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INNOVATIVE METHODS OF EARLY DIAGNOSIS OF TUBULOINTERSTITIAL LESIONS IN ACUTE PYELONEPHRITIS IN CHILDREN

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ANNOTATION

The COVID-19 pandemic has revealed previously unrecognized clinical features of infectious and inflammatory kidney diseases in pediatric patients. **Objective**: To determine the significance of tubuloglomerular abnormalities biomarkers (γ-GT, β2M, NGAL, KIM-1) in clinical and laboratory diagnostics in children with acute pyelonephritis after COVID-19. **Methods**. The study included 100 children with clinically and laboratory-confirmed acute pyelonephritis. The control group consisted of 25 apparently healthy children, matched for age and gender. **Results**. The study results revealed a statistically significant increase in urinary γ-GT, KIM-1, NGAL, and β_2-microglobulin concentrations compared to the control group. In patients in the first group, γ-GT levels were 4.57 times higher than the control group, KIM-1 levels were 3.77 times higher, NGAL levels were 25.96 times higher, and β_2M levels were 7.64 times higher. **Conclusion**. Determining the concentrations of these parameters allows us to identify early signs of tubulointerstitial damage, assess the activity of the pathological process in the renal tubular apparatus, and identify risk groups for chronic pyelonephritis.

Keywords: post-COVID complications, acute pyelonephritis, tubulointerstitial lesions, KIM-1, NGAL, β_2-microglobulin, γ-GT.

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ИННОВАЦИОННЫЕ МЕТОДЫ РАННЕЙ ДИАГНОСТИКИ ТУБУЛОИНТЕРСТИЦИАЛЬНЫХ ПОРАЖЕНИЙ ПРИ ОСТРОМ ПИЕЛОНЕФРИТЕ У ДЕТЕЙ

АННОТАЦИЯ

Пандемия COVID-19 выявила раннее неучтённые особенности клинического течения инфекционно-воспалительных заболеваний почек у педиатрических пациентов. **Цель исследования**: определить значимость биомаркеров тубуло-гломерулярных нарушений (γ-ГТ, β_2M, NGAL, KIM-1) в клинико-лабораторной диагностике у детей с острым пиелонефритом после перенесённого COVID-19. **Методы.** В исследование были включены 100 детей с клинически и лабораторно подтверждённым острым пиелонефритом. Контрольную группу составили 25 практически здоровых детей, сопоставимых по возрасту и полу. **Результаты.** Результаты исследования показали статистически значимое повышение концентраций γ-ГТ, KIM-1, NGAL и β_2-микроглобулина в моче по сравнению с контрольной группой. У пациентов первой группы уровень γ-ГТ превышал показатели контроля в 4,57 раза, KIM-1 — в 3,77 раза, NGAL — в 25,96 раза, а β_2M — в 7,64 раза. **Заключение.** Определение концентрации этих показателей позволяет выявить ранние признаки тубулоинтерстициального повреждения, оценить активность патологического процесса в канальцевом аппарате почек и выделить группы риска по хронизации пиелонефрита.

КЛЮЧЕВЫЕ СЛОВА: постковидные осложнения, острый пиелонефрит, тубулоинтерстициальные поражения, KIM-1, NGAL, β_2-микроглобулин, γ-ГТ.

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BOLALARDA O'TKIR PYELONEFRITDA TUBULOINTERSTITSIAL ZARARLANISHLARNI ERTA TASHXIS QILISHNING INNOVATSION USULLARI

ANNOTATSIYA

COVID-19 pandemiyasi bolalarda yuqumli va yallig'lanishli buyrak kasalliklarining ilgari tan olinmagan klinik xususiyatlarini aniqladi. **Maqsad**: COVID-19 dan keyin o'tkir pielonefritli bolalarda klinik va laboratoriya diagnostikasida tubuloglomerulyar anomaliyalar biomarkerlarining (γ-GT, β2M, NGAL, KIM-1) ahamiyatini aniqlash. **Usullar**. Tadqiqotga klinik va laboratoriya tomonidan tasdiqlangan o'tkir pielonefritli 100 bola kiritilgan. Nazorat guruhi yoshi va jinsi bo'yicha mos keladigan 25 nafar sog'lom boladan iborat edi. **Natijalar**. Tadqiqot natijalari nazorat guruhiga nisbatan siydikdagi γ-GT, KIM-1, NGAL va β_2-mikroglobulin konsentratsiyasining statistik jihatdan sezilarli darajada oshganligini ko'rsatdi. Birinchi guruhdagi bemorlarda γ-GT darajasi nazorat guruhiga qaraganda 4,57 baravar, KIM-1 darajasi 3,77 baravar, NGAL darajasi 25,96 baravar va β_2M darajasi 7,64 baravar yuqori bo'lgan. **Xulosa**. Ushbu parametrlarning konsentratsiyasini aniqlash bizga tubulointerstisial shikastlanishning dastlabki belgilarini aniqlash, buyrak naychalari apparatida patologik jarayonning faolligini baholash va surunkali pielonefrit uchun xavf guruhlarini aniqlash imkonini beradi.

KALIT SO'ZLAR: COVIDdan keyingi asoratlar, o'tkir pielonefrit, tubulointerstisial shikastlanishlar, KIM-1, NGAL, β_2-mikroglobulin, γ-GT.

Introduction: SARS-CoV-2 is capable of infecting various kidney structures, including glomerular cells and the epithelium of the proximal convoluted tubules. Morphological changes most often manifest as signs of focal segmental glomerulosclerosis and/or acute tubular necrosis [12].

However, invasive methods such as renal needle biopsy, especially in children, are associated with a high risk of complications, including severe hemorrhagic reactions. This significantly limits the capabilities of morphological analysis and requires the implementation of alternative, safer diagnostic methods [10].

One of the most promising approaches to noninvasive diagnosis of tubulointerstitial disorders is urinary enzyme analysis. This method allows for the assessment of the nephron tubular apparatus in the early stages of the pathological process, when changes are still reversible. Enzymuria is considered a sensitive marker, enabling earlier detection of functional disorders compared to traditional biochemical indicators [11].

γ-Glutamyl transferase (γ-GT) is localized primarily in the apical membrane of epithelial cells of the proximal tubules and the descending limb of the loop of Henle [6, 7, 9]. Increased activity in urine indicates damage to the tubular epithelium, since the enzyme is expressed on the cell surface and is rapidly released during sublethal or lethal destruction of the cells [8].

Modern approaches to diagnosing inflammatory and destructive changes in the tubulointerstitial zone include the use of biomarkers based on biologically active molecules. Neutrophil gelatinase-associated lipocalin-2 (NGAL), kidney injury molecule 1 (KIM-1), and β2-microglobulin (β2M) are considered the most clinically significant. These markers not only reflect the level of inflammatory activity but also enable the implementation of the concept of early, highly specific, and non-invasive diagnostics [1, 2, 3, 4, 5].

The aim: of the study was to determine the significance of biomarkers of tubuloglomerular disorders (γ-GT, β2M, NGAL, KIM-1) in clinical and laboratory diagnostics in children with acute pyelonephritis after COVID-19.

Materials and methods. The study involved 100 children aged 7–17 years suffering from acute pyelonephritis. The patients were divided into two groups, each containing 50 subjects. The control group consisted of 25 apparently healthy children of the same age range. Laboratory and instrumental studies were conducted at the L.M. Isaev Research Institute of Microbiology, Virology, Infectious and Parasitic Diseases of Samarkand State Medical University.

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

Urinary syndrome assessment included proteinuria (mg/day), leukocyturia (cells/field of vision), bacteriuria (CFU/ml), Nechiporenko test (cells/ml), and urine culture with clinically significant growth ($>10^5$ CFU/ml). Proximal tubular function was assessed by daily protein excretion (mg/l), and distal tubular function was assessed by the Zimnitsky test, titratable acid levels, and ammonia excretion (mmol/day).

Blood was collected from the cubital vein on an empty stomach. Serum was separated by centrifugation (3000 rpm, 15 min) and stored at –20°C for ≤3 months. Daily urine output was collected in a common container, from which 15 ml of samples were isolated and frozen at –20°C until analysis.

The study determined the levels of γ-glutamyl transferase (γ-GT, U/L), NGAL (ng/mL), β2-microglobulin (β2M, mg/L), and KIM-1 (ng/mL) in urine.

γ-GT activity (U/L) was determined 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 a γ-glutamyl group from a substrate to an acceptor, resulting in the formation of a colored compound. Stain intensity was measured spectrophotometrically at a wavelength of 405 nm. To improve the

informativeness of the results, the value was normalized to the urinary creatinine level and expressed as U/mmol creatinine.

Urine concentrations of NGAL (ng/mL), β2M (mg/L), and KIM-1 (ng/mL) were determined by enzyme-linked immunosorbent assay (ELISA) using sandwich diagnostic kits (ELISA). This method involves the specific binding of the antigen to monoclonal antibodies immobilized on a plate, after which secondary antibodies conjugated to the enzyme were added to the complex. After incubation with a chromogenic substrate (3,3',5,5'-tetramethylbenzidine, TMB), an enzymatic reaction occurred, forming a colored product. Changes in optical density were recorded using a plate spectrophotometer at a wavelength of 450 nm. Biomarker concentrations were calculated using calibration curves constructed using standard solutions. Before analysis, all urine samples were centrifuged at 3000 rpm for 10 minutes, and the supernatant was used for analysis. To exclude the influence of urine acidity on β2M levels, samples with a pH <6 were pre-neutralized to physiological levels.

Urine renal biomarker levels (γ-GT, NGAL, β2M, KIM-1) were analyzed upon admission of children to the hospital.

Statistical analysis of the study results was performed using Microsoft Excel (version 2016, USA) and StatPlus v.7 (AnalystSoft Inc., USA). Normal distribution of quantitative variables was tested using the Kolmogorov-Smirnov test. Differences between two groups were analyzed using the parametric Student's t-test, and comparisons between three groups were performed using a one-way analysis of variance using Fisher's exact test.

Quantitative values are presented as mean and standard deviation (M ± 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 and discussion: In this study, clinical and laboratory monitoring of patients in groups 1 and 2 was conducted during hospitalization, before the start of therapy. Two main groups were identified for comparative analysis. The first (main) group included 50 children diagnosed with acute pyelonephritis (AP) who had previously recovered from COVID-19. The comparison group (group 2, n=50) consisted of patients with AP who had no history of COVID-19. The interval between COVID-19 and hospitalization ranged from 3 weeks to 2.5 months. The control group included 25 apparently healthy children.

A comparative analysis of individual clinical and laboratory parameters, including total protein levels, body temperature on admission, white blood cell count, erythrocyte sedimentation rate (ESR), and glomerular filtration rate (GFR), was conducted between the two groups of patients. To assess the statistical significance of the differences, the parametric Student's t-test for independent samples was used. According to the obtained results, the average values of total serum protein in groups 1 and 2 did not differ significantly ($p > 0.05$) (Table 1). At the same time, the body temperature at the time of admission to the hospital in children of the comparison group (group 2) was statistically significantly higher (39.2 ± 0.5 °C) than in patients of the main group (38.2 ± 0.4 °C) ($p=0.003$). Statistically significant differences in the level of leukocytes, ESR and SCF were also noted between the groups ($p<0.001$ for all three parameters), which reflects the differences in the severity of the inflammatory process and the functional state of the kidneys in patients (Table 1).

Table 1

Mean values of individual clinical and laboratory parameters (M ± SD) in sick children in the compared groups

Parameter	Group 1 (n=50)	Group 2 (n=14)	p-meaning
Total protein, mg/ml	55,7 ± 2,9	56,2 ± 3,9	0,487
Temperature, °C	39,2 ± 0,5	38,2 ± 0,4	0,002
Leukocytes, 10^9/l	24,7 ± 2,8	12,8 ± 4,1	<0,001
ESR, mm/h	38,1 ± 5,1	25,3 ± 4,9	<0,001

Parameter	Group 1 (n=50)	Group 2 (n=14)	p-meaning
GFR, ml/min/1.73 m²	61,4 ± 7,2	82,4 ± 5,1	<0,001

Note: The table was compiled by the authors. Abbreviations: ESR – erythrocyte sedimentation rate; SCF – glomerular filtration rate.

In recent years, markers reflecting the activity of enzymes produced by kidney tissue and excreted in urine have become key diagnostic tools in assessing inflammatory processes. Determination of these biomolecules in urine is a non-invasive, clinically convenient approach, enabling the early diagnosis of pathological changes at the cellular level. The use of such methods facilitates more accurate prognosis of the course and outcome of the disease.

Analysis of γ-glutamyl transferase (γ-GT) levels revealed a significant increase in children with acute pyelonephritis. Specifically, in Group 1, the mean value was 35.10 ± 0.13 U/L, which exceeded the same value in children in Group 2 (11.44 ± 0.05 U/L; $p \leq 0.05$) and the control group (7.67 ± 0.01 U/L). It was found that, compared with healthy children, the level of γ-GT in patients of the 1st group increased by 4.57 times, and in the 2nd group – by 1.49 times (Table 2).

Table 2

Enzymuria values in children with pyelonephritis who recovered from COVID-19

Indicator	Healthy children (n = 25)	Group 1 (n = 50)	Group 2 (n = 50)
γ-GT, U/L	7,67 ± 0,01	35,10 ± 0,13 (p ≤ 0,001)	11,44 ± 0,05 (p ≤ 0,05)

Note: p is the level of reliability of differences in enzymuria indices between patients in the active phase of pyelonephritis and healthy children.

This study examined the expression of kidney injury molecule-1 (KIM-1) in children with acute pyelonephritis who had recovered from COVID-19.

Laboratory analysis results revealed a statistically significant increase in urinary KIM-1 concentrations in patients in Group 1 (5.73±0.03 ng/ml) compared to Group 2 (2.97±0.01 ng/ml; p≤0.01), as well as compared to healthy children (1.52±0.01 ng/ml). These data confirm the presence of severe tubular damage in children who had recovered from COVID-19, with the development of an inflammatory process in the tubulointerstitial zone of the kidneys (Table 3).

Table 3

Urine KIM-1 levels in children with pyelonephritis who recovered from COVID-19 (ng/ml)

Indicator	Healthy children (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)

Note: p is the level of statistical significance of differences when comparing KIM-1 expression indices in urine between children with active pyelonephritis and healthy children.

β2-microglobulin (β2M) levels were analyzed as a biomarker of renal function in the children included in the study.

In Group 1, urinary β₂-microglobulin (β₂M) levels were significantly higher than in healthy children—7.64 times (2.37±0.03 mg/L vs. 0.31±0.01 mg/L; p≤0.001). In Group 2, this indicator increased 3.74 times (1.16±0.04 mg/L; p≤0.001) compared to the control group.

We also analyzed levels of neutrophil gelatinase-associated lipocalin-2 (NGAL), or siderocalin. A statistically significant increase in the NGAL (neutrophil gelatinase-associated

lipocalin) level in the urine of patients in Group 1 was observed compared to children in Group 2. The average NGAL concentration in Group 1 patients was 111.64±0.24 ng/ml, which was significantly higher than the values in the comparison group (47.66±0.38 ng/ml; $p \leq 0.001$).

According to laboratory urine analysis data, the majority of patients with pyelonephritis (80.1%, n=97) had turbid urine, indicating severe inflammatory changes.

A statistically significant decrease in titratable acid excretion was observed in patients included in the study: 23.22±0.63 mmol/24 h in Group 1 and 32.04±0.24 mmol/24 h in Group 2; $p \leq 0.001$ (while in healthy subjects this indicator was equal to 49.0±1.8 mmol/24 h) and ammonia 30.12±1.22 and 35.08±1.12 mmol/24 h, respectively; $p \leq 0.001$ (while in healthy children this indicator was equal to 44.3±1.1 mmol/24 h), which indicates a pronounced impairment of hydrogen and ammonium ion transport in the distal tubules. At the same time, urine pH in patients remained within the normal range ($p \geq 0.1$), which may indicate compensatory mechanisms that maintain acid-base balance against the background of tubular dysfunction.

Elevated levels of γ-glutamyl transferase (γ-GT) observed in patients in the first group likely reflect the development of pathophysiological processes, including hypoxic renal tissue injury, endothelial dysfunction, and disturbances in the glutathione metabolism system induced by COVID-19 infection.

Patients in the first group showed a statistically significant increase in KIM-1 (Kidney Injury Molecule-1) levels compared to those in the second group, likely related to more severe ischemic and toxic damage to the proximal tubules, as well as activation of tubulointerstitial remodeling mechanisms, reflecting the extent of renal tissue damage.

The obtained results suggest that increased β2M levels in patients are associated with disruption of the glomerular barrier due to SARS-CoV-2 infection, reduced reabsorption capacity of proximal tubular cells, as well as the development of vasculopathy and disturbances in protein and electrolyte metabolism.

Due to its ability to bind and transport iron, NGAL is involved in maintaining redox homeostasis and limiting prooxidant effects, indicating its antioxidant and cytoprotective functions. Under conditions of virus-induced hypoxia and endothelial dysfunction, increased NGAL expression can be considered an element of the adaptive response aimed at stabilizing cell membranes, reducing apoptosis, and stimulating reparative processes. The obtained results demonstrate a pronounced correlation between the level of NGAL in urine and the severity of renal parenchyma damage, which confirms the fact of significant damage to renal tissue in children who have had SARS-CoV-2 infection.

CONCLUSION

Children who have recovered from SARS-CoV-2 infection are prone to more profound impairment of kidney function and structure, particularly in the tubulointerstitial component. Exacerbation of post-viral immune-inflammatory reactions, along with vascular dysfunction, hypovolemia, and fluid and electrolyte imbalances, contribute to the development of acute kidney injury. Accumulated data indicate an increased risk of developing latent or progressive tubulopathy in children who have recovered from COVID-19 compared to patients without a relevant history.

To early detect latent renal tubular damage and stratify patients according to the risk of chronicity, monitoring the condition of the proximal and distal tubules is essential. The most informative in this case are noninvasive biomarkers of kidney damage, such as KIM-1, NGAL, β2-microglobulin, and γ-glutamyl transferase (γ-GT), whose concentrations in urine allow us to assess the severity of tubulopathy.

Dynamic monitoring of these markers is recommended in patients with previous COVID-19 infection, even in the absence of pronounced clinical symptoms, to promptly detect subclinical disturbances in the renal tubulointerstitial structure and prevent the progression of the disease to a chronic form.

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