PELVIURETERAL JUNCTION OBSTRUCTION: CORRELATION OF RENAL CELL APOPTOSIS AND DIFFERENTIAL RENAL FUNCTION

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ABSTRACT

Purpose: We evaluated the relationship between renal biopsy changes and preoperative and postoperative renal scans in 29 male and 14 female infants with prenatal severe hydronephrosis and unilateral ureteropelvic junction obstruction. We also verified on immunohistochemical studies glomerular changes, degeneration of the epithelium of the proximal tubules, interstitial fibrosis and inflammation and apoptotic nuclei.

Materials and Methods: In the children, all with prenatal diagnosis of hydronephrosis, ureteropelvic junction obstruction was diagnosed with ^{99m}technetium mercaptoacetyltriglycine renal scan performed in all patients at ages 4 to 6 weeks to establish baseline differential renal function. All patients underwent renal biopsies at the time of pyeloplasty. The biopsy samples were examined with histological and immunohistochemical methods for antigens associated with apoptosis, such as clusterin, CD95, TDAG51 and bcl-2. Renograms were performed 3 months after surgical repair.

Results: The male-to-female ratio was 2.1:1. There was no difference between males and females in regard to baseline differential renal function of the affected kidney. All biopsy samples confirmed degeneration of the epithelium of the proximal tubules, interstitial focal fibrosis was found in 4 samples, mild chronic inflammation with lymphoid aggregates in 4 and focal Bowman's space dilatation in 1. No specimen demonstrated apoptotic nuclei as confirmed by immunohistochemical study which showed the presence of bcl-2 and absence of CD95, TDAG51 and clusterin, probably because there was no dysplasia in the samples examined.

Conclusions: These results indicate absence of apoptosis in the kidney with preoperative uptake less than 40% and minimal histological changes.

KEY WORDS: hydronephrosis, apoptosis, kidney

Obstruction is defined as restriction to urinary outflow that, when untreated to impair nephron growth and function, will cause progressive renal deterioration in children and adults. Obstructive uropathy can cause major changes in the tubulointerstitial compartment of the kidney, and the common consequence of long-standing obstruction is interstitial fibrosis.[1] Several studies have documented histological changes in children with ureteropelvic junction obstruction.[2–4]

Apoptosis of tubular, interstitial and glomerular cells is well documented in kidneys with chronic obstructive uropathy and probably has an important role in its pathogenesis.[5] Tubular cell apoptosis develops quickly after ureteral ligation, peaks between 7 and 24 days after obstruction, and tapers thereafter. Apoptosis initially involves the dilated collecting ducts but subsequently spreads to other tubules. Because the peak of tubular cell proliferation immediately precedes the onset of tubular cell apoptosis, a pathogenetic link may exist between these 2 processes. Interstitial cell apoptosis occurs with increasing frequency throughout the course of obstructive uropathy, while the proliferation appears in a bimodal pattern with the early peak coinciding with that of tubular cell proliferation and consisting mostly of fibroblasts, whereas the later peak consists mostly of inflammatory cells.[6, 7] Glomerular cell apoptosis and proliferation are no different from nonobstructed kidneys, which explains in part the structural integrity of the glomeruli throughout the disease course. Although the general pathways of cell apoptosis

and proliferation are well known, the molecular control of these processes in obstructive uropathy is poorly understood. In addition, whether apoptosis or proliferation of tubular and interstitial cells is differentially regulated remains to be determined.

However, several molecules known to be activated or over expressed in kidneys with obstructive uropathy may modulate cell apoptosis and proliferation. In contrast to necrosis, the more classically understood mode of cell death, apoptosis is a genetically active cell death, sometimes requiring the dying cell to synthesize ribonucleic acid and proteins for its occurrence. Apoptosis related proteins might be important in regulating cell apoptosis during the development of obstructive uropathy. This genetic activity is consistent with the ability to identify specific gene products whose expression is induced during hydronephrosis, including the sulfated glycoprotein-2 (SGP-2) or clusterin gene products.[8] The relevant functions of these molecules include induction of apoptosis (angiotensin II, reactive oxygen species, jun-N-terminal kinase, p53), inhibition of the cell cycle (transforming growth factor-β, p21), inhibition of apoptosis (epidermal growth factor, insulin-like growth factor, bcl-2, osteopontin) or promotion of interstitial fibroblast proliferation (platelet derived growth factor). Several reports show a lack of correlation between cell death and clusterin expression[9] but in several models of inducible tissue damage high levels of clusterin mRNA correlate well with the onset or occurrence of apoptosis. We evaluated differential renal function by radionuclide scan and histological findings on renal biopsies performed during pyeloplasty in patients with uni-

lateral ureteropelvic junction obstruction to determine the role of apoptosis in obstruction using immunohistochemical methods for the antigens associated with it, such as clusterin, CD95, TDAG51 and bcl-2.

MATERIAL AND METHODS

The charts of 29 male and 14 female infants with ureteropelvic junction obstruction and prenatal unilateral severe hydronephrosis with no predisposing conditions in maternal anamnesis were reviewed. Gestational age at diagnosis of hydronephrosis was 22 to 26 weeks. Ultrasonography was performed at least once every trimester and the last scan was obtained between 33 and 37 weeks of gestation. All infants were born at term, and ultrasound during week 1 of life (between 3 and 7 days) confirmed the hydronephrosis.

Mean patient age at presentation to our referral center was 12 days (range 4 to 20) and ultrasound was performed during month 1 of life. The definition and grading of hydronephrosis were assessed according to the Society for Fetal Urology.[10] Cystography was performed early to exclude vesicoureteral reflux. Ureteropelvic junction obstruction was diagnosed with [99m]technetium mercaptoacetyltriglycine (^{99m}Tc-MAG3) renal scan performed as soon as possible at ages 4 to 6 to establish baseline differential renal function.

Dynamic renal scintigraphy was performed using standard protocols.[11, 12] All studies were acquired with a small field gamma camera equipped with a high resolution, low energy collimator interfaced to a digital computer. Infants were studied in the supine position and a catheter was placed at the time of the renal scan. A 50-minute dynamic study began at the time of rapid intravenous injection of ^{99m}Tc-MAG3, and 1 mg./kg. furosemide (1 mg./kg.) was given 20 minutes into the study. All images were stored in a computer system. Split renal function at 2 minutes and time activity curves were obtained.

In patients with unilateral hydronephrosis, differential renal function was expressed as the percentage of renal counts acquired by an individual kidney relative to the total counts acquired by both kidneys. Kidney function was defined as poor—less than 20% contribution, moderate—20% to 39% and good—40% or greater.[13] Washout pattern as seen on post-furosemide curves was considered normal at half-time less than 10 minutes, uncertain at half-time 10 to 20 minutes, and pathological washout and probable obstruction at half-time greater than 20 minutes. In all cases a post-micturition image in orthostatic projection was performed to obtain further information about renal drainage, which was expressed as percentage of residual renal activity.[12]

Patients were included in the study only if the contralateral kidney was normal. They were followed for a mean of 3.6 years (range 1.5 to 5.9). Every 6 months during the study all infants underwent complete physical examination, measurement of blood pressure and biochemical analysis. Serum creatinine levels were measured by the Jaffe method and creatinine clearance was calculated using the formula of Schwartz et al[14] with K values of 0.55 for all subjects other than children younger than 1 year for whom a value of 0.45 was used. We also determined microalbuminuria on a random urine sample by enzyme immunoassay method (normal less than 20 mg./l.), urinary and serum β2-microglobulin by microparticle enzyme immunoassay method (normal urine 0.5 to 15 mg.%, serum 70 to 340 mg.%) to evaluate glomerular and tubular renal function. These data were correlated with a control sample obtained from 30 age matched healthy normal children. All patients were placed on amoxicillin-clavulanic acid or cefaclor prophylaxis during the first year of life or until they had significant improvement in hydronephrosis.

Surgery was performed if there was evidence of obstruction injury, defined as a reduction in differential function greater than 10%, ultrasonographic progression of hydronephrosis and/or symptoms (urinary tract infections, pain, colic, hematuria and hypertension). In patients who underwent pyeloplasty a small wedge biopsy was taken at the junction between the middle and lower thirds of the kidney in the thickest portion of the renal parenchyma. The specimens was fixed in 10% neutral buffered formalin, pH 7.4, embedded in histowax, sectioned at 4 μ., and stained with hematoxylin and eosin and hematoxylin, Van Gieson. Other tissue slices were used for staining with Dominici and Tricromica, and other specimens were collected for immunohistochemical studies using monoclonal antibodies CD95 (1.100) and bcl-2 (1:100), policlonal antibody TGAG51 (1:80) and clusterin (1:80). This process was performed with the streptavidin-biotin immunoperoxidase method, and to identify antibody we used as cromogen the 3,3 diaminobenzidin 0,2% in buffer phosphate at pH 7.4. The tissues were then stained with hematoxylin.

The detection of Apoptotic nuclei were detected by immunocytochemistry with the TUNEL technique. Other specimens were examined using immunohistochemical methods for the antigens associated with apoptosis (CD95, bcl-2, TDAG51 and clusterin). The specimens were analyzed by a pathologist with a high powered light microscopic who had no knowledge of the clinical status of any patient. Biopsy results (histological and immunohistochemical) were correlated with radionuclide renal scan differential function. ^{99m}Tc-MAG3 renal scans were repeated 3 months, 6 months and 1 year postoperatively, and yearly thereafter. Informed consent was obtained for all children before they entered the study. Statistical analysis was performed using Student's t test for unpaired data with p <0.05 considered statistically significant. All data are expressed as mean ± SD.

RESULTS

There was no difference between males and females for gestational age at diagnosis of hydronephrosis (males 7.3 ± 0.8 months, females 7.1 ± 0.8) and weight at birth (males 3.377 ± 0.5 kg., females 3.054 ± 0.6). The initial ultrasonogram performed at birth revealed that in both groups the left was significantly more affected than the right kidney (males 19/10, females 11/3, p <0.0005) and, therefore, the grade of hydronephrosis was 3 to 4 according to the Society for Fetal Urology, with mean pelvic dilatation greater than 20 mm. when using as measurements the maximum anteroposterior renal pelvic diameter (males 31.6 ± 12.9 mm., females 30.8 ± 10.2, not significant). There was no difference between males and females for initial differential renal function which was moderate in 12 males (41.4%) and in 6 females (42.8%) and good in 17 (58.6%) and in 8 (57.2%), respectively (table 1).

The patients underwent pyeloplasty because during followup they had evidence of obstructive injury, including decreased function, and increased hydronephrosis and symptoms. In our study 75% of kidneys required surgical treatment because of declining function with a mean differential renal function in the affected kidney of 32% which improved to 44% in all 3 months after pyeloplasty. There was no significant functional improvement, despite successful surgery, in the kidneys that were corrected because of increased hydronephrosis and symptoms, and final renal function was greater than 40%. Initial diuretic renogram washout half-time was 20 to 30 minutes in 23% and greater than 30 minutes in 76% of patients (p <0.05). After pyeloplasty half-time was greater than 30 minutes in 21% and 20 to 30 minutes in 79% of cases (p <0.05). In all cases there was significant improvement in the percentage of residual renal activity after surgical correction in males (p <0.0001) than in females (p <0.0005, table 1).

There was no statistically significant correlation between initial grade of hydronephrosis and initial renal function

TABLE 1. *Patient characteristics*

	Males			Females		
	Diagnosis	Postop.	p Value	Diagnosis	Postop.	p Value
Hydronephrosis ± SD (mm.)	31.6 ± 13	10.9 ± 3.6	<0.0001	30.8 ± 10.2	14.5 ± 5.1	<0.002
Mean % total uptake ± SD	44.4 ± 8.8	46.6 ± 5.3	<0.3	42.7 ± 6.2	46.8 ± 6.2	<0.03
Mean moderate function ± SD	34.5 ± 3.8	40.6 ± 4.3	<0.003	35.3 ± 3.5	48.7 ± 6.6	<0.005
Mean good function ± SD	48.2 ± 5.4	48.6 ± 3.4	Not significant	48.5 ± 4.4	48.7 ± 5.4	Not significant
Mean % washout*	23 ± 10	73.7 ± 8.8	<0.0001	20.5 ± 13.3	72.1 ± 11.5	<0.0005
Mean creatinine clearance ± SD (ml./min.)	68.8 ± 14.3	88.1 ± 14.8	<0.05	67.6 ± 15.7	87.6 ± 20.1	<0.04

* Renal drainage expressed as percentage of residual renal activity on orthostatic projection and post-micturition image.

indicating that ultrasound fails to estimate the potential relative kidney function in cases of severe hydronephrosis. Surgery was performed between ages 9 and 16 months (males mean 10.1 ± 6.1, females 9.3 ± 4.3, not significant) and there was no significant difference in postoperative results, which could be ascribed to patient age at surgery.

Mean creatinine clearance was normal range for age in all children (range 32 to 65 ml. per minute per 1.73 mg.; table 1). In these patients microalbuminuria values, which were greater than controls at diagnosis (34 ± 46 versus 10.4 ± 3.3 mg./l.) continued to increase during followup (48.2 ± 55 mg./ l.) and decreased 18 months after surgery in all but 16% (27.6 ± 16.5 mg./l., p <0.1) despite successful surgery. These patients underwent surgery because of increased hydronephrosis and symptoms. Urinary β2-microglobulin levels were greater than those of controls at first observation (39 ± 20 versus 7.4 ± 3.5 mg.%, p <0.005) and they decreased but not significantly after correction (15 ± 11 g.%). Seric β2-microglobulin remained in the normal range (174.4 ± 76 mg.%).

At biopsy all samples demonstrated hydropic degeneration of the epithelium of the proximal tubules, with large cells, and the cytoplasm was occupied by several small vacuoles. In table 2 the distribution of the histological changes is compared to the differential function on diuretic renal scan. Six males and 1 female had impaired renal parenchyma. Interstitial focal fibrosis was found in 4 samples, mild chronic inflammation with lymphoid aggregates in 4 and focal Bowman's space dilatation in 1. No sample demonstrated apoptotic nuclei as confirmed by immunohistochemical study which showed the presence of bcl-2 (see figure) and absence of CD95, TDAG51 and clusterin. Only 1 of the 7 patients (4%) with tubulointerstitial fibrosis had 37% preoperative renal function while the others had more than 40% renal function.

DISCUSSION

The treatment of children with ureteropelvic junction obstruction is controversial and decisions regarding surgery are often based on interpretation of the renal scan, evaluating the differential renal function and diuretic renogram half-time washout. However, it is generally recognized that the first objective is to prevent renal damage secondary to obstruction because one might expect that significant renal histological changes have occurred.[15] Krueger et al reported that 54% of children (7 of 13) who underwent pyeloplasty had normal renal biopsies.[2] However, Steinhardt et al performed
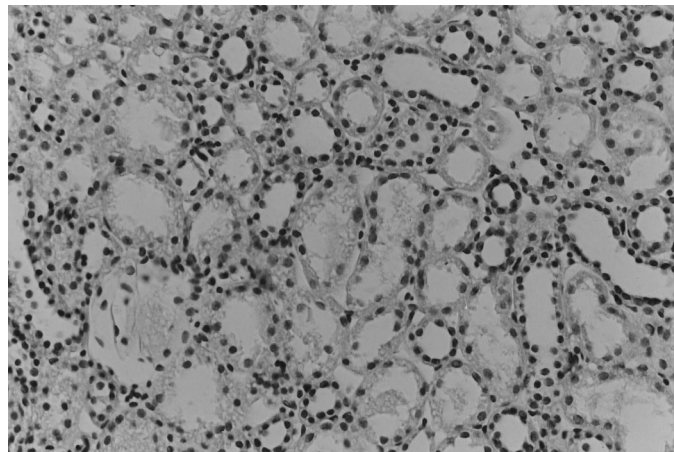
Bcl-2 staining in dilated tubules. Reduced from ×200

nephrectomy for severe ureteropelvic junction obstruction in 20 patients, and found severe abnormalities, such as interstitial fibrosis with inflammation in 75%, glomerulosclerosis with inflammation in 70%, medullary dysplasia in 30% and glomerular cystic changes in 15%.[3]

Elder and Dahms performed renal biopsy in 34 children who underwent pyeloplasty and 75% of the kidneys had histological parenchymal changes but they did not correlate biopsy results and postoperative renal function.[4] Subsequently they showed that in approximately 25% of children with ureteropelvic junction obstruction there was a disparity between preoperative differential renal function on diuretic renography and renal biopsy.[16] Indeed, they concluded that the damage produced by obstruction was progressive and that kidneys with reduced function are more likely to show significant alteration on renal biopsy.

In our patients differential renal function on diuretic renography correlated with histological changes since histologically normal kidneys had a higher differential renal function than abnormal kidneys. Apoptosis was absent in kidneys with preoperative uptake of less than 40% and minimal histological changes, which may explain the inverse correlation between degree of renal dysplasia and gestational age, whereas the opposite is true for cystic changes.[17] Even in our series renal anomalies might be related to the gestational age at which the injury occurred (a mean range more than 7 months) and to duration of the obstruction.

TABLE 2. *Characteristics of 7 patients with abnormal biopsies*

Pt. No.	Interstitial Fibrosis	Chronic Inflammation	Interstitial Edema	bcl-2	% Preop. Differential Function
1	Neg.	Pos.	Pos.	Neg.	51.2
2	Pos.	Pos.	Neg.	Pos.	37
3	Pos.	Neg.	Neg.	Pos.	46.6
4	Neg.	Pos.	Neg.	Pos.	50.1
5	Neg.	Neg.	Pos.	Pos.	45.9
6	Pos.	Neg.	Pos.	Neg.	48
7	Pos.	Pos.	Neg.	Pos.	48.9

Early complete obstructive uropathy results in renal dysplasia, possibly because of vasoconstrictive ischemia caused by increased production of vasoconstrictor prostanoids such as thromboxane A2 and progressive interstitial fibrosis of the obstructed kidney, in part because of activation of transforming growth factor-β1 by angiotensin system.[18] Recent studies have indicated that inhibition of angiotensin AT1 receptors in neonatal rats with unilateral ureteral obstruction markedly increases expression of clusterin.[5] In contrast, nonobstructive dilatation may be inconsequential to the child's well-being. Chronic obstructive uropathy in neonatal rats delays maturation possibly in part through suppressed expression of the epidermal growth factor.[18, 19] The response of the developing kidney to chronic obstructive uropathy is similar to that of cystic kidney disease, since both include a reduction in epidermal growth factor and increased apoptosis that may result from suppression of bcl-2, an oncoprotein that inhibits apoptosis in a complex cellular process consisting of multiple steps, each of which is mediated by families of related molecules.[20] Therefore, the possible explanation for the absence of renal expression of clusterin and apoptosis could be the presence of bcl-2 (in 5 of 7 kidney) as a critical in vivo renal cell survival factor for the developmentally mature kidney.[20] Thus, current investigations have revealed that the epidermal growth factor administration suppresses apoptosis related gene activity and, consequently, enhances renal tubular epithelial cell preservation and replication in the obstructed kidney. The presence of hydronephrosis at any stage of gestation is generally the first indicator of a potential urinary tract anomaly but that does not mean that, if left untreated, obstruction will cause progressive renal deterioration.

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