Prospective Study on Several Urinary Biomarkers as Indicators of Renal Damage in Children with CAKUT

Fabio Bartoli[1] Valentina Pastore[1] Isabella Calè[2] Gabriella Aceto[2] Vittoria Campanella[1] Carla Lasalandra[1] Simona Magaldi[1] Francesco Niglio[1] Angela Basile[1] Raffaella Cocomazzi[1]

[1] Pediatric Surgery Unit, University of Foggia, Foggia, Italy
[2] Pediatric Nephrology Unit, University of Bari, Bari, Italy

Address for correspondence Fabio Bartoli, MD, Pediatric Surgery Unit, Viale Pinto, Foggia 71100, Italy (e-mail: fabio.bartoli@unifg.it).

Eur J Pediatr Surg

Abstract

Purpose The aim of the study was to investigate urinary levels of monocyte chemotactic protein-1 (MCP-1), epidermal growth factor (EGF), β-2-microglobulin (β2M), and FAS-ligand (FAS-L) in children with congenital anomalies of kidney and urinary tract (CAKUT) disease at risk of developing glomerular hyperfiltration syndrome. For this reason, we selected patients with multicystic kidney, renal agenesia and renal hypodysplasia, or underwent single nephrectomy.

Materials and Methods This prospective, multicentric study was conducted in collaboration between the Pediatric Surgery Unit in Foggia and the Pediatric Nephrology Unit in Bari, Italy. We enrolled 80 children with CAKUT (40 hypodysplasia, 22 agenetic; 10 multicystic; 8 nephrectomy) who underwent extensive urological and nephrological workup. Exclusion criteria were recent urinary tract infections or pyelonephritis, age $>$ 14 years, presence of systemic disease, or hypertension. A single urine sample was collected in a noninvasive way and processed for measuring by enzyme-linked immunosorbent assay urine levels of MCP-1, EGF, β2M, and FAS-L. As control, urine samples were taken from 30 healthy children.

Furthermore, we evaluated the urinary ratios uEGF/uMCP-1 (indicator of regenerative vs inflammatory response) and uEGF/uβ2M (indicator of regenerative response vs. tubular damage).

Results These results suggest that urinary levels of MCP-1 are overexpressed in CAKUT patients. Furthermore, our findings clearly demonstrated that both uEGF/uMCP-1 and uEGF/uβ2M ratios were significantly downregulated in all patient groups when compared with the control group.

Conclusion These findings further support that CAKUT patients may, eventually, experience progressive renal damage and poor regenerative response. The increased urinary levels of MCP-1 in all groups of CAKUT patients suggested that the main factor responsible for the above effects is chronic renal inflammation mediated by local monocytes.

Keywords
- CAKUT
- urinary biomarkers
- children
- MCP-1
- EGF

Introduction

Congenital anomalies of kidney and urinary tract (CAKUT) are common findings during morphological prenatal ultrasound. Vesicoureteral reflux (VUR) and ureteropelvic junction obstruction (UPJO) are the most common. However, more sporadic forms are represented by renal agenesia, hypodysplasia, and multicystic kidney. The incidence of CAKUT has been reported as high as 5% of live birth neonates.[1] It is also a significant cause of chronic renal failure and related disorders

received
September 4, 2017
accepted after revision
March 22, 2018

DOI https://doi.org/10.1055/s-0038-1646960.
ISSN 0939-7248.

such as nephrogenic hypertension, urolithiasis, and pyelonephritis.[1] Patients with monolateral nonfunctioning/absent kidney experience a compensatory response in the opposite one. However, in the long term, these only functioning kidneys may develop a well-known form of renal damage named "glomerular hyperfiltration syndrome" (GHS). The first clinical sign of this syndrome is proteinuria, which, as matter of fact, is already the consequence of significant and progressive renal damage (sometimes just transitory). In fact, the physiopathology of GHS for nephron or for total kidney may be related to several medical conditions such as compensatory renal response, diabetes, pregnancy, sickle cell anemia, and polycystic kidney.[2] A better understanding of mechanisms involved in children at risk of developing GHS may contribute to developing new strategies in the prevention of progressive renal damage. The aim of this prospective study was to better understand the development of GHS in children with single or nearly single kidney function through the evaluation of urinary levels of epidermal growth factor (EGF, an important renal growth factor), monocyte chemotactic protein-1 (MCP-1, a proinflammatory cytokine), FAS ligand (FAS-L, a pro-apoptotic factor) and β-2-microglobulin (β2M, as marker of tubular damage) in children affected by renal agenesia, hypodysplasia, multicystic kidney or underwent single nephrectomy. We expect to find higher levels of these urinary biomarkers (especially MCP-1 and β2M) in groups with single functioning kidney compared with poorly functioning kidney (hypodysplasia) or control.

To our knowledge, there is no study in literature on children with CAKUT and single kidney or poorly functioning kidney where these biomarkers have been evaluated.

Materials and Methods

This is a prospective multicentric study between the Pediatric Surgery Unit of the University of Foggia, Italy, and the Pediatric Nephrology Unit of the University of Bari, Italy. Most of the clinical cases were provided by the Unit in Bari, while most of the laboratory analysis was performed in Foggia. Eighty children were included in the study. Their charts were evaluated for timing of diagnosis, history of infections, associated symptoms, and pathology. Laboratory analyses include urinalysis, urine culture, inflammatory index, urinary electrolytes, urinary osmolality, urinary β2M, and urinary creatinine. All had renal ultrasound, micturating cysto-urethrogram, and dimercaptosuccinic acid renal scan. The exclusion criteria were recent (< 3 months from urine sample collection) urinary tract infection (UTI) or pyelonephritis (< 1 year from urine sample collection), diagnosis of CAKUT or nephrectomy < 6 months from inclusion in the study, age > 14 years old, and presence of systemic diseases or nephrogenic hypertension. Presence of proteinuria was not considered an exclusion criterion.

►Table 1 reports the distribution of the five groups of children according to type of disease, sex, and age. Group 1= hypoplastic; Group 2= agenetic; Group 3= multicystic; Group 4= nephrectomy; Group 5= control. Furthermore, ►Table 2 reports significant clinical information on their renal and urinary situation at the time of urine sample collection.

Table 1 Division of children in groups according to the main diagnosis, number, sex, and mean age

Groups	Number (sex)/mean age (years)
Hypoplastic (group 1)	40 (28 M, 12 F)/6
Agenesic (group 2)	18 (10 M, 8 F)/5
Multicystic (group 3)	14 (4 M, 10 F)/3.2
Nephrectomy (group 4)	8 (6 M, 2F)/9.7
Controls (group 5)	30 (20 M, 10F)/5

Hypoplastic kidneys were considered those children with significantly reduced renal size with some residual function. In these children, split renal function ranged between 10 and 25%. Histological differentiation from dysplastic kidney could not be done because all parents denied consent for renal biopsy. Of the 40 patients, 11 had bilateral VUR, while 17 had monolateral VUR (13 in the hypoplastic kidney and 4 in the good functioning kidney). All of them had successful endoscopic treatment with Deflux or Macroplastique (after urine sample collection).

In the nephrectomy group, indications for nephrectomy were in four patients with severe VUR with recurrent UTIs and split renal function <10%; in two patients with nonfunctioning multicystic kidney and in two patients with nonfunctioning kidney following severe UPJO.

As control group, we collected urine samples from 30 healthy children with a mean age of 48 ± 24 months.

In all cases, informed consent was obtained from parents to collect urine sample in noninvasive way and to use relevant clinical information for the purpose of this study. This study was approved by ethical commission for patients data display.

Urine samples were collected early in the morning, centrifuged at 3500 rpm/g for 10 minutes, and frozen at −20° until tested. All urine samples were pH > 6 and free from consumption of nephrotoxic drugs.

Analysis of Urinary Markers

Urine samples were defrosted, centrifuged again at 3500 rpm for 10 minutes, and the surfactant was then separated.

Enzyme-Linked Immunosorbent Assay

MCP-1 and EGF urine concentrations were quantitated with a human MCP-1 and an EGF enzyme-linked immunosorbent assay (ELISA) kit (Quantikine; R&D Systems, Abingdon, United Kingdom, and Biotrak, Amersham, United Kingdom, respectively) whereas β2M was quantitated with a human kit (Human Beta-2 Microglobulin ELISA kit; Alpha Diagnostic International, San Antonio, Texas, United States), as previously reported.[3] FAS-L urinary concentrations were analyzed by using a human kit (Sigma-RAB0178, Human Fas Ligand ELISA Kit).

Urine concentrations of MCP-1, EGF, FAS-L, and β2M were normalized to urine creatinine excretion (CreatU: expressed in mg/dL). The levels of EGF, MCP-1, and FAS-L were expressed in pg/mL, while those of β2M in ng/mL.

Table 2 Reported clinical relevant information about present or past history (h/y) for all groups

Hypoplastic (group 1)	Agenesic (group 2)	Multicystic (group 3)	Nephrectomy (group 4)
24 patients with 1–3° VUR 8 patients with pyelectasis 2 patients with h/y of recurrent UTIs SRF in hypoplastic kidney range from 3% to 25%	2 patients with pyelectasis and moderate VUR 2 patients with hydronephrosis 2 patients with h/y of pyelonephritis	2 patients with UPJO 2 patients with omolateral VUR 2 patients with h/y of recurrent UTIs 4 patients with prenatal h/y of multicystic kidney disappeared	4 patients for 4–5° VUR and SRF < 10% 2 patients for multicystic kidney 2 patients for severe UPJO All with apparent normal residual kidney

Abbreviations: VUR, vesicoureteral reflux; UTIs, urinary tract infections; SRF, split renal function; UPJO, ureteropelvic junction obstruction.

Table 3 Descriptive statistics of urinary concentrations of EGF, MCP-1, FAS-L, and β2M normalized to urinary creatinine

	Hypoplastic (group 1)		Agenesic (group 2)		Multicystic (group 3)		Nephrectomy (group 4)		Control	
	Mean/SD	Min/Max	Mean/SD	Min/Max	Mean/SD	Min/Max	Mean/SD	Min/Max	Mean/SD	Min/Max
EGF (pg/mL)	754/435	252/1734	628/252	398/1260	794/243	611/1185	408/201	252/729	515/168	223/753
MCP-1 (pg/mL)	2.69/1.0	1.4/4.4	2.96/0.53	2.0/3.7	3.3/1.3	2.3/5.4	1.68/0.30	1.5/2.1	2.2/1.6	0.90/6.1
FAS-L (pg/mL)	0.008/0.01	0.00/0.012	0.014/0.015	0.00/0.03	0.001/0.007	0.00/0.01	0.007/0.01	0.00/0.19	0.012/0.02	0.00/0.027
β2M (ng/mL)	1.2/1.2	0.00/3.9	0.76/0.31	0.43/1.35	1.7/1.4	0.08 /3.3	1.0/1.03	0.16/2.65	0.39/0.32	0.01/1.2
EGF/ MCP-1	299/143	111/517	258/129	86/547	227/40	157/262	208/118	42/352	328/77	190/580
EGF/ β2M	1.23/1.26	0.17/4.81	1.58/1.51	0.18/5.1	0.48/0.19	0.27/ 0.73	0.98/0.76	0.09/1.94	4.4/2.2	2.61/6.6

Abbreviations: EGF, epidermal growth factor; FAS-L, Fas-ligand (FAS-L); β2M, β-2-microglobulin; MCP-1, monocyte chemotactic protein-1; SD, standard deviation.
Note: In addition are reported EGF/MCP-1 and EGF/β2M ratios (arbitrary units) in all groups.

Statistical Analysis

Data was expressed as mean ± standard deviation. Quantitative data was compared between groups by analysis of variance and a Mann-Whitney U test with $p < 0.05$ as the significance cutoff.

Results

►**Table 3** reports the descriptive statistics of urinary concentrations of urinary EGF (uEGF)/CreatU (pg/mL), urinary MCP-1 (uMCP-1)/CreatU (pg/mL), uFAS-L/CreatU (pg/mL), and urinary β2M (uβ2M) (ng/mL). The same table reports the values of the ratios EGF/MCP-1 and EGF/β2M, both expressed as arbitrary units.

Urinary Concentrations of uEGF/CreatU (pg/mL)

The lowest values in urinary concentrations of uEGF/CreatU were observed in the nephrectomy group (G4). The urinary concentrations were significantly downregulated in controls (G5) and nephrectomy (G4) groups when compared with hypoplastic (G1), agenetic (G2), and multicystic (G3) ones. There were no other statistical differences between the other groups. Also, when we matched the three groups (G2 + G3 + G4) with single functioning kidney with controls, we could not find significant statistical differences (►**Fig. 1**).

Urinary Concentrations of uMCP-1/CreatU (pg/mL)

The lowest values in urinary concentrations of uMCP-1/CreatU were observed in the nephrectomy group, while the highest

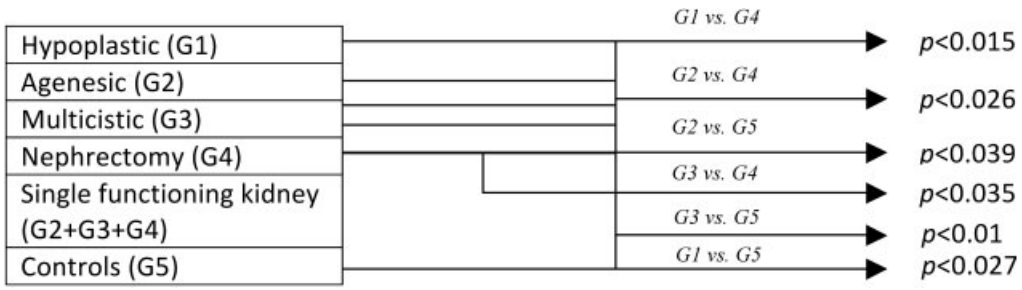

Fig. 1 Urinary concentrations of epidermal growth factor (EGF) normalized to urinary creatinine have been matched to find significant statistical differences.

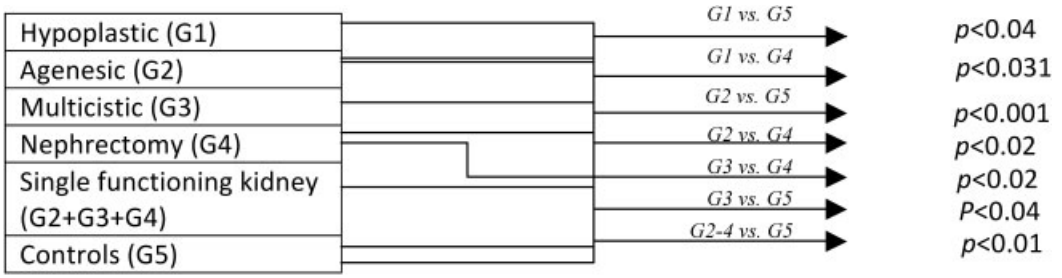

Fig. 2 Urinary concentrations of monocyte chemotactic protein-1 (MCP-1) normalized to urinary creatinine have been match to find significant statistical differences.

ones in the multicystic group. The hypoplastic, agenetic, and multicystic groups of patients had urinary concentration values significantly upregulated when compared with the controls and nephrectomy groups. In addition, the largest group with single functioning kidney had significant higher urinary levels of uMCP-1/CreatU when compared with controls (►**Fig. 2**).

Urinary Concentrations of uFAS-L/CreatU (pg/mL)

Surprisingly, we found that the lowest urinary concentrations of FAS-L were observed in the multicystic group, while the highest ones in the agenetic and control groups. In fact, the FAS-L/CreatU levels in the hypoplastic, multicystic, and nephrectomy groups were significantly downregulated when compared with the urinary concentrations found in the agenetic and control groups. The urinary levels of FAS-L in the multicystic group were also downregulated when compared with those observed in hypoplastic patients. However, none of the patient groups had urinary concentration of uFAS-L/CreatU above the control group levels (►**Fig. 3**).

Urinary Concentrations of uβ-2-Microglobulin/CreatU (ng/mL)

The urinary concentrations of uβ2M/CreatU were significantly upregulated in all patient groups when compared with controls. We could not find significant differences between patient groups (►**Fig. 4**).

Urinary Ratio uEGF/uMCP-1 (Arbitrary Units)

This ratio is a useful indicator of the relationship between regenerative versus inflammatory response within the kidney and urinary tract.

In the joined whole groups of patients with single functioning kidney (G2–G4), the uEGF/uMCP-1 ratio was downregulated when compared with controls. Furthermore, also the multicystic group expressed a significant downregulation only when compared with controls (►**Fig. 5**). However, we failed to demonstrate other significant statistical differences.

Urinary Ratio uEGF/uβ-2-Microglobulin (Arbitrary Units)

We believe that this ratio may reflect the renal regenerative response in relation to the degree of tubular damage.

The lowest values of uEGF/uβ2M ratio were observed in the multicystic group. All patient groups had a significant downregulation of this ratio when compared with controls. Furthermore, the multicystic group had a significant downregulation of uEGF/uβ2M when compared with the agenetic group. We failed to demonstrate other significant differences between patient groups (►**Fig. 6**).

Discussion

First of all, we would declare that this study has several biases. In fact, this is a clinical study and thus the population included in the study is not uniform being of different age, sex, number of patients/groups, and associated urological conditions. Furthermore, the timing of disease and the forms of treatment received by the patients may have been different. In experimental studies on animals, it is possible to set an exact study design and obtain quite a clear result. On the contrary, these studies often do not reflect the clinical situation, and sometimes may not provide useful information for clinicians. In addition, we did not compare the results

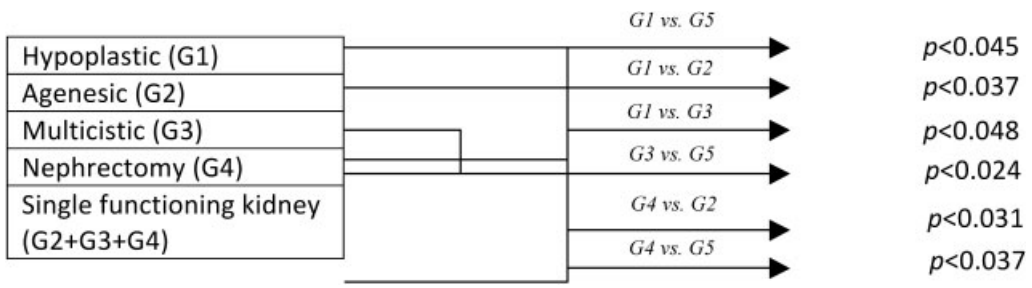

Fig. 3 Urinary concentrations of FAS-ligand (FAS-L) normalized to urinary creatinine have been matched to find significant statistical differences.

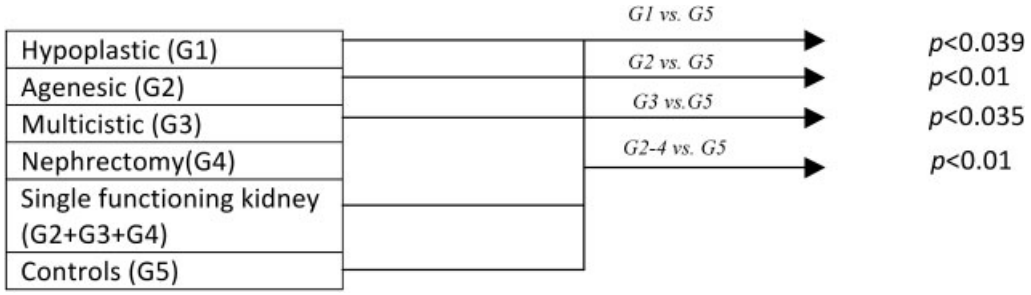

Fig. 4 Urinary concentrations of β-2-microglobulin (β2M) normalized to urinary creatinine have been matched to find significant statistical differences.

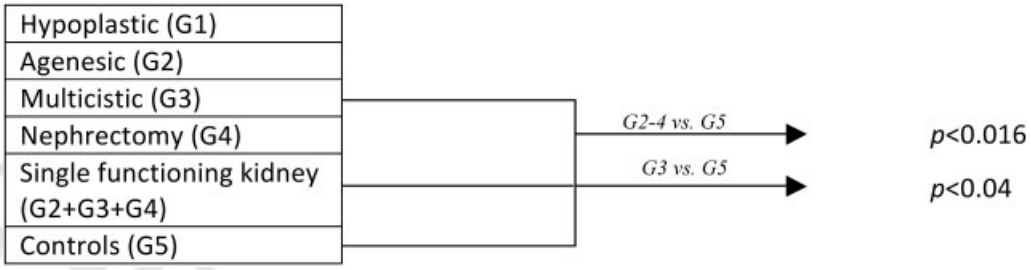

Fig. 5 Urinary concentrations of epidermal growth factor/monocyte chemotactic protein-1 (EGF/MCP-1) ratio (arbitrary units) normalized to urinary creatinine have been matched to find significant statistical differences.

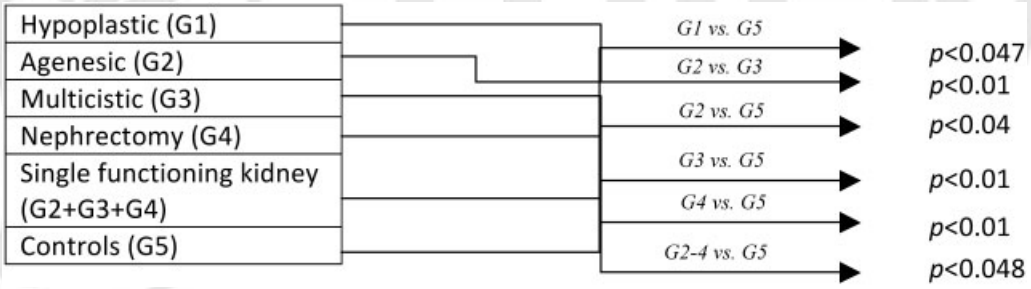

Fig. 6 Urinary concentrations of epidermal growth factor/β-2 microglobulin ratio (EGF/β2M) (arbitrary units) normalized to urinary creatinine have been matched to find significant statistical differences.

with patient's renal function parameters (except urinary creatinine) such as renal scan or glomerular filtration rates (GFRs) because most of the patients had renal function assessment done in different periods. Furthermore, parents and the hospital did not agree to conduct a new renal evaluation for the purpose of the study. As matter of fact, normal healthy children cannot be studied in the same way as for affected children. However, we are planning a follow-up study to collect urine samples at the same time of scheduled follow-up controls. This next study will require several years to be completed.

During the past 20 years, broad literature investigating several biomarkers has been published in major scientific journals. The extensive review of these studies is far beyond the aim of our research and will therefore focus on analyzing the most relevant studies in children with CAKUT disease. In a recent and extensive review,[4] it was underlined that research into biomarkers has reached great importance. Clinical and experimental lines of evidence leave no doubt about the role of inflammation in renal diseases. Understanding the effects of cytokines on the onset and progression of renal injury is thus paramount, as new prognostic markers and maybe as alternative therapeutic targets. In CAKUT patients, chronic obstructive or refluxing nephropathy is often characterized at histopathological level by glomerulosclerosis, tubular atrophy, interstitial inflammation and fibrosis, and monocyte infiltration. Several investigators have focused on these monocytes as a source of cytokines and growth factors in the interstitial space of the obstructed kidneys. MCP-1 is a powerful and specific chemotactic and monocyte-activating factor.[5] Grandaliano et al have demonstrated a striking increase in MCP-1 renal gene expression and urinary excretion in congenital UPJO patients.[6] This overexpression was directly correlated with the extent of monocyte infiltration, as previously shown by Diamonds et al in experimental unilateral ureteral obstruction,[7] and thus confirmed the role of MCP-1 in monocytes recruitment into the interstitial space of the obstructed kidney. Grandaliano et al also showed that MCP-1 is synthesized

locally in the interstitium and tubular epithelium,[6] and Vielhauer et al have confirmed these findings in a mouse model of obstructive nephropathy.[8] Lastly, close correlation of MCP-1 renal gene expression with the degree of tubular damage indicates that MCP-1 is involved in the pathogenesis of tubulointerstitial injury.[6] Recently, it has been confirmed MCP-1 as prognostic biomarker in children with prenatally diagnosed hydronephrosis.[9]

The kidney is an important site for the production of EGF,[10] which plays a major role in renal growth modulation and turnover of tubular cells, glomerular hemodynamics, renal metabolism, tubular transport, and eicosanoid synthesis.[11,12] Tissue EGF downregulation has been reported in several chronic renal diseases,[12–14] in obstructive[3] and in reflux nephropathy.[15]

Furthermore, it has been demonstrated that EGF, MCP-1 (plus their ratio EGF/MCP-1), and β2M may be used as possible indicators of progressive renal damage and recovery in children with urinary tract obstruction[3,6,16] and VUR.[17] Similar findings in obstructive nephropathy patients have been observed by other authors.[18] The separate monitoring of these urinary biomarkers may not be indicative of an ongoing process occurring in renal parenchyma,[19] which is most likely influenced by the interaction of MCP-1 and EGF so we studied also uEGF/uMCP-1 and uEGF/u β2M ratios.[3] The uEGF/uMCP-1 ratio may demonstrate the relationship between tubular regeneration and renal inflammation. Its role, in fact, may be explained by our previous observation that in children with UPJO, an inverse relationship exists between renal gene expression of EGF and MCP-1.[16] Furthermore, proinflammatory cytokines (MCP-1,TGF-β1, etc.) and EGF modulate their renal gene expression through apoptotic[20,21] or anti-apoptotic mechanisms,[22] respectively. In this sense, uEGF/uMCP-1 ratio may help to follow up the progression of parenchymal damage in kidneys.

Our research confirmed a significant upregulation of MCP-1/CreatU levels in hypoplastic, agenetic, and multicystic groups when compared with CTRL, while this finding was not observed in nephrectomy group. The main reason why the lowest uMCP-1/CreatU urinary concentrations were observed in the nephrectomy group is that none of those children had morbid conditions on the surviving kidney and the follow-up time after nephrectomy was short. We have shown that urinary tract obstruction and VUR are associated with increased urinary excretion of MCP-1.[6–8,17] Then, the presence of associated conditions such as urinary tract obstruction and vesicoureteral in hypoplastic, agenetic, and multicystic groups justified itself the increased urine excretion of MCP-1/Creat in those patients.

It is interesting that urine excretion of EGF/Creat was upregulated in hypoplastic, agenetic, and multicystic kidneys compared with CTRL and nephrectomy group. We believe that the lowest uEGF/CreatU urinary concentration in patients who underwent nephrectomy may be due to the limited time to establish compensatory renal growth. In fact, the other three groups of children with congenital disease may have had more time to develop the compensatory renal hypertrophy, which is mainly secondary to EGF production. Since the kidney is the main source of uEGF and EGF has been shown to induce renal growth both in vitro and in vivo, we conclude that EGF may have initiated renal hypertrophy.[23]

The uEGF/ uβ2M ratio, indeed, may reflect the balance between tubular regeneration and proximal tubular injury. As previously discussed, EGF is the most powerful regenerative factor for tubular epithelium and nearly all uEGF is produced by the renal tubular epithelium, while uβ2M excretion is directly correlated with the severity of tubular injury under specific conditions.[24] It is reasonable to suppose that renal expression of EGF may indirectly influence the excretion of uβ2M by improving the capacity of the proximal tubular epithelium to reabsorb this low molecular weight protein. In this sense, uEGF/uMCP-1 and uEGF/uβ2M may give a better idea of the tendency to progression or recovery from the parenchymal damage.

Our results clearly demonstrated that both uEGF/uMCP-1 and uEGF/uβ2M ratios were significantly downregulated in all patient groups when compared with the control group. These findings further support that CAKUT patients may experience progressive renal damage and poor regenerative response. The increased urinary levels of MCP-1 in all groups of CAKUT patients suggested that the main factor responsible for the above effects is chronic renal inflammation mediated by local monocytes and not GHS. This effect is even more evident in patient groups with associated urinary conditions such as VUR or nonobstructive urine flow impairment. Several authors have suggested that MCP-1 is involved in downregulation of renal EGF production[6] and is mediator for tubular damage (which is the main cause of increased β2M urinary excretion).

Our research showed an increased urinary excretion of β2M in all patient groups when compared with controls. The clear point is that significant and, most likely, progressive tubular damage is ongoing in those affected children. These data are consistent with previous reports in patients with UPJO[3] and VUR.[25,26]

One of the mechanisms suspected of being the cause of tubular damage was tubular apoptosis. Tubular cell apoptosis is a major event in the progression of tubular atrophy and renal mass loss in an experimental model of hydronephrosis.[27,28] Furthermore, apoptosis in the obstructed rat kidney is associated with marked reduction in the production of EGF messenger ribonucleic acid (mRNA) from the distal convoluted tubules.[20,21] However, with reversal of obstruction, the pre pro-EGF mRNA levels slowly return to baseline.[21] In chronic kidney disease (CKD), the balance between cell proliferation and apoptosis is disordered with excessive apoptosis of normal glomerular and tubular epithelial cells.[29] Abnormally abundant apoptotic stimuli such as TGF-β, tumor necrosis factor (TNF), FAS-L, and interferon-α result in increased cell death.[30,31] Despite strong scientific evidence of the role played by apoptosis, also FAS-L mediated, we failed to demonstrate overexpression of urinary FAS-L in this selected population of patients. However, we cannot exclude that tissue apoptosis is also present but not measurable by urinary excretion of FAS-L. These results are quite confusing and, in our opinion, the role of apoptosis still remains unclear (at least of FAS-L mediated

apoptosis) as urinary biomarker in this selected population of patients.

Furthermore, we were surprised that the lowest uEGF levels had been measured in nephrectomy patients. We would have expected a more intense regenerative response in this group of children following nephrectomy of affected kidney. We can only explain this finding because of the limited time from nephrectomy to urine sample collection (between 6 and 8 months) or the older age of these children at the time of nephrectomy (~9 years) which may both have contributed at a poorer (apparently) compensatory renal response. It is possible that this period of time is not enough to establish a proper compensatory response that should be mediated by increased tissue production and urinary excretion of EGF.

According to the Brenner theory,[32] low nephron number at birth explains why some individuals are prone to developing progressive renal damage later in life in the presence of other risk factors. Glomerular hyperfiltration has been observed in patients with unilateral renal agenesis,[33] congenitally reduced nephron number,[34] and acquired reduction in renal mass.[35] These individuals are prone to developing proteinuria early in life in association with glomerular sclerosis.

In a rat model of subtotal nephrectomy, it has been shown that the spared kidney (nephrons) maintains normal GFR by means of compensatory hyperfiltration; however, over time, decline in GFR, proteinuria, and CKD develops.[36] The compensatory response to nephron loss of hyperfiltration in the remaining nephrons leads to glomerular injury and secondary glomerulosclerosis. Glomerular cell proliferation, macrophage infiltration, and the progressive accumulation of extracellular matrix components may all contribute to the development of the glomerular sclerotic lesion.[29] Tissue hypoxia from decreased perfusion of the microvasculature also stimulates FAS-L-mediated apoptosis. An excess of cytokines in the kidney in CKD recruits macrophages into the kidney; macrophage-colony stimulating factor is overexpressed by the tubules as a response to injury. Macrophage infiltration of the interstitium correlates with renal dysfunction and the cells amplify the response by producing more cytokines, further inducing fibrosis and apoptosis.[29] Then, infiltrating monocytes/macrophages play a key role in the development of abnormalities associated to GHS.

In conclusion, this research underlines that CAKUT children already show clear signs of tubulointerstitial damage with poor regenerative response. Whether these changes may be considered an early sign of GHS or persisting chronic renal inflammation disease-related is still unclear. Furthermore, in our opinion, these children should seriously be considered at high risk of GHS because they have reduced renal mass and evidence of active chronic renal inflammation. Finally, we confirm that EGF, MCP-1, and β2M are promising biological markers useful to monitor progressive renal damage in this selected group of patients.

Conflict of Interest

None.

References

1 Lee RS, Cendron M, Kinnamon DD, Nguyen HT. Antenatal hydronephrosis as a predictor of postnatal outcome: a meta-analysis. Pediatrics 2006;118(02):586–593
2 Keller G, Zimmer G, Mall G, Ritz E, Amann K. Nephron number in patients with primary hypertension. N Engl J Med 2003;348(02): 101–108
3 Bartoli F, Penza R, Aceto G, et al. Urinary epidermal growth factor, monocyte chemotactic protein-1, and β2-microglobulin in children with ureteropelvic junction obstruction. J Pediatr Surg 2011; 46(03):530–536
4 Simões e Silva AC, Valério FC, Vasconcelos MA, Miranda DM, Oliveira EA. Interactions between cytokines, congenital anomalies of kidney and urinary tract and chronic kidney disease. Clin Dev Immunol 2013;2013:597920
5 Leonard EJ, Yoshimura T. Human monocyte chemoattractant protein-1 (MCP-1). Immunol Today 1990;11(03):97–101
6 Grandaliano G, Gesualdo L, Bartoli F, et al. MCP-1 and EGF renal expression and urine excretion in human congenital obstructive nephropathy. Kidney Int 2000;58(01):182–192
7 Diamond JR, Kees-Folts D, Ding G, Frye JE, Restrepo NC. Macrophages, monocyte chemoattractant peptide-1, and TGF-β 1 in experimental hydronephrosis. Am J Physiol 1994;266(6 Pt 2): F926–F933
8 Vielhauer V, Anders HJ, Mack M, et al. Obstructive nephropathy in the mouse: progressive fibrosis correlates with tubulointerstitial chemokine expression and accumulation of CC chemokine receptor 2- and 5-positive leukocytes. J Am Soc Nephrol 2001;12(06):1173–1187
9 Karakus S, Oktar T, Kucukgergin C, et al. Urinary IP-10, MCP-1, NGAL, cystatin-C, and KIM-1 levels in prenatally diagnosed unilateral hydronephrosis: the search for an ideal biomarker. Urology 2016;87:185–192
10 Singh B, Coffey RJ. Trafficking of epidermal growth factor receptor ligands in polarized epithelial cells. Annu Rev Physiol 2014; 76:275–300
11 Harris RC. Potential physiologic roles for epidermal growth factor in the kidney. Am J Kidney Dis 1991;17(06):627–630
12 Tang J, Liu N, Zhuang S. Role of epidermal growth factor receptor in acute and chronic kidney injury. Kidney Int 2013;83(05): 804–810
13 Gesualdo L, Di Paolo S, Calabró A, et al. Expression of epidermal growth factor and its receptor in normal and diseased human kidney: an immunohistochemical and in situ hybridization study. Kidney Int 1996;49(03):656–665
14 Fanos V, Pizzini C, Mussap M, Benini D, Pleban M. Urinary epidermal growth factor in different renal conditions in children. Ren Fail 2001;23(3-4):605–610
15 Chertin B, Farkas A, Puri P. Epidermal growth factor and monocyte chemotactic peptide-1 expression in reflux nephropathy. Eur Urol 2003;44(01):144–149
16 Bartoli F, Gesualdo L, Paradies G, et al. Renal expression of monocyte chemotactic protein-1 and epidermal growth factor in children with obstructive hydronephrosis. J Pediatr Surg 2000; 35(04):569–572
17 Pastore V, Bartoli F. Urinary excretion of EGF and MCP-1 in children with vesicoureteral reflux. Int Braz J Urol 2017;43(03): 549–555
18 Taranta-Janusz K, Wasilewska A, Dębek W, Waszkiewicz-Stojda M. Urinary cytokine profiles in unilateral congenital hydronephrosis. Pediatr Nephrol 2012;27(11):2107–2113
19 Taha MA, Shokeir AA, Osman HG, Abd El-Aziz Ael-A, Farahat SE. Pelvi-ureteric junction obstruction in children: the role of urinary transforming growth factor-β and epidermal growth factor. BJU Int 2007;99(04):899–903
20 Walton G, Buttyan R, Garcia-Montes E, Olsson CA, Hensle TW, Sawczuk IS. Renal growth factor expression during the early phase of experimental hydronephrosis. J Urol 1992;148(2 Pt 2): 510–514

21 Storch S, Saggi S, Megyesi J, Price PM, Safirstein R. Ureteral obstruction decreases renal prepro-epidermal growth factor and Tamm-Horsfall expression. Kidney Int 1992;42(01):89–94
22 Kennedy WA II, Buttyan R, Garcia-Montes E, D'Agati V, Olsson CA, Sawczuk IS. Epidermal growth factor suppresses renal tubular apoptosis following ureteral obstruction. Urology 1997;49(06):973–980
23 Robert C. Mouse pre-pro epidermal growth factor synthesis by kidney and other tissues. Nature 1985;313:12
24 Miyata T, Jadoul M, Kurokawa K, Van Ypersele de Strihou C. β-2 microglobulin in renal disease. J Am Soc Nephrol 1998;9(09): 1723–1735
25 Assadi FK. Urinary beta 2-microglobulin as a marker for vesicoureteral reflux. Pediatr Nephrol 1996;10(05):642–644
26 Konda R, Sakai K, Ota S, Takeda A, Orikasa S. Followup study of renal function in children with reflux nephropathy after resolution of vesicoureteral reflux. J Urol 1997;157(03):975–979
27 Gobe GC, Axelsen RA. Genesis of renal tubular atrophy in experimental hydronephrosis in the rat. Role of apoptosis. Lab Invest 1987;56(03):273–281
28 Truong LD, Petrusevska G, Yang G, et al. Cell apoptosis and proliferation in experimental chronic obstructive uropathy. Kidney Int 1996;50(01):200–207
29 Metcalfe W. How does early chronic kidney disease progress? A background paper prepared for the UK consensus conference on early chronic kidney disease. Nephrol Dial Transplant 2007;22 (Suppl 9):ix26–ix30
30 Savill J. Regulation of glomerular cell number by apoptosis. Kidney Int 1999;56(04):1216–1222
31 Schelling JR, Cleveland RP. Involvement of Fas-dependent apoptosis in renal tubular epithelial cell deletion in chronic renal failure. Kidney Int 1999;56(04):1313–1316
32 Brenner BM, Lawler EV, Mackenzie HS. The hyperfiltration theory: a paradigm shift in nephrology. Kidney Int 1996;49(06): 1774–1777
33 Rugiu C, Oldrizzi L, Lupo A, et al. Clinical features of patients with solitary kidneys. Nephron 1986;43(01):10–15
34 Brenner BM, Chertow GM. Congenital oligonephropathy and the etiology of adult hypertension and progressive renal injury. Am J Kidney Dis 1994;23(02):171–175
35 Solomon LR, Mallick NP, Lawler W. Progressive renal failure in a remnant kidney. Br Med J (Clin Res Ed) 1985;291(6509):1610–1611
36 Hostetter TH, Olson JL, Rennke HG, Venkatachalam MA, Brenner BM. Hyperfiltration in remnant nephrons: a potentially adverse response to renal ablation. Am J Physiol 1981;241(01):F85–F93