Biomarkers, 2012; 17(5): 471–476

ISSN 1354-750X print/ISSN 1366-5804 online
DOI: 10.3109/1354750X.2012.689009

informa
healthcare

RESEARCH ARTICLE

Urinary heme oxygenase-1 in children with congenital hydronephrosis due to ureteropelvic junction obstruction

ZhenZhen Li[1], XiangHua Liu[2], Shan Liu[3], ChaoHui Gu[4], FengYan Tian[5], and JianGuo Wen[1,4]

[1]*The Institute of Clinical Medicine, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China,* [2]*Pathological Experiment Center, Henan University of Traditional Chinese Medicine, Zhengzhou, China,* [3]*Neurology Department, Zhengzhou Children Hospital, Zhengzhou, China,* [4]*Urology Department and Pediatric Urodynamic Center, Zhengzhou, China, and* [5]*Pediatrics Department, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China*

Abstract

Context: Heme oxygenase-1 (HO-1) is implicated to be correlated with renal function in oxidative stress.

Objective: To determine whether urinary (u) HO-1 is associated with the progression of congenital obstructive hydronephrosis (HN).

Methods: A total 50 children with HN (study group and control 1) and 30 healthy children were enrolled in this study.

Results: The uHO-1/cr levels increased significantly and negatively correlated with split renal function in study group before and during surgery. One month after surgery, it decreased significantly.

Conclusion: Increased uHO-1 levels could be a potential biomarker for evaluating the progression of obstructive nephropathy.

Keywords: Heme oxygenase-1, urinary biomarker, children, hydronephrosis, ureteropelvic junction obstruction

Introduction

Ureteropelvic junction obstruction (UPJO) is a common cause of obstructive nephropathy. The prevalence of this abnormality in children is 1 in 1500 of the general population (Chevalier et al. 2010). The etiology of UPJO is multifactorial and polygenic, similar to most other anomalies of the urinary tract. Treatment of hydronephrosis ranges from early surgical repair to long-term conservative management. The nonoperative treatment approach to congenital UPJO, albeit justified, carries a 30% risk of deterioration necessitating subsequent surgery (Bajpai et al. 2002). This controversy has generated interest in UPJO as a possible cause of progressive renal damage in children.

Currently, available clinical tests, including renal ultrasonography, nuclide renal scans or plasma, and urine creatinine concentration are not good predictors of the future course of the disease, stressing the urgent need for new, simple markers of the obstructive nephropathy that may be useful in the clinical assessment of the suitability of patients for surgical therapy and renoprotective intervention. Renal biopsy is essential for assessing the degree of inflammation and tubulointerstitial damage. However, biopsy is invasive, associated with serious complications in rare cases, and cannot be frequently repeated to evaluate therapeutic response. In contrast, urine can be collected noninvasively and analyzed repeatedly (Mehta et al. 2007; Nguyen & Devarajan 2008). Thus, urinalysis would be highly advantageous if it could augment the insights gained from renal biopsy. Recently, emerging urinary biomarkers of renal disease or injury, such as neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule (KIM-1) (Sasaki et al. 2011; Tramonti & Kanwar 2011) have been reported that

Address for Correspondence: JianGuo Wen, The Institute of Clinical Medicine, Urology Department and Pediatric Urodynamic Center, The First Affiliated Hospital of Zhengzhou University, Henan, China. 450052. Tel.: +86 371 66295219. Fax: +86 371 66295219. E-mail: wenjjgg@163.com

(Received 09 March 2012; revised 21 April 2012; accepted 24 April 2012)

can act as noninvasive and relatively inexpensive tools for assessing the degree and characteristics of chronic inflammation and tubulointerstitial damage. More recently, the interest of clinicians has focused on the potential of heme oxygenase-1 (HO-1) as a biomarker (Yokoyama et al. 2011). In this study we assessed the urine level of HO-1 molecule as a marker of renal function in children with congenital hydronephrosis (HN).

The histopathology of chronic obstructive nephropathy is characterized by glomerulosclerosis, tubular atrophy, interstitial inflammation and fibrosis, and oxidative stress. Several investigators have focused on oxidative stress as a major contributor to the pathogenesis of renal fibrosis and epithelial mesenchymal transition (EMT) in unilateral ureteral obstruction (UUO) model, as well as renal tubular apoptosis (Dendooven et al. 2011; Yeh et al. 2011). The increased expression of HO-1 was found at very high levels in renal tubular epithelial cells under conditions of oxidative stress in ureteral obstruction models (Yang et al. 2003).

HO-1 is the rate-limiting enzyme in the degradation of heme and results in the release of equimolar quantities of biliverdin, iron, and carbon monoxide (CO) (Maines 1997). Normally, HO-1 protein is weakly expressed in the cortex and in the outer medulla of kidney. In oxidative stress, HO-1 plays a pivotal role in maintaining renal function and protecting renal structure, especially in renal tubular epithelial cells (Yang et al. 2003). Moreover, other studies have shown that HO-1 mRNA is induced in the kidney as early as 3–6 h in animal models of both ischemia/reperfusion and nephrotoxin-induced acute kidney injury (AKI) (Agarwal et al. 1995; Nath et al. 2000). It is discovered that HO-1 induction exerts a protective effect on renal function in animal models of rhabdomyolysis, cisplatin nephrotoxicity, and nephrotoxic nephritis (Nath 2006). Further, the products of heme degradation have a protective role in AKI. Another characteristic of HO-1 that makes it an ideal biomarker for kidney injury is its expression within the damaged renal tubular epithelial cells, which drop off into the cavity of renal tubules (Yokoyama et al. 2011). The uHO-1 level has been documented to be closely related to ongoing inflammation and oxidative damage in renal tubular epithelial cells and to correlate with kidney tissue damage. Clinical studies have documented that uHO-1 is upregulated in tubules of patients with inflammatory renal disease and IgA nephropathy and that it is associated with proteinuria and its consequence, such as tubulointerstitial inflammatory damage (Yang et al. 2003; Shimizu et al. 2005). These results have led to the suggestion that uHO-1 may be a promising, non-invasive biomarker of chronic tubulointerstitial damage.

The aim of the case–control study, reported here, was to evaluate uHO-1 levels in young children and adolescents affected by severe congenital HN caused by UPJO. We also assessed the possible clinical application of uHO-1 as non-invasive diagnostic and predictive biomarker in UPJO.

Materials and methods

Patients and subjects

This was a case–control prospective study performed in children with severe congenital HN caused by UPJO, who were diagnosed at the Department of Pediatrics and treated at the Urology Department of the First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China. The study was performed from January 2010 to October 2011. Healthy children were chosen from those referred to a pediatric outpatient clinic of the Zhengzhou Children Hospital, Zhengzhou, Henan, China, where all children are periodically monitored for their development and growth. All caregivers of the children were interviewed and gave informed consent for the children to participate in the study. The study was approved by the ethics committee of our Medical Faculty. Written informed consents were obtained from all patients' parents before study entry.

The children were categorized into three groups: one study group and two types of control groups. The study group included 25 children (19 boys, 6 girls; average age: 2.37 ± 0.66 years) with severe HN due to unilateral, critical degree ureteral stenosis, who underwent pyeloplasty by the Anderson-Hynes method. The first control group (control 1) included 25 children with mild, non-obstructive HN (17 boys, 8 girls; average age: 7.13 ± 0.65 years) who did not require pyeloplasty. The second group (normal control) consisted of 30 healthy children (16 boys, 14 girls; average age: 5.95±0.70 years). All the children had a routine renal ultrasound examination.

All 25 children in the study group had a prenatal diagnosis of unilateral hydronephrosis during the routine fetal ultrasound. All children were reevaluated after birth by abdominal ultrasound and technetium-99m diethyllenetriamine pentaacetic acid (^{99m}Tc-DTPA) diuretic renography. A postnatal diagnosis of UPJO was determined on the positive findings of renal ultrasonography and radionuclide renal scans. Then it was confirmed by the histologic findings of the obstructed segment, which included various histologic changes within the narrow segment, such as an increase of perifascicular fibrosis, thickening of muscularis propria thickness, and inflammatory cells infiltration in the lamina propria. The degree of HN was graded according to the Society for Fetal Urology (SFU) classification: Grade I represents a split pelvis; Grade II is further dilation of the renal pelvis, with a few visualized calyces permissible; Grade III is renal pelvis dilation, with many distended calyces; Grade IV is a Grade III appearance with the addition of thinned parenchyma (Fernbach et al. 1993). The diagnosis of non-obstructive HN (control 1) was made when there was no change in split renal function (SRF) and the clearance half-life ($t_{1/2}$) on the follow-up ^{99m}Tc-DTPA diuretic renography; and stationary HN on the renal ultrasonography.

Criteria for inclusion in the study group were: an age of 1 month–18 years; unilateral pelvicaliceal system dilation; a ^{99m}Tc-DTPA diuretic renography suggested

unilateral UPJO (study group) and SRF available. A SRF of the affected dilated kidney of ≥45% was considered normal and a SRF of <45% was considered abnormal. No patients had a urinary tract infection before surgery. Exclusion criteria were associated anomalies, including vesicoureteral reflux, ureterovesical junction obstruction and posterior urethral valves obstruction, bilateral hydronephrosis, previous operation on the urinary system and other deformations of the external genital organs, lower urinary tracts anomalies, urinary stones, neurogenic bladder dysfunction, and "supranormal function" of the affected kidney (SRF >55%).

Urine sample collection

In the study group, the urine samples were collected three times: the first morning voided urine samples obtained preoperatively (Test one); urine samples from affected pelvis obtained during the pyeloplasty (Test two); first morning voided urine samples collected 1 month after pyeloplasty (Test three). The clinical work-up included the analysis of medical charts to determine age, gender, laterality, and grade of hydronephrosis, anteroposterior (AP) pelvic diameter, age at diagnosis, method of treatment, as well as SRF. The biochemical work-up included the determination of serum creatinine concentration, urine creatinine concentration, and glomerular filtration rate (mL/min/1.73 m^2), estimated by the Schwartz formula (eGFR). The urine was aseptically collected between 7 and 8 am from the morning sample. The concentration of creatinine in the urine and blood samples was measured with a Modular P800 automatic biochemistry Analyzer (Roche Diagnostics, GmbH, Mannhein, Germany). Fresh urine samples (10 mL) were centrifuged for 5 min (500*g*, at 4°C). Supernatants were discarded. Sediment samples were washed twice with phosphate–buffered saline (PBS) (10 mL). These samples were maintained at –80°C for future uHO-1 ELISAs.

Enzyme-linked immunosorbent assay (ELISA) for uHO-1 evaluation

The uHO-1 levels in sediment lysates were evaluated by ELISA according to the manufacturer's instructions (StressXpress™ Human HO-1 ELISA Kit; Stressgen Biotechnologies Corp., Victoria, Canada). Lysates were prepared according to the guidelines of the HO-1 ELISA Kit. The urinary creatinine concentration was used to normalize HO-1 measurements to account for the influence of urinary dilution on its concentration. The uHO-1 levels were expressed as uHO-1/cr. ratio in nanograms per milligram creatinine (ng/mg cr.).

Statistical analysis

All statistical analyses were done with SPSS 16.0 (SPSS Inc, Chicago, IL,USA) for windows software. All values were presented as means ± SE. Nonparametric statistics was chosen, as the patient population of this study was relatively small. Statistical analysis was performed using the nonparametric Mann–Whitney *U* test. The receiver operating characteristic (ROC) curve was used to determine the cut-off values of uHO-1 that gave the best sensitivity and specificity. Correlation analysis was used for the comparisons. Bivariate analysis was presented as Pearson or Spearman correlation coefficient. *P* values less than 0.05 were considered to be statistically significant.

Results

Table 1 shows clinical and laboratory data. In our study, boys were more frequently affected with HN than girls, which have also been reported in the literature (Kajbafzadeh et al. 2010). The left kidney was more commonly involved than the right kidney (31:19, respectively). Mean age at pyeloplasty was approximately 2 years. Grade I HN was present in five children (10%), and Grade II, III, IV HN were present in 13 (26%), 18 (36%) and 14 (28%) children, respectively. The SRF in the study group was markedly lower than that in patients from control 1 ($p < 0.01$). A radionuclide scan was not performed in healthy children (normal control), so the SRF was not evaluated in this group. The AP pelvic diameter was found to be significantly higher in the study group than in control 1 group ($p < 0.05$).

Table 2 shows that the uHO-1 and uHO-1/cr. levels before surgery and during surgery were significantly greater in the study group than in control 1 and healthy control ($p < 0.01$). One month after surgery, uHO-1/cr. decreased significantly in the study group compared with

Table 1. Summary of the clinical parameters of all studied patients (mean ± SE).

Clinical parameters	Study group	Control 1	Normal control
Gender, *n* (male/female)	25 (19/6)	25 (17/8)	30 (16/14)
Age at diagnosis (years)	1.60 ± 0.58	5.39 ± 0.87	–
Age at the moment of examination (years)	2.37 ± 0.66	7.13 ± 0.65	5.95 ± 0.70
Clinical diagnosis (SFU grading)			
Grade I	–	5	–
Grade II	–	13	–
Grade III	11	7	–
Grade IV	14	-	–
Laterality (left/right)	17/8	14/11	–
Split renal function (%)	38.9 ± 1.3**	45.6 ± 0.7	–
AP at prepyeloplasty (mm)	39.2 ± 1.9*	32.8 ± 1.2	–
Estimated glomerular filtration rate (mL/min/1.73 m^2)	154 ± 5.5**	184.8 ± 7.2	155.5 ± 4.6

Data are shown as mean ± SE.
SFU, the society for fetal urology; AP, anteroposterior.
*$p < 0.05$ versus Control 1.
**$p < 0.01$ versus Control 1.

Table 2. Urine concentration of HO-1 in patients with UPJO (study group and control 1) and normal control (mean ± SE).

Test/group	uHO-1 (ng/mL)	uHO-1/cr (ng/mg cr.)
Test One (voided urine before pyeloplasty)	2.04 ± 0.33[a,c]	4.23 ± 0.53[a,c]
Test Two (urine sanples from affected pelvis during surgery)	2.07 ± 0.26[a,c]	4.43 ± 0.50[a,c]
Test Three (voided urine one month after surgery)	2.11 ± 0.22[a,c]	1.73 ± 0.25[b,c]
Control 1 (children with mild, non-obstructive hydronephrosis)	0.84 ± 0.11[c]	1.04 ± 0.21[d]
Normal control	0.36 ± 0.06	0.56 ± 0.1

Data are shown as mean ± SE.
uHO-1, urinary heme oxygenase-1; cr., creatinine; UPJO, ureteropelvic junction obstruction.
[a]$p < 0.01$ versus Control 1 group.
[b]$p < 0.05$ versus Control 1 group.
[c]$p < 0.01$ versus Normal control group.
[d]$p < 0.05$ versus Normal control group.

that before surgery ($p < 0.01$), but was still higher than that in control 1 group ($p < 0.05$). The uHO-1 and uHO-1/cr. levels were markedly lower in normal control than that in control 1 group ($p < 0.01$, $p < 0.05$, respectively).

A negative correlation between the uHO-1/cr. ratio and SRF was found before surgery ($r = -0.593$, $p = 0.002$) and during surgery ($r = -0.452$, $p = 0.023$) in the study group. No statistically significant correlation was found in control 1 group. Moreover, we did not find any significant correlation between uHO-1/cr. ratio with serum creatinine level and age of patients, eGFR, and initial AP diameter of the pelvis.

Receiver–operator curves (ROC) analysis were performed to define the diagnostic profile of uHO-1 in identifying children with an obstructive kidney condition (SRF <40%) among children with severe and mild HN (study group and control 1) and children with abnormal SRF (<45%) among all examined children. In the first analysis in children with HN, we found that uHO-1/cr. showed quite good diagnostic profile, describing an area under the curve (AUC) for uHO-1/cr. of 0.767 [95% confidence interval (CI) 0.624–0.910] with a best cut-off value of 1.92 ng/mg cr. (sensitivity 72.2%, specificity 78.1%). In a subsequent analysis, we assessed the sensitivity and specificity of uHO-1/cr. as biomarker of kidney injury (SRF <45%). In this analysis, the AUC for uHO-1/cr. was 0.812 (95% CI 0.686–0.937) with a best cut-off value 1.215 ng/mg cr. (sensitivity 76.7%, specificity 80%). ROC analysis for uHO-1 not corrected for urinary creatinine showed similar values.

Discussion

In clinical practice there are no clear indications for the timing of surgical intervention to prevent the kidney injury while evaluating and treating patients with obstructive nephropathy. It has been reported that only 19–29% of children with prenatally diagnosed UPJO require surgical intervention (Chertin et al. 2006). Although diuretic renography and ultrasound evaluation assist the clinician to establish the presence and degree of UPJO, there is an urgent need to look for potential biomarkers in patients' fluids, preferably urine, which would enable the early detection of obstructive nephropathy and renal function deterioration.

To assess the feasibility of HO-1 as a biomarker of obstructive nephropathy, we compared the magnitude of the alternation in its concentration in urine at different periods of time in children with confirmed HN with the levels in healthy children and in children with mild, non-obstructive HN. We also calculated the accuracy and sensitivity of uHO-1 in children with HN, according to the SRF of the affected kidney in a renal nuclide scan, using ROC analysis. To the best of our knowledge this study is the first report that assesses the concentration of uHO-1 in children with HN due to UPJO.

As we known, the mechanisms of obstructive nephropathy in UPJO have been identified to consist of the following cellular hallmarks: tubular dilatation, phenotypic cellular transition and cell apoptosis; interstitial inflammation; as well glomerulotubular injury and progressive renal fibrosis with loss of renal parenchymal (Chevalier 2006). Oxidative stress contributes importantly to the pathogenesis of unilateral ureteral obstruction (UUO) (Dendooven et al. 2011; Yeh et al. 2011). Among other pathways, oxidative stress has been shown to promote epithelial-to-mesenchymal transition. Various markers of oxidative stress are increased in UUO kidneys. Oxidant stress response molecules like heat shock protein-70 (HSP-70), heat-shock protein-27 and HO-1 are strongly expressed after UUO (Dendooven et al. 2011).

In our study, the uHO-1 level was significantly elevated in patients who had developed an obstructed kidney but not yet undergone pyeloplasty. One month after surgery, the concentration of uHO-1 had decreased significantly, but did not reach the value found in children with dilated, but not obstructed kidney (control 1) ($p < 0.05$). The uHO-1 levels in patients from the study group were significantly different from those of both control groups ($p < 0.05$). Moreover, we detected low concentrations of uHO-1 in the urine of healthy controls, which was contrary to the study reported by Yokoyama T et al (Yokoyama et al. 2011).

The mechanism for increase of HO-1 in patients with dilated and obstructed pelvis is not clear. HO-1 is constitutively expressed within distal tubular renal epithelial cells but is induced by oxidative stress within proximal tubular renal epithelial cells (Yang et al. 2003; Shimizu et al. 2005). It plays a pivotal role in maintaining renal function and protecting renal tubular epithelial cells under conditions of oxidative stress (Maines 1984; Kanwar 2001). Real-time monitoring of uHO-1 expression profiles may thus offer precise and valuable information

regarding oxidative stress endured by tubulointerstitial cells at a given moment. In UUO models, it has been found that transforming growth factor (TGF-β1) can induce a cytoprotective enzyme, HO-1 in renal proximal tubular cells (Traylor et al. 2007). Taha et al. (2007) confirmed that urinary TGF-β1 showed an initial significant increase in the first month after pyeloplasty in the children with UPJO, which might explain the fact that uHO-1 level was still elevated in our study patients 1 month after pyeloplasty. Interestingly, the difference in uHO-1 concentration in voided urine before pyeloplasty and pelvic urine from the affected kidney was not statistically significant ($p > 0.05$). One possible explanation for this observation may be that intact contralateral kidney undergoes compensatory growth that is proportional to the duration of the obstruction. In this situation, the source of the uHO-1 in the voided urine may also be the contralateral kidney. Moreover, the phenomenon that the urine from the obstructed kidney is diluted in the bladder with urine from the contralateral kidney is also inevitable. Although it has been shown that contralateral compensatory renal growth is dependent on the severity and duration of obstruction and takes place at the single nephron level (Yoo et al. 2006), in the clinical setting, these parameters are often unpredictable, so the contribution from the opposite kidney cannot be truly assessed and the expression of HO-1 in the contralateral kidney cannot be confirmed. Fortunately, in the present study, there was a positive correlation between the levels of uHO-1 in voided urine and those in the affected pelvis ($p < 0.01$, data not shown), which suggesting a valuable role for preoperative voided urine HO-1 in predicting intrapelvic level.

In the present study, the concentrations of uHO-1 in the voided urine before surgery and the pelvic urine from the affected kidney were negatively correlated with SRF in the radionuclide scan which was consistent with the observation of Elmarakby et al. (2012), who found that HO-1 was correlated with glomerular injury and apoptosis. The level of uHO-1 is indicative of its level within the damaged cells, which drop off into the cavity of renal tubules. Therefore, uHO-1 levels indicate ongoing inflammation and oxidative damage that induce HO-1 gene and protein expression of tubular epithelial cells.

If we consider uHO-1 to be an early marker of tubular injury, we can hypothesize that uHO-1 may express the degree of subclinical tubular impairment, thus representing an earlier measurable marker of its function. Recent data have demonstrated that uHO-1 levels in IgAN patients correlated with the degree of tubulointerstitial cellular infiltration, hematuria, and proteinuria (Yokoyama et al. 2011). The degree of renal impairment correlates more closely with tubulointerstitial damage than with glomerular injury, which is clinically significant. This has led to the proposal that uHO-1 is considered to be a valuable biomarker for studying the degree of tubulointerstitial inflammation and oxidative damage to assess whether the injury may progress to chronic kidney disease. This proposal is supported by data from our ROC analyses, which showed a good diagnostic profile for uHO-1 in HN children (study group and control 1 group, AUC: 0.767) and a slightly better profile for detecting kidney injury in HN children with SRF <45% (AUC 0.812). These results may suggest that uHO-1 may be useful in identifying the presence of obstruction in HN patients.

One limitation of our study was the study population was relatively small; thus, large prospective studies are required to confirm our findings. Moreover, we did not compare the examined parameters based on a histological diagnosis. The increased uHO-1 levels may not only reflect renal tubular cells damage, the extrarenal sources also should be taken into account. Finally, the follow-up measurement of uHO-1 concentration after pyeloplasty and correlation to the post-operative renal function and AP pelvic diameter are required in the future investigations.

Conclusions

The present study has clearly revealed that the children with UPJO had increased urinary HO-1 levels and that these correlated negatively with split renal function. Despite these above limitations, the results suggest that uHO-1 levels are associated with worsening obstruction. Future investigations are required to confirm a potential application of HO-1 as a useful biomarker for such a diagnosis and as a parameter to prevent the development and progression of chronic kidney disease.

Acknowledgments

Statistician, Ms Xiaoping Shang, is thanked for her great contribution to the statistical analysis in this study.

Declaration of interest

This study was supported by "The Youth Innovation Fund of the First Affiliated Hospital of Zhengzhou University" and Special Research Fund for the Doctoral Program of Colleges of Ministry of Education (No.20114101120010).

References

Agarwal A, Balla J, Alam J, Croatt AJ, Nath KA. (1995). Induction of heme oxygenase in toxic renal injury: a protective role in cisplatin nephrotoxicity in the rat. *Kidney Int* 48:1298–1307.

Bajpai M, Puri A, Tripathi M, Maini A. (2002). Prognostic significance of captopril renography for managing congenital unilateral hydronephrosis. *J Urol* 168:2158–61; discussion 2161.

Chertin B, Pollack A, Koulikov D, Rabinowitz R, Hain D, Hadas-Halpren I, Farkas A. (2006). Conservative treatment of ureteropelvic junction obstruction in children with antenatal diagnosis of hydronephrosis: lessons learned after 16 years of follow-up. *Eur Urol* 49:734–738.

Chevalier RL. (2006). Obstructive nephropathy: towards biomarker discovery and gene therapy. *Nat Clin Pract Nephrol* 2:157–168.

Chevalier RL, Thornhill BA, Forbes MS, Kiley SC. (2010). Mechanisms of renal injury and progression of renal disease in congenital obstructive nephropathy. *Pediatr Nephrol* 25:687–697.

Dendooven A, Ishola DA Jr, Nguyen TQ, Van der Giezen DM, Kok RJ, Goldschmeding R, Joles JA. (2011). Oxidative stress in obstructive nephropathy. *Int J Exp Pathol* 92:202–210.

Elmarakby AA, Faulkner J, Baban B, Saleh MA, Sullivan JC. (2012). Induction of hemeoxygenase-1 reduces glomerular injury and apoptosis in diabetic spontaneously hypertensive rats. *Am J Physiol Renal Physiol* 302:F791–F800.

Fernbach SK, Maizels M, Conway JJ. (1993). Ultrasound grading of hydronephrosis: introduction to the system used by the Society for Fetal Urology. *Pediatr Radiol* 23:478–480.

Kajbafzadeh AM, Elmi A, Talab SS, Emami H, Esfahani SA, Saeedi P. (2010). Urinary and serum carbohydrate antigen 19-9 as a biomarker in ureteropelvic junction obstruction in children. *J Urol* 183:2353–2360.

Kanwar YS. (2001). Heme oxygenase-1 in renal injury: conclusions of studies in humans and animal models. *Kidney Int* 59:378–379.

Maines MD. (1984). New developments in the regulation of heme metabolism and their implications. *Crit Rev Toxicol* 12:241–314.

Maines MD. (1997). The heme oxygenase system: a regulator of second messenger gases. *Annu Rev Pharmacol Toxicol* 37:517–554.

Mehta RL, Kellum JA, Shah SV, Molitoris BA, Ronco C, Warnock DG, Levin A; Acute Kidney Injury Network. (2007). Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. *Crit Care* 11:R31.

Nath KA. (2006). Heme oxygenase-1: a provenance for cytoprotective pathways in the kidney and other tissues. *Kidney Int* 70:432–443.

Nath KA, Haggard JJ, Croatt AJ, Grande JP, Poss KD, Alam J. (2000). The indispensability of heme oxygenase-1 in protecting against acute heme protein-induced toxicity in vivo. *Am J Pathol* 156:1527–1535.

Nguyen MT, Devarajan P. (2008). Biomarkers for the early detection of acute kidney injury. *Pediatr Nephrol* 23:2151–2157.

Sasaki D, Yamada A, Umeno H, Kurihara H, Nakatsuji S, Fujihira S, Tsubota K, Ono M, Moriguchi A, Watanabe K, Seki J. (2011). Comparison of the course of biomarker changes and kidney injury in a rat model of drug-induced acute kidney injury. *Biomarkers* 16:553–566.

Shimizu M, Ohta K, Yang Y, Nakai A, Toma T, Saikawa Y, Kasahara Y, Yachie A, Yokoyama H, Seki H, Koizumi S. (2005). Glomerular proteinuria induces heme oxygenase-1 gene expression within renal epithelial cells. *Pediatr Res* 58:666–671.

Taha MA, Shokeir AA, Osman HG, Abd El-Aziz Ael-A, Farahat SE. (2007). Pelvi-ureteric junction obstruction in children: the role of urinary transforming growth factor-beta and epidermal growth factor. *BJU Int* 99:899–903.

Tramonti G, Kanwar YS. (2011). Tubular biomarkers to assess progression of diabetic nephropathy. *Kidney Int* 79:1042–1044.

Traylor A, Hock T, Hill-Kapturczak N. (2007). Specificity protein 1 and Smad-dependent regulation of human heme oxygenase-1 gene by transforming growth factor-beta1 in renal epithelial cells. *Am J Physiol Renal Physiol* 293:F885–F894.

Yang Y, Ohta K, Shimizu M, Morimoto K, Goto C, Nakai A, Toma T, Kasahara Y, Yachie A, Seki H, Koizumi S. (2003). Selective protection of renal tubular epithelial cells by heme oxygenase (HO)-1 during stress-induced injury. *Kidney Int* 64: 1302–1309.

Yeh CH, Chiang HS, Lai TY, Chien CT. (2011). Unilateral ureteral obstruction evokes renal tubular apoptosis via the enhanced oxidative stress and endoplasmic reticulum stress in the rat. *Neurourol Urodyn* 30:472–479.

Yokoyama T, Shimizu M, Ohta K, Yuno T, Okajima M, Wada T, Toma T, Koizumi S, Yachie A. (2011). Urinary heme oxygenase-1 as a sensitive indicator of tubulointerstitial inflammatory damage in various renal diseases. *Am J Nephrol* 33:414–420.

Yoo KH, Thornhill BA, Forbes MS, Chevalier RL. (2006). Compensatory renal growth due to neonatal ureteral obstruction: implications for clinical studies. *Pediatr Nephrol* 21:368–375.