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Epidermal growth factor and monocyte chemotactic peptide-1: Potential biomarkers of urinary tract obstruction in children with hydronephrosis

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Abstract *Objective:* Hydronephrosis is diagnosed in 0.5% of all newborns, and ureteropelvic junction obstruction (UPJO) is a common cause. The aim of this study was to test whether specific urinary cytokines can be used as UPJO biomarkers in children with hydronephrosis.
Materials and methods: Twenty-eight children referred for pyeloplasty due to UPJO and 13 controls were included in this prospective study. Kidney function was assessed and urine samples collected pre-, peri-, and post-operatively. Urine levels of epidermal growth factor (EGF), monocyte chemotactic peptide-1 (MCP-1), macrophage inflammatory protein-1α (MIP-1α), interferon-γ-inducible protein-10 (IP-10), and RANTES were measured simultaneously by using a bead-based multiplex sandwich immunoassay.
Results: In hydronephrotic children, preoperative urine levels were significantly increased for EGF (median 7.4 [1.2—60.2] vs. median 4.0 [1.2—13.8] ng/mg creatinine) and MCP-1 (median 136.9 [47.7—545.5] vs. median 80.1 [28.8—149.9] pg/mg creatinine) compared to those of controls. Urine levels of EGF and MCP-1 were identical to controls at the postoperative 1-year follow-up exam.

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Conclusion: Urine levels of EGF and MCP-1 were preoperatively increased and postoperatively normalized. This study demonstrates that urine-excreted kidney cytokines may be potential biomarkers of obstruction in children with hydronephrosis.

Introduction

Hydronephrosis is diagnosed in 0.5% of all newborns, and ureteropelvic junction obstruction (UPJO) is a common cause [1]. Left untreated, UPJO may result in progressive renal tubular atrophy, interstitial fibrosis, and nephron loss, and since the obstruction occurs in early renal development, it may also affect the kidney's functional capacity [2]. The finding of hydronephrosis in a newborn is associated with a challenging year-long follow-up including repetitive monitoring of renal function to identify obstruction [3] and plan surgical treatment of the kidneys that are likely to lose function [4]. Today, this process requires repetitive ultrasonography, diuretic renography and, in selected cases, glomerular filtration rate (GFR) determination. These examinations can be time consuming and distressing to the child and are neither sensitive nor specific enough to identify all growing kidneys that require treatment. Consequently, there is a great need for additional sensitive methods to monitor specific changes in kidney function in these patients. The identification of one or more urinary biomarkers represents a promising approach for this purpose. Urinary protein levels offer a snapshot of the present physiological state and have the potential to be used as prognostic tools for early disease detection as well as optimal treatment and monitoring [5].

No urinary biomarkers have yet been clinically implemented, although potential candidate biomarkers have been suggested [6]. Transforming growth factor β1, epidermal growth factor (EGF), monocyte chemotactic peptide-1 (MCP-1), endothelin-1, and selected urinary tubular enzymes are currently among the promising biomarkers [6]. One of the challenging factors in the search for urinary biomarkers is the fact that no single biomarker is specific for upper urinary tract obstruction, and given the multifactorial nature of obstruction, it is unlikely to be identified using a single biomarker. Therefore, we hypothesize that a selected panel of biomarkers may be potential diagnostic and prognostic tools for UPJO.

In this study, we tested 5 cytokines as candidate biomarkers. We focused on the dynamics of the urinary excretion pattern after UPJO relief, and compared them to urinary levels in healthy controls. The chosen cytokines were: EGF, MCP-1, interferon-γ-inducible protein-10 (IP-10), macrophage inflammatory protein-1α (MIP-1α), and regulation on activation normal T-cell expressed and secreted (RANTES) (Supplementary Data, Table S1).

Materials and methods

Patients

Twenty-eight patients were included in a prospective study between 2007 and 2011. The study took place at our department when each patient was referred for a scheduled Anderson-Hynes pyeloplasty due to unilateral UPJO. The criteria for pyeloplasty included positive findings on renal ultrasonography, diuretic renography, and a relevant clinical story. The inclusion criteria in the study group were: age 3–15 years and surgical treatment of UPJO indicated by either a decreasing relative function of the hydronephrotic kidney of more than 5%, and to less than 40% of the differential renal function (DRF) or ipsilateral flank pain. The exclusion criteria were bilateral hydronephrosis; previous surgery of the urinary system; malformations of the lower ureter, bladder and urethra; urinary stones; vesicoureteral reflux; urinary tract infections; neurogenic bladder dysfunction; and non-compliance.

Prior to admission for pyeloplasty, a pediatric radiologist performed a renal ultrasonography and assessed the AP diameter and SFU grading. The patients also underwent a diuretic technetium-99m mercaptoacetyltriglycine (MAG3) renography. The labeled substance was injected (50 Ci/kg 99mTc MAG3), and after a 20–30 min observation, furosemide stimulation (0.5 mg/kg IV bolus) was given to the patients who did not eliminate at least 50% of the substance in the pelvis (T½). Elimination was monitored for another 20 min, and patients who did not achieve T½ by the end of the test were considered to have obstructive hydronephrosis. DRF of the obstructed kidney $<$40% was considered abnormal. At admission, each patient underwent a physical examination and a urine culture. All patients were operated on by a pediatric urologist with a robot-assisted retroperitoneoscopic pyeloplasty with the insertion of a thin stent (Salle Pyeloplasty Stent 4.7 Cook Urological, Spencer, IN, USA) to reduce the load on the anastomosis between the pelvis and the ureter. After the stent was inserted, the anastomosis was sutured and the stent was passed through the renal pelvis and guided through the skin. The stent was closed on the first postoperative day and removed without anesthetic at the outpatient clinic after 3 weeks. A follow-up including renal ultrasonography and ^{99m}Tc-MAG3 renography was scheduled at 3 months and 1 year after the procedure.

Material collection

In order to examine the urinary excretion pattern of the five cytokines over time, urine samples were collected 6 times from the patients: (1) preoperative bladder urine; (2) perioperative urine from the obstructed kidney and bladder urine (i.e., urine from non-obstructed kidney since it was collected after dismembering of the affected pelvis); (3) postoperative urine (1 day) from the stent (i.e., from the obstructed kidney) and bladder urine (i.e., from the non-obstructed kidney); (4) postoperative urine (3 weeks) from the stent (i.e., from the obstructed kidney) and bladder urine (i.e., from both kidneys since the stent had been closed prior to the collection); (5) postoperative bladder urine (3 months); and (6) postoperative bladder urine (1 year). Samples from times 1, 4, 5, and 6 were collected as voided midstream urine.