Pediatr Nephrol (2011) 26:275–280
DOI 10.1007/s00467-010-1677-9

ORIGINAL ARTICLE

Urinary transforming growth factor beta-1 as a marker of renal dysfunction in sickle cell disease

Davoud Mohtat · Rosemary Thomas · Zangfang Du · Yaa Boakye · Thomas Moulton · Catherine Driscoll · Robert Woroniecki

Received: 28 June 2010 / Revised: 14 September 2010 / Accepted: 17 September 2010 / Published online: 25 November 2010

Abstract Renal dysfunction affects 5–18% of patients with sickle cell disease (SCD). To date, no studies have described urinary levels of transforming growth factor β-1 (TGF-β1), a marker of fibrosis, and neutrophil gelatinase-associated lipocalin (NGAL), a marker of acute/chronic kidney disease, as biomarkers in identifying patients at risk of developing renal disease in SCD. We hypothesized that SCD subjects will have increased urinary excretion of TGF-β1 and NGAL compared with healthy controls (CTR). We examined 51 SCD subjects: 42 HbSS, 8 HbSC, and 1 HbSD. Sixteen out of 42 patients with HbSS were on hydroxyurea (HU). Urinary excretion of TGF-β1 was 26.4±1.5 pg/mgCr in SCD subjects vs 15.0±2.4 pg/mgCr in CTR ($p<0.00001$). SCD patients with hemoglobin<9 g/dl had higher urinary TGF-β1 than patients with milder anemia ($p=0.002$). Urinary TGF-β1 trended lower in HbSS patients treated with HU (23.61±2.6 pg/mgCr), vs patients not on HU (27.69±1.8 pg/mgCr; $p=0.055$). There was no correlation between urinary TGF-β1 and microalbuminuria or estimated glomerular function. There was no difference in urinary NGAL in SCD patients vs CTR. We suggest that urinary TGF-β1 may serve as a marker of early renal injury in SCD.

Keywords Fibrosis progression · Biomarkers · Sickle cell nephropathy

D.M. and R.T. contributed equally to this work.

D. Mohtat · R. Thomas · Z. Du · Y. Boakye · R. Woroniecki (✉)
Department of Pediatric Nephrology, Albert Einstein College of Medicine, Children's Hospital at Montefiore,
111 East 210th Street,
Bronx, NY 10467, USA
e-mail: rworonie@aecom.yu.edu

T. Moulton
Department of Pediatric Hematology/Oncology,
Bronx Lebanon Hospital,
Bronx, NY, USA

C. Driscoll
Department of Pediatric Hematology/Oncology, Albert Einstein College of Medicine, Children's Hospital at Montefiore,
Bronx, NY, USA

Introduction

In the United States, hemoglobin SS (HbSS) disease is more prevalent in African Americans, occurring in 1/400 births of children of African origin [1]. Sickle cell disease (SCD) is a genetic disorder with a single base-pair mutation resulting in an amino acid change of Glu > Val in the β globin gene. Patients with HbSS disease are homozygous for the β^S globin gene; they are characterized by severe anemia and morbidities involving multiple organ systems such as vaso-occlusive crises, acute chest syndrome, cerebrovascular events, retinopathy, and renal damage. Other hemoglobinopathies such as HbSC or HbSD disease are mixed heterozygote states with milder phenotypes than SS disease.

Sickle cell nephropathy (SCN) is a common complication that occurs in one-third of SCD patients [2–4]. Renal failure is a major cause of mortality in SCD [5, 6], developing in up to 18% of adult patients with SCD [7]. As SCN affects both the glomerular and tubular functions of the kidney, SCD patients have varied presentations [7]. Patients with glomerular dysfunction may present with proteinuria, nephrotic syndrome, hematuria, and renal failure. SCD patients with tubular dysfunction may present with urinary concentrating defects, impaired potassium secretion, renal tubular acidosis, and renal failure.

Microalbuminuria (MA) is an early marker of glomerular dysfunction and has a prevalence of up to 46% in children with SCN of between 10 and 18 years of age. It correlates with increasing age and lower hemoglobin levels [8–10]. Higher molecular weight proteinuria, evidenced by nephrotic syndrome is a predictor of progression to end-stage renal disease and correlates with early mortality [3, 11]. Tubular dysfunction is also very common in SCD. Recent studies suggest that tubular injury is independent of glomerular damage; Marsenic et al. reported increased urinary excretion of retinol binding protein as a marker of tubular injury without MA [12].

Currently, MA and creatinine are the only clinical markers of advanced renal injury. To find non-invasive urinary markers of early renal damage in SCD, we examined urinary levels of proteins involved in acute kidney failure and the initiation of fibrosis and tubular dysfunction, and correlated them with MA and the estimated glomerular filtration rate (GFR). We also aimed to study whether the urinary levels of these proteins correlated with the severity of SCD. If an association could be found, then we would propose that these urinary proteins could be used in future prospective studies to monitor the progression of sickle cell nephropathy and the effectiveness of its treatment.

Transforming growth factor β-1 (TGF-β1) is a ubiquitously expressed cytokine with roles in embryonic development, cellular homeostasis and extracellular matrix remodeling [13]. It causes renal fibrogenesis and nephron loss by various mechanisms, such as apoptosis of endothelial cells and podocytes; it also plays a role in epithelial to mesenchymal cell transition [14]. The TGFβ-1 pathway has been shown to be an important player in the development and progression of diabetic nephropathy [15, 16]. Recent studies have suggested that the TGF-β1 pathway may be activated by the renin–angiotensin aldosterone system (RAAS) [17]. The intrarenal RAAS system may be involved in the pathogenesis of sickle cell nephropathy, although there are no studies that directly address this. However, Falk et al. reported a reduction in proteinuria following treatment with an ACE inhibitor [18]. Single nucleotide polymorphisms in several genes of the TGF-β1/BMP superfamily have been shown to be associated with more severe renal disease and complications such as stroke, osteonecrosis, priapism, lower extremity ulcers, pulmonary hypertension, and in general as a measure of overall disease severity [19]. To date, to the best of our knowledge, there have been no published reports evaluating urinary excretion of TGFβ-1 in SCD.

Neutrophil gelatinase-associated lipocalin (NGAL) is expressed and secreted by immune cells, hepatocytes, and renal tubular cells in various pathological states. It is a member of the lipocalin family of proteins that has been extensively studied in acute kidney injury (AKI) [20, 21]. Mitsnefes et al. showed that serum NGAL correlates well with measured GFR, and may be a useful marker for monitoring and grading chronic kidney disease (CKD) even at GFR < 30 ml/min/1.73 m^2 [22]. We assessed urinary NGAL levels in patients with SCD to determine its usefulness in assessing chronic renal injury in SCD.

Subjects and methods

The study was conducted at The Children's Hospital at Montefiore/Albert Einstein College of Medicine. The Montefiore Medical Center Institutional Review Board approved the protocol for this study. Fifty-one children with SCD and 21 healthy controls between the ages of 2 and 21 years were enrolled from January 2009 to December 2009. We enrolled children with a diagnosis of sickle cell disease (HbSS, HbSC, HbSD or Sβ thalassemia) during their regular health maintenance visits to the outpatient hematology department. Control subjects were recruited and enrolled in the outpatient general pediatric clinics. The controls were primarily African Americans and Hispanics. Patients with a recent hospitalization within the last 30 days, history of blood transfusions within the last 90 days prior to enrollment, history of current pregnancy, neoplasm, hypertension, any known glomerular disease, patients who were on antihypertensive or steroid therapy for any reason were excluded. A total of 42 patients had HbSS, 8 HbSC, and 1 HbSD.

Clinical and laboratory data, obtained during routine medical care, was gathered by chart review of the visit at which the patients were enrolled. Estimated GFR was measured by the Schwartz formula [23]. Urinary microalbumin and creatinine were determined by turbidimetric analysis (Olympus 640). Microalbumin excretion was determined by the urinary microalbumin to urinary creatinine ratio. MA was defined as albumin/creatinine > 20 mg/g, as previously used in other studies [8]. Data analysis was performed using descriptive statistics and correlation analysis. Fresh urine samples were collected at room temperature within 2 h, centrifuged (10,000 rpm for 10 min) and the supernatant was stored at −80°C for analysis. Enzyme-linked immunosorbent assay (ELISA) kits were used to detect urinary excretion of activated TGF-β1 and NGAL (R&D Systems, Minneapolis, MN, USA). Values were normalized to measured urine creatinine. Mean arterial pulse index (MAPI) was calculated as the average MAP (calculated from systolic and diastolic values) for a given patient divided by the MAP value for the 95th percentile for age, gender, and height based on the Fourth Report of the National High Blood Pressure Education Program Working Group [24]. Self-reported

patient ethnicity was confirmed from the medical record of each patient.

Statistical analysis was performed using STATA (Stata Corporation, College Station, TX, USA) and Microsoft Office Excel 2003 (Microsoft Corporation, Redmond, WA, USA). $P<0.05$ was considered statistically significant.

Results

We analyzed the urine samples of 51 SCD subjects: 42 had HbSS, 8 had HbSC, and 1 had HbSD. Of the 42 HbSS subjects, 16 (38%) were receiving treatment with hydroxyurea (HU). We compared all the SCD subjects against 21 healthy controls (CTR). There were no statistically significant differences with regard to mean age, gender, and race between the SCD group and the CTR group. Demographic information for the SCD subjects and the CTR group are shown in Table 1.

All SCD patients had elevated eGFR (174.7 ± 40.3 ml/min/1.73 m^2 in the HbSS group and 137.8±40.0 ml/min/1.73 m^2 in the HbSC group, $p=0.01$); eGFR data on control subjects was not available. Further clinical and laboratory parameters characterizing the HbSS and HbSC/SD groups are shown in Table 2.

Urinary excretion of TGF-β1 was 26.4±1.5 pg/mgCr in SCD subjects vs 15.0±2.4 pg/mgCr in CTR, $p<0.00001$). TGF-β1 was higher in both HbSS and HbSC subjects (HbSS vs CTR, $p<0.00001$, and HbSC vs CTR, $p<0.01$), but there was no statistical difference in TGF-β1 levels between HbSS and HbSC patients (Fig. 1). We found a negative correlation between urinary TGF-β1 levels and age and a positive correlation between urinary creatinine and age in CTR (Fig. 2, Spearman's rho=−0.8, and Spearman's rho=0.87 respectively, $p<0.00001$), but this correlation was not observed in SCD subjects. SCD patients with hemoglobin less than 9 g/dl had higher urinary TGF-β1 than SCD patients with milder anemia ($p=0.002$). Urinary TGF-β1 was lower in HbSS patients on HU (23.61±2.6 pg/mgCr) vs HbSS patients who were not on HU (27.69±1.8 pg/mgCr), although this difference did not reach statistical significance ($p=0.055$).

MA (urine microalbumin >30 mg/gCr) was detected in 10% of subjects with SCD. All of the subjects with MA were older than 10 years. Urinary TGF-β1 was 33.9±19.7 pg/mgCr in patients with MA vs 25.8±9.5 pg/mgCr in patients with no MA ($p=0.49$, Mann–Whitney test). There was no correlation between urinary TGF-β1 and levels of urinary microalbumin (Spearman's rho=0.2, $p=0.17$) or eGFR (Spearman's rho=−0.07, $p=0.64$).

We found no statistical difference in urinary NGAL excretion between healthy controls and SCD subjects; 7.8±5.3 ng/mgCr in controls, 7.9±6.6 ng/mgCr in HbSS and 7.8±5.3 ng/mgCr ($p=0.85$, Kruskal–Wallis). Mean arterial pressure index (MAPI) was 0.8±0.08 in HbSS, 0.8±0.07 in HbSC/SD, and 0.7±0.09 CTR ($p=0.21$). Hospitalizations per person-year in HbSS group was 1.4 per year vs 0.3 in the HbSC/SD group ($p=0.02$).

Discussion

TGF-β1 is a known profibrogenic cytokine that plays an important role in the progression of CKD [13]. TGF-β1 has been shown to directly induce both increased synthesis and reduced degradation of matrix proteins, leading to a net accumulation of pathological matrix, thereby playing a role in the fibrotic process. In our study, we found elevated levels of urinary TGF-β1 in patients with SCD compared with controls. However, we did not observe statistical difference in urinary TGF-β1 levels and MA between HbSS and HbSC patients. One explanation for this finding may be the limited number of HbSC patients in our study. While there was a trend toward MA in SS patients more than in SC/SD patients, TGF-β1 levels were elevated in both groups. Although we observed that lower Hgb is associated with higher TGF-β1 urine levels, we were unable to demonstrate that less anemic patients (SC group) had lower urinary TGF-β1 levels. This could be explained by the relatively small SC sample size; or alternatively, it

Table 1 Clinical parameters

	Control ($n=21$)	SS ($n=42$)	SC/SD ($n=29$)	p
Age in years (minimum–maximum)	9.4±4.7 (2–18)	12.3±5.1 (3–20)	10.7±5.9 (3–19)	0.1*
Female	12 (57%)	20 (48%)	5 (56%)	0.75**
BMI (kg/m^2)	21.1±4.8	20.8±9.7	20.8±5.2	0.9*
MAPI	0.7±0.09	0.8±0.08	0.8±0.07	0.21*

BMI, body mass index; MAPI, mean arterial pressure index

±SEM for non-normally distributed data

*Kruskal–Wallis test

**Fisher's exact test

Table 2 Clinical parameters in subjects with sickle-cell disease (SCD)

	SS	SC/SD	*p*
eGFR (ml/min/1.73 m^2)	177.3±38.0	137.8±40.0	0.01*
Hemoglobin (gm/dl)	8.65±1.5	11.0±1.6	<0.00001*
Urinary microalbumin/creatinine (mg/g/Cr)	48.5±26.3	7.7±2.4	0.32**
Number of hospitalizations/person/year	1.4 (range 0–10)	0.3 (range 0–3)	0.02**

eGFR, estimated glomerular filtration rate

±SD for normally distributed data, ±SEM for non-normally distributed data

*t test

**Mann–Whitney

may be that the relationship between Hb and TGF-β1 is more complex and other factors may modulate it, i.e. SS patients were treated with hydroxyurea. Urinary TGF-β1 in SCD subjects did not correlate with age, eGFR or MA. Our finding of increased prevalence of MA in older children with SCD (all were older than 10 years) and urinary TGF-β1 excretion that did not correlate with age (p=0.14), suggests that greater renal injury was present earlier than would be recognized based on MA testing alone. There are no systematic studies examining urine excretion of TGF-β1 over the course of childhood. However, in Japanese patients Okamoto et al., demonstrated that the serum TGF-β1 level of children (aged 0–14 years) was significantly higher than that of adults (over 15 years of age; p<0.01) [25]. Interestingly in our study, we did note a strong negative correlation between urinary TGF-β1 (normalized to urine creatinine) and age, and a positive correlation between urinary creatinine and age in CTR subjects. This may suggest that in controls the higher ratio of TGF-β1/urine Cr is determined by lower urinary creatinine excretion.

Close follow-up of SCD patients is important to identify those who are at increased risk of developing renal dysfunction. For example, lower hemoglobin has been associated with a higher risk of proteinuria and renal dysfunction [26, 27]. In our SCD cohort, lower hemoglobin was correlated with higher urinary TGF-β1 levels, and it is likely that these are the patients most at risk of developing renal disease. The small sample size of HbSC patients makes it difficult to interpret our findings in the SC group. Long-term monitoring of this cohort is necessary to determine the clinical relevance of our findings. Alvarez et al. reported that blood transfusions protected against MA in patients with SCD [28]. As we excluded patients who had received blood transfusions in the 90 days prior to enrollment, which excludes patients receiving chronic transfusion therapy, we did not assess MA in that subset of patients. Hydroxyurea with ACE inhibitors may further reduce proteinuria in SCD subjects [29]. In our HbSS group, patients being treated with HU tended to have lower urinary TGF-β1 excretion compared with those not receiving treatment with HU; the difference was not

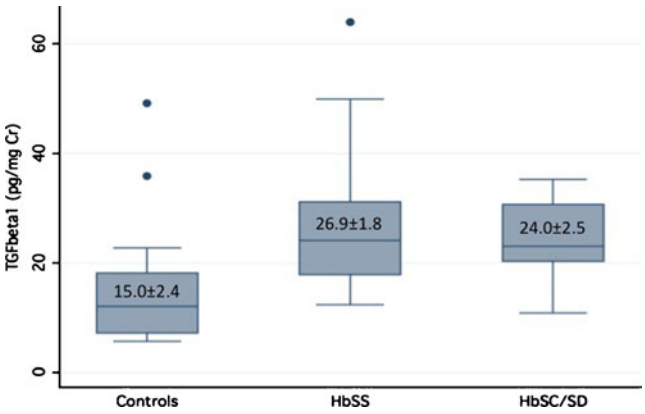

Fig. 1 Box plot of urinary transforming growth factor (TGF) beta-1 levels in controls and subjects with sickle cell disease (SCD). ±SEM standard error of the mean. p=0.0001 comparison amongst three groups (Kruskal–Wallis). p<0.00001 comparison between controls (CTR) and HbSS (Mann–Whitney). p=0.71 comparison between HbSS and HbSC/SD (Mann–Whitney). p<0.01 comparison between CTR and HbSc/SD (Mann–Whitney)

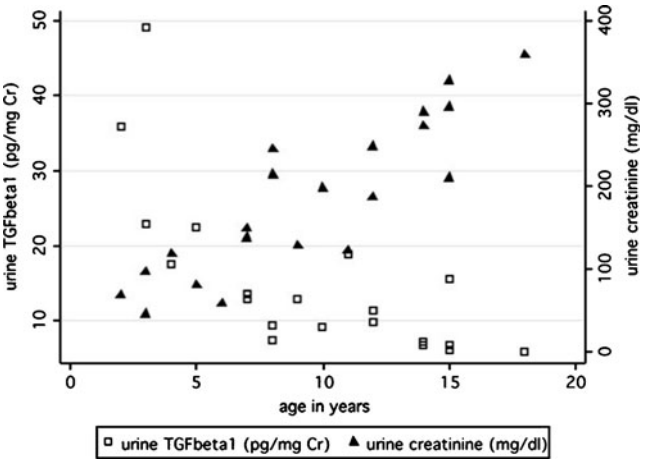

Fig. 2 Negative correlation between urinary TGF-β1 levels (*open squares*) and age in CTR (Spearman's rho=−0.8, p<0.00001), and positive correlation between urinary creatinine (*triangles*) and age in CTR (Spearman's rho=0.87, p<0.00001)

statistically significant, which may be attributed to our small sample size. Serum TGF-β1 protein levels have been shown to be positively and significantly associated with the systolic and diastolic blood pressure in African Americans after controlling for age, gender, and body mass index [30]. However, in our population MAPI was not increased in the SCD group compared with CTR.

Urinary NGAL is a diagnostic biomarker that has been shown to predict the development of acute kidney injury (AKI) and has been associated with CKD [20–22]. In our population, we found no difference in urinary excretion of NGAL between SCD patients and CTR. This could be attributed to the fact that, based on our exclusion criteria, we excluded patients who were clinically less stable, likely patients experiencing frequent and/or severe vaso-occlusive crises and resultant episodes of AKI. In addition, none of our SCD subjects had CKD.

Reports in adults have shown that asymptomatic proteinuria can progress to overt proteinuria and ultimately to renal failure [1, 11, 31]. Renin and angiotensin II have been shown to directly stimulate the expression of renal TGF-β1 independent of renal hemodynamics [32–34]. Therefore, blockade of the RAAS is an important therapeutic strategy for prevention of the progression of renal disease; for example, angiotensin receptor blockers or angiotensin converting enzyme inhibitors (ACEI) have been shown to decrease albuminuria and preserve renal function in diabetic nephropathy [35, 36]. ACEI have been successfully used in SCD, suggesting that glomerular capillary hypertension may be a pathogenic factor in SCN [18]. In our cohort of SCD patients, we did show evidence of glomerular hyperfiltration, which may support the use of ACE inhibitors even before the advent of MA. It is unclear if such a treatment would correspondingly decrease urinary TGF-β1 excretion. Interestingly, blockade of TGF-β1 directly in experimental models has been shown to prevent the progression of CKD [37, 38], suggesting a role for new treatment strategies. The long-term effectiveness of these therapies in prevention of renal disease in SCD patients needs to be further studied [18, 39, 40].

One of the limitations of our study is that the urine microalbumin evaluation was not performed on urine collected in a first morning void to exclude elements of orthostatic proteinuria. Also, as we recruited CTR subjects during routine well child visits, we neither had laboratory values available to calculate GFR nor noted hemoglobin or urine microalbumin.

Although serum creatinine is currently the clinically available tool used to estimate renal function, more and more evidence points to the fact that creatinine clearance may not be an accurate assessment of renal function in general [41]. Creatinine clearance compared with inulin clearance is significantly increased in SCD individuals due to the oversecretion of creatinine by the proximal tubules, above the amount filtered by the glomerulus [39]. In SCD subjects, even those with normal GFR, the presence of albuminuria is indicative of a decreased ultrafiltration coefficient, suggesting an injury to the filtration barrier [42]. Serum cystatin C has been suggested as a marker of GFR in children and adults with SCD [27, 43, 44], but further long-term studies are required to assess the reliability of serum cystatin C as an early marker of renal dysfunction in SCD patients. Urinary TGF-β1 is elevated in SCD subjects and may serve both as a marker to detect patients at risk of developing impending renal injury and as a treatment target. Our finding needs to be evaluated in long-term prospective trials.

Acknowledgements This work was supported by NIH training grant to R.T. DK007110-34, and an Emerald Foundation, Inc. grant to R.P. W. We thank Drs. Peter Belamarich and Andrew Racine and the nurses from the Pediatric Outpatient Clinics at The Children's Hospital at Montefiore for helping with subject recruitment.

References

1. Guasch A, Navarrete J, Nass K, Zayas CF (2006) Glomerular involvement in adults with sickle cell hemoglobinopathies: prevalence and clinical correlates of progressive renal failure. J Am Soc Nephrol 17:2228–2235
2. Powars DR, Elliott-Mills DD, Chan L, Niland J, Hiti AL, Opas LM, Johnson C (1991) Chronic renal failure in sickle cell disease: risk factors, clinical course, and mortality. Ann Intern Med 115:614–620
3. Falk RJ, Jennette JC (1994) Sickle cell nephropathy. Adv Nephrol Necker Hosp 23:133–147
4. Pham PT, Pham PC, Wilkinson AH, Lew SQ (2000) Renal abnormalities in sickle cell disease. Kidney Int 57:1–8
5. Thomas AN, Pattison C, Serjeant GR (1982) Causes of death in sickle-cell disease in Jamaica. Br Med J (Clin Res Ed) 285:633–635
6. Platt OS, Brambilla DJ, Rosse WF, Milner PF, Castro O, Steinberg MH, Klug PP (1994) Mortality in sickle cell disease. Life expectancy and risk factors for early death. N Engl J Med 330:1639–1644
7. Wesson DE (2002) The initiation and progression of sickle cell nephropathy. Kidney Int 61:2277–2286
8. Dharnidharka VR, Dabbagh S, Atiyeh B, Simpson P, Sarnaik S (1998) Prevalence of microalbuminuria in children with sickle cell disease. Pediatr Nephrol 12:475–478
9. McBurney PG, Hanevold CD, Hernandez CM, Waller JL, McKie KM (2002) Risk factors for microalbuminuria in children with sickle cell anemia. J Pediatr Hematol Oncol 24:473–477
10. Wigfall DR, Ware RE, Burchinal MR, Kinney TR, Foreman JW (2000) Prevalence and clinical correlates of glomerulopathy in children with sickle cell disease. J Pediatr 136:749–753
11. Bakir AA, Hathiwala SC, Ainis H, Hryhorczuk DO, Rhee HL, Levy PS, Dunea G (1987) Prognosis of the nephrotic syndrome in sickle glomerulopathy. A retrospective study. Am J Nephrol 7:110–115
12. Marsenic O, Couloures KG, Wiley JM (2008) Proteinuria in children with sickle cell disease. Nephrol Dial Transplant 23:715–720

13. Gordon KJ, Blobe GC (2008) Role of transforming growth factor-beta superfamily signaling pathways in human disease. Biochim Biophys Acta 1782:197–228
14. Blumenberg M, Gao S, Dickman K, Grollman AP, Bottinger EP, Zavadil J (2007) Chromatin structure regulation in transforming growth factor-beta-directed epithelial-mesenchymal transition. Cells Tissues Organs 185:162–174
15. Chen S, Jim B, Ziyadeh FN (2003) Diabetic nephropathy and transforming growth factor-beta: transforming our view of glomerulosclerosis and fibrosis build-up. Semin Nephrol 23:532–543
16. Nicholas SB (2003) Advances in pathogenetic mechanisms of diabetic nephropathy. Cell Mol Biol (Noisy-le-grand) 49:1319–1325
17. Wolf G (2006) Renal injury due to renin-angiotensin-aldosterone system activation of the transforming growth factor-beta pathway. Kidney Int 70:1914–1919
18. Falk RJ, Scheinman J, Phillips G, Orringer E, Johnson A, Jennette JC (1992) Prevalence and pathologic features of sickle cell nephropathy and response to inhibition of angiotensin-converting enzyme. N Engl J Med 326:910–915
19. Steinberg MH (2009) Genetic etiologies for phenotypic diversity in sickle cell anemia. ScientificWorldJournal 9:46–67
20. Mishra J, Ma Q, Prada A, Mitsnefes M, Zahedi K, Yang J, Barasch J, Devarajan P (2003) Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury. J Am Soc Nephrol 14:2534–2543
21. Mishra J, Dent C, Tarabishi R, Mitsnefes MM, Ma Q, Kelly C, Ruff SM, Zahedi K, Shao M, Bean J, Mori K, Barasch J, Devarajan P (2005) Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. Lancet 365:1231–1238
22. Mitsnefes MM, Kathman TS, Mishra J, Kartal J, Khoury PR, Nickolas TL, Barasch J, Devarajan P (2007) Serum neutrophil gelatinase-associated lipocalin as a marker of renal function in children with chronic kidney disease. Pediatr Nephrol 22:101–108
23. Schwartz GJ, Haycock GB, Edelmann CM Jr, Spitzer A (1976) A simple estimate of glomerular filtration rate in children derived from body length and plasma creatinine. Pediatrics 58:259–263
24. National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents (2004) The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics 114:555–576
25. Okamoto Y, Gotoh Y, Uemura O, Tanaka S, Ando T, Nishida M (2005) Age-dependent decrease in serum transforming growth factor (TGF)-beta 1 in healthy Japanese individuals; population study of serum TGF-beta 1 level in Japanese. Dis Markers 21:71–74
26. McKie KT, Hanevold CD, Hernandez C, Waller JL, Ortiz L, McKie KM (2007) Prevalence, prevention, and treatment of microalbuminuria and proteinuria in children with sickle cell disease. J Pediatr Hematol Oncol 29:140–144
27. Alvarez O, Lopez-Mitnik G, Zilleruelo G (2008) Short-term follow-up of patients with sickle cell disease and albuminuria. Pediatr Blood Cancer 50:1236–1239
28. Alvarez O, Montane B, Lopez G, Wilkinson J, Miller T (2006) Early blood transfusions protect against microalbuminuria in children with sickle cell disease. Pediatr Blood Cancer 47:71–76
29. Fitzhugh CD, Wigfall DR, Ware RE (2005) Enalapril and hydroxyurea therapy for children with sickle nephropathy. Pediatr Blood Cancer 45:982–985
30. Suthanthiran M, Gerber LM, Schwartz JE, Sharma VK, Medeiros M, Marion R, Pickering TG, August P (2009) Circulating transforming growth factor-beta1 levels and the risk for kidney disease in African Americans. Kidney Int 76:72–80
31. Schmitt F, Martinez F, Brillet G, Giatras I, Choukroun G, Girot R, Bachir D, Galacteros F, Lacour B, Grunfeld JP (1998) Early glomerular dysfunction in patients with sickle cell anemia. Am J Kidney Dis 32:208–214
32. Yu L, Border WA, Anderson I, McCourt M, Huang Y, Noble NA (2004) Combining TGF-beta inhibition and angiotensin II blockade results in enhanced antifibrotic effect. Kidney Int 66:1774–1784
33. Huang Y, Noble NA, Zhang J, Xu C, Border WA (2007) Renin-stimulated TGF-beta1 expression is regulated by a mitogen-activated protein kinase in mesangial cells. Kidney Int 72:45–52
34. Gaedeke J, Peters H, Noble NA, Border WA (2001) Angiotensin II, TGF-beta and renal fibrosis. Contrib Nephrol (135):153–160
35. Lewis EJ, Hunsicker LG, Bain RP, Rohde RD (1993) The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. The Collaborative Study Group. N Engl J Med 329:1456–1462
36. Brenner BM, Cooper ME, de Zeeuw D, Keane WF, Mitch WE, Parving HH, Remuzzi G, Snapinn SM, Zhang Z, Shahinfar S (2001) Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. N Engl J Med 345:861–869
37. Zeisberg M, Hanai J, Sugimoto H, Mammoto T, Charytan D, Strutz F, Kalluri R (2003) BMP-7 counteracts TGF-beta1-induced epithelial-to-mesenchymal transition and reverses chronic renal injury. Nat Med 9:964–968
38. Deelman L, Sharma K (2009) Mechanisms of kidney fibrosis and the role of antifibrotic therapies. Curr Opin Nephrol Hypertens 18:85–90
39. Voskaridou E, Terpos E, Michail S, Hantzi E, Anagnostopoulos A, Margeli A, Simirloglou D, Loukopoulos D, Papassotiriou I (2006) Early markers of renal dysfunction in patients with sickle cell/beta-thalassemia. Kidney Int 69:2037–2042
40. Foucan L, Bourhis V, Bangou J, Merault L, Etienne-Julan M, Salmi RL (1998) A randomized trial of captopril for microalbuminuria in normotensive adults with sickle cell anemia. Am J Med 104:339–342
41. Ataga KI, Orringer EP (2000) Renal abnormalities in sickle cell disease. Am J Hematol 63:205–211
42. Guasch A, Cua M, Mitch WE (1996) Early detection and the course of glomerular injury in patients with sickle cell anemia. Kidney Int 49:786–791
43. Alvarez O, Zilleruelo G, Wright D, Montane B, Lopez-Mitnik G (2006) Serum cystatin C levels in children with sickle cell disease. Pediatr Nephrol 21:533–537
44. Marouf R, Mojiminiyi O, Abdella N, Kortom M, Al Wazzan H (2006) Comparison of renal function markers in Kuwaiti patients with sickle cell disease. J Clin Pathol 59:345–351