

Earlier detection of glomerular dysfunction in β -thalassemia major patients

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Abstract

Chronic transfusions program in β -thalassemia patients will inevitably lead to iron overload with a significant morbidity and mortality. Glomerular filtration rate (GFR) is progressively declined in relation to iron overload as well as chronic anemia. Objective is to define levels of Cystatin C in transfusion dependent β -thalassemia major patients as a sensitive marker for detection of earlier glomerular dysfunction in addition to understand the effect of iron overload, chelating therapy and hepatitis infection. A cross sectional study conducted at Al-Basrah Hemoglobinopathy Centre for the period from September 2017 to January 2018 to enroll 75 β -thalassemia major patients. Data collected included duration of the disease, total transfusion requirement, details of chelation therapy and its therapeutic index. In addition to blood urea, serum creatinine and Cystatin C with estimated GFR (eGFR). The mean Cystatin C was 1.075 mg/L where 66.6% of patients had abnormal renal function which is higher proportion than those with renal (42.6%) detected according to serum creatinine level. Cystatin C was significantly higher in patients who received desferrioxamine as compared to those received deferasirox ($P=0.007$), in accordance with GFR which is significantly higher in patients receiving the latter chelation therapy ($P=0.009$). A significant inverse relationship between Cystatin C, and GFR, while positive relationship between ferritin and Cystatin C ($P=0.0001$, 0.001 respectively). Cystatin C is better for detection and monitoring of glomerular dysfunction in β -thalassemia major patient which is already not uncommon complications for the disease and iron chelation therapy.

Introduction

β -thalassemias are a group of autosomal recessive hereditary hemoglobinopathies,

characterized by quantitative deficiency of beta-globin chains, ineffective erythropoiesis, and anemia.¹

In Iraq, the prevalence of thalassemia had increased from 33.5/100,000 in 2010 to 37.1/100,000 in 2015, while the incidence rate had decreased from 72.4/100,000 live births to 34.6/100,000 live births between 2010 and 2015.²

Chronic transfusions program will inevitably lead to iron overload. The cumulative effects of iron overload lead to significant morbidity and mortality, if untreated.³

When serum transferrin saturation exceeds 70%, free iron species - have been found in the plasma. These iron species are mainly responsible for generating reactive oxygen species with eventual tissue damage, organ dysfunction, and death.⁴

Iron chelation therapy has proven to be the only option to reduce morbidities and prolong survival into the fourth and fifth decades of life.⁴

Chronic anemia and hypoxia as well, are associated with oxidative stress and lipid peroxidation that are correlated to functional abnormalities in tubular cells of renal system.⁵

A negative correlation between serum ferritin levels and glomerular filtration rate (GFR), has been reported in patients with β -thalassemia major.⁶ Additional factors that contribute to a decreased GFR in these patients include transfusion-related hepatitis B or C virus or HIV infections leading to glomerulonephritis, as well as iron-induced hepatic and cardiac dysfunction.⁷

Serum creatinine is less reliable for making therapeutic decisions in critically ill patients.⁸

Serum Cystatin C is a non-glycosylated, 13.3-kDa protein belonging to Cystatin C protease inhibitors. It is produced at a constant rate by all nucleated cells, freely filtered by the glomeruli, minimally bound to proteins, and totally reabsorbed and metabolized in the proximal tubule.⁹ It represents an early indication of renal impairment, possibly superior to serum creatinine.⁸ Concentration of serum cystatin C is not affected by gender, age, race, protein intake, and muscle mass, unlike serum creatinine. When GFR decreases, cystatin C level begins to rise proportionately.¹⁰ Confidence in the use of Cystatin C-based eGFR has increased since the 2012 Kidney Disease Improving Global Outcomes (KDIGO) guideline was published; results of a large meta-analysis show that Cystatin C-based eGFR detects increased risk of adverse outcomes that is not detected by creatinine-based eGFR.¹¹

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Objectives

The objectives were: i) to define levels of Cystatin C in transfusion dependent β -thalassemia major patients as a sensitive marker for detection of earlier glomerular dysfunction in relation to estimated GFR; ii) to evaluate the effect of iron overload, chelating therapy and hepatitis infection on Cystatin C level.

Materials and Methods

A cross sectional study conducted at Al-Basrah hemoglobinopathy Centre for the period from September 2017 to January 2018. Seventy-five β -thalassemia major patients were enrolled in the study, and assessed during their regular follow up for iron chelation therapy and blood transfusions. They had no clinical or laboratory evidence of renal impairment at time of

study. Verbal consents had been taken from all patients under approval of IRB rules.

Those who known to have renal failure, heart failure, longstanding diabetes mellitus, active infection or malignancy were excluded. For each patient, both demographic and clinical data were recorded like - age, sex, body mass index, duration of the disease, total transfusion requirement, type of chelation therapy, the dose and its therapeutic index, in addition to hepatitis C, B virology status. Therapeutic index calculated according to Daily dose (mg/kg) / current serum ferritin ($\mu\text{g/L}$).¹² At same time other laboratory parameters were registered like (Hemoglobin level, blood urea, serum creatinine, ferritin and Cystatin C). Estimated glomerular filtration rate (eGFR) was calculated by using CKD-EPI Cystatin and Creatinine 2012 equation adjusted for age, sex, and race.¹³

$$\text{eGFR} = 177.6 \times \text{SCr}^{-0.65} \times \text{CysC}^{-0.57} \times \text{age}^{-0.20} \times 0.80 \text{ (if female)} \times 1.11 \text{ (if African American)}$$

eGFR (estimated glomerular filtration rate) = mL/min/1.73 m²

SCr (serum creatinine) = mg/dL

SCys (standardized serum cystatin C) = mg/l, age = years.

Pretransfusion hemoglobin level between (9.5-10.5 g/dL) was arbitrarily considered achievable target for TM patients,¹² other parameters were considered normal according to the following cutoffs: serum creatinine (50-110 $\mu\text{mol/L}$ or 0.6-1.2 mg/dL), blood urea (4-8.2 $\mu\text{mol/L}$ or 24-50 mg/dL),¹⁴ and the patients were grouped according to serum ferritin into two groups, >1000 $\mu\text{g/L}$, <1000 $\mu\text{g/L}$, on the bases that it is the target level to start chelation therapy.¹²

Renal dysfunction was defined as eGFR <90 mL/min/1.73 m², and/or abnormally elevated serum Cystatin C according to the reference ranges (mg/L) for age ≤ 50 year (male: 0.31-0.79 and female: 0.40-0.99) and for age >50 year (for both :0.40-0.99).¹⁵

Data were analyzed with IBM SPSS statistical package for windows version 23 (SPSS Inc. Chicago, Illinois, USA). They expressed as mean \pm standard deviation (SD), while categorical data were expressed as frequencies and percentages. Chi-square test was used to study the correlation between the variables, and T-test for testing the significant differences P value of ≤ 0.05 is considered significant, and P value < 0.001 is considered highly significant.

Results

The female: male ratio of (1.08:1), their age ranged between 16-43 year with mean age 21.9 ± 6.1 year. The mean transfusion requirement was 23.5 units/year ranged between (12-34), over their disease durations that ranged between (8-35) years as in Table 1.

Thirty-five patients (47%) were receiving desferrioxamine (desferal®), while the remaining 40 patients (53%) were kept on deferasirox (Exjad®) with dose of (20-40 mg/kg) and (30-40 mg/kg) per day respectively. Nearly all patients who receiving desferrioxamine, had an expected therapeutic index < 0.025 .

The laboratory data which are shown in Table 2 reveal that all patients had apparent-

Table 1. Demographic data of β -thalassemia major.

Variable	Frequency or range	Mean \pm SD
Age (year)	16-43	21.9 \pm 6.1
	Male	16-43
	Female	17-34
Transfusion requirement (units/year)	12-34	23.5 \pm 5.8
	Male	12-34
	Female	13-30
Disease duration (years)	8-35	15.8 \pm 5.5
	Male	10-35
	Female	8-22
Type of chelation (number of patients)		
Desferrioxamine No. (%)	35 (47%)	-
Deferasirox No. (%)	40 (53%)	

Table 2. Laboratory data.

Variable	Range	(mean \pm SD)
Hemoglobin(g/dL)	5.4-10.3	7.7 \pm 0.9
	Male	5.6-9.7
	Female	5.4-10.3
Blood urea ($\mu\text{mol/L}$)	4-6.2	5.50 \pm 4.4
	Male	4-6.1
	Female	4-6.2
Creatinine ($\mu\text{mol/L}$)	60-98	77.30 \pm 7.5
	Male	63-98
	Female	62-93
Ferritin ($\mu\text{g/L}$)	540-7600	2111 \pm 1784
	Male	1000-7600
	Female	540-7000
Cystatin C (mg/L)	0.64-1.99	1.07 \pm 0.26
	Male	0.64-1.98
	Female	0.64-1.99
GFR (mL/min/1.73m ²)	53-115	91.3 \pm 14
	Male	58-115
	Female	53-115

Table 3. Relationship of laboratory data with different lines of treatment.

Biochemical marker	Line of treatment	No.	Mean \pm SD	P value
Bloodurea ($\mu\text{mol/L}$)	Deferasirox	39	4.94 \pm 0.6	0.43
	Desferrioxamine	36	5.06 \pm 0.7	
Creatinine ($\mu\text{mol/L}$)	Deferasirox	39	76.64 \pm 8.3	0.09
	Desferrioxamine	36	76.84 \pm 6.3	
Cystatin C (mg/L)	Deferasirox	39	0.99 \pm 0.16	0.007*
	Desferrioxamine	36	1.14 \pm 0.28	
GFR (mL/min/1.73m ²)	Deferasirox	39	97.31 \pm 14	0.009*
	Desferrioxamine	36	87.78 \pm 15	

*P value of < 0.05 is considered significant.

ly normal renal functions, with lower GFR in female than in male, the minimum GFR was 53 mL/min/1.73 m², and maximum 115 mL/min/1.73 m². Thirty-two patients (42.6%) had impaired renal function according to eGFR <90 mL/min/1.73 m².

The mean Cystatin C was 1.075 mg/L ranged between (0.64-1.99). Fifty patients (66.6%) had abnormal level indicating that there were higher proportion of patient having an impaired renal function than those detected by calculation of eGFR.

The maximum ferritin level was 7600 µg/L, while the minimum was 540 µg/L. Sixty patients (79.7%) had serum ferritin >1000 µg/L, nineteen patients of them were HCV positive, with statistically significant positive relation between them (P=0.04), as shown in Figure 1.

Cystatin C was significantly higher in patients who received desferrioxamine as compared to those received deferasirox (P=0.007), as shown in Figure 2, in accordance with GFR which is significantly higher in patients receiving the latter chelation therapy (P=0.009), as shown in Table 3.

The results showed significant inverse relationship between Cystatin C, and GFR, while positive relationship between ferritin and Cystatin C (P=0.0001, 0.001 respectively), also there were significant positive relationship between Cystatin C and number of blood transfusion/year, (P=0.001).

No significant correlations were found between Cystatin C, blood urea, serum creatinine, (p value 0.43, 0.09) respectively as well as no significant correlation with sex, BMI (P=0.1, 0.15 respectively), however

there is significant correlation with age (P=0.01).

Discussion

The mechanism of renal dysfunctions in patients with β-thalassemia major is multifactorial, resulted mainly from long-standing anemia, chronic hypoxia, iron overload and desferrioxamine toxicity.^{12,14} The present study - demonstrated that 66.6% of β-thalassemia major patients had abnormally high serum Cystatin C level, which may indicate- that Cystatin C is more sensitive marker for early detection of glomerular dysfunction.

It is also shown that there was no statistically significant relation between Cystatin C level, blood urea and serum creatinine (P=0.43, 0.09) respectively, and that is why the routine investigation can't be more reliable parameter for assessment of renal function in case of use nephrotoxic medication in Thalassemia Major patients.

Cystatin C has characteristic independency on BMI and sex (P=0.1, 0.15 respectively), which makes it superior on serum creatinine, which is in agreement with the results of Banfi G *et al.*¹⁶ study, and Hannemann A *et al.*¹⁷ Cystatin C level did not correlate statistically with sex, BMI. These results were compatible with Papassotiriou *et al.*¹⁸ results, but contrasted with Groesbeck *et al.*¹⁹ who found statistically significant differences between genders, with men having higher Cystatin C levels than women.

A significant correlation found between cystatin C and age in this study (P=0.01), in contrast with Papassotiriou *et al.*,¹⁸ who assumed that there is no significant relation between Cystatin C and age, There is an inevitable decline in renal function with age even in healthy people, due progressive loss of nephrons with aging, and Cystatin C is freely filtered by the glomeruli, and totally reabsorbed by proximal tubules, hence it's level increased with age. This significant relationship was supported also by Groesbeck D *et al.*¹⁹ and Delanaye P *et al.*²⁰

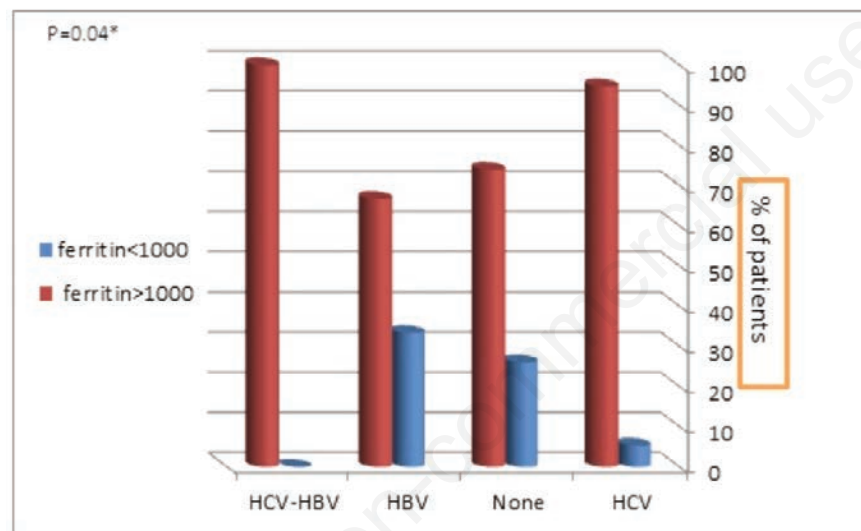


Figure 1. Diagrammatic representation of virology status versus ferritin level.

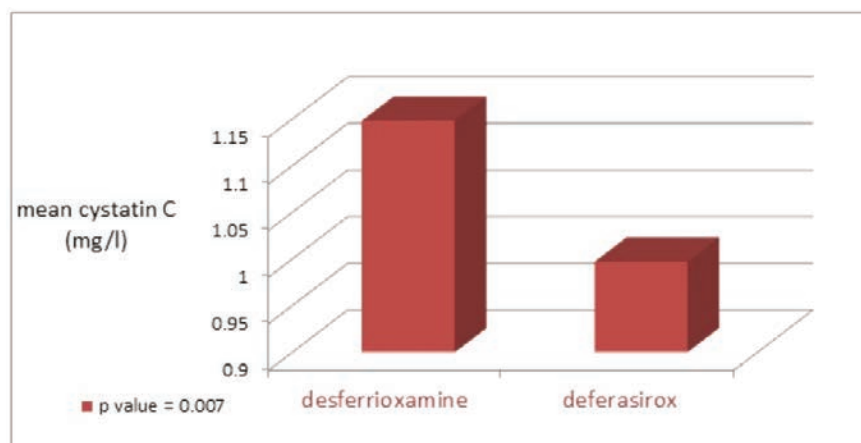


Figure 2. Mean cystatin C level according to line of treatment.

Conclusions

The results showed highly significant inverse relationship between Cystatin C and GFR (P<0.000), despite normal range of conventional biochemical parameters (blood urea and serum creatinine), the results were coming in agreement with Ali and Mahmoud *et al.*²¹ These results support the idea that cystatin C gives a good estimate of

GFR, more accurate and precise than other methods.²¹ This significant decline in eGFR over time suggesting that tubular damage acquired in childhood caused by either TM or its treatment may eventually result in abnormal eGFR.²²

Positive highly significant relationship were found in the present study between Cystatin C and serum ferritin, the transfusion requirement(units/year), and disease duration (years) ($P < 0.001$ for all), that may indicate the effect of iron overload in increasing the risk of renal impairment. These results are in accordance with Papassotiriou *et al.*,¹⁸ Ali and Mahmoud *et al.*,²¹ and Eliana Lai.²²

Regarding the type of chelation therapy, Cystatin C level significantly higher in those kept on desferrioxamine ($P = 0.007$), as compared to deferasirox chelation therapy, in accordance with GFR that is significantly higher in patients receiving the later ($P = 0.009$), and this is expected as the former chelation therapy is mainly renal excreted, and overdosing is predicted possibly because of administration pump malfunction and inadequate dosage monitoring.²³

In conclusion it can be recommended to use Cystatin C for earlier detection and follow up of glomerular dysfunction in B thalassemia major patient as it is an accurate, sensitive endogenous marker keeping in mind that this complication is not an uncommon in those patients that may aggravated by iron overload and iron chelation therapy.

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