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Impact of cytokine genetic polymorphisms on the risk of renal parenchymal infection in children

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Summary

Background
Acute pyelonephritis is associated with renal scarring in up to 30% of patients. Renal scarring may cause significant long-term morbidity. The pathogenesis of acute pyelonephritis remains unclear, although it involves interaction among uroepithelium, the immune system cells, and the locally produced cytokines. That some UTI-prone children develop acute pyelonephritis, and eventually renal parenchymal scarring, suggests a genetic role. Interleukin-6, interleukin-8, chemokine receptor-1 (CXCR1), and tumor necrosis factor-alpha (TNFα), the key regulators of the host immune responses, are proteins whose secretion is controlled by genes. We postulated that functional polymorphic variants of their genes might have a role in APN susceptibility.

Objectives
We sought to investigate a possible association of the common functional polymorphisms in genes encoding IL-6, IL-8, CXCR1, and TNFα with the risk of APN in children.

Methods
Urine culture was used to diagnose 300 children with UTI, of mean age of 51.31 $\pm$ 37.4 months (2–180 months). 99Tc-DMSA scans diagnosed 86 children with APN. Follow-up scans identified new renal scars in 18 children. Six functional single-nucleotide polymorphisms (SNPs) in genes encoding IL-6, IL-8, CXCR1, and TNFα were genotyped in all subjects (IL-6 rs1800795 (−174G/C), IL-6 rs1800796 (−572G/C), IL-8 rs2227306 (781C/T), IL8 rs4073 (−251A/T), CXCR1 rs2234671 (2607G/C), and TNFα rs1800629 (−308G/A)).

Results
TT genotype of IL-8 −251A/T polymorphism was significantly higher in APN patients (26.7%) than those with lower UTI (11.7%, $p = 0.01$) and control individuals (12.2%, $p = 0.002$). T allele was significantly more common in APN than in lower UTI ($p = 0.025$) and was significantly more common in APN (46%) than in the controls ($p = 0.001$). Similarly, TT genotype of IL-8 781C/T polymorphism was significantly more common in APN patients (31.4%) than those with lower UTI (17.3%, $p = 0.003$) and the controls (14.3%, $p = 0.001$). T allele was significantly more common in APN (55%) than lower UTI (40%, $p = 0.005$) and controls (37%, $p = 0.001$). However, IL-8 −251A/T and +781C/T SNPs did not qualify as an independent risk for parenchymal infection (OR 1.9, 95% CI 0.68–2.6, $p = 0.13$ and OR 2.3, 95% CI 0.89–3.7, $p = 0.091$, respectively). Lower UTI did not differ from the controls. The frequency of the genotypes and alleles of IL-6, CXCR1, and TNFα SNPs did not differ significantly among the different groups of the study.

Conclusion
IL-8 −251A/T and +781C/T SNPs are associated with susceptibility to renal parenchymal infection in children and could be implicated in APN risk. However, none of these variants could clearly and independently predict this risk.

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Introduction

Urinary tract infection (UTI) is the second most common bacterial infection in childhood, with an incidence of 5% in children younger than 12 years [1,2]. Acute pyelonephritis (APN) is a severe upper UTI and a common cause of morbidity in children. It is an important risk factor for late complications such as renal scarring, hypertension, and chronic renal insufficiency [3]. Bacterial growth is usually prevented by host factors (which collectively constitute the innate immune system) such as the barrier formed by the epithelial cells, the locally produced proteins, chemokines, and cytokines [4]. UTI begins when bacteria gain access to the urinary tract and attack the urinary mucosa. Exposure of the uroepithelial cells to the uropathogens triggers a robust immune response with upregulation of the pro-inflammatory cytokines and chemokines (including interleukin-6 (IL-6) and IL-8) by multiple mechanisms [5]. IL-8, the most important chemokine and activator of human neutrophils, interacts with the chemokine receptors CXCR1 and CXCR2 on the surface of epithelial cells and neutrophils to promote chemotaxis [6]. Neutrophils are then recruited to the site of infection and the phagocytosis of bacteria increases the production of IL-1 and tumor necrosis factor-alpha (TNF-α). IL-1 and TNF-α stimulate further production of cytokines to augment the inflammatory responses [7]. In animal models of UTI, the homozygous deletion of a single gene for the murine IL-8 receptor homologue precipitated the syndrome of APN and renal scarring [8]. Cytokine responses in humans, however, showed high interindividual variability; Individuals were classified according to their cytokine responses into "high responders" or "low responders" [9]. In the high responders, the excessive inflammatory host response to infection is directly related to the severity of APN and renal parenchymal scarring (RPS). Both APN and RPS can therefore be regarded as infection-driven inflammatory disorders of the kidneys [10]. The 99mTc-dimercaptosuccinic acid (DMSA) scan, a scintigraphic method for detecting the parenchymal inflammatory lesions, is the gold standard for diagnosis of APN and RPS [11]. Some children develop APN with the possibility of renal scarring, despite having anatomically normal urinary tract (no evidence of vesicoureteral reflux), suggesting a genetic predisposition [12]. Although certain genetic variations in cytokines and their receptors have been implicated in different diseases [13,14], none has been identified as a causative polymorphism for APN/RPS and their role in UTI susceptibility remains elusive [6]. Although early diagnosis of APN and its differentiation from lower UTI (which is less likely to cause RPS) are very important, DMSA scanning exposes the patients to radiation; and there is no consensus on its clinical usefulness. Identifying children at greater risk for APN and RPS based on genetic predisposition might encourage the clinician to follow more strict imaging protocols in genetically predisposed children. In addition, replication studies in different populations may help to evaluate the genotype—phenotype association observed in independent samples from previous studies. The aim of the present study was to investigate the role of the common functional polymorphisms of IL-6, IL-8, CXCR1, and TNFα genes as risk factors for development and progression of APN in children.

Methods and study design

This study was conducted at Assiut University Children's Hospital (Assiut, Egypt). The study was approved by the ethics committee of Assiut University Hospitals, and the Faculty of Medicine. We consecutively enrolled 300 Egyptian children at the time of their first documented UTI over a period of 3 years (from Jan 2013 to Dec 2015). We collected urine specimens for urinalysis and culture by clean-catch or urinary catheter. In two young infants, however, we collected urine by sonographic-guided suprapubic aspiration. We suspected APN in the children if they had symptoms related to the urinary tract and body temperature $\geq$38 °C with or without flank pain, nausea, vomiting, and chills. APN was confirmed in the culture-positive children by typical renal scintigraphic findings on DMSA-scan. The inclusion criteria for the study were: fever defined as body temperature $\geq$38 °C; pyuria with a count of more than 5 WBC per high-power field; and a positive urine culture with growth of a single organism of more than 10^5 colony-forming units/mL collected from clean catch urine, or more than 10^4 colony-forming units/mL collected via a catheter. Children were excluded from the study if they had any of the following: evidence of kidney or urinary tract anomalies on expert ultrasound, a previously documented UTI, ongoing antibiotic treatment, severe concomitant infections, or indwelling catheter. To test the effect of genetic polymorphisms as a risk for APN without any confounding factors, children with vesicoureteral reflux (VUR) or established old renal scar with any volume loss at the initial DMSA scans were excluded from the study. Fig. 1 shows a flowchart representation of the methodology and design of this study.

Control group

The control group comprised 300 blood samples (130 males and 170 females) for generic study obtained consequently from healthy volunteers living in Assiut province as they attended an outpatient clinic, well-child clinic for routine check-up, or were admitted for minor elective surgeries such as minor cosmetic repair, minor fractures, or circumcision. They had negative history of urinary infection, negative family history of urinary troubles, and normal urinalysis and renal ultrasound scans. Their mean age was 19.8 $\pm$ 13.4 years, range 3—31 years.

Imaging study

Abdominal ultrasound was performed within 2 days to exclude structural anomalies of the urinary tract and confirm the final enrolment. If APN was suspected based on body temperature (febrile UTI) and/or clinical presentation (suggesting APN), DMSA-scans were performed within the first 72 h of presentation to assess the presence of the typical parenchymal lesions of acute pyelonephritis. UTI children were divided into two groups based on the findings of the DMSA scans: APN or lower UTI. A normal scan was defined as normal radioactive marker uptake in the kidneys, and an abnormal scan was defined as the presence of impaired (focal or multifocal) uptake defects (photopenia)

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