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Serum and urine levels of interleukin-6 and interleukin-8 in children with acute pyelonephritis

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Abstract

Urinary tract infection (UTI) is a common clinical disorder in younger infants and children and may result in permanent renal damage. The inflammatory cytokines interleukin (IL)-6 and IL-8 play an important role in response to bacterial infection. This prospective study investigated the association between serum and urine IL-6 and IL-8 levels and acute pyelonephritis confirmed by ^{99m}Tc-dimercaptosuccinic acid (DMSA) scan. A total of 78 children aged 1–121 months with a diagnosis of first-time febrile UTI were included. The following inflammatory markers were assessed: fever; white blood cells count (WBC); C-reactive protein (CRP); and serum and urine IL-6 and IL-8. The patients were divided into the acute pyelonephritis group ($n = 42$) and the lower UTI group ($n = 36$) according to the results of DMSA scan. Fever, WBC and CRP levels were significantly higher in children with acute pyelonephritis than in those with lower UTI (all $p < 0.001$). Significantly, higher initial serum and urine IL-6 and IL-8 levels were found in children with acute pyelonephritis than in those with lower UTI (all $p < 0.001$). Serum and urine IL-6 in children with acute pyelonephritis were positively correlated with fever, CRP and leucocyturia. These results indicate that both serum and urine IL-6 and IL-8 levels, particularly IL-6, are useful diagnostic tools for early recognition of acute pyelonephritis in febrile children.

Keywords: Acute pyelonephritis; Children; Interleukin-6; Interleukin-8; Vesicoureteral reflux

1. Introduction

Urinary tract infection (UTI) is a common pediatric disorder which may be localized to the kidney or bladder or spread to the tissue outside the urinary tract. Children after acute pyelonephritis may result in renal scarring, which has been estimated to occur in 10–65% of cases [1,2]. Renal scarring later in life may lead to the development of hypertension, proteinuria and renal insufficiency [3,4]. The level diagnosis of a UTI in younger infants and children has an important bearing on treatment and follow-up. The primary distinction between acute pyelonephritis and lower UTI is based on clinical manifestations and indirect laboratory tests [5,6]. However, fever is not a reliable indicator of the presence of acute inflammatory lesions in the kidneys of younger infants and children. Inflammatory markers including C-reactive protein (CRP), white blood cells count (WBC) and erythrocyte sedimentation rate (ESR) are also unreliable diagnostic indicators in the acute phase of pyelonephritis [7,8].

Cytokines are small soluble proteins which play a major role in mediating the inflammatory process in response to bacterial pathogens [9]. Elevated levels of the inflammatory

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cytokines such as interleukin (IL)-6 and IL-8 have been found in the serum and urine of younger infants and children with UTI [10–14]. IL-6 is a proinflammatory cytokine that acts as a pyrogen and appears early during the inflammatory process, which is responsible for the acute-phase reaction including the development of fever and increased production of acute phase proteins such as CRP [15,16]. IL-8 is a potent chemotactic factor for neutrophils, responsible for the migration of granulocytes into the site of infection and the release of storage enzymes and toxic metabolites [15,17]. Both IL-6 and IL-8 can activate local and systemic inflammatory processes and these two cytokines are major mediators of inflammation in response to bacterial infection [15,18,19].

^{99m}Tc-dimercaptosuccinic acid (DMSA) uptake reflects the functional integrity of renal parenchyma especially in the renal cortex. DMSA scan is a widely accepted standard method for detecting the acute inflammatory lesions after pyelonephritis and the subsequent occurrence of renal scarring [20,21]. Bacterial infection of renal parenchyma in children may lead to various inflammatory lesions, and may be identified by reduced focal or multifocal perfusion defects on unilateral or bilateral kidneys on DMSA scan. DMSA scintigraphy offers great value in distinguishing pyelonephritis from lower UTI in pediatric patients.

The aims of this prospective study were to investigate the local and systemic IL-6 and IL-8 responses in younger infants and children with acute pyelonephritis confirmed by DMSA scan and also to assess the association of serum and urine IL-6 and IL-8 levels with inflammatory markers and vesicoureteral reflux (VUR).

2. Patients and methods

2.1. Subjects and study design

A total of 78 children (ages from 1 month to 10 years) with first-time febrile UTI were enrolled into this prospective study during a 2-year period. The diagnostic criteria required for study entry were as follows: temperature $\geqslant 38$ °C; leucocyturia defined as $\geqslant 5$ WBC per high-power field; and a positive urine culture, defined as growth of a single organism $>10^5$ colony-forming units/ml collected from clean midstream urine or $>10^4$ colony-forming units/ml collected via catheter. Children were excluded from the study if they had a history of previous UTI or ongoing antibiotic treatment. We also collected 12 healthy children with age and sex matched served as controls. No control child was febrile at the time of samples collection. Informed consent for participation was obtained from all parents of the participants. The protocol was approved by the Institutional Review Board of Chung Shan Medical University Hospital.

All patients were treated empirically with broad-spectrum antibiotics and the regimen was later adjusted according to the results of antibiotic susceptibility testing of the isolates. In all episodes, a subsequent urine specimen for culture was performed after 3 days of treatment. All patients underwent renal ultrasound examination for the detection of urinary tract anomalies within the first 3 days of admission. DMSA scans were routinely performed within 1 week of admission to verify the presence of pyelonephritic lesions on the kidney parenchyma. Normal scan was defined as normal radioactive marker uptake in the kidneys, and abnormal scan was defined as the presence of areas of impaired uptake (focal or multifocal) with or without changes in kidney size [21]. According to the findings on DMSA scan, the patients were divided into two groups based on the diagnosis of acute pyelonephritis or lower UTI. Voiding cystourethrography (VCUG) was performed 1–2 weeks after the completion of treatment for infection (negative urine culture). The presence of VUR was graded 0–V using an international classification scheme [22].

2.2. Laboratory analysis

Serum and urine samples were collected from all children with suspected UTI. Laboratory tests routinely performed for the identification of infection included WBC and differential count, platelet count, CRP, urinalysis and urine culture, as well as routine biochemical tests.

2.3. Measurement of cytokines

Serum and urine samples for IL-6 and IL-8 determination were collected from all children before the initiation of antibiotic treatment and approximately 2 weeks after the start of antibiotic treatment for the infection. Serum and urine samples for IL-6 and IL-8 assay obtained were immediately centrifuged, separated, frozen and stored at −70 °C until they were tested in batches. IL-6 and IL-8 levels were measured by enzyme-linked immunosorbent assay (ELISA) using commercially available kits according to the manufacturer's instructions (R&D Systems, Minneapolis, Minnesota, USA), which employs the quantitative sandwich enzyme immunoassay technique. Cytokine levels (pg/ml) were calculated from standard curves generated with recombinant cytokines. The differences between duplicate wells were consistently less than 10% of the mean values. The lower limits of detection were 0.5 pg/ml for IL-6 and 1.0 pg/ml for IL-8. Urine interleukin-to-urine creatinine quotients were determined in order to standardize the urine samples and were expressed as pg/mg. All samples were measured in duplicate by ELISA.

2.4. Statistical analysis

All statistical analyses were performed using SPSS for Windows, version 10.0 (SPSS Inc., Chicago, IL, USA). The data were expressed as means ± SD. Mann–Whitney U test was used for intergroup comparisons. Wilcoxon's