THE USE OF MEASURING CYTOKINE CONCENTRATIONS IN URINE IN MEDICAL PRACTICE

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Abstract. *The paper presents data on the study of the content of cytokines (IL-1β, RAIL-1β, IL-2, IL-4, IL-10, IL-17A, TNF-α, IFN-γ) in morning urine using enzyme immunoassay in healthy individuals (n=20) and in patients with acute glomerulonephritis (n=93). Determination of cytokine levels in patients was carried out at the onset of the disease and 12 months after the manifestation of the disease. The obtained indicators of cytokine content in urine are presented as absolute values in pg/ml and values normalized to creatinine, calculated using the formula: cytokine level (pg/ml) / urine creatinine (μmol/ml). A study was made of changes in the content of cytokines in urine during glomerulonephritis relative to a group of healthy individuals, as well as the dynamics of cytokine content in urine over a 12-month observation period. The results of the study showed that absolute values of cytokines in urine can distort the true picture of the cytokine profile of urine in kidney pathology. Normalized values of the predominant number of pro- and anti-inflammatory cytokines (IL-1β, IL-2, IL-8, IL-10, IL-17A and TNF-α) in patients with glomerulonephritis were significantly higher than the corresponding indicators in healthy individuals. Normalized cytokine values were shown to be more sensitive indicators than absolute values when analyzing differences in the cytokine profile in patients with glomerulonephritis depending on the acute and chronic course of the disease. These indicators influenced the outcome of glomerulonephritis, assessed, as a rule, 12 months after the manifestation of the disease. Thus, low levels of IL-1β, IL-8 and IL-17A found at the onset of the disease in combination with a high level of RAIL-1β determined the chronicity of glomerulonephritis. So, values of cytokine content in urine normalized by creatinine expand the possibilities of using assessment of the cytokine profile of urine to establish changes in the content of cytokines in urine in kidney pathology and predict the nature of the clinical course of glomerulonephritis.*

Keywords: *glomerulonephritis, cytokines, chronic glomerulonephritis, diabetes mellitus, chronic kidney disease.*

Introduction. In recent years, much attention has been paid to the study of the cytokine profile in patients with various pathologies, including kidney diseases [1–4]. At the same time, the authors were mainly involved in studying the content of cytokines in blood serum [1–3]. However, cytokines circulating in the blood can be blocked by their own soluble receptors or receptor antagonists [4], which limits the use of cytokine levels in the blood to assess cytokine status in various pathological conditions. There is no doubt that cytokines exert their effects at the site of their production, therefore, studying the content of cytokines in urine in kidney diseases may be more informative than determining their serum levels. In connection with the above, the present study was undertaken, the purpose of which was to assess the informativeness of indicators of the content of pro and anti-inflammatory cytokines in the urine in healthy

individuals and in kidney pathology, an example of which was the widespread glomerular disease - glomerulonephritis (GN).

Material and methods. During the study, urine samples were taken to determine the content of cytokines - IL-1β (interleukin-1β), RAIL-1β (receptor antagonist of interleukin-1β), IL-2 (interleukin-2), IL-4 (interleukin-4), IL-10 (interleukin-10), TNF-α (tumor necrosis factor-α), IFN-γ (interferon-γ) in apparently healthy individuals (20 people aged 23 to 60 years, average age 34.2 ±2.9 years) and in patients with post-infectious glomerulonephritis (PIGN) who received inpatient treatment in the nephrology department of the Republican Scientific and Practical Center of Nephrology in 2012 - 2017. (93 people, aged from 21 to 63 years, average age 36.4±2.1 years). Selected portions of urine were centrifuged for 10 minutes at 1000 g, then the supertalent was separated for study into plastic tubes. To study the relationship between urinary and serum levels of cytokines, in addition to the study of cytokine levels in urine, the content of cytokines above the specified spectrum in the blood serum was determined. The urine and blood serum samples selected for the study were stored at -70 C until the study.

In patients with PIGN (Post-infectious glomeronephritis), cytokine testing in urine was carried out twice - during the onset of the disease upon admission to hospital treatment (1-2 days from hospitalization) and 12 months after discharge from the hospital. The patients were retrospectively distributed after 12 months into 2 groups depending on the results of a general clinical examination. The first group included patients who were convalescents after suffering from acute PIGN, the second group included patients with chronic PIGN, who, a year after the manifestation of the disease, continued to have clinical symptoms and changes in urine characteristic of GN (hypertension, edema, decreased glomerular filtration rate - GFR - below 60 ml/min, proteinuria, hematuria, leukocyturia, in some cases cylindruria). For the next stage of the study, 30 people from each group of patients were selected. When selecting patients, we sought to randomize groups according to gender, age, social composition, and morphological forms of PIGN. Diagnosis of PIGN was carried out on the basis of an analysis of the clinical status of patients, anamnestic data, results of laboratory tests of blood and urine, examination of the kidneys using an SSD ultrasound machine from Aloka (Japan), and a morphological study of nephrobiopsy samples of patients using light and fluorescent microscopy. Quantitative determination of cytokines was carried out by enzyme immunoassay using test systems of Cytokin LLC (St. Petersburg) according to the methods of the manufacturer of laboratory reagents on the Immune enzyme immunoassay analyzer “Institute Virion/Serion GmbH”, Germany).

Results. The results obtained during the study were processed using the statistical apparatus of the computer program “Statistica - v. 10.0". The distribution of the obtained values of cytokine levels in urine and blood was first checked for compliance with the normal (Gaussian) distribution using the Kolmogorov–Smirnov test with the Lilliefors correction. Provided that the achieved significance levels (p) were below the critical value of 0.05, the null hypothesis about the similarity of the studied characteristics with a normal distribution was rejected. The use of this approach made it possible to establish the asymmetry of the distribution of the total values of cytokine content in urine and blood serum in the studied groups of patients and in healthy individuals, and therefore the obtained data were presented in the form Me (P – P), where Me is the median, P is the value 10 -th percentile, P90 – 90th percentile value. To assess differences in indicator values in study groups, the non-parametric Mann-Whitney test (p) was used. When conducting a correlation analysis of the studied indicators, the Spearman

rank correlation coefficient (r) was calculated; the correlation was considered significant at $p < 0.05$

The absolute values of urinary content of 5 cytokines - IL-1β, IL-2, IL-8, IL-10 and TNF-α - were increased in patients at the onset of PIGN relative to the values of healthy ones, while the absolute levels of IL-4 , IL-17A, and IFN-γ were lower than those of healthy people (Table 2). The normalized values of the predominant number of pro- and anti-inflammatory cytokines (IL-1β, IL-2, IL-8, IL-10, IL-17A and TNF-α) significantly exceeded the corresponding values of healthy individuals. The exceptions were IL-4, RAIL-1β and IFN-γ, the urinary levels of which in normalized terms did not differ from those in healthy controls. In a retrospective analysis of the nature of the clinical course of PIGN 12 months after the onset of the disease, 2 groups of patients were identified - with acute PIGN and chronic PIGN, a comparative study of the urinary levels of cytokines in which revealed a number of differences (Table 3). It was noteworthy that the normalized values of cytokine content had more pronounced differences than their absolute values. Thus, the normalized levels of typical pro-inflammatory cytokines - IL-1β, IL-8, IL-17A - in patients with chronic PIGN were lower at the onset of the disease compared to the corresponding indicators in patients with an acute course of the disease, and the level of RAIL-1β was higher. At the same time, the levels of IL-8 and RAIL-1β in absolute terms did not respond to the nature of the clinical course of the disease and had almost identical values in the acute and chronic course of PIGN. Levels of IL-2, IL-4, IL-10, and TNF-α and IFN-γ did not differ between groups of patients with acute and chronic PIGN. In patients with chronic PIGN, 12 months after the onset of the disease, the level of IL-1β in the urine in absolute and normalized values became higher, while the level of RAIL-1β (both in absolute and normalized terms) was lower than in patients with favorable outcome – acute course of the disease (Table 4). The levels of IL-2 and IL-4 began to exceed in patients with a chronic course of the disease similar indicators in acute PIGN. In patients with chronic PIGN, the absolute and normalized values of IL-10 became lower than those of patients with acute PIGN, while the normalized value of IL-17A became higher.

Discussion. As a result of a correlation analysis in healthy individuals, the existence of direct connections between urinary and serum levels of RAIL-1β and IL-2 was revealed. For the remaining cytokines, there was no correlation between serum and urinary levels. The total number of correlations between urinary levels of cytokines was 14, which is much greater than the number of those between serum levels, equal to 8, which indicates a closer connection between urinary levels than serum levels. This may be due to the fact that cytokines excreted in the urine are formed locally in the kidneys and obey the general laws of cytokine production, one of the principles of which is the cascade of activation of their production, when some cytokines induce the synthesis of others [5, 6]. In addition, the existence of many correlations between the levels of cytokines excreted in the urine may reflect the dependence of urinary cytokine levels on the state of renal excretory function. However, correlation analysis did not reveal an association between urinary levels of most cytokines and urinary creatinine; only the absolute value of urinary RAIL-1β was positively associated with urinary creatinine levels. The lack of association of other cytokines with serum and urinary creatinine levels can be explained in part by their small absolute values, short lifetime, and rapid utilization in the liver and kidneys. The excess of urinary levels of cytokines in practically healthy individuals and in patients with PIGN compared to the corresponding serum levels also suggests that the source of cytokines excreted in the urine is the kidneys. This position is confirmed by data from other authors on the local production of cytokines in the affected organ [7, 8]. The results of the study

showed that absolute values of cytokines in urine can distort the true picture of the cytokine profile of urine in kidney pathology. Thus, in the group of patients with PIGN at the onset of the disease, a decrease in the levels of IL-4, IL-17A and IFN-γ was found relative to healthy levels, which does not fit into the picture of activation of the cytokine network characteristic of kidney damage. At the same time, an increase in the levels of most pro- and anti-inflammatory cytokines normalized by creatinine was detected, which is consistent with the data of other authors on increased levels of pro-inflammatory cytokines in the urine in glomerular pathology [4], as well as a simultaneous increase in the urine content of both pro-, and anti-inflammatory cytokines in various forms of GN [10]. Quite a lot of work has been published indicating the important role of pro-inflammatory cytokines in damage to the renal glomeruli [11-18]. It has been shown that one of the main pro-inflammatory cytokines, IL-1β, is produced by glomerular macrophages and mesangial cells activated as a result of the interaction of pattern recognition receptors (PRRs) of these cells with pathogen-associated molecular patterns of infectious pathogens (PAMPs) or products of damage to their own tissues (DAMPs).). Under experimental conditions, IL-1β produced in the kidneys promoted glomerular necrosis, glomerular crescent formation, and renal tubular damage [19]. IL-8 (chemokine CXCL-8) caused an increase in glomerular basement membrane permeability and proteinuria in rats by reducing the synthesis of heparan sulfate proteoglycans [20]. TNF-α produced in the kidneys initiated damage to glomerular podocytes and also activated the renin-angiotensin system [21,22]. IL-17A is considered one of the main proinflammatory cytokines in GN; it is produced by T lymphocytes, is a chemoattractant for neutrophils, promotes the accumulation of macrophages in glomeruli, and blocks the suppressive effect of Treg cells on autoimmune processes [23–25]. There are no data on the direct damaging effect of IL-2 on the kidneys, but positive results have been published on the use of high doses of methylprednisolone and cyclosporine in the treatment of patients with GN, which are known to suppress the production of IL-2 [26, 27]. IFN-γ contributed to the development of oxidative stress, mesangial cell damage, accumulation of extracellular matrix, and ultimately the development of nephrosclerosis [18, 28, 29]. IL-4 induced kidney damage and proteinuria in mice [30]. Two other cytokines, IL-10 and RAIL-1β, have pronounced anti-inflammatory and nephroprotective properties.

In previous experiments, administration of RAIL-1β to laboratory animals with different forms of GN inhibited the development of the inflammatory process in the kidneys, reduced proteinuria, and restored renal function [31, 32]. IL-10 prevented the formation and deposition of immune complexes in the renal glomeruli, and also prevented the progression of GN [33–35]. Normalized cytokine values were shown to be more sensitive indicators than absolute values in the analysis of differences in the cytokine profile in patients with PIGN depending on the nature of the clinical course - acute or chronic. Thus, already at the onset of PIGN during the chronic course of the disease, certain features could be noted in the cytokine profile of urine - low levels of pro-inflammatory cytokines - IL-1β, IL-8 and IL-17A against the background of a high level of RAIL-1β. Low levels of IL-1β and IL-17A were indicated by both absolute and normalized values of urinary cytokine levels. A decrease in the level of IL-8 and an increase in the level of RAIL-1β were detected only through the use of normalized values, since the absolute values of these cytokines did not respond to the nature of the clinical course of the disease at the onset of PIGN. After 12 months of observation, there was an inversion of the nature of the identified differences in the levels of IL-1β, IL-17A and RAIL-1β: thus, the

absolute and normalized values of the initially low level of the proinflammatory cytokine IL-1β became higher in chronic PIGN, and the normalized value of IL-1β also became higher. 17A, and the level of the anti-inflammatory cytokine RAIL-1β is lower than in patients with a favorable outcome – acute course of the disease. The persistence of high levels of cytokines with pro-inflammatory and nephrotoxic properties (IL-1β, IL-2, IL-17A and IL-4) in patients with PIGN against the background of a decrease in the levels of anti-inflammatory cytokines - RAIL-1β and IL-10, apparently contributes to chronic the course of glomerular damage. The literature has previously described changes in the content of cytokines in the blood serum of patients at the onset and dynamics of GN, depending on the nature of the clinical course of the disease [2, 36]. In particular, in children with chronic GN, a decrease in the level of RAIL-1β was found relative to healthy individuals, with an unchanged value of this indicator in patients with acute GN. At the same time, the latter showed an increase in the level of IL-4 in the blood serum, while maintaining it at the level of healthy people in patients with chronic GN. The formation of chronic GN was associated with an increase in the content of pro-inflammatory cytokines circulating in the blood - IL-1β, TNF-α and IL-8, in the absence of dynamics in the content of anti-inflammatory cytokines. In the literature, we did not find data on the study of differences in urinary levels of cytokines in patients with acute and chronic PIGN. Our data suggest that the chronic course of PIGN is associated with an initially reduced functional activity of innate immune cells - a decrease in the ability to produce the “early” pro-inflammatory cytokine - IL-1β and associated other pro-inflammatory cytokines - IL-8 and IL-17A against the background of increased RAIL-1β production. As a result, the immune response turns out to be ineffective, the pathogen is not removed, and the activity of innate immune cells remains almost at the original level, continuing to synthesize the pro-inflammatory cytokines IL-1β, IL-17A, while in patients with a favorable outcome the absolute and normalized IL-17A value as well as normalized IL-1β value.

Conclusion. So, the use of creatinine-normalized values of cytokine content in urine expands the possibilities of using assessment of the cytokine profile of urine to establish changes in the content of cytokines in urine in kidney pathology, as well as to predict the nature of the clinical course of PIGN.

REFERENCES

1. Simmons E.M., Himmelfarb J., Sezer M.T., Chertow G.M., Mehta R.L., Paganini E.P. et al. Plasma cytokine levels predict mortality in patients with acute renal failure. Kidney Int. 2004; 65(4): 1357-65.
2. Zhiznevskaya I.I., Khmelevskaya I.G., Razinkova N.S., Kalinina Z.N. Dynamics of immunological parameters in acute and chronic glomerulonephritis in children. Fundamental´nye issledovaniya. 2014; 4 (2): 269-73. (in Russian)
3. Zhang Z., Wang H., Zhang L., Crew R., Zhang N., Liu X. et al.. Serum Levels of Soluble ST2 and IL-10 Are Associated with Disease Severity in Patients with IgA Nephropathy. J. Immunol. Res. 2016; 2016:6540937.
4. Al-Eisa A.A., Al Rushood M., Al-Attiyah R.J. Urinary excretion of IL-1β, IL-6 and IL-8 cytokines during relapse and remission of idio-pathic nephrotic syndrome. J. Inflamm. Res. 2017; 10:1-5.

5. Beal A. L., Cerra F. B. Multiple organ failure syndrome in the 1990s. Systemic inflammatory response and organ dysfunction. JAMA. 1994; 271(3): 226-33.
6. Suzuki K., Nakaji S., Yamada M., Totsuka M., Sato K., Sugawara K. Systemic inflammatory response to exhaustive exercise. Cy-tokine kinetics. Exerc. Immunol. Rev. 2002; 8: 6-48.
7. Wada T., Furuichi K., Segawa-Takaeda C., Shimizu M., Sakai N., Takeda S.I. et al. MIP-1alpha and MCP-1 contribute to crescents and interstitial lesions in human crescentic glomerulonephritis. Kid-ney Int. 1999; 56(3): 995–1003.
8. Stangou M., Bantis C., Skoularopoulou M., Korelidou L., Kou-loukouriotou D., Scina M. et al. Th1, Th2 and Treg/T17 cytokines in two types of proliferative glomerulonephritis. Indian J. Nephrol. 2016; 26(3):159-66.
9. Sugama K., Suzuki K., Yoshitani K., Shiraishi K., Kometani T. Uri-nary excretion of cytokines versus their plasma levels after endur-ance exercise. Exerc. Immunol. Rev. 2013; 19: 29-48.
10. Kalavrizioti D., Gerolymos M., Rodi M., Kalliakmani P., Pro-vatopoulou S., Eleftheriadis T. et al. “T helper (Th)-cytokines in the urine of patients with primary glomerulonephritis treated with immunosuppressive drugs: can they predict outcome?” Cytokine. 2015; 76(2): 260–9.
11. Cho B.S., Yoon S.R., Jang J.Y., Pyun K.H., Lee C.E. Upregulation of interleukin-4 and CD23/Fc epsilon RII in minimal change neph-rotic syndrome. Pediatr. Nephrol. 1999; 13(3): 199–204.
12. Noronha I.L., Niemir Z., Stein H., Waldherr R. Cytokines and growth factors in renal disease. Nephrol. Dial. Transplant. 1995; 10(6): 775–86.
13. Pereira Wde F., Brito-Melo G.E., Guimarres F.T., Carvalho T.G., Mateo E.C., Simxes e Silva A.C. The role of the immune system in idiopathic nephrotic syndrome: a review of clinical and experimen-tal studies. Inflamm. Res. 2014; 63(1): 1–12.
14. Shao X.S., Yang X.Q., Zhao X.D., Li Q., Xie Y.Y., Wang X.G. et al. The prevalence of Th17 cells and FOXP3 regulate T cells (Treg) children with primary nephrotic syndrome. Pediatr. Nephrol. 2009; 24(9): 1683–90.
15. Kanai T., Yamagata T., Momoi M.Y. Macrophage inflammatory protein-1beta and interleukin-8 associated with idiopathic steroid-sensitive nephrotic syndrome. Pediatr. Int. 2009; 51(4): 443–7.
16. Meng X.M., Nikolic-Paterson D.J., Lan H.Y. Inflammatory process-es in renal fibrosis. Nat. Rev. Nephrol. 2014; 10(9): 493–503.
17. Kurts C., Panzer U., Anders H.J., Rees A.J. The immune system and kidney disease: Basic concepts and clinical implications. Nat. Rev. Immunol. 2013; 13(10): 738–53.
18. Bai J., Wu L., Chen X., Wang L., Li Q., Zhang Y. et al. Suppressor of Cytokine Signaling-1/STAT1 Regulates Renal Inflammation in Mesangial Proliferative Glomerulonephritis Models. Front. Immu-nol. 2018; 9: 1982.
19. Lichtnekert J., Kulkarni O.P., Mulay S.R., Rupanagudi K.V., Ryu M., Allam R. et al. Anti-GBM glomerulonephritis involves IL-1 but is independent of NLRP3/ASC inflammasome-mediated activation of caspase-1. PLoS One. 2011; 6(10): e26778.
20. Pan Q., Wu J., Tao J., Chen Y., Li L., Deng Z. et al. Role of baso-phils in the pathogenesis of minimal change nephrotic syndrome: a literature review. Exp. Ther. Med. 2014; 8(4): 1027–31.

21. Zhang J., Patel M.B., Griffiths R., Mao A., Song Y.S., Karlovich N.S. et al. Tumor necrosis factor-α produced in the kidney contrib-utes to angiotensin II-dependent hypertension. Hypertension. 2014; 64(6): 1275-81.
22. Pedigo C.E., Ducasa G.M., Leclercq F., Sloan A., Mitrofanova A., Hashmi T. et al. Local TNF causes NFATc1-dependent cholesterol-mediated podocyte injury. J. Clin. Invest. 2016; 126(9): 3336-50.
23. Velden J., Paust H.J., Hoxha E., Turner J.E., Steinmetz O.M., Wolf G. et al. Renal IL-17 expression in human ANCA-associated glomerulo-nephritis. Am. J. Physiol. Renal Physiol. 2012; 302(12): 1663–73.
24. Zhou L., Lopes J.E., Chong M.M., Ivanov II., Min R., Victora G.D. et al. TGF-beta-induced Foxp3 inhibits T (H) 17 cell differentiation by an-tagonizing RORgammat function. Nature. 2008; 453(7192): 236–40.
25. Korn T., Reddy J., Gao W., Bettelli E., Awasthi A., Petersen T.R. et al. Myelin-specific regulatory T cells accumulate in the CNS but fail to control autoimmune inflammation. Nat. Med. 2007; 13(4): 423–31.
26. Shin J.I. Inverse relationship between soluble urokinase receptors and estimated glomerular filtration rate: a role for IL-2? Kidney Int. 2015; 87(5): 1074.
27. Shishido S., Satou H., Muramatsu M., Hamasaki Y., Ishikura K., Hataya H. et al. Combination of pulse methylprednisolone infu-sions with cyclosporine-based immunosuppression is safe and ef-fective to treat recurrent focal segmental glomerulosclerosis after pediatric kidney transplantation. Clin. Transplant. 2013; 27(2): 143–50.
28. Hua K. F., Yang S. M., Kao T. Y., Chang J. M., Chen H. L., Tsai Y. J. et al. Osthole mitigates progressive IgA nephropathy by inhibiting reactive oxygen species generation and NF-κB/NLRP3 pathway. PLoS One. 2013; 8(10): e77794.
29. Gao J., Wei L., Liu X., Wang L., Niu D., Jin T. et al. Association Between IFN-γ Gene Polymorphisms and IgA Nephropathy in a Chinese Han Population. Kidney Blood Press. Res. 2017; 42(1): 136-44.
30. Kim A.H., Chung J.J., Akilesh S., Koziell A., Jain S., Hodgin J.B. et al. B cell-derived IL-4 acts on podocytes to induce proteinuria and foot process effacement. JCI Insight. 2017; 2(21): e81836.
31. Lan H.Y., Nicolic-Paterson D.J., Zarama M., Vannice J.L., Atkins R.C. Suppression of experimental crescentic glomerulonephritis by the interleukin-1 receptor antagonist. Kidney Int. 1993; 43(2): 479–85.
32. Chen A., Sheu L.F., Chou W.Y., Tsai S.C., Chang D.M., Liang S.C. et al. Interleukin-1 receptor antagonist modulates the progression of a spontaneously occurring IgA nephropathy in mice. Am. J. Kidney Dis. 1997; 30(5): 693–702.
33. Yin Z., Bahtiyar G., Zhang N., Liu L., Zhu P., Robert M.E. et al. IL-10 regulates murine lupus. J. Immunol. 2002; 169(4): 2148–55.
34. 34.Zhang R., Li Q., Chuang P.Y., Lu G., Liu R., Yang J. et al. Regulation of pathogenic Th17 cell differentiation by IL-10 in the development of glomerulonephritis. Am. J. Pathol. 2013; 183(2): 402-12.
35. Ostmann A., Paust H.J., Panzer U., Wegscheid C., Kapffer S., Huber S. et al. Regulatory T cell-derived IL-10 ameliorates crescentic GN. J. Am. Soc. Nephrol. 2013; 24(6): 930-42.

36. Zhiznevskaya I.I., Khmelevskaya I.G. Features of cytokine profile in children with glomerulopathy. Kurskiy nauchno-prakticheskiy vestnik Chelovek i ego zdorov´e. 2013; 1: 62–6. (in Russian)