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Impact of Splenectomy and Chelating Agents on Serum Cystatin C Levels in Egyptian Children With Beta-Thalassemia

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Abstract: The outlook for patients with thalassemia has improved in recent decades with the use of modern transfusion practice and iron chelation, but patients continue to be at high risk for iron overload and its toxicities. Cystatin C (CysC) has been suggested as a sensitive marker of glomerular filtration rate providing an early indication of renal impairment, possibly superior to serum creatinine. This study was conducted to observe changes in cystatin C serum concentration in β-thalassemia patients treated with chelating therapy and to determine whether splenectomy could change serum cystatin C concentration. This study recruited 40 pediatrics patients with transfusion-dependent β-thalassemia from Hematology Clinic of Pediatric Hospital, Faculty of Medicine, Cairo University. A control group of 31 age- and sex-matched Egyptian children was also included. Results: 72.5% of thalassemia patients had cystatin C values greater than the upper limit of normal (ULN, 0.99 mg/L) whereas 2.5% of the patients had increased serum creatinine (ULN, 1.4 mg/dL). Patients on subcutaneous infusion chelation were 10 (25%). They had significant higher serum levels of urea, creatinine, CysC and duration of chelation (P = .016, P = 0.007, P = 0.001, P = 0.000 respectively) than patients on oral chelation therapy. No significant difference was found between patient's subgroups of oral chelation therapy regarding disease onset, disease duration, blood transfusion rate, splenectomy duration, serum levels of urea, creatinine and CysC (P > 0.05) for all. Patients with splenectomy were 22 (55%), with significant higher serum levels of urea, creatinine, CysC and duration of chelation (P = 028, P = 0.003, P = 0.000, P = 0.001 respectively). In thalassemia patients, CysC showed significant positive correlation with serum levels of ALT, AST, urea, creatinine, ,systolic BP,BMI ,duration of chelation and age (r = 0. .380, P = 0.016; r = 0. .432, P = 0.005) , r = .746, P = *.000 ;r* = .696, P = .000 ; r =.530, P = .000; r = .356, P = .024 ; r = .515, P = .001 and r = .543, P = .000 respectively). Conclusion: These findings suggest that slight changes of cystatin C serum levels in *β*-thalassemia major patients during chelating therapy or after splenectomy may not reflect renal impairment and, therefore, measurements of this biomarker should be interpreted with caution.

Key words: *β*-thalassemia major, cystatin C, chelating therapy, splenectomy.

INTRODUCTION

β-thalassaemia major (βTM), is a type of chronic hemolytic, inherited, microcytic anemia that is characterized by impaired biosynthesis of the β-globin chain leading to accumulation of unpaired α-globin chain. Despite advances in chelating therapy, some major complications of thalassemia are inevitable. A large number of studies have been performed on different complications of thalassemia, however, there are a few studies on the renal involvement in this disease (Aldudak *et al*., 2000) .Recently there have been some concerns about renal complications in (βTM), but some controversies have been demonstrated. There is evidence, mainly from studies in the pediatric population, of tubular dysfunction and glomerular filtration rate abnormalities in this patient population (Ponticellia *et al*., 2010). Derakhshan *et al*., (2008) concluded that significant renal involvement is not a frequent complication in children and young adults suffering from thalassemia. Recently Enas *et al*., (2010) confirm that glomerular and tubular dysfunctions exist in children and adolescent with TD-βTM. These abnormalities are mainly sub-clinical, but, with prolonged repeated tubule damage, tubulointerstitial fibrosis may occur.

The conventional treatment for transfusion related iron overload is chelation therapy aimed at reducing iron stores or maintaining an iron balance. Treatment with iron chelators is primarily governed by the degree of iron overload and the transfusional requirements. Patients presenting with transfusion-related iron overload are treated with Deferoxamine (DFO). Deferoxamine has been the standard iron chelator since the 1970s. DFO is both safe and effective for transfusional hemosiderosis. It is given as an overnight subcutaneous infusion 5 to 7 nights/wk (Davis *et al*., 2000; Borgna *et al*., 2004).

About 20 years ago, the first oral iron chelator (deferiprone, DFP) was presented. Concerns about potential side effects were responsible for the late acceptance and license of this drug. Typical dosage for deferiprone is 75 mg/kg/d in 3 divided doses, up to 100 mg/kg daily (Pennell *et al*., 2005; Hider *et al*., 2005).

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Patients have splenectomy when the transfusion requirements of packed red blood cells increased to 180 mL/kg/year, presence of hypersplenism signs such as leucopenia or thrombocytopenia, or presence of large spleen (Al-Salem *et al*., 2002).

Human Cystatin C (CysC) is a non-glycosylated protein with low molecular mass (13kDa) in the cystatin super family. Cystatin C is produced at a constant rate in all nucleated cells. This protein is secreted from the cells and thus found in detectable amounts in most body fluids. Cystatin C belongs to the cysteine proteinase inhibitors. It is freely filtered in the renal glomeruli and almost completely reabsorbed and catabolized in the renal proximal tubular cells (Zahran *et al*., 2007). Many studies have indicated that serum cystatin C can be used as an endogenous marker of GFR in adults and children (Roos *et al*., 2007; Enas *et al*., 2010).

Recently Papassotiriou *et al*. (2010) reported that any changes in cystatin C do not reflect renal impairment and, therefore, measurements of this biomarker should be interpreted with caution.

This study was conducted to estimate cystatin C serum concentration in β thalassemia patients treated with different chelating therapy. Also we aimed to determine the effect of splenectomy on cystatin C serum concentration.

Subjects and Methods:

Subjects:

This study recruited 40 pediatrics patients with transfusion-dependent β-thalassemia from Hematology Clinic of Pediatric Hospital, Faculty of Medicine, Cairo University. The diagnosis of βTM was based on standard criteria. Patients with either history of nephrotic syndrome or severe systemic diseases, (such as cardiovascular, renal, and hepatic disease, were excluded from the study. A control group of 31 age- and sex-matched Egyptian children was also included in the study. The study was approved by the Ethical Committee of National Research Centre and informed consent was obtained in every case from their legal guardians. Blood from patients was collected before routine transfusion. Patients were divided into 3 groups, group I (n = 10) were on regular iron DFO chelation therapy deferoxamine (desferal) 20-40mg/kg subcutaneous infusion via a battery-operated portable pump over a period of 8-12 hours overnight, for 5 nights per week) and group II (n = 22) were treated with deferiprone (Kelfer)75mg/kg/day. Group 111(n = 8) were on deferiprone (Ferriprox) 75mg/kg/day (Cohen *et al*., 2000).

All patients were subjected to the following: detailed history and clinical examination including Anthropometric measurements: The weight was measured to the nearest 0.1 kg with a digital balance and with the patients in light clothes and without shoes. Height was measured to the nearest 0.5 cm with a stadiometer. Body mass index was calculated as weight divided by height squared for each patient (kg/m^2).

Blood samples: Five-millilitres of fasting (for 12-14 hours) venous blood sample was collected from each child participating in the study. The blood was left to clot and centrifuged for 10 minutes at 3000 rpm for separation of serum that stored at -20ºC until analysis of serum alanine (ALT) and aspartate (AST) aminotransferases, serum urea, serum creatinine, serum ferritin and serum cystatin-C.

The determination of serum aminotransfrases, urea and creatinine were carried out using colorimetric methods on Olympus auto analyser AU 400 (Life Science, Hamburg, Germany).

The determination of serum ferritin was carried out using radioimmunoassay (Rimon *et al*., 2002).

Serum cystatin C was measured by BN 100 nephelometer (Dade Behring Inc., Deerfield, Illinois, U.S.A) using a particle- enhanced immunonepholemetric assay (Klein *et al*., 2009).

Data Analysis:

SPSS for Windows, version 10.0 computer program was used for statistical analysis. Data were represented as frequency, percent, range and mean ± standard deviation. The *t*-test was used to compare between 2 independent means. Spearman's correlation coefficient rho was used to correlate between non-normally distributed continuous variables. Chi-square test was used to compare between independent proportions. A *p* value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Overall we enrolled 40 *β*-thalassemia major patients in the study. Twenty two patients out of 40 (55%) were female and 18 patients (45%) were male. They were divided into three groups based on chelation therapy: Group 1n = (10) were on deferoxamine (desferal) 20-40mg/kg 5 days a week SC infusion and group II (n = 22) were on deferiprone (Kelfer) 75mg/kg/day. Group 111(n = 8) were on deferiprone (Ferriprox) 75mg/kg/day.

Table I shows some descriptive data of the studied βTM children. Their mean age was 9.98 ± 4.69 years (2-18 years) and the mean duration of chelation was 4.955 ± 3.77 years (0.5-13). Twenty four cases out of 40 (60%) had splenectomy. Thirty one patients were in pre-pubertal stage/stage I except 9 cases that were in pubertal stages/stages 11-1V.

Table 1: Descriptive Statistics descriptive data of the studied of β-thalassemia major children.

	N	Minimum	Maximum	Mean	Std. Deviation
Age(years)	40	2.00	18.00	9.99	4.69
Diastolic BP(mm Hg)	40	50	70	62.88	5.97
Systolic BP (mm Hg)	40	80	100	87.62	6.69
Age of onset (months)	40	8	24	14.65	4.83
Splenectomy duration(years)	24	3.00	12.00	7.17	2.77
NO of Transfusion/ (years)	40	3	6	4.25	.67
Puberty age (years)	9	13.00	15.00	14.33	0.70
Stage of Puberty	9	2	4		
BMI (Kg/m^2)	40	13.50	24.50	17.62	3.09
Duration of Chelating (years)	40	0.5	13.0	4.96	3.77

Table 2 shows Biochemical data of the studied βTM children and controls. There were no significant differences regarding age and BMI between patients and controls ($P > 0.05$). In patients significant lower level of hemoglobin and significant higher levels of serum urea, creatinine, ALT, AST, ferritin and CysC ($P = 0.0001$ for all). 72.5% had cystatin C values greater than the upper limit of normal (ULN, 0.99 mg/L) whereas 2.5% of the patients had increased serum creatinine (ULN, 1.4 mg/dL).

Patients with splenectomy were 22 (55%), with significant higher serum levels of urea, creatinine, CysC and duration of chelation (P = 028, $P = 0.003$, $P = 0.000$, $P = 0.001$ respectively). Also 20/22 (90.9%) had serum cystatin C values greater than the upper limit of normal (ULN, 0.99 mg/L) whereas in non-splenectomized group 8/18 (44.5%) of the patients had increased serum cystatin C value. Chi-square test was used to compare between independent proportions, the result was statistically significant (P = 0.001).

Table 2: Biochemical parameters in serum of β-thalassemia major children and controls.

	2=Thalassemic children 1=Controls	N	Mean	Std. Deviation	P-value
AGE (years)	1	31	11.81	3.98	.088
	2	40	9.99	4.69	
BMI (kg/m^2)	1	31	18.37	2.77	.291
	2	40	17.62	3.09	
Hemoglobin (g/dl)	1	31	12.46	0.95	.000
	2	40	5.82	0.34	
FERITTIN (µg/l)	1	31	33.61	8.61	.000
	2	40	2023.55	914.02	
ALT (U/l)	1	31	16.48	5.71	.000
	2	40	52.23	35.14	
AST (U/l)	1	31	15.00	5.01	.000
	2	40	47.23	33.04	
UREA (mg/dL)	1	31	12.26	4.41	.000
	2	40	26.50	9.38	
CREATININE (mg/dL)	1	31	0.27	0.15	.000
	2	40	0.68	0.38	
CYSTATIN-C (mg/L)	1	31	0.74	0.13	.000
	2	40	1.33	0.36	

There were no significant differences regarding other items as shown in table 3.

Table 4 shows clinical and biochemical data of the studied βTM children with oral and subcutaneous infusion chelation therapy. Patients on subcutaneous infusion chelation were 10 (25%).They had significant higher serum levels of urea, creatinine, CysC and duration of chelation (P = .016, $P = 0.007$, $P = 0.001$, P = 0.000 respectively).

No significant difference was found between patient's subgroups of oral chelation therapy regarding disease onset, disease duration, blood transfusion rate, splenectomy duration, serum levels of urea, creatinine and CysC ($P > 0.05$) for all (table 5).

In thalassemia patients, serum CysC showed significant positive correlation with serum levels of ALT, AST, urea, creatinine, ,systolic BP, BMI, duration of chelation and age (r = 0. .380, $P = 0.016$; r = 0. .432, P = 0.005), r = .746, P = .000; r = .696, P = .000; r = .530, P = .000; r = .356, P = .024; r = .515, P = .001 and r = .543, P = .000 respectively) as shown in table 6. While in normal children serum cystatin c showed no significant correlations with ALT, AST, urea, creatinine, BMI and age (r = .o38, .028, .094, .148, .117, .076 and P > 0.05 for all).

Table 3: Clinical and Biochemical data of the studied β-thalassemia major children with and without Splenectomy.

	0=splenectomy 1=no splenectomy	N	Mean	Std. Deviation	P Value
AGE (years)	0	22	11.41	4.72	.030
	1	18	8.25	4.15	
Diastolic BP (mm Hg)	0	22	63.64	5.16	.395
	1	18	61.94	6.89	
Systolic BP (mm Hg)	0	22	88.41	6.79	.419
	1	18	86.67	6.64	
Age of onset (months)	0	22	14.73	4.61	.914
	1	18	14.56	5.24	
Splenectomy duration(years)	0	22	7.18	2.84	.943
	1	2	7.00	2.83	
No of transfusion/year	0	22	4.18	0.59	.496
	1	18	4.33	0.77	
BMI (kg/m^2)	0	22	18.00	3.39	.386
	1	18	17.15	2.71	
Ferritin (µg/l)	0	22	2179.45	951.22	.233
	1	18	1833.00	853.78	
ALT (U/l)	0	22	49.82	20.82	.663
	1	18	55.17	47.76	
AST (U/l)	0	22	47.41	26.02	.971
	1	18	47.00	40.85	
UREA (mg/dL)	0	22	29.36	9.50	.028
	1	18	23.00	8.16	
Creatinine (mg/dL)	0	22	0.83	0.37	.003
	1	18	0.49	0.30	
Cystatin C (mg/L)	0	22	1.51	0.31	.000
	1	18	1.11	0.30	
Hemoglobin (g/dL)	0	22	5.80	0.36	.829
	1	18	5.83	0.32	
Duration of chelation(years)	0	22	6.64	4.15	.001
	1	18	2.90	1.82	

Table 4: Clinical and biochemical data of the studied β-thalassemia major children with oral and subcutaneous infusion chelation therapies.

	1=SC injection 0=oral chelation	N	Mean	Std. Deviation	P Value
AGE (years)	0	30	8.38	4.01	.000
	1	10	14.80	3.05	
Diastolic BP (mmHg)	0	30	62.17	6.39	.116
	1	10	65.00	4.08	
Systolic BP (mmHg)	0	30	86.00	5.93	.017
	1	10	92.50	6.77	
Age of onset (months)	0	30	13.80	4.49	.085
	1	10	17.20	5.18	
Splenectomy duration (years)	0	14	6.00	2.22	.016
	1	10	8.80	2.74	
No of transfusion /year	0	30	4.33	0.71	.105
	1	10	4.00	0.47	
BMI (kg/m^2)	0	30	16.82	2.88	.004
	1	10	20.00	2.50	
Ferritin (µg/l)	0	30	1939.27	914.07	.328
	1	10	2276.40	912.82	
ALT (U/l)	0	30	52.17	39.10	.981
	1	10	52.40	20.59	
AST(U/l)	0	30	48.17	37.26	.657
	1	10	44.40	15.69	
UREA (mg/dL)	0	30	24.20	8.27	.016
	1	10	33.40	9.50	
Creatinine (mg/dL)	0	30	0.58	0.32	.007
	1	10	0.99	0.37	
Cystatin C (mg/L)	0	30	1.22	0.33	.001
	1	10	1.64	0.26	
Hemoglobin (g/dL)	0	30	5.83	0.37	.641
	1	10	5.78	0.23	
Duration of chelation (years)	0	30	3.07	1.75	.000
	1	10	10.60	2.17	

Table 5: Comparison between the two oral chelation therapies in β- thalassemia major children.

	Oral chelation	N	Mean	Std. Deviation	P-value
Age (years) BP	2	22	7.88	3.96	.285
	3	8	9.750	4.06	
Diastolic (mmHg)	2	22	61.36	6.01	.319
	3	8	64.38	7.28	
Systolic BP (mmHg)	2	22	86.14	6.34	.820
	3	8	85.63	4.95	
Age of onset (months)	2	22	13.59	4.57	.681
	3	8	14.38	4.50	
Splenectomy duration(years)	2	11	5.81	2.31	.579
	3	3	6.66	2.08	
No of transfusion/year	2	22	4.36	.72	.706
	3	8	4.25	.70	
BMI (kg/m^2)	2	22	16.5318	2.69	.437
	3	8	17.6125	3.41	
Ferritin (µg/l)	2	22	1804.0000	798.24	.280
	3	8	2311.2500	1155.08	
ALT (U/l)	2	22	41.27	20.08	.103
	3	8	82.12	61.03	
AST (U/l)	2	22	38.54	22.15	.118
	3	8	74.62	56.46	
Urea (mg/dL)	2	22	23.63	8.88	.488
	3	8	25.75	6.51	
Creatinine (mg/dL)	2	22	.61	.32	.252
	3	8	.46	.31	
Cystatin C (mg/L)	2	22	1.25	.33	.474
	3	8	1.15	.33	
Hemoglobin (g/dL)	2	22	5.85	.33	.563
	3	8	5.75	.45	
Duration of chelation(years)	2	22	3.00	1.64	.777
	3	8	3.25	2.12	

P>0.05 means insignificant 2= deferiprone (Kelfer) 3= deferiprone (Ferriprox)

Table 6: Spearman's rho correlations in β-thalassemia major children.

		ALT	AST	UREA	Creatinine	Cystatin C	Systolic BP	BMI	Duration of chelation	AGE
ALT	r	1.000	.727(**)	.456(**)	.374(*)	.380(*)	.475(**)	.358(*)	.277	.510(**)
	P	.	.000	.003	.017	.016	.002	.023	.084	.001
	No.	40	40	40	40	40	40	40	40	40
AST	r	.727(**)	1.000	.512(**)	.363(*)	.432(**)	.304	.271	.218	.412(**)
	P	.000	.	.001	.021	.005	.056	.091	.176	.008
	No.	40	40	40	40	40	40	40	40	40
UREA	r	.456(**)	.512(**)	1.000	.688(**)	.746(**)	.450(**)	.403(**)	.499(**)	.496(**)
	P	.003	.001	.	.000	.000	.004	.010	.001	.001
	No.	40	40	40	40	40	40	40	40	40
Creatinine	r	.374(*)	.363(*)	.688(**)	1.000	.696(**)	.424(**)	.390(*)	.597(**)	.547(**)
	P	.017	.021	.000	.	.000	.006	.013	.000	.000
	No.	40	40	40	40	40	40	40	40	40
Cystatin C	r	.380(*)	.432(**)	.746(**)	.696(**)	1.000	.530(**)	.356(*)	.515(**)	.543(**)
	P	.016	.005	.000	.000	.	.000	.024	.001	.000
	No.	40	40	40	40	40	40	40	40	40
Systolic BP	r	.475(**)	.304	.450(**)	.424(**)	.530(**)	1.000	.601(**)	.554(**)	.674(**)
	P	.002	.056	.004	.006	.000	.	.000	.000	.000
	No.	40	40	40	40	40	40	40	40	40
BMI	r	.358(*)	.271	.403(**)	.390(*)	.356(*)	.601(**)	1.000	.633(**)	.840(**)
	P	.023	.091	.010	.013	.024	.000	.	.000	.000
	No.	40	40	40	40	40	40	40	40	40
Duration of chelation	r	.277	.218	.499(**)	.597(**)	.515(**)	.554(**)	.633(**)	1.000	.819(**)
	P	.084	.176	.001	.000	.001	.000	.000	.	.000
	No.	40	40	40	40	40	40	40	40	40
AGE	r	.510(**)	.412(**)	.496(**)	.547(**)	.543(**)	.674(**)	.840(**)	.819(**)	1.000
	P	.001	.008	.001	.000	.000	.000	.000	.000	.
	No.	40	40	40	40	40	40	40	40	40

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Although measurement of serum creatinine is simple and easily available, almost 50% of patients with impaired GFR have normal creatinine (Zahran *et al*., 2007; Roos *et al*., 2007) even though specific enzymatic creatinine measurements are used. Some studies report that cystatin C is a potentially superior indicator of renal function than either creatinine or creatinine-based formulae (Filler *et al*., 1997; Helin *et al*., 1998). Cystatin C has been proposed to be such a marker because it purportedly is produced by all nucleated cells at a constant rate, is filtered at the glomerulus, and is taken up and degraded by the proximal tubular cells of the kidney. Unfortunately, despite the early enthusiasm, cystatin C is at best only a slightly better predictor and discriminator than creatinine (Knight *et al*., 2004). For example, other factors are associated with and likely affect the cystatin C concentration, including age, weight, smoking, gender, and C-reactive protein (CRP) (Knight *et al*., 2004). In addition, the cystatin C concentration may be affected by other conditions such as liver disease (Chu *et al*., 2004*)* and thyroid disease (hypo- or hyperthyroidism) (Fricker *et al*., 2003; Wiesli *et al*., 2003). However, there are limited data on factors that may influence serum cystatin C level in thalassemic .This study aimed to investigate the effect of chelation therapy and splenectomy on cystatin C plasma concentration.

This study is a cross sectional examination from a single time point and employs only simple bivariate comparisons in the analysis. The findings in this study indicate that the mean serum cystatin C level as well as serum creatinine were significantly elevated in thalassemia patients compared to control group. Similar to our study, Economou *et al*., (2010) study showed that serum cystatin C level was elevated in thalassemia children.

Cystatin C levels of healthy children were considered as normal when their levels are less than 0.99 mg/L, based on mean + 2SD of our controls. Considering the cut-off value of 0.99 mg/L, 11/40 (27.5%) patients had normal cystatin C level, while 29 (72.5%) had high levels of cystatin C. So 29 (72.5%) patients had mild to moderate decreased glomerular filtration rate based on cystatin C levels. Furthermore, increased Cys-C levels were strongly associated with normal serum creatinine values that were observed in 28(70%) of βTM children. Only 1 (2.5%) patient had high serum levels of cystatin C and creatinine. Economou *et al*. (2010) reported 36% of their βTM patients had elevated serum CysC, while Dimitriadou *et al*. (2010) reported that (29.41%) of their βTM patients demonstrated impaired renal function with increased serum cystatin C levels. Therefore, CysC seems to be a better parameter than creatinine to reveal renal impairment and is possibly an earlier marker reflecting the oncoming renal failure.

We studied other factors might affect the cystatin concentration. The findings in this study indicate that gender had no effect on serum cystatin C levels neither in thalassemia children nor in normal controls, but it had significant effect on serum creatinine. Same result reported by other others (Economou *et al*., 2010). Taher *et al*. (2009) observed a clear gender difference in serum cystatin C levels with female individuals having lower levels than male individuals. No significant correlations were observed between cystatin C with age and BMI in normal children, but they had significant positive effect on serum cystatin C levels in thalassemia children. In contrast with others (Economou *et al*., 2010) who did not find any correlation of CysC with age, weight and height. But in consistence with MacDonald *et al*., (2006) who reported that serum CysC is not independent of body composition, as previously thought. They showed that past studies were based on the fact that CysC is produced at a constant rate by all nucleated cells, but 60% of cellular mass comes from muscles, which varies with anthropometric measures, diet, genetics and physical activity.

Another notable finding in this study was the effect of splenectomy on serum cystatin C levels. To our knowledge, such study is not available in the literature. Serum CysC was significantly higher in splenectomized patients than non-splenectomized patients. Also, urea and creatinine were significantly higher in splenectomized patients. Taher *et al*. (2009) reported that splenectomized patients had higher serum non-tranferrin-bound plasma iron (NTBI) levels than non-splenectomized patients, which could be attributed to the function of the spleen in scavenging iron free radicals, including NTBI. NTBI has been suggested to be toxic to iron-susceptible tissues (Porter *et al*., 2005; Kontoghiorghes *et al*., 2009).This could be the cause of kidney injury.

In addition, the levels of the liver enzymes ALT and AST were significantly raised in βTM children compared with controls. ALT is fairly specific marker of liver function, and the finding of raised levels of this enzymes in βTM children indicates liver involvement, which was expected based on previous reports However, the raised levels of AST in in βTM children could have the same explanation, but could also be of muscle origin.

The cystatin C concentration was affected by liver injury in βTM children as it was positively correlated with ALT and AST. Iron overload as a side effect of treatment can cause damage to the heart, liver, and endocrine systems. No significant difference was found between patient's subgroups of oral chelation therapy regarding ALT or AST. Hepatic fibrosis has been suggested in one small retrospective study to be a consequence of deferiprone therapy (Bulaj *et al*., 2000). However, other evidence based study on 56 repeat biopsies in patients treated for a mean of 3.1 years shows no evidence for this. Most studies of deferiprone have found fluctuations in alanine aminotransferase (ALT) levels, particularly in the first months of treatment and in hepatitis C antibody–positive patients. In the study of Ceci *et al*, (2002) ALT levels did not change over time in the analysis of 151 patients who completed 3 years of treatment. Olivieri *et al*. (1995) and Beutler *et al*. (2002) reported a reduction in ALT levels in most patients receiving deferiprone.

In this study, Serum CysC was significantly higher in patients with subcutaneous infusion chelation than with oral chelation therapy. The possibility that chronic administration of DFO caused duration dependent kidney damage could be an explanation. (Mohkam *et al*., 2008). In consistency, Koren *et al*. (1991) reported that subcutaneous administration of DFO was associated with a clinically significant decrease in GFR in 40% of βTM patients and with a mild decrease in another 40%. Furthermore, the short half-life of deferoxamine in the body after stopping an infusion results in a prolonged period each day when non-transferrin bound iron (NTBI) can be detected at elevated levels in the circulation (Gosriwatana *et al*., 1999). Others (Sumboonnanonda *et al*., 1998) reported lower level of urinary malondialdehyde (UMDA) in groups treated with DFO than those without treatment which supports the hypothesis of direct suppressive effect of DFO on peroxidation.

In a previous study in randomly selected transfused patients with β-thalassemia treated or not treated with deferasirox, cystatin C levels correlated significantly with N-acetyl-beta-D glucosaminidase (NAG) concentrations which is a sensitive indicator of deferasirox treatment and that any changes observed do not reflect renal injury but appear to be a consequence of the effects of deferasirox on hemodynamic signals such as LVEF alterations and iron mobilization do appear to affect changes in cystatin C concentration .It is possible that removal of iron in some body compartments exceeds that of transfusional iron intake, which could result in exclusion of iron from iron-dependent enzymes and transporters that are associated with kidney function. Cystatin C concentration may be influenced by hemodynamic parameters as a result of therapy with deferasirox. So changes in cystatin C do not always reflect renal impairment. There was no significant difference between the two oral chelation therapies.

Conclusion:

These findings suggest that slight changes of cystatin C serum levels in *β*-thalassemia major patients during chelating therapy or after splenectomy may not reflect renal impairment and, therefore, measurements of this biomarker should be interpreted with caution. Significant positive correlations were observed between cystatin C with age and BMI. Also clinicians should bear in mind that variation in cystatin C levels may be influenced by hemodynamic parameters as a result of therapy with chelating agents and iron mobilization. The administration of selective antioxidants, along with an appropriate, nutritionally balanced diet would represent a promising approach towards counteracting oxidative damage and its deleterious effects on β-thalassemia especially in splenectomized patients.

REFERENCES

Aldudak, B., K.A. Bayazit, A. Noyan, *et al*. Renal function in pediatric patients with beta-thalassemia major, 2000. Pediatr Nephrol., 15(1-2): 109-12.

Al-Salem, A.H., Z. Nasserulla, 2002. Splenectomy for children with thalassemia, Int Surg., 87(4): 269-73.

Beutler, E., A.V. Hoffbrand, J.D. Cook, 2003. Iron Deficiency and Overload Hematology, 1(1): 40- 61.

Borgna-Pignatti, C., S. Rugolotto, P. De Stefano, *et al*., 2004. Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine, Haematologica., 89: 1187-1193.

Bulaj, Z.J., R.S. Ajioka, J.D. Phillips, *et al*., 2000. Disease-related conditions in relatives of patients with hemochromatosis. N Engl J Med., 343:1529-1535.

Ceci, A., P. Baiardi, M. Felisi, *et al*., 2002. The safety and effectiveness of deferiprone in a large-scale, 3-year study in Italian patients, Br J Haematol., 118: 330-336.

Chu, S.C., C.P. Wang, Y.H. Chang, Y.S. Hsieh, S.F. Yang, J.M. Su, *et al*., 2004. Increased cystatin C serum concentrations in patients with hepatic diseases of various severities, Clin Chim Acta., 341: 133-8.

Dimitriadou, M., A. Christoforidis, M. Economou, A. Teli, N. Printza, V. Tzimouli, *et al*., 2011. Fok-I Polymorphism of Vitamin D Receptor Gene and the Presence of Renal Dysfunction in Patients with β-Thalassemia Major, Pediatr Hematol Oncol., 28(6): 459-60.

Economou, M., N. Printza, A. Teli, V. Tzimouli, I. Tsatra, F. Papachristou, 2010. Athanassiou-Metaxa M: Renal Dysfunction in Patients with Beta-Thalassemia Major Receiving Iron Chelation Therapy either with Deferoxamine and Deferiprone or with Deferasirox, Acta Haematol, 123(3):148-152.

Enas, A., Hamed and T. Nagla, 2010. ElMelegy Renal functions in pediatric patients with beta thalassemia major: relation to chelation therapy: original prospective study. Italian Journal of Pediatrics 36:39.

Filler, G., I. Witt, F. Priem, J.H.H. Ehrich, K. Jung, 1997. Are cystatin C and b2-microglobulin better markers than serum creatinine for prediction of a normal glomerular filtration rate in pediatric subjects? [Technical Brief], Clin Chem, 43: 1077-8.

Fricker, M., P. Wiesli, M. Brandle, B. Schwegler, C. Schmid, 2003. Impact of thyroid dysfunction on serum cystatin C, Kidney Int., 63: 1944-7.

Helin, I., M. Axenram, A. Grubb, Serum cystatin C as a determinant of glomerular filtration rate in children.,1998. Clin Nephrol, 49: 221-5.

Hider, R.C., T. Zhou, 2005. The design of orally active iron chelators, Ann NY Acad Sci., 1054: 141-154.

Kattamis, A., E. Margeli, G. Hantzi, A. Paleologos, A. Sergounioti, Lourida, *et al*. 2007. Assessment of renal function in transfused beta-thalassemia patients based on cystatin C measurements and equations, Blood., 110(11): abst 2796.

Klein, R., M.D. Knudtson, M.S. Lee and B.E.K. Klein, 2009. Serum Cystatin C, Kidney Disease Markers, and the Incidence of Age-Related Macular Degeneration: The Beaver Dam Eye Study. Arch Ophthalmol., 127(2): 193-199.

Kontoghiorghes, G.J., 2006. Iron mobilization from transferrin and non-transferrin-bound-iron by deferiprone. Implications in the treatment of thalassemia, anemia of chronic disease, cancer and other conditions., Hemoglobin, 30: 183-200.

Kuwabara, T., K. Mori, M. Mukoyama, M. Kasahara, H. Ykoi, Y. Saito, T. Yoshioka, *et al*., 2009. Urinary neutrophil gelatinase-associated lipocalin levels reflect damage to glomeruli, proximal tubules, and distal nephrons, Kidney Int., 75: 285-294.

MacDonald, J., S. Marcora, M. Jibani, G. Roberts, M. Kumwenda, R. Glover, J. Barron, A. Lemmey, 2006. GFR estimation using cystatin C is not independent of body composition, Am J Kidney Dis., 48: 712-719.

Mohkam, M., B.S. Shamsian, A. Gharib, S. Nariman, M.T. Arzanian, Early markers of renal dysfunction in patients with beta thalassemia major, 2008. Pediatr Nephrol., 23: 971-976.

Olivieri, NF., GM. Brittenham, D. Matsui, *et al*., 1995. Ironchelation therapy with oral deferiprone in patients with thalassemia major, 1995. N Engl J Med., 332: 918-922.

Olynyk, J.K., D.J. Cullen, S. Aquilia, E. Rossi, L. Summerville, L.W. Powell, 1999. A population-based study of the clinical expression of the hemochromatosis gene, 1999. N Engl J Med., 341: 718-724.

Papassotiriou, I., A. Margeli, E. Hantzi, P. Delaporta, A. Sergounioti, E. Goussetis, V. Ladis, A. Kattamis, 2010. Cystatin C levels in patients with β-thalassemia during deferasirox treatment, Blood Cells, Molecules, and Diseases, 44: 152-155.

Pennell, D.J., V. Berdoukas, M. Karagiorga, *et al*., 2005. Randomized controlled trial of deferiprone or deferoxamine in beta - thalassemia major patients with asymptomatic myocardial siderosis, Blood., 107: 3738-3744.

Porter, J.B., R. Rafique, S. Srichairatanakool, B.A. Davis, F.T. Shah, T. Hair, *et al*. 2005. Recent insights into interactions of deferoxamine with cellular and plasma iron pools: Implications for clinical use. Ann Y Acad Sci., 1054: 155-68.

Koren, G., Y. Kochavi-Atiya, Y. Bentur, N.F. Olivieri, The effects of subcutaneous deferoxamine administration on renal function in thalassemia major, 1991. *Int J Hematol.,* 54: 371-375.

Rimon, E., S. Levy, A. Sapir, G. Gelzer, R. Peled, D. Ergas and Z.M. Sthoeger, 2002. Diagnosis of Iron Deficiency Anemia in the Elderly by Transferrin Receptor-Ferritin Index, Arch Intern Med., 162: 445-449.

Roos, J.F., J. Doust, S.E. Tett, C.M. Kirkpatrick, 2007. Diagnostic accuracy of cystatin C compared to serum creatinine for the estimation of renal dysfunction in adults and children-a meta-analysis, Clin. Biochem., 40: 383-391.

Stevens, L.A., J. Coresh, C.H. Schmid, H.I. Feldman, M. Froissart, J. Kusek, J. Rossert, F. Van Lente, R.D. Bruce 3rd, Y.L. Zhang, T. Greene, A.S. Levey, Estimating GFR using serum cystatin C alone and in combination with serum creatinine: a pooled analysis of 3,418 individuals with CKD,2008. Am J Kidney Dis., 51: 395-406.

Sumboonnanonda, A., P. Malasit, V.S. Tanphaichitr, S. Ong-ajyooth, S. Sunthornchart, S. Pattanakitsakul, S. Petrarat, A. Assateerawatt, A. Vongjirad, 1998. Renal tubular function in beta-thalassemia, Pediatr Nephrol, 12: 280-283.

Taher, Ali, Khaled M Musallam, 2009. Fouad El Rassi, Lorena Duca, Adlette Inati, Suzane Koussa and Maria D Cappellini, 2009. Br J Haematol., 146(5): 569-72.

Wiesli, P., B. Schwegler, GA. Spinas, C. Schmid Serum cystatin C is sensitive to small changes in thyroid function;2003. Clin Chim Acta., 338: 87-90.

Zahran, A., A. El-Husseini, A. Shoker, 2007. Can cystatin C replace creatinine to estimate glomerular filtration rate? A literature review, 2007. Am J Nephrol., 27: 197-205.