SERUM CYSTATIN-C VERSUS URINARY ALBUMIN CREATININE RATIO AS AN EARLY INDICATOR OF KIDNEY DYSFUNCTION IN CHILDREN AFFECTED BY B-THALASSEMIA MAJOR

By

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ABSTRACT

Background: *β-thalassemia is one of the most common hereditary diseases in Egypt. The leading causes of renal dysfunction in thalassemic patients include chronic anemia, iron overload from repeated blood transfusions, and kidney-induced damage from the use of iron chelators.*

Objective: *We aimed to investigate the association of serum cystatin-C in children with β-thalassemia major and albumin/creatinine (A/C) ratio and to assess its accuracy as an early indicator of nephropathy.*

Methods: *This case-control study enrolled 41 children diagnosed as β-thalassemia major on chelation therapy and 41healthy, age- and sex-matched controls. The kidney function was assessed using blood urea and serum creatinine to calculate the glomerular filtration rate (eGFR) by Schwartz formula, A/C ratio, and serum cystatin-C.*

Results: *We found that 26.8 % of β-thalassemia patients had kidney dysfunction (eGFR below 90 mg/min/1.73 m2). These patients were significantly different from thalassemic patients with normal eGFR regarding the frequency of blood transfusion (P-value < 0.0001), duration of chelation therapy (P-value = 0.002), blood urea (P-value = 0.001), serum creatinine (P-value < 0.0001), A/C ratio (P-value < 0.0001), serum cystatin-C (P-value = 0.009), and eGFR (P-value < 0.0001).*

Conclusion: *Serum cystatin-C might be a good diagnostic test for early detection of glomerular dysfunction like A/C ratio and may precede it.*

Key word: *albumin/creatinine ratio; β-thalassemia major; cystatin-C; kidney dysfunction.*

INTRODUCTION

Thalassemia is a genetic disease characterized by quantitative disorder of hemoglobin resulting in an imbalance between the alpha and β chains. The defect in the alpha and β globin genes causes an imbalance between both chain types resulting in ineffective erythropoiesis and anemia. The degree of mutations in the β-globin genes determines the phenotypic expression of patients as being either thalassemia intermedia or thalassemia major (TM). However, the degree of insufficiency in erythropoiesis divides thalassemic patients into transfusion-dependent and non-transfusion-dependent **(Taher et al., 2018)**.

Significant morbidity and mortality in thalassemia patients may result from excess iron deposition in different body tissues. Several factors are responsible for the iron overload including chronic transfusion and increased gastrointestinal iron absorption because of ineffective erythropoiesis **(Nemeth, 2010)**. Enhanced survival of thalassemia patients allowed the previously unrecognized renal complications to emerge. Therefore, it becomes essential to identify early predictors of renal dysfunction in young patients with β-thalassemia major to provide them with early management **(Borgna-Pignatti et al., 2004)**.

Renal impairment was found to be less severe in patients on hyper transfusion and iron chelation therapy, suggesting that kidney injury might be caused by the anemia and the iron-induced oxidative damage **(Ponticelli et al., 2010)**.

Also, there is an increase in renal plasma flow caused by glomerular hyper filtration and renal hyper perfusion as a result of anemia. However, a significant decrease in glomerular filtration rate (GFR) will occur with time and persistent anemia. Therefore, anemia leads to an alteration in renal hemodynamics disturbing renal plasma flow and GFR **(Ponticelli, 2012)**.

Cystatin-C is a low-molecular-weight, non-glycosylated protein synthesized and secreted by all human nucleated cells. It is a sensitive biomarker for GFR as it is neither secreted nor reabsorbed by the renal tubules. It is not affected by height, sex, diet, or muscle mass. Therefore, relative to creatinine clearance, it is considered a better indicator of renal function **(Tanaka et al., 2007)**.

This study was conducted on children with β-thalassemia major in Fayoum Governorate to investigate the association of serum cystatin-C with albumin/creatinine (A/C) ratio and to assess its accuracy as an early indicator of nephropathy.

METHODS

Ethical considerations:

1. The study was approved by the Ethics Committee of the Faculty of Medicine, Fayoum University, Egypt.
2. Informed, verbal consents were obtained from the participants or their legal guardians.
3. All the data of the study is confidential and the patients have the right to keep or withdraw from study at any time,
4. The authors declared no potential conflict of interest with respect to the research, authorship and /or publication of the article,
5. No financial disclosure regarding the study or publication.

Sample size:

Sample size was calculated using (G power version 3.1.9.4). Minimal sample size of patients was 40 in each group needed to get power level of 0.80, alpha level of 0.05 and medium effect size of 0.64 in serum cystatin-C between the two groups.

Study design:

This case control study was conducted at the Pediatric Department of Fayoum University Hospital between April 2019 and April 2020. The study included 82 children who were enrolled into two groups: the thalassemia (41 children (diagnosed as β-thalassemia major) and the control (41 age and sex matched, healthy children) groups and the studied groups were selected by simple random method.

Eligibility criteria:

The study recruited male and female children (3-15 years-old) with β-thalassemia major who had been on regular blood transfusion. We excluded patients who had another type of hemoglobinopathy (e.g., sickle cell anemia), primary renal disease, or type-1 diabetes mellitus as well as those using diuretics or anti-epileptic drugs.

Procedures:

All the study participants were subjected to:

I. Full History taking with stress on age of first transfusion; frequency of transfusion; type, dose, complication, duration, and compliance to chelation therapy; history of

splenectomy; and symptoms of renal insufficiency.

II. Complete clinical examination including anthropometric measures (height, weight, and body mass index were obtained and plotted on Egyptian Growth Chart); general physical examination with stress on blood pressure (measured and plotted against its levels for boys and girls by age and height percentile), general look, presence of jaundice or pallor, and clinical evidence of iron overload (e.g., skin hemosiderosis); and abdominal examination to detect hepatomegaly, splenomegaly, or the scar of splenectomy.

III. Laboratory investigations included complete blood picture using sysnex xn 1000 device, methode: electrical impedence, laser light scattering and dye bonding. Serum levels of ferritin, urea, creatinine, electrolytes (sodium, potassium, phosphorus). Serum cystatin-C was determined using Elisa plate reader statfax chromate 4300 (USA) **Ramazan et al., 2018**; and fresh morning midstream urine sample for A/C using Gem premier device. An A/C value between 30 and 299 mg/g was defined as microalbuminuria, and a value of 300 mg and over was defined as macro albuminuria **(liu et al., 2018)**.

The glomerular filtration rate (eGFR) was estimated using Schwartz formula: eGFR (mL/min/1.73 m2) = 0.55 x (height [centimeters]/serum creatinine [mg/dL]) (Schwartz and Work 2009). Renal dysfunction was graded according to K/DOQI guidelines as shown in table 1 **(Hogg et al., 2003)**.

Table (1): K/DOQI stages of chronic kidney disease

Stage	Description	GFR (ml/min/1.73 m2)
1	Kidney damage with normal or increased GFR	≥ 90
2	Kidney damage with mild decrease in GFR	60–89
3	Moderate decrease in GFR	30–59
4	Severe decrease in GFR	15–29
5	Kidney failure	<15 (or dialysis)

RESULTS

Our study results are demonstrated in the following tables and figures

Table (2): Socio-demographic features of cases and controls

Variable	Cases (N=41)		Control (N=41)		P-value
	Mean	SD	Mean	SD	
Age (year)	9.1	2.5	9.4	2.1	0.518
Variable	**N**	**%**	**N**	**%**	**P-value**
Gender					
Male	23	56.1%	21	51.2%	0.582
Female	18	43.9%	20	48.8%	
Residence					
Rural	20	48.8%	17	41.4%	0.612
Urban	21	51.2%	24	58.6%	
Consanguinity					
+ve	25	61.0%	20	48.8%	0.02
-ve	16	39.0%	21	51.2%	

This table shows no significant differences between cases and controls as regarding age, gender or residence but shows statistically significant difference as regarding positive consanguinity (P-value = 0.02).

Table (3): Clinical manifestations in patients group

Clinical findings	N	Percentage
Pallor	40	97.6 %
Mongoloid face	29	70.7 %
Clubbing	3	7.3 %
Jaundice	40	97.6 %
Skin hemosiderosis	6	14.6 %
Splenomegaly	29	70.7 %
Hepatomegaly	41	100%

As shown in the previous table examination of the patients group, hepatomegaly was present in all patients show, followed by pallor, jaundice and mongoloid face.

Table (4): Laboratory findings of studied groups

	Cases (N=41)	Control (N=41)	P-value
	Mean ± SD	Mean ± SD	
Pre-transfusion hemoglobin (g/dl)	6.7 ± 1.3	11.2 ± 1.1	<0.0001*
Serum ferritin (µg/dl)	1818.8 ± 929.1	39.8 ± 31.9	<0.0001*
White blood cells	$9.1x10^3$ ± 7	5.6×10^3 ± 1	0.002*
Platelets	480.8 ± 152.3	335 ± 58.5	<0.0001*
Blood urea (mg/dl)	26.2 ± 6.7	19.6 ± 3.4	<0.0001*
Serum creatinine (mg/dl)	0.6 ± 0.1	0.5 ± 0.1	0.085
Albumin/creatinine ratio (mg/g)	39.1 ±17.1	9.5 ± 0.9	<0.0001*
Serum cystatin-C (ng/ml)	1.5 ± 0.5	0.4 ± 0.1	<0.0001*
Glomerular filtration rate (ml/min/1.73)	98.7 ± 16.4	106.6 ± 8.0	0.008*

*Significant

This Table shows significant differences between cases and controls regarding all laboratory data.

Table (5): The percentage of renal symptoms and asymptomatic chronic kidney disease among the thalassemic patients

Renal symptoms	N	Percentage
No	38	92.7 %
Polyuria	3	7.3 %
Chronic kidney disease grade II		
Yes	11	26.8 %
No	30	73.2 %

This table shows that 92.7 % of the cases have no renal symptoms in the form of oliguria, edema, hypertension, or renal dialysis but only 7.3 % complain of polyuria. By calculating eGFR of the thalassemic patients, 26.8 % of the cases appeared to have chronic kidney disease (CKD) grade II according to K/DOQI guidelines 2003.

Table (6): Comparison between disease characteristics of thalassemic patients with normal glomerular filtration rate and those with chronic kidney disease grade II.

Chronic kidney disease grade II / Disease characteristics	Yes (N=11)		No (N=30)		P-value
	Mean ± SD		Mean ± SD		
Age of first transfusion (month)	9.9 ± 12.8		8.4 ± 7.2		0.636
Interval between consecutive transfusions (day)	22.8 ± 4.6		34.4 ± 8.1		<0.0001*
Duration of chelation therapy (year)	3.4 ± 0.8		2.8 ± 1.8		0.002*
Iron chelator type					
Deferoxamine	0	0.0 %	3	10.3 %	0.158
Deferasirox	8	72.7 %	24	82.8 %	
Deferasirox + deferiprone	0	0.0 %	1	3.4 %	
Deferiprone	3	27.3 %	2	6.8 %	

*Significant

This table shows that thalassemic patients with with chronic kidney disease grade II had significantly shorter intervals between consecutive transfusions (P-value < 0.0001) and longer durations of chelation therapy (P-value = 0.002). The majority of the cases used Deferasirox as an iron chelator.

Table (7): Comparison of laboratory findings of thalassemic patients with normal glomerular filtration rate and those with chronic kidney disease grade II

Chronic kidney disease grade II / Laboratory findings	Yes (N=11)	No (N=30)	P-value
	Mean ± SD	Mean ± SD	
Pre-transfusion hemoglobin (g/dl)	6.8 ± 0.9	6.6 ± 1.4	0.814
Serum ferritin (µg/dl)	21463 ± 978.2	1698.8 ± 897.4	0.175
White blood cells	11.8 $x10^3$ ± 8.8	8.2 $x10^3$ ± 3.8	0.360
Platelets	485.4 ± 173.1	479.1 ± 147.1	0.909
Serum sodium (mmol/dl)	134.5 ± 2.0	134.7 ± 2.3	0.757
Serum potassium (mmol/dl)	4 ± 0.4	3.9 ± 0.4	0.677
Serum phosphorus (mmol/dl)	4.8 ± 0.9	4.6 ± 0.9	0.529
Blood urea (mg/dl)	31.5 ± 6.2	24.3 ± 5.9	0.001*
Serum creatinine (mg/dl)	0.7 ± 0.1	0.5 ± 0.1	<0.0001*
Albumin/creatinine ratio (mg/g)	54.7 ± 20.9	33.1 ± 11.2	<0.0001*
Serum cystatin-C (ng/ml)	1.9 ± 0.6	1.3 ± 0.3	0.009*
Glomerular filtration rate (ml/min/1.73)	77.6 ± 10.7	106.4 ± 10.1	<0.0001*

*Significant

This table shows that thalassemic patients with CKD grade II were significantly different from those with normal eGFR regarding blood urea, serum creatinine, A/C ratio, and eGFR. These patients had also a significantly higher level of serum cystatin-C in (P-value = 0.009).

Table (8): Correlations of albumin/creatinine ratio, serum cystatin-C, and glomerular filtration rate with other parameters in thalassemic patients with chronic kidney disease grade II

	Albumin/creatinine ratio		Serum cystatin-C		Glomerular filtration rate	
	R	P-value	R	P-value	R	P-value
Age (year)	0.116	0.468	0.093	0.564	-0.031	0.847
Age of first transfusion (month)	-0.068	0.674	-0.029	0.855	-0.001	0.995
Interval between consecutive transfusions (day)	-0.419	0.006*	-0.363	0.020*	0.553	<0.0001*
Duration of chelation (month)	0.022	0.891	0.108	0.509	-0.015	0.929
Serum sodium (mmol/dl)	-0.130	0.418	-0.021	0.895	0.179	0.262
Serum potassium (mmol/dl)	-0.018	0.909	-0.073	0.648	0.120	0.455
Serum phosphorus (mmol/dl)	0.254	0.109	0.014	0.932	-0.083	0.606
Blood urea (mg/dl)	0.619	<0.0001*	0.105	0.513	-0.579	<0.0001*
Serum creatinine (mg/dl)	0.685	<0.0001*	0.378	0.016*	-0.835	<0.0001*
Serum ferritin (µg/dl)	0.617	<0.0001*	0.336	0.032*	-0.372	0.017*

*Significant

This table shows that the A/C ratio had a significant negative correlation with the interval between consecutive transfusions but positive correlations with blood urea, serum creatinine, and serum ferritin levels. Serum cystatin-C levels had comparable significant correlations with all the aforementioned parameters except the urea blood level and eGFR. Also, the duration of chelation therapy in the affected patients was significantly higher than the thalassemic patients with normal eGFR.

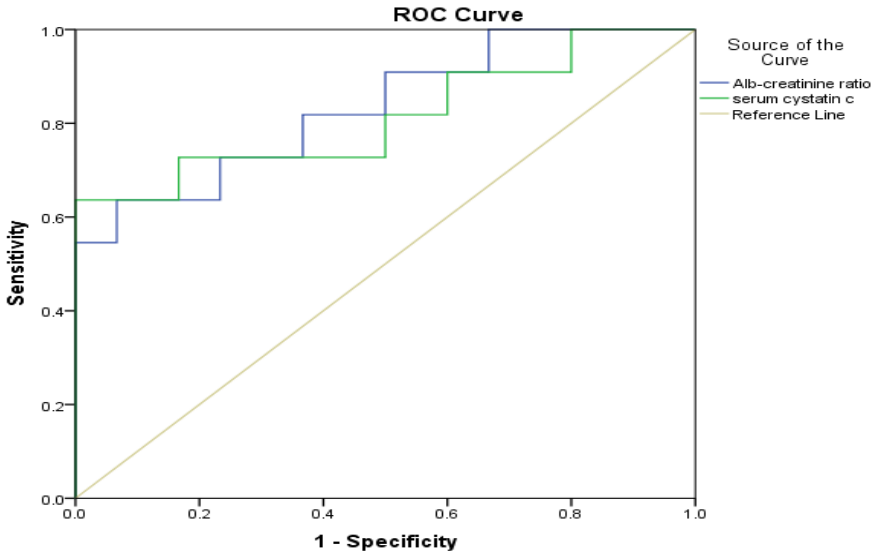

Figure (1): Receiver Operating Characteristic (ROC) curve for Prediction of patients with CKD grade II among the thalassemic patients using serum cystatin C in comparison with urinary albumin creatinine ratio.

Table (9): Sensitivity, Specificity and ROC curve of Albumin/creatinine ratio And Serum cystatin-C in thalassemic patients

Variables	Area under the curve	P-value	Cut-off point	Sensitivity (%)	Specificity (%)
Albumin/creatinine ratio	0.833	0.001	45.61	72.7	93.3
Serum cystatin-C	0.812	0.002	1.57	72.6	83.3

The ROC curve in figure 1 and table 9 revealed that at a cut-off point of 1.57 ng/ml the serum cystatin-c gave 72.6 % sensitivity and 83.3 % specificity with an area under the curve of 0.812, while at a cut-off point of 45.6 mg/g the urinary A/C ratio gave 72.7 % sensitivity and 93.3 % specificity with an area under the curve of 0.833.

DISCUSSION

Cystatin-C is a cysteine protease inhibitor that is produced by all nucleated cells. Cystatin-C is an ideal marker of GFR because its levels are not affected by age, sex, race, or muscle mass contrary to creatinine. In addition, cystatin-C is a reliable marker for acute kidney injury **(Barrera and Bobadilla, 2012)**.

Murty et al., 2013 reported that serum cystatin-C was superior to creatinine for detection of impaired kidney function. Additionally, β-thalassemia patients had a high frequency of glomerular dysfunction, and cystatin-C was a promising marker for monitoring the glomerular function in these patients **(Ali and Sultan, 2014)**.

Renal dysfunction caused by iron overload is characterized by increased ferritin and cystatin-C levels **(Sen et al., 2015)**. Our study showed that serum cystatin-C had a significant positive correlation with serum levels of creatinine and ferritin but a negative correlation with the interval between consecutive blood transfusions and eGFR. Also, the duration of chelation therapy was significantly higher in the affected patients compared to thalassemic patients with normal eGFR.

We observed a significantly high level of serum cystatin-C in patients with CKD grade II. Likewise, **Saghir et al., 2020** reported a highly significant positive correlation between serum ferritin and cystatin-C as compared to creatinine among thalassemic patients as well as a significant negative relationship between cystatin-C and eGFR. These findings indicated the role of cystatin-C in thalassemia major children as an early marker of glomerular dysfunction better than creatinine.

In a cross sectional study conducted by **Permadi et al., 2019** correlations were found between serum ferritin and each of the eGFR and cystatin-C. Also, serum cystatin-C presented a positive correlation with renal function. Also, a strong correlation was found between ferritin and cystatin-C. Serum ferritin and cystatin-C are promising biomarkers to assist monitoring of the renal functions in children with β-thalassemia major. This promotes our result regarding the iron overload as being one of the causes of renal injury.

Contrarywise, Kaçar et al., 2015 found no significant differences between urea and neither creatinine clearance nor cystatin-C. There was no significant relationship between

cystatin-C and levels of creatinine and B-2 microglobulin, which is used as a marker for tubular dysfunction.

Similar to our study, Shimizu-**Tokiwa et al., 2002** demonstrated that serum cystatin-C is more sensitive than serum creatinine and creatinine clearance in various CKD stages, but they didn't compare with the A/C ratio.

In concordance with our result, **Behairy et al., 2017** demonstrated that cystatin-C had higher sensitivity and specificity (91.4 %, and 85.7 %, respectively) than serum creatinine and creatinine clearance (83.0%, 100% and 81.4%, 100%, respectively) for small changes in GFR.

The main limitation of our study is the size of the study group. We had only 11 thalassemic patients with renal impairment. Larger sample size is needed to determine the risk factors of renal dysfunction in thalassemic patients.

CONCLUSION

Serum cystatin-C was found to be very useful as an early predictor of renal glomerular dysfunction in thalassemic patients besides the A/C ratio, which is one of the reliable conventional methods estimating glomerular dysfunction and may precede it.

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