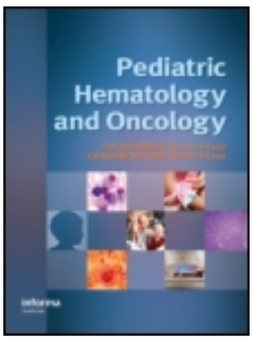

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ORIGINAL ARTICLE

Cystatin C, Beta2 Microglobulin, N-Acetyl-beta-D-glucosaminidase, Retinol-Binding Protein, and Endothelin 1 Levels in the Evaluation of Sickle Cell Disease Nephropathy

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Objectives: Renal involvement is common in sickle cell disease (SCD). Early demonstration of renal injury and commencement of appropriate treatment will increase survival and quality of life in these patients. We investigated renal manifestations in our pediatric and adult SCD patients and evaluated the role of cystatin C, Beta2 microglobulin (B2M), retinol-binding protein (RBP), N-acetyl-beta-D-glucosaminidase (NAG), and endothelin-1 (ET-1) to indicate renal damage. *Methods*: The study involved 45 pediatric and 10 adult patients with SCD and 20 healthy children and 10 healthy adults as a control. All the patients were questioned for possible renal manifestations. 24-hour urine samples were collected and glomerular filtration rates (GFRs) were calculated by using creatinine ($GFR_{creatinine}$), Schwartz formula ($GFR_{Schwartz)}$, and cystatin C ($GFR_{cystatin\ C}$). Blood and urine samples were collected and serum cystatin C, urine B2M, RBP, NAG, and ET-1 levels were measured. *Results*: Nocturnal enuresis and proteinuria were the most common renal manifestations in SCD patients. When the groups were compared in terms of GFR, $GFR_{creatinine}$ and $GFR_{Schwartz}$ levels were higher in group 1 and 2 patients than in control 1 and 2 patients ($P < .05$). Cystatin C, B2M, RBP, NAG, and ET-1 values were normal in both the patient and the control groups. However, B2M/creatinine levels were higher than 160 μg/mg creatinine levels in 10 patients. *Conclusions*: Serum cystatin C, urine NAG, RBP, and ET-1 levels were found to be insufficient for the evaluation of SCD nephropathy. Increased B2M/creatinie levels can be valuable in estimating possible glomerular and tubular damage in SCD.

Keywords beta2 microglobin, cystatin C, endothelin-1, N-acetyl-beta-D-glucosaminidase, nephropathy, retinol-binding protein, sickle cell disease

INTRODUCTION

Sickle cell disease (SCD) is a genetic hemoglobin (Hb) disorder in which abnormal sickle Hb (HbSS) partially or completely replaces normal Hb. SCD is characterized by periodic vaso-occlusive crisis, chronic hemolysis, and frequent infections, accompanied by pain and organ damage [1].

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Kidney is one of the most frequently affected organs in these patients and functional and structural abnormalities of the kidneys have been observed during the course of the disease. Sickling of erythrocytes in the hypertonic and hypoxic medium of renal medulla produces various complications. Hyposthenuria, renal acidification defects, K^+ secretion defects, hypertension, hematuria, proteinuria, nephritic syndrome, renal papillary necrosis, renal medullary carcinoma, and acute and chronic renal insufficiency may all develop in the clinical course of SCD [1–3].

Biochemical markers have been investigated for the early diagnosis of renal injury due to various pathologies. One of them is cystatin C, which is produced with a constant rate in the body and filtered from the glomeruli due to its lower molecular weight [4]. It is a non-glycosylated, 122 amino acid protein produced by all nucleated cells. Its rate of production increases in inflammatory events. Cystatin C is reabsorbed by tubular epithelial cells and metabolized quickly by the kidneys. Serum cystatin C levels strongly correlated with the glomerular filtration rate (GFR) in many studies [4–6]. It was documented that GFR calculation based on cystatin C ($GFR_{cystatin\ C}$) had not been affected by parameters such as age, sex, body muscle mass, besides being more sensitive for the diagnosis of early stage renal failure than GFR calculations based on other parameters such as creatinine [6].

Beta2 microglobulin (B2M) is a small, non-glycosylated peptide. In situations where its synthesis was not altered, increased urine B2M levels indicated disturbed reabsorption capacity of proximal tubules; therefore, B2M was proposed to be used in the follow-up of GFR [7].

Retinol-binding protein (RBP) is a single polypeptide chain with a molecular weight of 21,200 Dalton. RBP is in complex with prealbumin while carrying vitamin A in body secretions. RBP is filtrated from the glomeruli, reabsorbed from the proximal tubular cells, and destroyed in these cells. In case of a renal disease, RBP and B2M levels increase in urine as a result of decreased tubular reabsorption [8].

N-acetyl-beta-D-glucosaminidase (NAG) is a lysosomal enzyme in the proximal renal tubular cells. Being a large molecule, it cannot be filtered from the glomeruli and for that reason NAG in urine is mostly of tubular origin. Increased NAG activity in urine has been detected in many diseases of the proximal tubules and injuries caused by anephrotoxic agents [9].

Endothelin-1 (ET-1) is the major endothelin in the blood synthesized by human vascular endothelium. It is a strong vasoconstrictor and mitogen. ET-1 is 10 times more active in renal tissue than the others. It contributes to the regulation of renal blood flow, GFR, sodium, and water transport. Many of the different cell types in renal, vascular and glomerular endothelium, mesenchymal cells, and tubular epithelial cells synthesize ET-1. Physiological levels of ET-1 are considered to have a multi-directional role in regulating both vascular and tubular functions [10].

Urine proteins and enzymes such as B2M, RBP, NAG, and ET-1 were previously evaluated in monitoring renal function in various diseases and increased levels of these proteins in urine were suggested as early indicators of renal disorders [11].

Renal manifestations are common in SCD. Early demonstration of renal injury and commencement of appropriate treatment will increase survival and quality of life in these patients. In this study, the above-mentioned parameters were investigated in SCD patients to evaluate their role as indicators of renal damage in these patients.

PATIENTS AND METHODS

The study involved 55 SCD patients that were under follow-up by Pediatric Hematology Department, Mersin University Hospital. The Ethics Committee of Mersin

University approved the study protocol, and all of the patients gave informed consent. Group 1 involved 45 pediatric patients (ages between 1 and 15), and group 2 involved 10 adult patients (ages between 16 and 45). Patients who had transfusion in the last three months, who had a history of vaso-occlusive crisis, and infection within the last month were not included in the study. Twenty healthy children (Control 1) and 10 adults (Control 2) were included in the control groups. They had no disease, their renal function tests were in normal range, and had normal Hb electrophoresis pattern. After a detailed physical examination, all of the patients were questioned about the history of painful crisis, transfusion, stroke, acute chest syndrome (ACH), splenic sequestration, splenectomy, chelation, and hydroxyurea treatment. Hypertension, urinary stone, urinary tract infection, polyuria, pollakuria, hematuria, enuresis, and family history for renal disease were investigated and recorded to evaluate possible renal abnormalities.

Laboratory Investigation

Complete urinalysis, involving the presence of leukocytes and bacteria, was performed in all groups. Urine samples had been collected for 24 hours and GFR was calculated for all patients by using creatinine levels [$GFR_{Creatinine} = (urine_{Creatinine}/serum_{Creatinine}) \times (24\text{-hour urine volume}/1440) \times (1.73/m^2)$], Schwartz formula [$GFR_{Schwartz\ boy} = (height/serum_{Creatinine}) \times 0.55$), $GFR_{Schwartz\ girl} = (height/serum_{Creatinine}) \times 0.47)$], and cystatin C [$(GFR_{cystatin\ C} = -4.32 + (80.35/cystatin\ C)$]. To evaluate the tubular function in each patient, levels of fractional excretion of urea (FE_{urea}), sodium (FE_{Na}), potassium (FE_K), chloride (FE_{Cl}), tubular protein reabsorption (TPR), and presence of proteinuria were investigated. Urine total protein was measured by the biuret method (COBAS Integra 800 Analyzer, Roche Diagnostic, USA). Urine samples were stored at $-20^{\circ}C$ for the evaluation of renal markers. The patients who had urine protein levels <4 mg/m^2/hr were considered to have mild proteinuria, 4–40 mg/m^2/hr were considered to have moderate, and >40 mg/m^2/hr were considered to have heavy proteinuria. Abdominal ultrasonograms were performed in all of the patients to evaluate the kidneys in detail.

Veneous blood samples were collected from each patient and serum samples were obtained after centrifugation. Serum samples were stored at $-80^{\circ}C$ until the laboratory work-up. Cystatin C in serum, and RBP and B2M in urine were determined by the microelisa method using an automated instrument, TekTIME (bioMerieux, France). Urine NAG was measured by spectrophotometer (Varian, USA) using a commercial kit. Urine ET-1 levels were determined by an enzyme immunoassay technique using the Triturus ELISA analyzer (Grifols, USA). The accepted reference range for cystatin C is 0.25–25 ng/mL in serum, for RBP is 0.01–0.54 mg/L in urine, for B2M is <0.4 mg/L in urine, for NAG is 0.3–12 IU/L in urine, and for ET-1 is 0–250 pg/mL in urine.

Statistical Evaluation

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) 11.5 for Windows. Biochemical parameters were expressed as mean and standard deviation. Data related to clinical findings and renal complications were given as frequencies and ratios. A one-way ANOVA test was used if parameters were normally distributed parameters and the Kruskal–Wallis test was used otherwise. A Chi-square test was used to compare categorical variables such as clinical findings and renal complications. If $P < .05$, then the difference between the groups was expressed as significant.

TABLE 1 Demographic and Clinical Characteristics of the Patient Group

	Group 1 ($N = 45$)	Group 2 ($N = 10$)
Age (years)	8.4 ± 1.21	31 ± 10.11
Gender		
Female	15 (30%)	
Male	30 (60%)	10 (100%)*
Type of SCD		
HbSS	29 (64%)	7 (70%)
HbSß	16 (35%)	3 (30%)
Hydroxyurea therapy	32 (71%)	7 (70%)
Renal complications		
Nocturia	22 (56%)	3 (30%)
Proteinuria	13 (28%)	5 (50%)*
History of urinary tract infection	20 (44%)	6 (60%)*
Polyuria	6 (13%)	3 (30%)
Hematuria	1 (2%)	2 (20%)*
Hypertension	1 (2%)	2 (20%)*
Nephrolithiasis	0	2 (20%)*
History of chelation therapy	6 (13%)	3 (30%)

*$P < .05$.

RESULTS

Both serum and urine electrolytes were within the normal ranges in groups 1 and 2 compared with controls. All the patients had normal FE_{urea}, FE_{Na}, FE_K, FE_{Cl}, and TPR levels.

General characteristics and renal findings of the patients were summarized in Table 1. Proteinuria was found to be more common in patients having 10 or more painful crises in a year ($P < .05$). Proteinuria was not related to other clinical findings such as chelation, stroke, ACH, and hydroxyurea treatment.

In both groups 1 and 2, the mean levels of $GFR_{creatinine}$ and $GFR_{Schwartz}$ were significantly ($P < .05$) higher, where mean cystatin C, B2M, NAG, RBP, and ET-1 levels were not different from the respective control groups (Table 2). However, B2M/creatinine levels in urine were higher than 160 μg/mg creatinine levels in 10 patients. Clinical characteristics and GFR_{CRE}, $GFR_{Schwartz}$, $GFR_{cystatin\ C}$, CystatinC, B2M, RBP, NAG, ET-1 levels in SCA patients with and without proteinuria were shown in Table 3.

TABLE 2 Levels of GFR_{CRE}, $GFR_{Schwartz}$, $GFR_{cystatin\ C}$, Cystatin C, B2M, RBP, NAG, and ET-1 Levels in Patient and Control Groups

	Group 1 ($N = 45$)	Group 2 ($N = 10$)	Control 1 ($N = 20$)	Control 2 ($N = 10$)
$GFR_{Creatinine}$ (mL/min/1.73/m^2)	177.6 ± 36.54*	171.07 ± 51.88†	114.6 ± 36.54	116.53 ± 32.41
$GFR_{Schwartz}$ (mL/min/1.73/m^2)	262 ± 65.4	226.50 ± 73.35	147.70 ± 45.16	158.30 ± 16.91
$GFR_{cystatin\ C}$ (mL/min/1.73/m^2)	$103,6 \pm 24.55$	$110,6 \pm 32.16$	99.32 ± 15.09	$114,64 \pm 24.13$
B2M (mg/L)	0.23 ± 0.09	0.27 ± 0.11	0.22 ± 0.08	0.31 ± 0.12
NAG (IU/L)	8.75 ± 4.51	7.24 ± 1.32	7.57 ± 0.8	6.25 ± 1.3
RBP (mg/L)	0.36 ± 0.16	0.38 ± 0.05	0.28 ± 0.15	0.17 ± 0.14
ET-1 (pg/mL)	1.59 ± 1.08	1.22 ± 0.62	1.75 ± 1.64	1.58 ± 1.09
Cystatin C (ng/mL)	0.77 ± 0.23	0.75 ± 0.42	0.63 ± 0.31	0.78 ± 0.07

*$P < .05$ between group 1 and control 1.
† $P < .05$ between group 2 and control 2.

TABLE 3 Clinical Characteristics and GFR_{CRE}, $GFR_{Schwartz}$, $GFR_{cystatin\ C}$, Cystatin C, B2M, RBP, NAG, and ET-1 Levels in SCA Patients with and Without Proteinuria

	SCA patients with proteinuria ($N = 18$)	SCA patients without proteinuria ($N = 37$)
Type of SCD		
HbSS	16 (88%)*	20 (54%)
HbSß	2 (22%)	17 (46%)
Hydroxyurea therapy	11 (61%)	28 (76%)
History of chelation therapy	5 (28%)	4 (11%)
$GFR_{Creatinine}$ (mL/min/1.73/m^2)	176.24 ± 33.46	173.22 ± 41.15
$GFR_{Schwartz}$ (mL/min/1.73/m^2)	268.5 ± 83.14	225.6 ± 24.12
$GFR_{cystatin\ C}$ (mL/min/1.73/m^2)	108.5 ± 12.54	103.6 ± 15.24
B2M (mg/L)	0.24 ± 0.25	0.27 ± 0.18
NAG (IU/L)	8.23 ± 4.34	7.94 ± 3.14
RBP (mg/L)	0.37 ± 0.24	0.38 ± 0.23
ET-1 (pg/mL)	1.55 ± 0.16	1.48 ± 0.22
Cystatin C (ng/mL)	0.78 ± 0.35	0.74 ± 0.48

*$P < .05$.

DISCUSSION

We investigated renal manifestations of SCD in our pediatric and adult patients and evaluated the role of cystatin C, B2M, RBP, NAG, and ET-1 in indicating renal damage.

Considering renal manifestations of the patients, nocturia was detected in 25 (53%) of 47 patients and there was no difference between pediatric and adult patients ($P > .05$). Although the frequency of nocturnal enuresis in SCD is reported to be 28–37%, we found a higher value than the literature [3]. Because renal tubular functions were in normal range in all the patients with nocturia, psychogenic and familial causes may be important for the development of nocturia.

Proteinuria is observed in about 20–25% of the patients with SCD and this percentage is increased with age [12, 13]. We found 32% of the patients to have mild and moderate proteinuria. Similar to the literature, proteinuria was higher in adult patients than in the pediatric group ($P < .05$). Regarding clinical findings of the patients, proteinuria was found to be more common in patients having 10 or more painful crises in a year ($P < .05$). Therefore, we suggest that older patients and patients with frequent crises should be under close follow-up for proteinuria.

Painless macroscopic hematuria is a complication observed both in SCD patients and carriers. We detected painless macroscopic hematuria in three patients (6%). Two of these patients were HbSS and one was HbSß. Although its incidence is proposed to be around 3–4% in carriers [14], a higher prevalence (8.5%) was reported in Saudi Arabian patients with SCD[15].

Measuring the serum creatinine level is easy and cheap and creatinine clearance is still the most frequently used method in evaluating renal function in SCD patients [16]. GFR increases during early childhood and adolescence and often reaches a value of 200 mL/min/1.73 m^2 in SCD patients [2]. The mean GFR was 177 mL/min/1.73 m^2 with a range of 97–400 mL/min/1.73 m^2 in a study of 120 subjects with SCD [17]. However, in another study of 57 children with SCD, the GFR estimated by 24-hour urine collection was not significantly different in children with SCD (mean GFR of 131.7) compared with the normal controls (mean GFR 147) [18]. We also found higher $GFR_{creatinine}$ levels in the patient groups (childhood and adult patients) in comparison with the control groups ($P < .05$) (Table 2). $GFR_{Schwartz}$ is another means of evaluating GFR by using Schwartz formula. We found higher $GFR_{Schwartz}$ values in both Group 1 and 2 patients

compared to control group. $GFR_{Schwartz}$ had a wide range of 119–320 mL/min/1.73 m^2 in the study with the pediatric SCD patients [19].

Serum cystatin C has been used in evaluating renal function since the 1980s [20]. It was in good correlation with serum creatinine in children with various renal pathologies [21]. In our study, we found normal levels of cystatin C for all the pediatric and adult patients and there was no significant difference with the control groups. $GFR_{cystatin\ C}$ is also used to evaluate renal function and is proposed to be a reliable measure of glomerular function [22]. Twenty-two SCD patients were evaluated to compare $GFR_{creatinine}$, $GFR_{Schwartz}$, and $GFR_{cystatin\ C}$ levels. $GFR_{creatinine}$ was 149, $GFR_{Schwartz}$ was 176 and $GFR_{cystatin\ C}$ was 114 mL/min/1.73 m^2, suggesting $GFR_{cystatin\ C}$ to be more sensitive in evaluating GFR than the others [23]. All the patients in our study groups had normal or increased $GFR_{creatinine}$ levels, except three patients who had $GFR_{cystatin\ C}$ levels lower than 90 mL/min/1.73 m^2. These three patients had normal renal and tubular functions with moderate proteinuria. This is an interesting finding and it may be a result of the small number of adult patients who have frequent renal complications. For this reason, the role of $GFR_{cystatin\ C}$ in evaluating glomerular function should be investigated in a larger number of adult patients having SCD. Another method of measuring GFR in SCD patients is isotope clearance studies using substances such as mannitol, inulin, and Cr^{51}-EDTA. Adult patients were preferably involved in these studies and GFR of adult SCD patients had increased GFR compared with control [24].

Another marker of proximal tubular dysfunction is increased urinary B2M levels [25]. Urinary B2M levels greater than 160 μg/mg creatinine were found to be associated with proximal tubular damage [23]. In our study, all the patients had normal urinary B2M levels. However, the B2M/urine creatinine ratio is found to be greater than 160 μg/mg creatinine in 10 of the 55 patients (18%). Detecting nocturia in eight of these patients implies that they may have tubular dysfunction. Although other markers of tubular dysfunction were normal in these eight patients, increased urinary B2M levels were thought to be a more sensitive marker of tubular dysfunction.

There is only one study in the literature evaluating urinary excretion of RBP in patients with SCD. Between the ages of 7 and 14 years, 5 patients (16%) were found to have increased urinary RBP [26]. Although RBP levels were not increased in the urine of our patients, we suggest that RBP is not a marker in evaluating early tubular dysfunction in SCD.

Increased NAG excretion in urine is suggested to be a good indicator of proximal tubular damage [27]. Urinary NAG levels were found to increase in diabetic nephropathy patients [28]. In a recent study, urinary NAG levels were found to increase in SCD patients and correlated with urinary albumin. Therefore, urinary NAG was suggested to be an important marker of SCD nephropathy [29]. Contrary to these findings, we found no increase in urinary NAG levels in none of our SCD patients and NAG levels were not correlated to urine protein levels as well. This may be due to having mostly pediatric patients and limited number of adult patients in this study.

ET-1 is another marker that we investigated for demonstrating renal damage in SCD patients. ET-1 synthesis is very sensitive to cellular damage and renal ischemia, where its release increased from renal endothelium and tubular cells due to hypoxia [10]. Urine ET-1 levels increased significantly in a study involving 17 adult SCD patients compared with controls [30]. Contrary to the literature, we found no difference between the patient and control groups in terms of urinary ET-1 levels.

In conclusion, SCD patients are under high risk of morbidity and mortality due to renal complications, nocturnal enuresis and proteinuria being the most frequent. Routine laboratory tests are insufficient in evaluating tubular and glomerular functions in the early stages of the disease when the patients had normal renal function. Therefore,

we investigated some new markers of renal function in our SCD patients. In our study, serum levels of cystatin C, urine NAG, RBP, and ET-1 were not helpful for the evaluation of SCD nephropathy. Increased B2M/creatinine levels can be valuable in estimating possible glomerular and tubular damage in SCD. New studies with a larger number of patients are needed to evaluate the role of these parameters in clinical decision-making in SCD.

Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

[1] Nathan DG. (ed.) *Hematology of Infancy and Childhood*. Philadelphia, PA: WB Saunders; 2009:949–1014.
[2] Sharpe CC, Thein SL. Sickle cell nephropathy: a practical approach. *Br J Haematol.* 2011;155:287–297.
[3] da Silva GB Jr, Liborio AB, Daher Ede F. New insights on pathophysiology, clinical manifestations, diagnosis, and treatment of sickle cell nephropathy. *Ann Hematol.* 2011;90:1371–1379.
[4] Randers E, Erlandsen EJ. Serum cystatin C as an endogenous marker of the renal function: a review. *Clin Chem Lab Med.* 1999;37:389–395.
[5] Coll E, Botey A, Alvarez L, et al. Serum cystatin C as a new marker for noninvasive estimation of glomerular filtration rate and as a marker for early renal impairment. *Am J Kidney Dis.* 2000;36:29–34.
[6] Laterza OF, Price CP, Scott MG. Cystatin C: an improved estimator of glomerular filtration rate? *Clin Chem.* 2002;48:699–707.
[7] Revillard JP, Vincent C. Structure and metabolism of beta-2-microglobulin. *Contrib Nephrol.* 1988;62:44–53.
[8] Bernard A, Vyskocyl A, Mahieu P, Lauwerys R. Effect of renal insufficiency on the concentration of free retinol-binding protein in urine and serum. *Clin Chim Acta.* 1988;171:85–93.
[9] Ring E, Zobel G, Erwa W, Haim-Kuttnig M. Urinary excretion of N-acetyl-beta-D-glucosaminidase in proteinuric states. *Child Nephrol Urol.* 1992;12:15–18.
[10] Richter CM. Role of endothelin in chronic renal failure: developments in renal involvement. *Rheumatology*. 2006;45:336–338.
[11] Koyner JL, Vaidya VS, Bennett MR, et al. Urinary biomarkers in the clinical prognosis and early detection of acute kidney injury. *Clin J Am Soc Nephrol.* 2010;5:2154–2165.
[12] McBurney PG, Hanevold CD, Hernandez CM, et al. Risk factors for microalbuminuria in children with sickle cell anemia. *J Pediatr Hematol Oncol.* 2002;24:473–477.
[13] McKie KT, Hanevold CD, Hernandez C, et al. Prevalence, prevention, and treatment of microalbuminuria and proteinuria in children with sickle cell disease. *J Pediatr Hematol Oncol.* 2007;29:140–144.
[14] Kiryluk K, Jadoon A, Gupta M, Radhakrishnan J. Sickle cell trait and gross hematuria. *Kidney Int.* 2007;71:706–710.
[15] Aleem A. Renal abnormalities in patients with sickle cell disease: a single center report from Saudi Arabia. *Saudi J Kidney Dis Transpl.* 2008;19:194–199.
[16] Allon M. Renal abnormalities in sickle cell disease. *Arch Intern Med.* 1990;150:501–504.
[17] Alvarez O, Montane B, Lopez G, et al. Early blood transfusions protect against microalbuminuria in children with sickle cell disease. *Pediatr Blood Cancer*. 2006;47:71–76.
[18] Bayazit AK, Noyan A, Aldudak B, et al. Renal function in children with sickle cell anemia. *Clin Nephrol.* 2002;57:127–130.
[19] Becker AM. Sickle cell nephropathy: challenging the conventional wisdom. *Pediatr Nephrol.* 2011;26:2099–2109.
[20] Simonsen O, Grubb A, Thysell H. The blood serum concentration of cystatin C (gamma-trace) as a measure of the glomerular filtration rate. *Scand J Clin Lab Invest.* 1985;45:97–101.
[21] Stickle D, Cole B, Hock K, et al. Correlation of plasma concentrations of cystatin C and creatinine to inulin clearance in a pediatric population. *Clin Chem.* 1998;44:1334–1338.
[22] Hoek FJ, Kemperman FA, Krediet RT. A comparison between cystatin C, plasma creatinine and the Cockcroft and Gault formula for the estimation of glomerular filtration rate. *Nephrol Dial Transplant*. 2003;18:2024–2031.

[23] Alvarez O, Zilleruelo G, Wright D, et al. Serum cystatin C levels in children with sickle cell disease. *Pediatr Nephrol.* 2006;21:533–537.
[24] Arlet JB, Ribeil JA, Chatellier G, et al. Determination of the best method to estimate glomerular filtration rate from serum creatinine in adult patients with sickle cell disease: a prospective observational cohort study. *BMC Nephrology.* 2012;13:83.
[25] Diraimondo CR, McCarley P, Stone WJ. Beta-2 microglobulin in peritoneal dialysis patients: serum levels and peritoneal clearances. *Perit Dial Int.* 1988;8:43–47.
[26] Marsenic O, Couloures KG, Wiley JM. Proteinuria in children with sickle cell disease. *Nephrol Dial Transplant.* 2008;23:715–720.
[27] Price RG. The role of NAG (N-acetyl-beta-D-glucosaminidase) in the diagnosis of kidney disease including the monitoring of nephrotoxicity. *Clin Nephrol.* 1992;38 (Suppl 1):S14–S19.
[28] Basturk T, Altuntas Y, Kurklu A, et al. Urinary N-acetyl B glucosaminidase as an earlier marker of diabetic nephropathy and influence of low-dose perindopril/indapamide combination. *Ren Fail.* 2006;28:125–128.
[29] Sundaram N, Bennett M, Wilhelm J, et al. Biomarkers for early detection of sickle nephropathy. *Am J Hematol.* 2011;86:559–566.
[30] Tharaux PL, Hagege I, Placier S, et al. Urinary endothelin-1 as a marker of renal damage in sickle cell disease. *Nephrol Dial Transplant.* 2005;20:2408–2413.