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Serum and Urinary MIP-1α and IP-10 Levels in Children with Urinary Tract Infections*

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Objectives. Urinary tract infection (UTI) is a common bacterial disease in infants and children, with potentially serious complications, including kidney damage. The aim of this study was to test whether serum and urinary levels of interleukin-6 (IL-6), macrophage inflammatory protein-1α (MIP-1α) and interferon-γ-inducible protein-10 (IP-10) can be used as biomarkers in children with urinary tract infections.
Material and Methods. The study group consisted of 22 children with UTI and 20 controls. Blood and urine samples were collected in the acute phase and the convalescent phase, on the eighth day after the onset of antibiotic therapy. Serum and urine levels of MIP-1α, IP-10 and IL-6 were measured.
Results. In children with UTI in the acute phase, serum MIP-1α and IL-6 levels were significantly higher compared to the controls ($p < 0.05$ and $p < 0.005$, respectively). A correlation between the serum levels of the chemokines MIP-1α and IP-10 in the acute phase was found.
Conclusions. The findings suggest that the chemokines MIP-1α or IP-10 respond to infection, but they cannot be used as biomarkers for UTI in childhood (**Adv Clin Exp Med 2014, 23, 6, 933–938**).

Key words: chemokines, children, urinary tract infection.

Urinary tract infection (UTI) is a common bacterial disease in infants and children, with potentially serious complications, including kidney damage and chronic renal failure. UTI is diagnosed in approximately 5% of infants who are brought to hospital emergency departments because of a fever without any obvious cause [1].

Recent European standards regarding the investigation and treatment of UTI are based on the National Institute for Health and Clinical Excellence (NICE) guidelines, which require a clinical examination, urinalysis, urine culture and imaging tests to diagnose UTI [2]. Some authors have attempted to identify markers of inflammation in urine and to use them in the diagnosis of infection of the urinary tract and in monitoring response to therapy [3–6].

Escherichia coli is the most common cause of UTI, but *Klebsiella, Proteus, Enterobacter, Staphylococcus saprophyticus* and *Enterococcus* also grow in the urine culture [7]. Bacteria that enter the urinary tract cause an activation of the host inflammatory response. In the results of bacterial stimulation, renal epithelial cells have been shown to produce a number of cytokines and chemokines, among other substances [8]. Cytokines, especially interleukin-6 (IL-6), participate in the local inflammatory response to infections [4, 9–11]. Chemokines (chemotactic cytokines) mediate this process locally and systemically; they are important mediators of leukocyte extravasation and chemotaxis [12]. Chemokine concentration results in an influx of leukocytes including

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neutrophils, natural killer cells, macrophages and lymphocytes.

The importance of chemokines during kidney inflammation has been described in various studies [5, 13–15], but little is known about the role of each chemokine in the kidney. Chemokine macrophage inflammatory proteins-1alpha (MIP-1α, CCL3), produced by macrophages, may be involved in the development of cellular crescents in the acute phase of kidney pathology in bacterial infections [8]. Interferon-gamma-induced protein 10 (IP-10, CXCL10) is produced by several cell types, such as fibroblasts, endothelium cells and monocytes, and its secretion is stimulated by tumor necrosis factor alpha (TNFα), interleukin-1 beta (IL-1β) and macrophage colony-stimulating factor (M-CSF) [12]. Plasma IP-10 is considered to be a predictor of serious bacterial infection in infants [16] and has recently been associated with acute kidney injury [13]. Both chemokines have been detected in urine [17, 18]. Evaluating the response of chemokines MIP-1α and IP-10 to infection could help to explain whether they can be markers in a febrile urinary tract infection in children.

The aim of this pilot study was to investigate and compare serum and urinary levels of MIP-1α, IP-10 and IL-6 in children with urinary tract infections on admission, in the acute phase, and after eight days of parenteral antibiotic therapy. This information could be useful for diagnosing UTI.

Material and Methods

Patients with Urinary Tract Infections

The study involved 22 children of both sexes (aged from 4 to 17 years; median age 10 years; 18 female, 4 male) diagnosed with a UTI at the Department of Pediatric Immunology and Rheumatology, Wroclaw Medical University (Wrocław, Poland) between 2006 and 2009. To qualify for the study a diagnosis of UTI according to NICE clinical guideline [2] and significant bacteriuria (>10^5 colony-forming units (CFU)/L of a single uropathogenic bacteria in a clean catch sample) were required. Children who have bacteriuria and a fever of 38°C or higher, and children presenting a fever lower than 38°C with loin pain/tenderness and bacteriuria were considered to have acute pyelonephritis/upper urinary tract infection [19]. All other children with bacteriuria but no systemic symptoms or signs (a peripheral white blood cell [WBC] count of > 15,000 cells/μL, or C-reactive protein [CRP] level of ≥ 6.0 g/dL) were considered to have cystitis/lower urinary tract infection.

Healthy Control Group

The study also included 20 healthy controls (aged from 4 to 17 years; median age 11.5; 35 female, 7 male) without any symptoms of UTI and with one negative urinary culture. These controls were selected from 2 distinct populations: outpatients seen at the hospital for routine medical follow-up, and children whose parents (or guardians) volunteered them as participants in the study.

Exclusion Criteria for the Group with UTI and the Control Group

Patients with a history of previous UTIs, those suffering from vesicoureteral reflux or severe renal function impairment, those who had undergone bladder catheterization within the previous two weeks, those who had had sexual activity within the previous 2 weeks, those using immunosupression or being treated with nephrotoxic medications, and those who had signs of any other infection requiring antibiotic treatment within 2 weeks prior to the screening were excluded from the study.

Detection of Inflammatory Mediators

In the group with UTI, blood and urine samples were obtained twice:

– in the acute phase, while admitting the patients to the hospital, while the symptoms were presenting; and

– in the convalescent phase, on the 8th day after the onset of antibiotic therapy.

In the control group, blood and urine samples were collected one time only.

All the blood samples were drawn between 8 and 9 am. The sera received were aliquoted and stored at a temperature of –70°C until the assay. Urine samples obtained by clean catch sampling were centrifuged (7000 g, 15 min, 4°C), divided into portions and immediately frozen at –70°C.

Serum levels of MIP-1α and IP-10 were analyzed with Duo-Set ELISA systems (R&D, Abingdon, UK). Briefly, flat-bottom 96-well MaxiSorp microtiter plates (Nunc) were coated overnight at room temperature with 100 μL per well of relevant capture antibodies diluted in PBS buffer (pH 7.4). The wells were blocked with 300 μL of 1% BSA in PBS for 1 h at 37°C. Dilutions of the serum samples in the 1:2–1:16 range (for MIP-1α and IP-10), undiluted urine samples and standards. PBS buffer containing 0.05% Tween 20 and 1% BSA (TPBS buffer) was used for all dilutions. All samples were run in duplicate. After washing 4 times with TPBS,

100 µL of the relevant biotinylated detection antibodies were added and the plate was incubated for 2 hrs at room temperature. After subsequent washing with TPBS, the wells were filled with streptavidin-HRP (diluted ×200) and the plate was incubated for 20 min at room temperature and in darkness. After washing, 100 µL per well of TMB substrate (R&D Abingdon, UK) was added and the plate was incubated 20 min at room temperature and in darkness. The enzymatic reaction was stopped with 50 µL 1M H_2SO_4. The optical density was read at 450 nm with a Dynatech 5000 photometric reader.

Serum IL-6 levels were measured using a commercial kit according to the manufacturer's instructions (R&D, Abingdon, UK) with a detection limit of 1 pg/mL.

Additionally, the following markers of inflammation were measured in the UTI group: serum CRP level, peripheral WBC, urine dipstick analysis and microscopic exam, using standardized methods. Pyuria was defined as ≥ 5 WBC/high power field (hpf) or ≥ 10 WBC/mm^3 on a hemocytometer with an enhanced urinalysis, and bacteriuria was defined as the presence of any bacteria per hpf [19].

The study protocol was approved by the Research Ethics Committee of Wroclaw Medical University. The parents (or guardians) of the participants and participants older than 16 years signed written consent forms before participating in the study.

Statistical Analysis

The results are expressed as median values and interquartile ranges. The statistical analyses were performed using non-parametric tests for data without a normal distribution. The Mann-Whitney *U*-test was used for independent variables and the Wilcoxon paired rank test was used for dependent variables. Spearman's rank correlation coefficient was used to investigate any relationship between the parameters. The level of statistical significance was assumed to be $p < 0.05$. The statistical analyses were carried out using StatSoft software (STATISTICA 9).

Results

The group of patients with UTI and the control group were similar in terms of age and gender. The results of the urinalysis and of urine collections as well as selected results of the blood tests are presented in Table 1. Most patients (n = 15, 68.2%) were suffering from pyelonephritis. The results of the urine cultures revealed that *E. coli* was the cause of UTI in most cases (n = 14, 63.6%).

Table 1. Clinical data of the patients with urine tract infection, n = 22

Parameter	n	%
Diagnosis		
Pyelonephritis	15	68.2
Cystitis	7	31.8
Urine culture		
Escherichia coli	14	63.6
Proteus vulgaris	4	18.2
Enterococcus faecalis	2	9.1
Citrobacter sp.	1	4.5
Candida albicans	1	4.5
Urine sample		
Pyuria (≥ 10 WBC/high-powered field)	13	59.0
Hematuria (≥ 10 cells/high-powered field)	10	45.5
Proteinuria	4	18.2
pH ≥ 7	4	18.2
Serum creatinine level (>1.1 mg/dL)	none	
Leucocytosis (WBC count >15,000 cells/µL)	6	27.3
High CRP level (> 6.0 mg/L)	15	68.2
Anemia	2	9.1

CRP – C-reactive protein, UTI – urine tract infection, WBC – white blood cell.

The serum and urine concentrations of the chemokines MIP-1α and IP-10 and the cytokine IL-6 in the acute and convalescent phases of UTI compared with the healthy controls are presented in Table 2. The median serum MIP-1α and IL-6 level was found to be significantly higher in the acute phase of UTI as compared to the control group ($p < 0.05$ and $p < 0.005$, respectively) but no such difference was observed in the convalescent phase. Although a similar trend was observed for IP-10 levels, it was not statistically significant ($p = 0.058$). No statistically significant differences in the urinary levels of MIP-1α and IP-10 were found in the patients in the acute or convalescent phases, or in the healthy controls.

A correlation was found between the serum level of MIP-1α and IP-10 in the acute phase ($S_r = 0.522$, $p = 0.01$, Fig. 1). No correlation between the urinary levels of MIP-1α and IP-10 was observed.

Table 2. The median values and interquartile ranges of the examined parameters in UTI patients and the control group

Parameter	Median values (interquartile ranges) of the analyzed parameters		
	acute UTI (n = 23)	**convalescent UTI (n = 13)**	**control (n = 20)**
MIP-1α (pg/mL)			
Serum	25.84* (14.12.–138.71)	22.55 (7.86–112.02)	16.45 (10.87–41.54)
Urine	1.84 (1.07–2.36)	1.67 (0.866–1.77)	1.57 (1.39–1.82)
IP-10 (pg/mL)			
Serum	60.91 (34.03–144.42)	44.14 (29.86–70.51)	45.40 (13.28–75.0)
Urine	18.39 (15.51–27.77)	16.47 (9.67–21.55)	16.33 (15.95–17.61)
IL-6 (pg/mL)			
Serum	7.4** (4.13–26.71)	3.56# (3.17–4.27)	2.84 (2.67–3.36)

* $p < 0.05$ acute UTI vs. control; ** $p < 0.0001$ acute UTI vs. control; # $p < 0.005$ convalescent UTI vs. control.

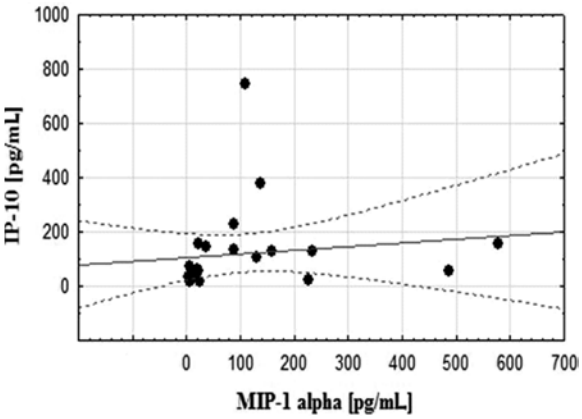

Fig. 1. Correlation between the serum concentration of MIP-1α and IP-10 in children with urinary tract infections during the acute phase of the disease ($S_r = 0.522$, $p = 0.01$)

No correlation was found between the serum and urinary levels of the chemokines MIP-1α and IP-10 and leukocytosis, CRP level, serum IL-6 level, a positive urine nitrite test or leucoctyuria (data not present).

Discussion

Early diagnosis and treatment of acute UTI in children is of particular importance since the majority of children examined by the authors (68%) had already developed pyelonephritis, which can result in serious complications such as renal scarring, hypertension and renal failure. Diagnosis of urinary infection in patients with acute urinary tract symptoms is usually done on the basis of urinalysis, pyuria and urine collection [20]. Screening methods including commonly used inflammatory markers such as leukocyte count, procalcitonin and C-reactive protein, as well as serum IL-6 levels, are helpful. IL-6 promotes the growth and differentiation of T and B lymphocytes. Kassir et al. reported that elevated IL-6 confirmed the inflammatory process, and that IL-6 levels rapidly decreased after appropriate antibiotic treatment [6]. The findings in the current study are not consistent with this observation. In the current study, elevated serum levels of IL-6 in children were already present at the time of diagnosis; they decreased on the 8th day of antibiotic treatment, but were still significantly higher than in the control group. These results suggest that the inflammatory process does not cease as rapidly as described in the report by Kassir et al.

Recently, several immune mediators present in the peripheral circulation have been detected in the urine of patients with kidney dysfunctions. Some studies have focused on a number of proteins and enzymes normally present in urine or released to urine as a direct response to infection, which have been suggested as potential biomarkers [21, 22]. Efforts to identify novel, sensitive urinary biomarkers, however, have led to the conclusion that a combination of mediators could be even more useful.

Febrile urinary tract infections are accompanied by a complex chemokine response [23]. According to Godolay et al. *E. coli* strains isolated from patient with pyelonephritis caused an increase *in vitro* of epithelial secretion of the chemokines CXC (CXCL1, CXCL8, CXCL9, CXCL10) and CC (CCL2, CCI3, CCL5) [24, 25].

In this study the relationship between the chemokine level in plasma and urine of children with UTI was investigated. The results revealed that serum MIP-1α levels are significantly higher in the acute phase of UTI than in the convalescent phase and in the control group. There was no significant difference between the median serum level of MIP-1α in the convalescent phase and the control group. Elevated MIP-1α in serum at the time of diagnosis suggests a chemokine release in response to UTI, and is followed by a decrease in serum MIP-1α after treatment. The proinflammatory activities of MIP-1α induced chemotaxis of CD8+ T lymphocytes [26], while CD4+ T cells migrated in response to IP-10 [27]. In the field of respiratory infection, the evidence suggests that the response to microorganism invasion is regulated by a distinct chemokine expression profile involving MIP-1α [28].

Unfortunately, in the current study no such relationship was found in the urine in the patients with UTI. The results of this study indicate that the chemokine MIP-1α is not a good marker in urine samples. Other authors have reported that chemokines – but not MIP-1α – were elevated in infected uro-epithelial cells *in vitro* [24], in patients on admission in urosepsis and experimental endotoxemia [21] and in children with hydronephrosis [3].

Previous studies have documented that CXCL10 correlates positively with fever and CRP [23]. *E. coli* infection stimulates *in vitro* production of mucosal chemokines, including CXCL10 [24]. In the current study, no differences were found between the levels of IP-10 in the serum or in urine of children with UTI before or after antibiotic treatment, or in comparison with the control group, which is consistent with studies mentioned above [3, 23, 24].

However, the data from the current study revealed a positive correlation between serum levels of MIP-1α and IP-10. It can be concluded that both chemokines are involved in inflammation of the urinary tract in children.

In summary, neither chemokine MIP-1α or IP-10 are good biomarkers in UTI. Despite significant progress in understanding the dynamics of cytokine release during the infectious process, the search continues for reliable biomarkers for the presence of UTI and the results of treatment.

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