551	2694 ±411	4913 ± 1543	0.01**

Table (6):- comparison of creatinine and urea in regard to different ranges of serum ferritin in group A patients.

Parameters	*Mean ± SD*			*P - value*
	< 4000 N=12	*4000-6000 N=16*	*> 6000 N=12*	
Creatinine µmol/l	38.8 ± 11.21	48.7 ± 17.2	53.8 ± 11.15	0.05*
Urea mmol/l	4.69 ± 1.4	5.96 ± 1.6	5.25 ± 1.2	NS
Ferritin ng/ml	2541 ±930	4783 ± 633	8192 ± 1758	0.01**

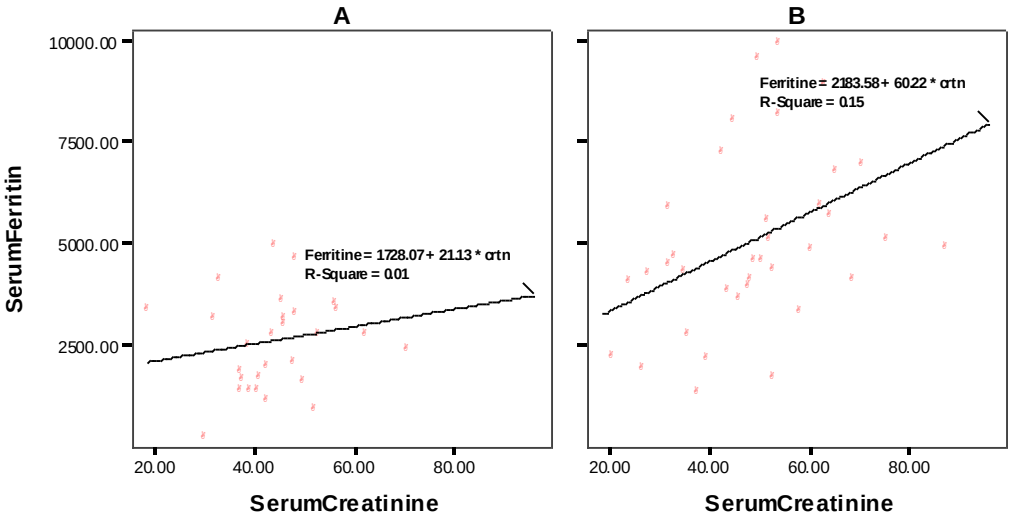

Figure (1):- correlation between serum creatinine and the level of serum ferritin in group A and B β-thalassemia patients.

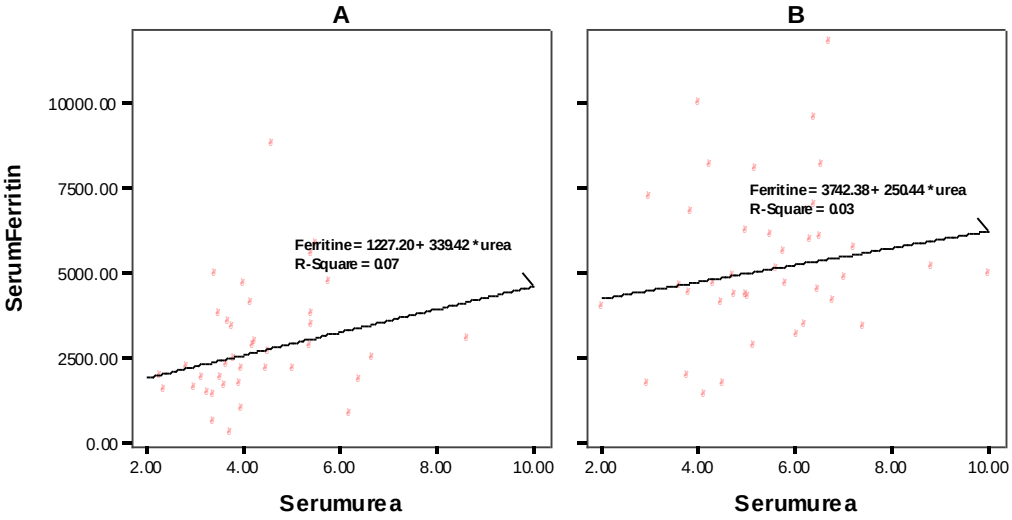

Figure (2):- correlation between serum urea and serum ferritin levels in groups A and B of β-thalassemia patients.

Discussion

The significant decrease in the mean serum creatinine level may be related to the lower body mass index, due to growth retardation and lower muscle mass, usually encountered in β-thalassemia patients [19]. This result was in agreement with that stated by Jafari, who found a significant decrease in the level of serum creatinine in β-thalassemia patients compared to control healthy subjects, and concluded that there was no evidence of renal tubular and glomerular damage in β-thalassemia patients as demonstrated by the results of the study (20). The result of the present study demonstrated a progressive significant increase in the mean level of serum creatinine with increased age of the patients in between subgroups B (group B), and according to different ranges of elevated serum ferritin. However, the mean serum creatinine level still within normal range. The increased level of serum creatinine up to normal level may prove that treatment of β-thalassemia patients with blood transfusion and iron chelating therapy provide the chance for normal growth with increasing body mass index[21]. These results may also indicate that some deterioration in glomerular functions regarding creatinine filtration might be expected in these individuals [22]. Finally, this work showed that the reduction of iron overload by iron chelating therapy may be a factor contributing to changes in serum creatinine level, this results is in accordance with that of Ponticelli *et al*, [23]. The mean serum urea was within normal range in both groups of β-thalassemia patients (A and B). However, when group B was compared with its control group, it showed a significantly increased level ($P \leq 0.01$). Any how, it seems that no apparent deterioration in glomerular function regarding the filtration of urea was seen in β-thalassemia patients. These results are in agreement with other studies [20, 24]. The significantly higher mean serum ferritin levels seen in β-thalassemia patients of groups A and B when compared with group C (C1 and C2 respectively), can be attributed to the repeated blood transfusion regimens in these patients[9]. In addition, the mean serum ferritin showed a significant increase with increased age of the patients in between subgroups in both groups A and B; this can be caused by the poor compliance of patients to deferroxamine iron chelating therapy[9]. The results of the present study indicate no significant changes in the level of microalbuminuria between β-thalassemia patients and their corresponding control subjects. It seems that no apparent deterioration in glomerular function was observed in β-thalassemia patients concerning the levels of proteinuria. In this concern the results was in agreement with Mula-Abed[24], who studied the indicators of glomerular and tubular function in β-thalassemia patients, & he concluded that normal level of microalbuminuria beside other parameters had reflected the intact renal function in these patients. In conclusion, no changes in renal function tests were observed in both groups of β-thalassemia patients (A and B) as indicated by the normal levels of serum urea and creatinine in addition to microalbuminuria. Monitoring of renal functions are recommended to be performed using more specific and early markers of renal dysfunction.

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