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THE ROLE OF URINARY BIOMARKERS IN THE PREDICTION OF TUBULOINTERSTITIAL KIDNEY INJURY IN CHILDREN FOLLOWING COVID-19



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ABSTRACT. The COVID-19 pandemic has demonstrated the vulnerability of the kidneys as target organs due to high ACE2 expression in renal tubular epithelial cells. The aim of this study was to develop a diagnostic and prognostic approach for pediatric acute pyelonephritis based on the assessment of urinary biomarkers of kidney injury. A total of 100 children aged 7–17 years with acute pyelonephritis were included and divided into two groups according to previous COVID-19 history, along with 25 clinically healthy children as a control group. All participants underwent urinalysis, and measurement of urinary KIM-1, NGAL, β_2 -microglobulin, and L-FABP levels. Children with a history of SARS-CoV-2 infection showed significantly elevated urinary biomarker levels and more pronounced subclinical signs of tubulointerstitial injury, including leukocyturia, microhematuria, proteinuria, as well as reduced titratable acidity and ammonia excretion ($p < 0.001$). These findings indicate predominant tubulointerstitial kidney damage even in the absence of clinical manifestations. Early assessment of urinary biomarkers is essential for timely diagnosis and optimization of patient management.

KEYWORDS: acute pyelonephritis, children, COVID-19, KIM-1, NGAL, β_2 -microglobulin, L-FABP.

РОЛЬ МОЧЕВЫХ БИОМАРКЕРОВ В ПРОГНОЗИРОВАНИИ ТУБУЛОИНТЕРСТИЦИАЛЬНОГО ПОВРЕЖДЕНИЯ ПОЧЕК У ДЕТЕЙ ПОСЛЕ COVID-19.

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АННОТАЦИЯ. Пандемия COVID-19 продемонстрировала уязвимость почек как органов-мишеней вследствие высокой экспрессии ACE2 в эпителиальных клетках почечных канальцев. Цель исследования заключалась в разработке диагностического и прогностического подхода при остром пиелонефрите у детей на основе оценки мочевых биомаркеров повреждения почек. В исследование были включены 100 детей в возрасте 7–17 лет с острым пиелонефритом, которые были разделены на две группы в зависимости от



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перенесённой инфекции COVID-19, а также 25 клинически здоровых детей контрольной группы. Всем участникам проводились общий анализ мочи, а также определение уровней KIM-1, NGAL, β_2 -микроглобулина и L-FABP в моче. У детей с перенесённой SARS-CoV-2 инфекцией выявлено значительное повышение уровней мочевых биомаркеров и усиление субклинических признаков тубулоинтерстициального поражения, включая лейкоцитурию, микрогематурию, протеинурию, а также снижение титруемой кислотности и экскреции аммиака ($p < 0,001$). Полученные данные свидетельствуют о преобладании тубулоинтерстициального поражения даже при отсутствии клинических проявлений. Раннее определение мочевых биомаркеров имеет важное значение для своевременной диагностики и оптимизации клинического ведения пациентов.

Ключевые слова: острый пиелонефрит, дети, COVID-19, KIM-1, NGAL, β_2 -микроглобулин, L-FABP.

COVID-19 DAN KEYIN BOLALARDA TUBULOINTERSTISIAL BUYRAK SHIKASTLANISHINI BASHORAT QILISHDA SIYDIK BIOMARKERLARINING ROLI

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ANNOTATSIYA. COVID-19 pandemiyasi buyraklarning ACE2 ning yuqori ifodalanishi tufayli nishon a'zolar sifatida sezgirlikni ko'rsatdi. Tadqiqotning maqsadi bolalarda o'tkir pielonefritda buyrak shikastlanishini erta aniqlash va prognoz qilish uchun siydik biomarkerlariga asoslangan diagnostik yondashuvni ishlab chiqish edi. Tadqiqotga 7–17 yoshdagi 100 nafar bemor bola kiritildi va ular COVID-19 bilan kasallanganlik tarixiga ko'ra ikki guruhga ajratildi, shuningdek 25 nafar sog'lom bola nazorat guruhini tashkil etdi. Barcha ishtirokchilarda umumiy siydik tahlili, hamda siydikdagi KIM-1, NGAL, β_2 -mikroglobulin va L-FABP darajalari aniqlandi. Statistik tahlil Student t-testi, bir omilli ANOVA va χ^2 mezoni yordamida amalga oshirildi. SARS-CoV-2 infeksiyasini boshdan kechirgan bolalarda siydik biomarkerlarining sezilarli oshishi va tubulointerstitsial shikastlanishning subklinik belgilarining kuchayishi, jumladan leykotsituriya, mikrogematuriya, proteinuriya hamda titrlanadigan kislotalik va ammiak ajralishining kamayishi kuzatildi ($p < 0,001$). Olingan natijalar klinik belgilar bo'lmaganda ham tubulointerstitsial zararlanish ustunligini ko'rsatadi. Siydik biomarkerlarini erta aniqlash o'z vaqtida tashxis qo'yish va klinik boshqaruvni optimallashtirish uchun muhimdir.

Kalit so'zlar: o'tkir pielonefrit, bolalar, COVID-19, KIM-1, NGAL, β_2 -mikroglyubulin, L-FABP.

INTRODUCTION. SARS-CoV-2 is capable of infecting various renal structures, including glomerular cells and the epithelium of the proximal convoluted tubules. The resulting morphological alterations most commonly manifest as features of focal segmental glomerulosclerosis and/or acute tubular necrosis.

However, the use of invasive diagnostic procedures, such as percutaneous kidney biopsy—especially in pediatric patients—is associated with a high risk of complications, including severe



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hemorrhagic events. This significantly limits the feasibility of morphological assessment and necessitates the development of alternative, safe diagnostic approaches.

One of the most promising directions in the non-invasive diagnosis of tubulointerstitial disorders is the assessment of urinary enzyme activity. This method allows evaluation of the tubular apparatus of the nephron at the early stages of the pathological process, when the changes are still potentially reversible. Enzymuria is considered a sensitive marker that enables earlier detection of functional impairment compared with conventional biochemical parameters.

Modern approaches to the diagnosis of inflammatory and destructive changes in the tubulointerstitial region involve the use of biomarkers based on biologically active molecules. Among the most clinically significant are neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), and β 2-microglobulin (β 2M). These indicators not only reflect the activity of the inflammatory process but also support the concept of early, highly specific, and non-invasive diagnosis.

KIM-1 is a type I transmembrane glycoprotein that is minimally expressed in intact renal tissue. In cases of ischemic or toxic injury to the proximal tubular epithelium, urinary KIM-1 levels increase markedly, reflecting the extent of parenchymal damage. A significant correlation has been established between urinary concentrations of this marker and its tissue expression.

NGAL is also regarded as an important indicator of acute kidney injury. Clinical studies have demonstrated that its levels in both urine and serum rise substantially in prerenal as well as renal forms of kidney failure. NGAL exhibits high sensitivity and specificity and can be used to monitor the effectiveness of therapy.

β 2-microglobulin has demonstrated greater accuracy in assessing renal function compared with serum creatinine levels. It is actively investigated as a potential marker of impaired glomerular filtration, particularly in the context of kidney transplantation. According to data reported by E.O. Bogdanova and co-authors, β 2-microglobulin concentrations may also reflect the degree of fibrotic changes in the glomerular apparatus, tubulointerstitial tissue, and vascular wall.

Objective. Assessment of morphofunctional changes in renal tubulointerstitial tissue in children with a history of COVID-19 using urinary biomarkers.

Methods. The patients were divided into three groups: Group 1 (control group) included 25 healthy children; Group 2 consisted of 50 patients with acute pyelonephritis who had a history of COVID-19; and Group 3 comprised 50 children with acute pyelonephritis without a history of COVID-19.

Methods for measurement of target parameters. Evaluation of the urinary syndrome included assessment of proteinuria (mg/day), leukocyturia (cells per field of view), bacteriuria (CFU/mL), Nechiporenko test parameters (cells/mL), and bacteriological urine culture with clinically significant bacterial growth ($>10^5$ CFU/mL).

Proximal tubular function was assessed by daily protein excretion (mg/L), whereas distal tubular function was evaluated using the Zimnitsky test, titratable acidity, and ammonia excretion (mmol/day).

Blood samples were collected from the cubital vein after overnight fasting. Serum was separated by centrifugation at 3000 rpm for 15 minutes and stored at $-20\text{ }^{\circ}\text{C}$ for no longer than 3



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months. Twenty-four-hour urine samples were collected in a single container, from which 15 mL aliquots were taken and stored at -20°C until analysis.

Urinary levels of γ -glutamyl transferase (γ -GT, U/L), NGAL (ng/mL), β 2-microglobulin (β 2M, mg/L), and KIM-1 (ng/mL) were determined.

γ -GT activity (U/L) was measured using a colorimetric enzymatic method on an automated biochemical analyzer. This method is based on the ability of γ -GT to catalyze the transfer of the γ -glutamyl group from a substrate to an acceptor, resulting in the formation of a colored compound. The absorbance was measured spectrophotometrically at 405 nm. To improve diagnostic accuracy, γ -GT activity was normalized to urinary creatinine levels and expressed as U/mmol creatinine.

Urinary concentrations of NGAL (ng/mL), β 2-microglobulin (β 2M, mg/L), and KIM-1 (ng/mL) were determined using a solid-phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The method relies on the specific binding of the target antigen to monoclonal antibodies immobilized on a microplate, followed by the addition of enzyme-conjugated secondary antibodies. After incubation with a chromogenic substrate (3,3',5,5'-tetramethylbenzidine, TMB), an enzymatic reaction resulted in the formation of a colored product. Optical density was measured using a microplate spectrophotometer at 450 nm. Biomarker concentrations were calculated from calibration curves constructed using standard solutions.

Prior to analysis, all urine samples were centrifuged at 3000 rpm for 10 minutes, and the supernatant was used for testing. To eliminate the effect of urine acidity on β 2M levels, samples with $\text{pH} < 6$ were neutralized to physiological pH before analysis.

Assessment of urinary renal biomarkers (γ -GT, NGAL, β 2M, and KIM-1) was performed upon hospital admission.

Statistical methods. Statistical analysis was performed using Microsoft Excel (version 2016, USA) and StatPlus v.7 (AnalystSoft Inc., USA). The normality of distribution of quantitative variables was assessed using the Kolmogorov–Smirnov test. Differences between two groups were analyzed using Student's *t*-test, while comparisons among three groups were performed using one-way analysis of variance (ANOVA).

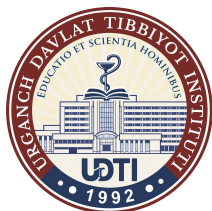
Quantitative data are presented as mean $\pm$ standard deviation ($M \pm SD$). The χ^2 (chi-square) test was used to analyze categorical variables and compare proportions. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Study sample formation

In this study, clinical and laboratory assessment of patients was performed upon hospital admission, prior to the initiation of therapy. For comparative analysis, two main groups were identified. The main group included 50 children diagnosed with acute pyelonephritis (AP) with a history of COVID-19. The comparison group ($n = 50$) consisted of patients with AP without a history of COVID-19. The interval between COVID-19 infection and hospitalization ranged from 3 weeks to 2.5 months. The control group included 25 apparently healthy children.

All children included in the main, comparison, and control groups were comparable in terms of sex, age, and basic anthropometric parameters. In Group 1 ($n = 50$), there were 16 boys (32.0%) and 34 girls (68.0%); in Group 2 ($n = 50$), 19 boys (38.0%) and 31 girls (62.0%); and in the control group ($n = 25$), 12 boys (48.0%) and 13 girls (52.0%).



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According to Pearson's χ^2 test, no statistically significant differences in sex distribution were found between the groups ($p = 0.568$). The mean age ($M \pm SD$) in Group 1 was 10.2 ± 1.7 years, in Group 2 — 11.7 ± 1.3 years, and in the control group — 11.4 ± 1.9 years. One-way analysis of variance (ANOVA) demonstrated no statistically significant differences in age between the groups ($p = 0.294$).

Comparative analysis of selected clinical and laboratory parameters, including total serum protein levels, body temperature at admission, leukocyte count, erythrocyte sedimentation rate (ESR), and glomerular filtration rate (GFR), was performed between the two patient groups.

Statistical significance of differences was assessed using Student's t -test for independent samples. Mean serum total protein levels did not differ significantly between Groups 1 and 2 ($p > 0.05$) (Table 2).

Body temperature at hospital admission was significantly higher in the comparison group (Group 2) (39.2 ± 0.5 °C) than in the main group (38.2 ± 0.4 °C) ($p = 0.003$). Statistically significant differences were also found in leukocyte count, ESR, and GFR between the groups ($p < 0.001$ for all parameters), reflecting differences in the severity of the inflammatory process and renal functional status.

In recent years, biomarkers reflecting the activity of enzymes produced by renal tissue and excreted in urine have gained key diagnostic importance in the assessment of inflammatory processes. The determination of these biomolecules in urine represents a non-invasive and clinically convenient approach that enables early detection of pathological changes at the cellular level. The application of such methods contributes to more accurate prediction of disease course and outcomes.

Analysis of γ -glutamyl transferase (γ -GT) levels demonstrated a significant increase in children with acute pyelonephritis. In Group 1, the mean value was 35.10 ± 0.13 U/L, which was significantly higher than in Group 2 (11.44 ± 0.05 U/L; $p < 0.05$) and the control group (7.67 ± 0.01 U/L). Compared with healthy children, γ -GT levels increased 4.57-fold in Group 1 and 1.49-fold in Group 2.

Table 1

Urinary Enzyme Levels in Children with Pyelonephritis and a History of COVID-19.

parameter	Control group (n = 25)	Group 1 (n = 50)	Group 2 (n = 50)
γ -GT, U/L	$7,67 \pm 0,01$	$35,10 \pm 0,13$ ($p \leq 0,001$)	$11,44 \pm 0,05$ ($p \leq 0,05$)

In the present study, the urinary expression of kidney injury molecule-1 (KIM-1) was assessed in children with acute pyelonephritis who had a history of COVID-19.

The laboratory results demonstrated a statistically significant increase in urinary KIM-1 levels in Group 1 (5.73 ± 0.03 ng/mL) compared with Group 2 (2.97 ± 0.01 ng/mL; $p < 0.01$) and healthy children (1.52 ± 0.01 ng/mL). These findings indicate pronounced tubular injury in children with a history of COVID-19, associated with inflammatory changes in the renal tubulointerstitial compartment.



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Table 2

Urinary KIM-1 levels in children with pyelonephritis and a history of COVID-19 (ng/ml).

parameter	Control group (n = 25)	Group 1 (n = 50)	Group 2 (n = 50)
KIM-1 (ng/mL)	1,52 ± 0,01	5,73 ± 0,03 (p ≤ 0,01)	2,97 ± 0,01 (p ≤ 0,05)

Urinary β 2-microglobulin (β 2M) levels were evaluated as a biomarker of renal functional status in the included children.

In Group 1, urinary β 2M levels were significantly higher compared with healthy children, increasing 7.64-fold (2.37 ± 0.03 mg/L vs 0.31 ± 0.01 mg/L; $p < 0.001$). In Group 2, an increase of 3.74-fold was observed (1.16 ± 0.04 mg/L; $p < 0.001$) compared with the control group (Figure 1).

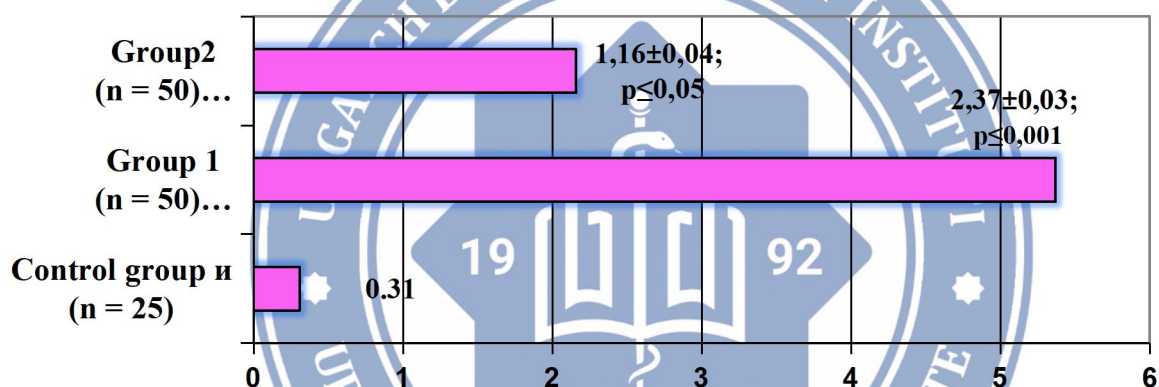


Figure 1. Comparative analysis of urinary β 2M concentrations in children with acute pyelonephritis with and without a history of COVID-19.

Urinary levels of neutrophil gelatinase-associated lipocalin (NGAL), also known as siderocalin, were evaluated.

A statistically significant increase in urinary NGAL levels was observed in Group 1 compared with Group 2. The mean NGAL concentration in Group 1 was 111.64 ± 0.24 ng/mL, which was significantly higher than in the comparison group (47.66 ± 0.38 ng/mL; $p < 0.001$) (Figure 2).

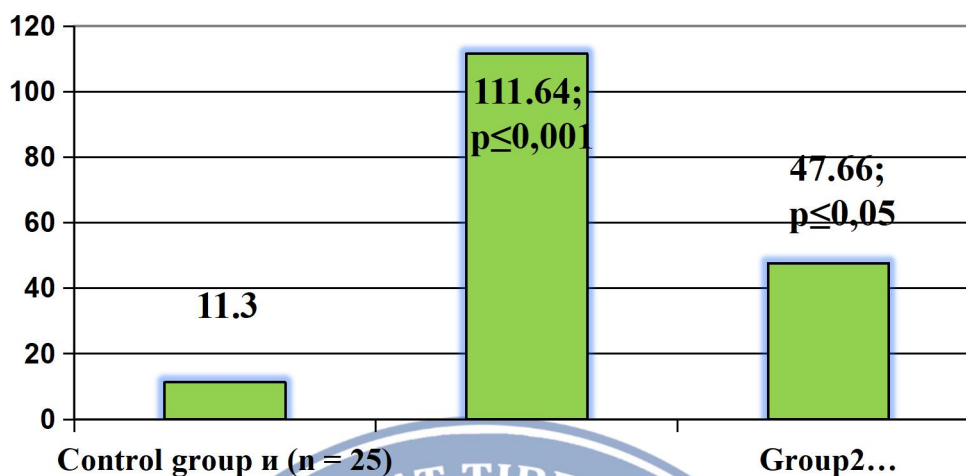


Figure 2. Comparative analysis of urinary NGAL concentrations in children with acute pyelonephritis with and without a history of COVID-19.

Proteinuria of varying degrees was detected: in Group 1 it was 404.1 ± 18.2 mg/day, whereas in Group 2 it was 264.08 ± 12.6 mg/day.

Urinalysis using the Nechiporenko test revealed characteristic changes across all study groups. In Group 1, leukocyturia was 6850 ± 165.0 cells/mL, in Group 2 — 4350 ± 168.0 cells/mL, while in the control group this parameter did not exceed 1900 ± 55.0 cells/mL. The differences were statistically significant ($p < 0.001$, one-way ANOVA).

Microhematuria was detected in 90% of patients in the main group (2650 ± 132 erythrocytes/mL) and in 12% of children in the comparison group (1390 ± 130 erythrocytes/mL), indicating a more pronounced inflammatory process in patients with a history of COVID-19. In the control group, erythrocyturia did not exceed 980 ± 50.0 cells/mL. These differences were also statistically significant ($p < 0.001$, one-way ANOVA).

In addition, all children with acute pyelonephritis demonstrated impaired reabsorption function of the proximal tubules, which was most pronounced in Group 1.

A statistically significant decrease in titratable acidity was observed: 23.22 ± 0.63 mmol/24 h in Group 1 and 32.04 ± 0.24 mmol/24 h in Group 2 ($p < 0.001$), compared with 49.0 ± 1.8 mmol/24 h in healthy children. Ammonia excretion was also reduced: 30.12 ± 1.22 and 35.08 ± 1.12 mmol/24 h in Groups 1 and 2, respectively ($p < 0.001$), compared with 44.3 ± 1.1 mmol/24 h in controls. These findings indicate impaired hydrogen and ammonium ion transport in the distal tubules. However, urinary pH remained within normal limits ($p \geq 0.1$), suggesting compensatory mechanisms maintaining acid–base balance despite tubular dysfunction.

Conclusion

Children who have experienced SARS-CoV-2 infection demonstrate a tendency toward more pronounced functional and structural renal alterations, particularly affecting the tubulointerstitial compartment. Exacerbation of post-viral immunoinflammatory responses, along with vascular dysfunction, hypovolemia, and disturbances in water–electrolyte balance, contributes to the development of acute kidney injury.



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Accumulated evidence indicates an increased risk of latent or progressive tubulopathy in children with a history of COVID-19 compared with patients without such history. To enable early detection of subclinical tubular injury and to stratify patients according to the risk of chronicity, monitoring of proximal and distal tubular function is essential.

The most informative non-invasive biomarkers of renal injury in this context include KIM-1, NGAL, β 2-microglobulin, and γ -glutamyl transferase (γ -GT), urinary concentrations of which reflect the degree of tubulopathy severity.

Dynamic monitoring of these biomarkers is recommended in patients with a history of COVID-19, even in the absence of overt clinical symptoms, to facilitate early identification of subclinical tubulointerstitial damage and prevent progression to chronic kidney disease.

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