RESEARCH PAPER

Effect of Enalapril on Glomerular Filtration Rate and Proteinuria in Children with Chronic Kidney Disease: *A Randomized Controlled Trial*

PANKAJ HARI, JITENDER SAHU, ADITI SINHA, *RAVINDER MOHAN PANDEY, #CHANDRA SHEKHAR BAL AND ARVIND BAGGA

*From the Departments of Pediatrics, *Biostatistics and #Nuclear Medicine, All India Institute of Medical Sciences, Ansari Nagar, New Delhi, India.*

Correspondence to: Dr Pankaj Hari, Professor, Department of Pediatrics, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110 029, India. pankajhari@hotmail.com

Received: January 08, 2013; Initial review: January 28, 2013; Accepted: February 21, 2013.

Objective: To evaluate the efficacy of enalapril treatment on decline in glomerular filtration rate and reduction in proteinuria in children with chronic kidney disease (CKD).

Design: Open-label, randomized controlled trial.

Setting: Pediatric nephrology clinic at a tertiary-care referral hospital.

Intervention: Children with GFR between 15-60 mL/min/1.73 m^2 were randomized to receive either enalapril at 0.4 mg/kg /day or no enalapril for 1 year.

Outcome measures: Change in GFR using 99mTc-DTPA and urine protein to creatinine ratio. Secondary outcomes included occurrence of composite outcome (30% decline in GFR or end stage renal disease) and systolic and diastolic blood pressure SDS during the study period.

Results: 41 children were randomized into two groups; 20 received enalapril while 21 did not receive enalapril. During 1 year, GFR decline was not different in the two groups (regression coefficient (r) 0.40, 95% CI -4.29 to 5.09, *P*=0.86). The mean proteinuria reduction was 65% in the enalapril group, significantly higher than control group. The difference was significant even after adjustment for blood pressure was 198.5 (CI 97.5, 299.3; *P*<0.001). 3 (17.6%) patients in enalapril and 7 (36.8%) in non-enalapril group attained the composite outcome.

Conclusions: Enalapril is effective in reducing proteinuria in children with CKD and might be renoprotective in proteinuric CKD.

Keywords: *Chronic kidney disease, Enalapril, GFR, Proteinuria*.

Chronic kidney disease (CKD) with glomerular filtration rate (GFR) below 60 ml/min/1.73 m^2 invariably progresses to end stage renal disease (ESRD). Causes of CKD in children differ substantially from those in adults [1,2]. Large studies in adults have demonstrated protective effect of angiotensin converting enzyme inhibitor (ACEI) in retarding disease progression in diabetic and other proteinuric nephropathies [3]. However, comparable data from randomized controlled trials involving children are lacking. While renin-angiotensin system (RAS) antagonists are used in 80% of CKD children with glomerular etiology its use has been reported in 47% of the non-glomerular CKD [4]. The Italian Pediatric Registry of chronic renal insufficiency concluded that it was unclear whether ACEI retarded progression of CKD in hypodysplastic CKD [5]. Since these diseases are associated with reduction in nephron mass with consequent hyperfiltration, there is a potential rationale for use of ACEI for renoprotection. Although ACEI are frequently used as antihypertensive and antiproteinuric agents in children, their efficacy in slowing progression of renal disease has not been prospectively examined. Recently, a large randomized controlled (ESCAPE) trial using ACEI in children showed that intensified blood pressure control was renoprotective as compared to conventional blood pressure control[6]. Since both groups received ramipril, the trial demonstrated benefits of strict blood pressure control rather than efficacy of ACEI in retarding progression of CKD.

The present study was conducted to evaluate whether ACE inhibition by enalapril retards rate of decline in GFR and decrease proteinuria in children with CKD.

METHODS

This prospective, single-center, open-label, randomized controlled trial was conducted at the Pediatric Nephrology Clinic of a tertiary care hospital over 48-month period ending in October 2008. The study protocol was approved by the Institute Ethics Committee. Written informed consent was obtained from the parents before inclusion in the study.

Children from 2 to 18 years of age with estimated

GFR between 15 and 60 mL/min/1.73 m^2 [7] with or without hypertension and proteinuria were screened for the study. Those with radionuclide GFR between 15 and 60 ml/min/1.73 m^2 were eligible for the study. Patients were excluded if they had stage II hypertension, serum potassium >5.5 mEq/L, renal artery stenosis, therapy with ACEI or angiotensin receptor blockers (ARB) or non-steroidal anti-inflammatory drugs in the last 2 months, surgery for obstructive uropathy or therapy with immunosuppressive agents in the last 6 months. Prior to inclusion, no patient underwent washout of enalapril.

Initial evaluation included measurement of blood pressure, blood counts and blood levels of creatinine, electrolytes, urinary protein and creatinine, and estimation of radionuclide GFR.

Blood pressure was measured by auscultatory technique and hypertension was defined based on criteria of the Fourth Report on Hypertension[8]. GFR estimation was performed by two sample plasma disappearance method after intravenous administration of 1 mCi ^{99m}Tc-DTPA, followed by collection of blood samples at 60 and 180 minutes [9]. Urinary protein concentration was measured using pyrogallol red-molybdate complex method on autoanalyzer. Serum and urinary creatinine concentrations were measured by modified Jaffé reaction [10]. Proteinuria was expressed as the ratio of protein to creatinine (Up/Uc), as determined in spot urine samples.

Eligible patients were randomized to either enalapril or non-enalapril group. Enalapril was given in a single bedtime dose of 0.4 mg/kg for 12 months. Permuted block randomization was performed using block size of four by an individual not involved in trial implementation. The investigators were blinded to the randomization schedule and allocation was concealed in opaque sealed envelopes. Standard therapy for CKD was continued in both groups. Therapy with antihypertensives other than ACEI, ARB and calcium channel blockers was continued to maintain blood pressure <90th percentile for age, height and gender.

Follow up: Clinical evaluation, consisting of physical examination, blood pressure, renal function tests, electrolytes, complete blood count, Up/Uc were performed at the beginning of the study and after 15 days, and 3, 6, and 12 months. Compliance was assessed at each visit by pill count. DTPA GFR was repeated at 6 and 12 months. Patients were withdrawn from the study if two consecutive serum potassium levels were >6 mEq/L or serious adverse events occurred.

The primary outcomes were decline in GFR and percentage change in Up/Uc during 1 year. Secondary outcome measures included occurrence of composite outcome and systolic and diastolic blood pressure Standard deviation scores (SDS). Composite outcome was defined as decline in GFR by >30% or attainment of ESRD. The end point of the study was attainment of composite outcome or completion of 1-year follow up.

Outcomes were also assessed in the subgroup of children with proteinuria defined as Up/Uc >1.5 and without proteinuria (Up/Uc <1.5).

Blood pressure values were normalized to SDS [7]. All adverse events and serious adverse events were recorded.

Statistical analysis: In a previous study, the mean monthly rate of decline of GFR in patients not treated with ACEI was reported as 0.29 ml/min/1.73 m^2 [11]. Assuming a 50% difference in decline in GFR from baseline between enalapril treated and untreated group, and considering a drop-out rate of 10%, power of 0.80 and an α error of 0.05 the study required 20 patients in each group. This sample size was also sufficient to detect 50% proteinuria reduction by ACEI [12]. Data are presented as mean ± SD and analyzed using intention-to-treat principle. The missing data for patients who were lost to follow up was computed using the mean decline of GFR and percentage reduction of urine protein to creatinine ratio as observed in the non-enalapril group. Since the outcomes in the study were correlated and longitudinal, we used generalized estimating equations for analysis [13]. P<0.05 was considered significant.

RESULTS

Sixty six patients with CKD stages 3 and 4 were screened for inclusion in the study and 41 patients (2 girls) were randomized into two groups (***Fig*.1**). The baseline characteristics of the two groups were similar (***Table* I**). The chief underlying causes of CKD were reflux nephropathy and obstructive uropathy. At inclusion, 6 patients had systolic and/or diastolic blood pressure above 95th percentile, and 4 were receiving antihypertensive drugs (amlodipine in 3, prazosin in 1). Twenty three patients had proteinuric and 18 had non-proteinuric CKD. The baseline mean Up/Uc was significantly higher (5.1±3.8 *vs* 0.82±0.56, P<0.001) and the mean baseline GFR lower (28.9±8.7 *vs* 22.4 ±6.5 ml/min/1.73 m^2, P=0.01), in the proteinuric as compared to non-proteinuric patients respectively.

At 1 year, the rate of decline in GFR was 3.0±4.2 in the enalapril and 4.2±5.1 mL/min/1.73 m^2 in the non-enalapril group (P=0.51). The treatment with enalapril was associated with slower GFR decline (regression

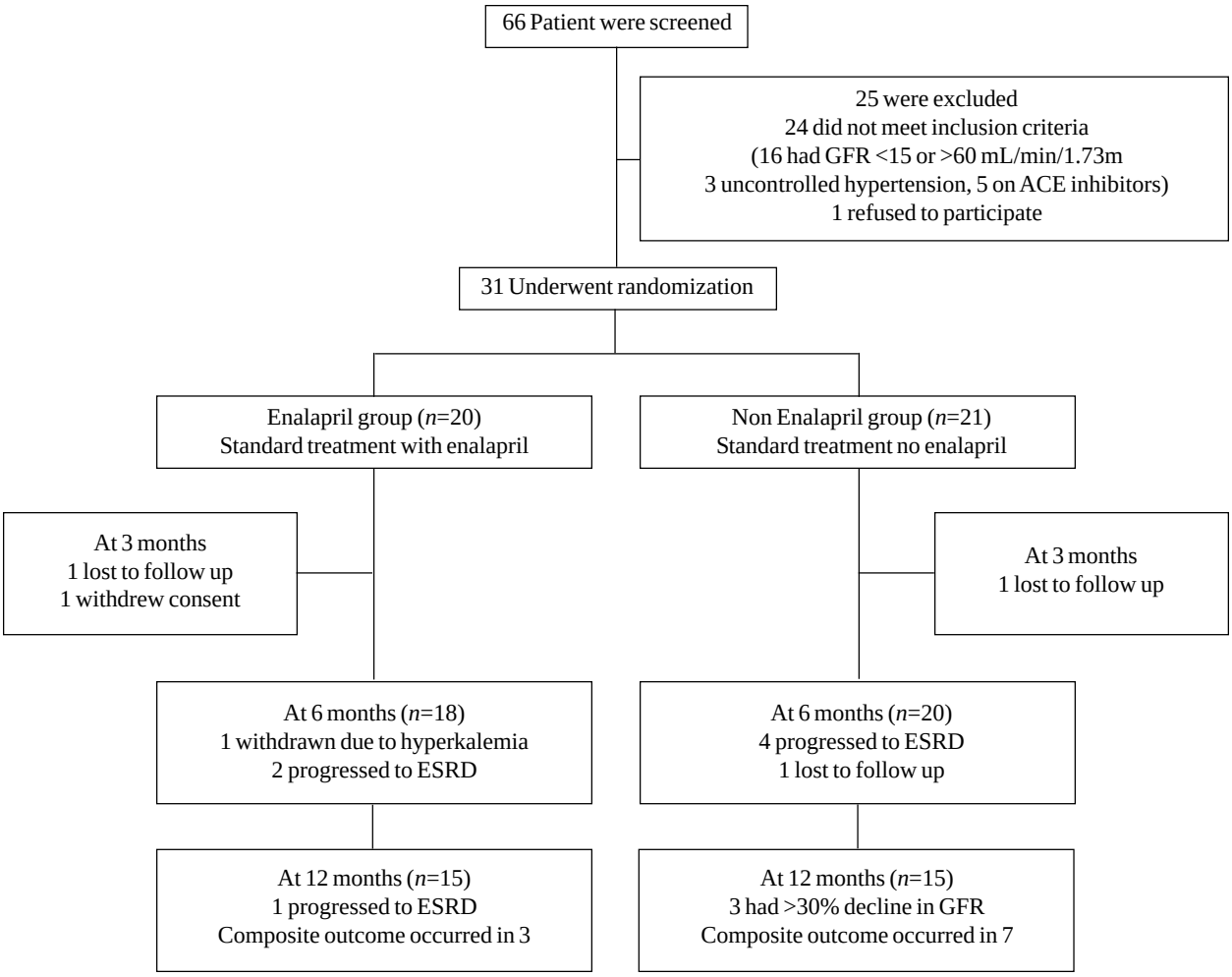

Fig. 1 *Flow of participants during the study.*

coefficient -0.78, 95% CI -3.59, 2.03; *P*=0.58), but not statistically significant during 1-year period (***Table* III**). In the subgroup of patients with proteinuria (*n*=23), the rate of GFR decline was 3.8±5.2 *vs.* 8.6±7.9 mL/min/1.73 m^2 per year in enalapril and non-enalapril group, respectively.

The percentage change in proteinuria was significantly higher in enalapril treated group as compared to non enalapril group at both 6 months and 12 months (***Table* II**). Proteinuria reduction was correlated to baseline protein excretion (r=0.72, P<0.001) and baseline GFR (r=-0.43, *P*=0.03). The difference in proteinuria reduction remained significant after adjustment for proteinuria and GFR at baseline (*P*=0.02). In the subgroup with proteinuria, there was significantly higher percentage reduction in proteinuria in enalapril group which remained significant after adjustment for blood pressure (***Web Table* I**). In the subgroup with Up/Uc <1.5 (*n*=18) the percentage reduction in proteinuria was also higher in the enalapril treated patients (regression coefficient 200, 95% CI 21.4 to 379; *P*=0.03).

Secondary outcomes: The composite outcome was assessed in 36 (87.8%) patients; 17 in enalapril and 19 in non-enalapril group. Three (17.6%) patients in enalapril group and 7 (36.8%) in non-enalapril group attained the composite outcome (***Fig.* 1**). In the proteinuric subgroup, 1 of 13 (7.7%) patients treated with enalapril and 6 of 10 (60%) patients in the non-enalapril group attained composite outcome (*P*=0.01). Occurrence of composite outcome was significantly lower in the proteinuric patients treated with enalapril (*P*=0.003) and remained significant after adjustment for proteinuria and blood pressure. However, the composite outcome was not

TABLE I BASELINE CHARACTERISTICS OF INCLUDED PATIENTS

	Enalapril (N=20)	*Non-enalapril (N=21)*
Age, yr	8.4 ± 4.3	9.5 ± 4.7
Male sex (%)	20 (100)	19 (90.5)
Underlying renal disorder (%)		
Glomerulonephritis	0 (0)	3 (14.3)
Reflux nephropathy	7 (35)	6 (28.7)
Obstructive uropathy	6 (30)	6 (28.7)
Other, unknown	7 (35)	6 (28.7)
Systolic BP (mm Hg)	105 ± 9.0	109 ± 15.3
Diastolic BP (mm Hg)	66 ± 8.8	69 ± 12.9
>95th percentile of BP (%)		
Prior antihypertensive treatment (%)	2	2
GFR (ml/min/1.73 m^2)#	26.5 ± 7.4	25.3 ± 8.8
Creatinine (mg/dL)	1.8 ± 0.12	2.1 ± 0.23
Potassium (mEq/L)	4.4 ± 0.08	4.3 ± 0.16
Up/Uc (mg/mg)*	3.0 ± 2.7	2.8 ± 4.2
>1.5 Up/Uc	13 (65)	10 (47.6)

*Values are mean±SD; *Up/Uc - Urinary protein to creatinine ratio; #^{99m}Tc-DTPA; *Urinary protein-to-creatinine ratio; BP = Blood pressure.*

significantly different in the non-proteinuric group (regression coefficient 0.13, 95% CI -0.23 to 0.49, *P*=0.4).

At 3 months, mean reduction in systolic and diastolic blood pressures from the baseline was 6.1±8.4 mm Hg and 5.5±5.8 mm Hg in the enalapril group as compared to 1.7±1.5 mmHg and 0.8±0.7 mm Hg in the non-enalapril group. The systolic and diastolic blood pressure SDS were lower in the enalapril treated patients as compared to non-enalapril group during the study (***Table* III**). Serum potassium was higher in enalapril treated patients during the study period (*P*=0.07); the mean increase from baseline was 0.6±0.5 mEq/L at 1 year.

Adverse events were similar in the two groups. One patient in the enalapril group was withdrawn due to hyperkalemia.

DISCUSSION

Majority of patients had congenital abnormalities of the kidney and urinary tract; about half of the patients had moderate proteinuria (Up/Uc>1.5). ACE inhibition when started early in CKD may be more renoprotective as compared to late CKD stages. Considering slow decline in GFR in early CKD and short follow-up period of the study, children with CKD stage II were unlikely to have outcome and hence were not included in the study. The study was unable to demonstrate a significant benefit of enalapril treatment in the rate of decline of GFR or occurrence of composite outcome. However, occurrence of composite outcome was significantly lower in subgroup of patients with proteinuria treated with enalapril which persisted after adjustment for proteinuria and blood pressure.

There is limited data on the efficacy of RAS inhibition for renoprotection in children. Small uncontrolled studies have shown beneficial effects of ACEI in children with proteinuric CKD and hemolytic uremic syndrome [14,15]. Litwin, *et al*. [16] showed add-on renoprotection with losartan added to ACEI in 11 patients with CKD. A retrospective analysis of the Italkid project did not show improved renal survival after an average 5-year follow-up with ACEI [5]. The major limitations of this study were selection bias and lack of

TABLE II CHANGES IN THE PARAMETERS (MEAN±SD) DURING TREATMENT IN ENALAPRIL AND NON-ENALAPRIL GROUPS

	Enalapril		*Non-enalapril*		*P value*	
	6 Months	*12 Months*	*6 Months*	*12 Months*	*6 Months*	*12 Months*
DTPA GFR (mL/min/1.73 m^2)	22.4±7.6	22.6±5.8	20.1±9.5	25.3±10.7	0.53	0.42
GFR decline (mL/min/1.73 m^2)		3.0±4.2		4.2±5.1		0.51
Urine protein/creatinine (mg/mg)	1.2±1.6	0.57±0.56	1.9±1.0	1.7±1.5	0.38	0.01
Percentage change in proteinuria	57.3±40.1	65.8±40.5	-56.9±97	-199±345	0.01	0.0005
Systolic BP SDS	0.54±1.0	0.56±0.89	1.29±1.26	1.16±0.82	0.05	0.07
Diastolic BP SDS	0.68±0.85	0.81±0.81	1.26±0.95	1.45±0.68	0.06	0.03
Serum creatinine (mg/dL)	2.2±0.9	2.1±0.9	2.5±1.7	2.5±1.5	0.44	0.46
Serum potassium (mEq/L)	5.1±0.4	5.1±0.5	4.7±0.6	4.8±0.9	0.01	0.23

GFR: Glomorular filtration rate; BP: Blood pressure; SDS: standard scores.

TABLE III OUTCOMES WITH ENALAPRIL AS COMPARED TO NON ENALAPRIL TREATMENT (*N*=41)

Outcome	**Regression coefficient ± SE*	*95% CI*	*P value*
GFR decline	-0.78±1.43	-3.59, 2.03	0.58
Composite outcome	-0.83±0.80	-2.40±0.73	0.29
Percentage change in proteinuria *(unadjusted)*	179.4±48.4	84.6, 274.3	<0.001
adjusted for blood pressure	198.5±51.5	97.5, 299.3	<0.001
Systolic BP SDS	- 0.54±0.27	-1.07, -0.01	0.04
Diastolic BP SDS	- 0.45±0.24	-0.92, 0.02	0.06
Serum potassium	0.23±0.13	-0.02, 0.49	0.07

information on dosage of ACEI. Due to lack of data on proteinuria, the study could not assess the benefits of ACEI in proteinuric CKD.

ACEI reduce the risk of doubling of serum creatinine or ESRD by 30-40% in adults, which is related to the degree of proteinuria [17,18]. Proteinuria and hypertension have been shown to be independent predictors of decline in renal function in children with CKD [19]. ESCAPE trial also showed that residual proteinuria was associated with progression of renal failure [6].

There was a significant reduction in proteinuria in the enalapril group in our study. Studies have shown reduction in proteinuria ranging from 30-50% in children with CKD treated with ACEI and ARB [6,20-22]. ESCAPE trial demonstrated equally effective reduction in proteinuria with ramipril in children with glomerulopathy and hypoplasia-dysplasia. However the proteinuria increased later to nearly baseline level at three years. This late increase in proteinuria which is attributed to "aldosterone breakthrough" phenomenon was not observed in our study and proteinuria steadily decreased over 12-month period.

The renoprotection observed in the proteinuric subgroup could be due to both antiproteinuric and antihypertensive effect of enalapril. However, on multivariate regression, better renal outcome in enalapril treated group with proteinuric CKD was independent of blood pressure and proteinuria reduction. Diminished local release of cytokines, inhibition of inflammatory pathways and reduced oxidative stress could explain the renoprotective effect of RAS inhibitors independent of proteinuria and blood pressure control [23].

More than 90% of the study subjects were males. This could have been due to gender bias in seeking medical care, and also preponderance of genitourinary anomalies, which are common in boys. The sample size was small, which did not allow detection of smaller differences in GFR decline in the two groups. As the number of patients with glomerular disease was small, the effect of enalapril in children with glomerular *versus* non-glomerular disease could not be examined. The study included patients with GFR below 30 ml/min/ $1.73m^2$ who could potentially develop hyperkalemia with ACE inhibitors. Therefore we used enalapril in submaximal doses which could have obscured the renoprotective effect. However, it seems unlikely as the antiproteinuric effect of enalapril was substantial. Single-center trial, non-blinding and short follow-up period are other limitations of the study. The subgroup analysis was not decided *a priori* and not taken into consideration for the sample size calculation. Thus findings of subgroup analysis are at best limited to hypothesis generation. We conclude that enalapril appears to be effective in reducing proteinuria in children with CKD and might retard progression to end stage renal failure in proteinuric CKD. While proteinuria reduction with ACEI is a fairly well established finding, its renoprotective efficacy needs to be confirmed in non-proteinuric children with CKD by large well-designed multi-centric trials.

Contributors: PH: conceived and designed the study, interpreted the data and drafted the manuscript; JS: collected the data and helped in manuscript writing; AS: helped in analysis of the data and in manuscript writing; RMP: analysed the data and helped in manuscript writing; CSB: conducted the laboratory test and interpreted them; AB: designed the study and revised the manuscript for important intellectual content. He will also act as guarantor of the study. The final manuscript was approved by all authors.
Funding: None; *Competing interest*: None stated.

REFERENCES

1. Warady BA, Chadha V. Chronic kidney disease in children: the global perspective. Pediatr Nephrol. 2007;22:1999-2009.
2. Hari P, Singla IK, Mantan M, Kanitkar M, Batra B, Bagga A. Chronic renal failure in children. Indian Pediatr. 2003;40:1035-42.
3. Bakris GL. Slowing nephropathy progression: focus on proteinuria reduction. Clin J Am Soc Nephrol. 2008;3:S3-10.
4. Wong CS, Pierce CB, Cole SR, Warady BA, Mak RH, Benador NM, *et al*. CKiD Investigators. Association of proteinuria with race, cause of chronic kidney disease, and glomerular filtration rate in the chronic kidney disease in children study. Clin J Am Soc Nephrol. 2009;4:812-9.
5. Ardissino G, Viganò S, Testa S, Daccò V, Paglialonga F, Leoni A, *et al*. Italkid Project. No clear evidence of ACEi efficacy on the progression of chronic kidney disease in children with hypodysplastic nephropathy—report from

WHAT IS ALREADY KNOWN?

- Angiotensin converting enzyme inhibitors slow decline in glomerular filtration rate and reduce proteinuria in adults with chronic kidney disease.

WHAT THIS STUDY ADDS?

- Enalapril was found to be effective in reducing proteinuria in children with CKD.
- Enalapril might retard progression in proteinuric CKD which needs to be confirmed by large well-designed multi-centric trial.

the ItalKid Project database. Nephrol Dial Transplant. 2007;22:2525-30.
6. ESCAPE Trial Group, Wühl E, Trivelli A, Picca S, Litwin M, Peco-Antic A, Zurowska A, *et al*. Strict blood-pressure control and progression of renal failure in children. N Engl J Med. 2009;22:1639-50.
7. Schwartz GJ, Haycock GB, Edelmann CM Jr, Spitzer A. A simple estimate of glomerular filtration rate in children derived from body length and plasma creatinine. Pediatrics. 1976;58:259-63.
8. National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics. 2004;114:555-756.
9. Piepsz A, Colarinha P, Gordon I, Hahn K, Olivier P, Sixt R, *et al*. Guidelines for glomerular filtration rate determination in children. Eur J Nucl Med. 2001;28:BP31-6.
10. Romeo J. Evaluation of a kinetic method for creatinine. Lab Med. 1975;6:15-8.
11. Ruggenenti P, Perna A, Gherardi G, Garini G, Zoccali C, Salvadori M, *et al*. Renoprotective properties of ACE-inhibition in non-diabetic nephropathies with non-nephrotic proteinuria. Lancet. 1999;31:359-64.
12. Seeman T, Dusek J, Vondrák K, Flögelová H, Geier P, Janda J. Ramipril in the treatment of hypertension and proteinuria in children with chronic kidney diseases. Am J Hypertens. 2004;17:415-20.
13. Zeger SL, Liang KY. Longitudinal data analysis for discrete and continuous outcomes. Biometrics. 1986;42:121-30.
14. Ellis D, Vats A, Moritz ML, Reitz S, Grosso MJ, Janosky JE. Long-term antiproteinuric and renoprotective efficacy and safety of losartan in children with proteinuria. J Pediatr. 2003;143:89-97.
15. Van Dyck M, Proesmans W. Renoprotection by ACE inhibitors after severe hemolytic uremic syndrome. Pediatr Nephrol. 2004;19:688-90.
16. Litwin M, Grenda R, Sladowska J, Antoniewicz J. Add-on therapy with angiotensin II receptor 1 blocker in children with chronic kidney disease already treated with angiotensin-converting enzyme inhibitors. Pediatr Nephrol. 2006;21:1716-22.
17. Ruggenenti P, Schieppati A, Remuzzi G. Progression, remission, regression of chronic renal diseases. Lancet. 2001;19:1601-8.
18. Jafar TH, Schmid CH, Landa M, Giatras I, Toto R, Remuzzi G, *et al*. Angiotensin-converting enzyme inhibitors and progression of nondiabetic renal disease. A meta-analysis of patient-level data. Ann Intern Med. 2001;17:73-87.
19. Wingen AM, Fabian-Bach C, Schaefer F, Mehls O. Randomised multicentre study of a low-protein diet on the progression of chronic renal failure in children. European Study Group of Nutritional Treatment of Chronic Renal Failure in Childhood. Lancet.1997;19:1117-23.
20. Webb NJ, Shahinfar S, Wells TG, Massaad R, Gleim GW, Santoro EP, *et al*. Losartan and enalapril are comparable in reducing proteinuria in children. Kidney Int. 2012;Jun 27. doi: 10.1038/ki.2012.210.
21. Webb NJ, Lam C, Loeys T, Shahinfar S, Strehlau J, Wells TG, *et al*. Randomized, double-blind, controlled study of losartan in children with proteinuria. Clin J Am Soc Nephrol. 2010;5:417-24.
22. Webb NJ, Lam C, Shahinfar S, Strehlau J, Wells TG, Gleim GW, *et al*. Efficacy and safety of losartan in children with Alport syndrome—results from a subgroup analysis of a prospective, randomized, placebo- or amlodipine-controlled trial. Nephrol Dial Transplant. 2011;26:2521-6.
23. Izuhara Y, Nangaku M, Inagi R, Tominaga N, Aizawa T, Kurokawa K, *et al*. Renoprotective properties of angiotensin receptor blockers beyond blood pressure lowering. J Am Soc Nephrol. 2005;16:3631-41.