Levels of Beta-2 Microglobulin and Cystatin C in Beta Thalassemia Major Patients

Beta Talasemi Major Hastalarında Beta-2 Mikroglobulin ve Sistatin C Düzeyleri

Beta-2 Mikroglobulin ve Sistatin-C Düzeyleri / Levels of Beta-2 Microglobulin and Cystatin-C

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Özet

Amaç: Talaseminin dünyadaki en yaygın genetik hastalık olduğu düşünülmektedir. Gerek hastalık, gerekse tekrarlayan transfüzyonlar nedeniyle birçok sistemde komplikasyon ortaya çıkmaktadır. Bu çalışma; üzerinde daha az durulan böbrek glomerüler hasarının beta talasemi majorlü hastalarda mevcut olup olmadığının saptanması ve glomerüler hasarı saptarken üre, kreatinin, kreatinin klirensinin erken böbrek hasarı göstergeçlerinden olan sistatin c, B2 mikroglobulin ile kıyaslanması amacıyla yapıldı. Gereç ve Yöntem: Bu çalışma beta talasemi major tanısıyla çocuk hematoloji polikliniğimizce düzenli takip edilen hastalarda prospektif olarak yapılmıştır. Bulgular: Üre ile kreatinin klirensi ve sistain C arasında istatistiksel olarak anlamlı bir ilişki bulunmamaktadır(p>0.05). Kreatinin ile kreatinin klirensi arasında negatif yönde, %53.7 düzeyinde ve istatistiksel olarak ileri düzeyde anlamlı bir ilişki bulunmaktadır (p:0.002; p<0.01). Kreatinin ile sistain C ve Beta 2 mikroglobulin düzeyleri arasında istatistiksel olarak anlamlı bir ilişki bulunmamaktadır (p>0.05). Sistain C ile β-2 mikroglobulin arasında pozitif yönde, %86.1 düzeyinde ve istatistiksel olarak ileri düzeyde anlamlı bir ilişki bulunmaktadır (p:0.001; p<0.01). Tartışma: Literatürde GFR>40 ml/dk/1.73 m² olduğunda kreatinin ile β-2 mikroglobulin ve sistatin c arası ilişkinin azaldığı belirtilmiştir. Bizim çalışmamız da bu literatür sonuçlarıyla benzerlik göstermektedir. Ancak renal parametrelerin birbiriyle korelasyonu hakkında kesin sonuçlara varmak için daha geniş populasyonda yapılacak daha fazla sayıda çalışmalara ihtiyaç vardır.

Anahtar Kelimeler

Sistatin C; Böbrek; Talasemi; Beta 2-Mikroglobulin

Abstract

Aim: Thalassemia is accepted to be the most common genetic disease in the world. This study was performed to establish whether there was a glomerular renal damage, which was usually a less mentioned subject in patients with Beta Thalassemia Major, and to compare urea, creatinine and creatinine clearance with early indicators of kidney damage as Cystatin-C and β-2 microglobulin as on determining the glomerular damage. Material and Method: This study was prospectively performed in patients, who were regularly followed in the children hematology outpatient clinic with a diagnosis of Beta Thalassemia Major. Results: There was no statistically significant difference between urea and levels of creatinine clearance and Cystatin-C. There was a statistically negative relationship between creatinine and creatinine clearance at an advanced level as 53.7% (p: 0.002, p<0.01). There was not any significant relationship between Cystatin-C and levels of creatinine and B-2 microglobulin. There was a significant high positive relationship between Cystatin-C and B-2 microglobulin at level of 86.1% (p: 0.001; p<0.01). Discussion: The results of our study were also similar to the literature. However, new studies are required to carry out in wider populations in order to reach definite conclusions about correlation of renal parameters with each other.

Keywords

Cystatin C; Kidney; Thalassemia; Beta 2-Microglobulin

DOI: 10.4328/JCAM.1968 Received: 15.07.2013 Accepted: 12.08.2013 Printed: 01.05.2015 J Clin Anal Med 2015;6(3): 292-296
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Introduction

Thalassemia is an autosomal recessive hereditary anemia that is characterized by incomplete production of one globulin chain forming hemoglobin molecule, or by having a small amount of globulin [1]. Most cases require blood transfusion in the first year of life. Some complications are encountered in these patients due to the free oxygen radical, which is emerged by ineffective erythropoiesis (as well as chronic anemia), iron accumulation, which emerges due to frequent blood transfusions, and side effects of chelate treatments.

There are not many studies related to this subject, because the affected kidney is lesser seen in the patients with Beta Thalassemia major comparison to other complications. Free oxygen radicals, caused by ineffective erythropoiesis (as well as chronic anemia), iron accumulation, emerging due to frequent blood transfusions, and the side effects of chelate treatments are able to make glomerular tubular and interstitial damage in kidney. There are some studies related to cell damage of free radicals in urine by leading to lipid peroxidation. This is because N-Acetyl glicosaminidase and β-2 microglobulin, recognized as an indicator of proximal tubular damage, increased in urine. However, issue of glomerular damage was mentioned in these studies [2, 3].

Cystatin-C is a cysteine protease inhibitor that is synthesized from all human cells and secreted into the blood. Cystatin-C is a better parameter on revealing impairment of renal function in comparison with creatinine clearance [4]. The advantage of Cystatin-C measurement in comparison with creatinine clearance is that it is not affected from height, sex, diet and muscle mass.

β-2 microglobulin is a low-molecular-weight protein that is freely filtered by glomeruli, reabsorbed by renal tubule and destroyed. The amount of serum β-2 microglobulin is very low in the healthy individuals. Its levels increase in case of inflammatory, immunologic and neoplastic events. Therefore, Cystatin-C is more sensitive than β-2 microglobulin in case of proteinuria, considerably accompanied with glomerular injury [5, 6].

This study was performed to establish whether there was a glomerular renal damage, which was usually a less mentioned subject in patients with Beta Thalassemia Major, to illuminate correlation of this damage with blood ferritin, hemoglobin and chelating treatment, and to compare urea, creatinine and creatinine clearance with parameters of early kidney damage as Cystatin-C and β-2 microglobulin as on determining the glomerular damage.

Material and Method

30 children, who needed regular blood transfusions for 2-3 weeks and were regularly followed in the hematology outpatient clinic with diagnosis of Beta Thalassemia Major, were included to this study in addition to 10 healthy children who admitted to our outpatient clinic for follow-up without having any health problems.

We individually interviewed with patients and their families, and they were informed about the study and permissions. Hereafter, we gathered information of these patients from outpatient follow-up cards, including their, age, sex, height, weight, age at onset of diagnosis and blood transfusion, annual number of transfusions, existing of a splenectomy operation history, bone marrow transplantation and range of the chelate treatments.

Blood samples were taken to two dry tubes and centrifuged at 3000 rpm for 10 minutes to work β-2 microglobulin and Cystatin-C. The separated serum samples were sent to the lab for analyzing in same day. Both parameters were measured by nephelometric quantitative method using N-Latex Cystatin-C kit in BN-Pro-SPECT (Dade Behring) device. Normal reference range between the ages of 1-50 was considered to be 0.53-0.95 mg/L for serum Cystatin-C and 0.7-1.8 mg/L for serum beta-2 microglobulin.

Because the control group was created from individuals who suffered from any disease, we prepared a separate research sheet for processing identity and physical examination findings. Blood samples were worked similar to patient group and then stored.

Descriptive statistical methods (mean, standard deviation and frequency) were used for evaluating the findings of this study. Kruskal-Wallis test was used in comparison of quantitative data, which included parameters with non-normal distribution. Student's t-test was used in comparison of two groups for parameters with normal distribution, as Mann-Whitney U test was used for parameters with abnormal distribution. The chi-square test was used to compare qualitative data. Pearson's correlation analysis was used for assessing relationship between parameters with normal distribution, as Spearman's rho correlation analysis was performed for the abnormally distributed parameters. NCSS 2007 & PASS 2008 Statistical Software (Utah, USA) was used for statistical analysis. p values lower than 0.05 were considered statistically significant for 95% confidence interval.

Results

This study was performed on a total of 40 children and included in 13 female (32.5%) and 27 male (67.5%). After the patient and control groups were compared in terms of their demographic characteristics, any difference was not observed between these two groups (Table 1). General characteristics of the patients were given in table 2.

Table 1. Assessment of Demographic Characteristics by Groups

		Thalassemia	Control	P
		Mean±SD	Mean±SD	
Age		19,30±6,48	17,0±6,98	0,346
Height (cm)		154,40±21,45	154,9±22,13	0,950
Weight		48,0±15,48	50,9±20,39	0,639
		n (%)	n (%)	
+Gender	Female	9 (%30)	4 (%40)	0,559
	Male	21 (%70)	6 (%60)	

Student's t-test was used + Chi-Square Test

Patients with β-Thalassemia Major were grouped according to transfusion frequency. After pre-transfusional ferritin levels of these groups were evaluated, any statistical difference was not observed between the groups. When the patient group was evaluated according to type of chelate, any significant difference was not observed in terms of pre-transfusional ferritin levels (Table 2).

Table 2. General characteristics of the patient group and pre-transfusional ferritin levels

		N	%	Pre-Transfusional Ferritin Levels (ng/ml)	P
Frequency of transfusion	Biweekly	11	36,7	1585,72±1014,21	
	Triweekly	13	43,3	1369,78±1048,84	0.591
	A four-week	6	20	1868,17±1135,47	
Chelation Therapy	Depheriprone	5	16,7	1107,64±744,31	
	Despheroxamine	10	33,3	1786,90±1164,48	0.509
	Depheriprone+ Despheroxamine	15	50	1536,80±1035,61	
Splenectomy	Rendered	21	70		
	Not Rendered	9	30		
Bone Marrow Transplantation	Rendered	-	-		
	Not Rendered	30	100		

The patient and control groups were evaluated in terms of their ferritin levels. Pre-transfusional ferritin values in thalassemia were considerable high comparison to ferritin values of the control group (p<0.01) (Table 3). Urea, phosphorus and β-2 microglobulin levels were proved to be significant, when the patient and control groups were compared in terms of biochemical parameters. No significant difference was observed in terms of Cystatin-C and other blood parameters (Table 4). Any correlation was not observed among hemoglobin, hematocrit, pre-transfusional ferritin values and parameters, indicating kidney functions in the patients (Table 5).

Table 3. Evaluation of patient and control groups in terms of ferritin levels

	Thalassemia	Control	P
	Mean±SD (Median)	Mean±SD (Median)	
++ Pre transfusional ferritin(ng/ml)	1548,64±1033,53 (1276)	48,38±31,11 (38,90)	0,001**
+Hematocrit (%)	24,59±2,06	37,15±3,53	0,001**
+Heamoglobin (g/dl)	8,78±0,71	12,68±0,85	0,001**

++Student t-test ++Mann-Whitney-U test **p<0.01

Table 4. Comparison of the groups in terms of biochemical values.

	Thalassemia	Control	P
	Mean±SD (Median)	Mean±SD (Median)	
++ Urea (mg / dL)	29,27±6,61	23,0±7,05	0,015*
++ Creatine clearance (Schwartz)	184,03±51,58	155,20±41,15	0,118
++ Beta 2 Microglobulin (mg / l)	2,40±0,64	1,07±0,31	0,001**
++ Sodium (mmol / l)	137,27±2,91	139,10±2,08	0,074
++ Potassium (mmol / l)	4,41±0,36	4,39±0,38	0,898
++ Uric acid (mg / dl)	4,54±1,05	4,12±0,58	0,237
++ Phosphorus (mg / dl)	4,57±0,63	3,34±0,59	0,001**
+ Creatinine (mg / dl)	0,56±0,12 (0,55)	0,62±0,11 (0,60)	0,172
+ Cystatin-C (mg / l)	0,76±0,12 (0,75)	0,72±0,13 (0,75)	0,628
+ Alkaline Phosphatase (U / L)	209,20±135,45 (161,5)	135,70±60,41 (132,5)	0,151

++Student t- test ++Mann-Whitney U test **p<0.01 *p<0.05

Table 5. Correlation of hematological parameters with parameters indicating renal function in the patient group

	+Pre-transfusional Ferritin Level	++Hemoglobin	++Hematocrit
	R	R	R
Urea	-0,120	-0,126	-0,230
Creatinine	-0,259	-0,075	-0,005
Crea. clearance	0,089	-0,191	-0,099
Cystatin-C	0,218	0,001	0,060
B2-Microglobulin	0,192	-0,198	-0,157

+ Spearman's rho correlation test
++ Pearson's correlation test

There was a significant positive relationship between urea and creatinine levels at the rate of 38.2% (p: 0.037; p<0.05). There was also another positive relationship between levels of urea and β-2 microglobulin at the rate of 41.8% (p: 0.021; p<0.05). There was no statistically significant difference between urea and levels of creatinine clearance and Cystatin-C. There was a statistically negative relationship between creatinine and creatinine clearance at an advanced level as 53.7% (p: 0.002, p<0.01). There was not any significant relationship between Cystatin-C and levels of creatinine and B-2 microglobulin. There was also not any significant relationship between Cystatin-C and levels of creatinine clearance and B-2 microglobulin. However, there was a significant high positive relationship between Cystatin-C and B-2 microglobulin at level of 86.1% (p: 0.001; p<0.01) (Table 6).

Table 6. Correlation of tests indicating impairment of renal function

	++Urea	+Creatinine	++Creatinine Clearance	+Cystatin- C	++β2-Microbulin
	r	R	r	r	R
Urea	-				
Creatinine	0,382*	-			
Creatinine Clearance	-0,221	-0,537**	-		
Cystatin-C	0,330	0,261	-0,222	-	
B2-Microglobulin	0,418*	0,204	-0,152	0,861**	-

+ Spearman's rho correlation test ++ Pearson's correlation test
* p<0.05 ** p<0.01

Discussion

The main complications of Beta Thalassemia Major include increased gastrointestinal iron absorption and deposition of the overloaded iron in heart, liver and endocrine organs due to transfusions [7]. Despite accumulation of hemosiderin and lipofuscin in the kidneys, early clinical signs are not observed. Development of these complications are delayed, and life expectancy of patients are extended providing adherence to treatment by the help of close follow-up, hyper-transfusion regimes, early diagnosis of the disease, using new generation chelating agents. Thus, in later years, problems associated with kidney damage will arise in patients with thalassemia because of extension of life expectancy. In addition, it was shown that use of Desferoksamin caused dose-dependent dysfunction in the proximal tubules via unknown mechanisms [8].

Many reasons are proposed, although mechanism of kidney dysfunction in thalassemia is not exactly known [9, 10]. Increased

mezengial matrix, focal global glomerulosclerosis, tubular atrophy, interstitial fibrosis were found on glomeruli in a histopathological study by Landing et al. Hemosiderin accumulation was found in glomerular visceral epithelial cells, but a bit less in parietal epithelial cells of mezengium and bowman capsule [11, 12].

In recent years, the research studies about effects of thalassemia over kidneys were started to perform. Because these studies were focused over tubular dysfunction, glomerular dysfunction was not sufficiently investigated. In a study of Leena Ong-ajyooth et al., the renal function in Beta Thalassemia Major was investigated among the study groups, including 95 patients with Beta Thalassemia Major and 27 healthy control subjects in the same ages. Creatinine clearance was only analyzed in terms of glomerular dysfunction, and no difference was found between patient and control groups. However, low-molecular-weight protein was detected in urine of 16% of patient group in addition to low osmolarity of first morning urine. NAG and β-2 microglobulin levels were high in urine of the patients, and MDA was found increased in both urine and plasma.

We considered that glomerular diseases had low incidence in the patients with thalassemia, because findings, consistent with glomerular damage as severe proteinuria and low creatinine clearance, were not detected in this study. Glomerular damages were identified in autopsies of patients with thalassemia and end-stage renal failure. The table of <2 g/day proteinuria in 95% of cases indicated that proteinuria originated from tubular structure. This study also demonstrated proximal tubular defect in the patient group. These defects were identified in the patients with splenectomy, which blood ferritin levels were also high. High serum levels of MDA showed role of oxidative stress in the pathogenesis [13]. In a study, Voskaridou et al. [14] investigated glomerular dysfunction in patients with Beta Thalassemia Major measuring both glomerular and tubular filtration via new markers.

According to results of the present study, serum Cystatin-C, beta-2 microglobulin and urinary NAG levels were detected increased in patient group comparison to control group. Increase rates of NAG, Cystatin-C and beta-2 microglobulin in the patient group were 32.1%, 74.7% and 70.1%, respectively, while serum creatinine only increased in 6.8% of patients.

High values of Cystatin-C were only observed in 36% of patients whose creatinine clearance decreased. Low levels of hemoglobin raised beta 2-microglobulin, NAG and Cystatin-C. This was the evidence of that chronic hypoxia due to anemia disrupted kidney function [15, 16]. In our study, urea levels of thalassemia group were found significantly high in the control group ($p<0.05$). However, no significant difference was rated, because levels of urea were within normal limits in both these groups. Creatinine and creatinine clearance did not statistically differ among the groups ($p> 0.05$). The results were similar to findings of both Sumboonnanonda et al, and Leena Ong-ajyooth et al. [12, 13].

Although the viewed glomerular filtration rates according to creatinine clearance in thalassemia group were detected within normal range in all patients, beta-2 microglobulin levels were significantly high in the control group ($p <0.01$). Beta-2 microglobulin levels, in a study of E. Voskaridou et al, were identified as elevated in 70.1% of all patients in comparison with control group of the same study [14]. In the studies of E. H. Cooper et al., the highest level of beta-2 microglobulin was identified in the patients with thalassemia, who had hypersplenism and the treatment was not begun [17]. This was because RES activity and expansion in erythroid series increased.

In our study, the increase was related to impaired renal function, because the treatment was recently started and there were no patients with hypersplenism. Because increase occurred in patients with normal glomerular filtration rate according to creatinine clearance, that should be assessed as a predictive value for detection of early renal dysfunction.

Cystatin-C levels were not statistically different than the other groups ($p> 0.05$). In the study of E. Voskaridou et al., increase rate of Cystatin-C in the patient group was 32.1%, while serum creatinine only increased in 6.8% of patients [14]. High values of Cystatin-C were encountered only in 36% of patients whose creatinine clearance fell. Thus, the increased Cystatin-C levels were identified in patients with normal creatinine level and creatinine clearance. In our study, creatinine, creatinine clearance and Cystatin-C showed no difference in the patients in comparison to control group. This showed that new studies with a larger group of patients and controls were necessary.

Levels of serum sodium, potassium, uric acid and alkaline phosphate were not statistically different than other groups ($p> 0.05$). Although proximal tubular damage in the patients with thalassemia was a foreground finding, any change was not observed in sodium homeostasis. No difference was observed between two groups in our study, although levels of serum potassium and uric acid were detected as high due to hemolysis and increased red blood cell cycle, respectively.

In the study of Lapatsanis et al., increased hemoglobin level of patient group over 7.5 mg/dl was accompanied by increasing level of serum ALP; that increase was linked to improved osteoblastic dysfunction. Although mean hemoglobin level was 8.78 ± 0.71 mg/dl in our patient group, such an increase was not detected [9]. Phosphorus levels of thalassemia group were significantly higher in the control group ($p <0.05$). This result was consistent with study of Lapatsanis et al, which examined phosphorus balance in patients with thalassemia. Despite normal serum phosphorus levels, the table of increased phosphaturia was linked to hemolysis [18].

According to the literature [9, 10], oxygen radicals, caused by non-hemoglobin iron (Fenton reaction), generate lysosomal damage by accumulation of iron in lysosomes of renal cells. MDA (Malondialdehyde), yielding as a result of lipid peroxidation of polyunsaturated fatty acids which are catalyzed by iron, covalently bonds to protein, phospholipids and DNA. Hereby, renal dysfunction occurred due to chronic hypoxia related to anemia. Contrary to all this results, there was no significant relationship between average of annual pre-transfusional ferritin and levels of urea, creatinine, creatinine clearance, Cystatin-C, β-2 microglobulin in outcomes of our study.

In comparison of hemoglobin and hematocrit levels with urea, creatinine, creatinine clearance, Cystatin-C, β-2 microglobulin, there was not any relationship. These results, not correlated with literature information, indicated that studies with a larger group of patients and controls should be performed.

Another purpose of this study was to compare parameters, in-

dicating glomerular filtration rate, with each other, which alerts renal dysfunction and includes urea, creatinine, creatinine clearance, Cystatin-C and β-2 microglobulin. In the study of Donido Carlo et al., Cystatin-C, β-2microglobulin and retinol binding protein (RBP) rapidly increased by a reduction in GFR. The increase rate of parameters occurred in the order of β-2 microglobulin, Cystatin-C and RBP, particularly as GFR was lower than 20 ml/min/1.73 m². Although diagnostic accuracy of Cystatin-C and β-2 microglobulin was similar to creatinine in different degrees of GFR, RBP was not a proper marker for measuring GFR [19].

In the study of Donido Carlo et al, when GFR levels were measured higher than 80ml/dk/1.73 m², all renal parameters (creatinine, Cystatin-C and β-2 microglobulin) were detected close to the upper limits, but within normal values.

Serum values of Cystatin-C, beta-2 microglobulin and creatinine were detected within normal levels in little deteriorated renal functions. Reducing relationship between creatinine and levels of β-2 microglobulin and Cystatin-C was reported, as GFR was higher than 40 ml/min/1.73 m² (20). Results of our study were also similar to the literature. In the study of Elsabeth Coll et al, Cystatin-C was found increased, as GFR was lower than 88 ml/min/1.73 m². Creatinine increased in case of GFR <75 ml/min/1.73 m² [21].

In our study, creatinine and Cystatin-C levels were normal in accordance with the literature, because level of GFR was higher than 88 ml/min/1.73 m² in all groups. However, greater numbers of studies in wider population are needed in order to reach definite conclusions about correlation of renal parameters with each other.

Competing interests

The authors declare that they have no competing interests.

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