CHANGES IN IMMUNOLOGICAL INDICATORS OF BLOOD AND URINE IN GLOMERULONEPHRITIS

Ibragimova Nadiya Sabirovna,
Isakulova Muhabbat Mardanovna
assistants at the Department of Clinical and Laboratory Diagnostics with a course of clinical and laboratory diagnostics at the Faculty of Postgraduate Education,
G'aniyeva Mahliyo Xurram qizi
cadet of the Department of Clinical and Laboratory Diagnostics with a course of clinical and laboratory diagnostics of FOPE,
Samarkand State Medical University,
Uzbekistan, Samarkand

ИЗМЕНЕНИЯ ИММУНОЛОГИЧЕСКИХ ПОКАЗАТЕЛЕЙ КРОВИ И МОЧИ ПРИ ГЛОМЕРУЛОНЕФРИТЕ

Ибрагимова Надия Сабировна,
Исакулова Мухаббат Мардановна
ассистенты кафедры Клинико-лабораторной диагностики с курсом клинико-лабораторной диагностики ФПДО,
Ганиева Махлиё Хуррам кизи
курсант кафедры Клинико-лабораторной диагностики с курсом клинико-лабораторной диагностики ФПДО,
Самаркандского Государственного Медицинского Университета,
Узбекистан, Самарканд

Annotation. The timeliness of diagnosis and treatment of post-infectious glomerulonephritis is currently limited by the inactivity and low-symptoms of the disease, which prompts the search for informative biological markers of the disease, which can be immunological parameters of blood and urine. Previously, studies have been undertaken to study individual components of the immune status in patients with PIGN [1, 4], but there is still no complete understanding of the nature of changes in the immunological parameters of blood and urine in patients with PIGN. In connection with the above purpose of the study, it was to establish characteristic changes in the immunological parameters of blood and urine in patients with PIGN.

Key words: immunological parameters of blood and urine, glomerulonephritis, immune complex damage to the glomeruli.

Аннотация. Своевременность диагностики и лечения постинфекционного гломерулонефрита в настоящее время ограничивают стертость и малосимптомность заболевания, что побуждает к поиску информативных биологических маркеров заболевания, в роли которых могут выступать иммунологические показатели крови и мочи. Ранее предпринимались исследования по изучению отдельных компонентов иммунного статуса у пациентов с ПИГН [1, 4], однако до сих пор нет полного представления о характере изменений в иммунологических показателях крови и мочи у больных ПИГН. В связи с приведенной целью исследования явилось установление характерных изменений в иммунологических показателях крови и мочи у больных ПИГН.

Ключевые слова: иммунологические показатели крови и мочи, гломерулонефрит,
иммунокомплексное повреждение клубочков.

Introduction. One of the important problems of modern medicine is the task of reducing the number of patients with chronic kidney disease (CKD), which causes the development of end-stage renal failure (ESRD), for the treatment of which enormous amounts of money are spent worldwide [1, 16]. The main cause of the development of ESRD is glomerulonephritis (GN), a group of diseases based on immunoinflammatory damage to the renal glomeruli. The ancestor of glomerulopathies is Bright's disease - post-streptococcal glomerulonephritis (PSGN), associated with a previous streptococcal infection. Fundamental changes in the epidemiology of PSGN occurred about three decades ago. The incidence of PSGN has decreased dramatically in industrialized countries due to effective antibiotic therapy for streptococcal infections and improved sanitation, but PSGN continues to be a common disease in developing countries [2]. The etiological structure of GN associated with infection has changed. Staphylococci (particularly Staphylococcus aureus) and viruses have become the most common causative agents of PIGN. Taking this into account, glomerulopathies, the development of which is associated with infection, began to be called “post-infectious glomerulonephritis” (PIGN) [3, 14].

Material and methods. An observational, single-center, cross-sectional study recruited PIGN patients receiving inpatient treatment in the nephrology department. The study included patients of both sexes, aged 18 to 65 years with a confirmed diagnosis of PIGN. The inclusion criteria were: 1) an established diagnosis of GN that developed after an infection (acute respiratory disease, infections of the oropharynx, nasopharynx, skin, urogenital infection, etc.) or during the manifestation of these infectious diseases; 2) debut of PIGN.

PIGN was diagnosed when three of the following signs were detected: 1) clinical or laboratory signs of an infection preceding the development of GN or the presence of infection during the development of GN, 2) diffuse endocapillary proliferative/exudative GN, 2) a decrease in the level of complement components in the blood serum - C3 and /or C4, 33) deposition of C3 in the renal glomeruli in combination with or without immune complexes, 4) detection by electron microscopy of hump-like subepithelial formations at the site of deposits of immune complexes [3, 5]. When selecting patients, persons with concomitant diseases (gastrointestinal, cardiovascular) were excluded from the study; secondary GN as part of systemic autoimmune diseases; renal failure (serum creatinine above 200 mcg/l, glomerular filtration rate - GFR below 60 ml/min); age over 65 years; pregnancy. The control group included practically healthy individuals of both sexes from 18 to 65 years old, with normal indicators of the functional state of the kidneys and blood pressure, who had no chronic diseases and no indications of acute diseases during the last month.

Mononuclear cells (MNC) were isolated on a Ficoll-Verografin density gradient (p=1.077 g/cm3). To identify lymphocytes and their subpopulations, immunophenotyping of MNCs was performed using direct immunofluorescence on a flow cytofluorimeter. In this case, monoclonal antibodies (mAb) to the differentiation markers CD3, CD3/CD4, CD3/CD8, CD20, CD4/CD25, CD95 (Beckman Coulter) were used according to the method of the mAb manufacturer.

Determination of the concentration of IgM, IgG, IgA in blood serum and urine was carried out by the immunoturbidimetric method using an automatic biochemical analyzer.

The levels of pro-inflammatory and anti-inflammatory cytokines - IL-1ß, Ra-IL-1ß (IL-1ß receptor antagonist), IL-2, IL-4, IL-8, IL-10, IL-17A and complement components (C3 and C4) were determined) in blood serum and morning urine by ELISA in a bideterminant antigen determination system using perosidase as an indicator enzyme using standard kits.

Results. The number of patients with PIGN selected for the study was 60 people, the control group included 30 practically healthy individuals. The group of patients included 19 women and 41 men. The average age of the patients was 31±8.5 years. In a typical case, the diagnosis of PIGN was made to patients who had such clinical manifestations as edema of varying severity (in 61.6% of patients), increased blood pressure (37% of patients), oliguria (26%), darkening of the color of urine (34%), which occurred 1-3 weeks after a staphylococcal, streptococcal, Klebsiella or viral infection or during the manifestation of these infections. 37% had no clinical manifestations of the disease.

Infection associated with PIGN manifested itself in the form of pharyngitis, tonsillitis, pyoderma, phlegmon, acute respiratory infection of the upper respiratory

tract, cystitis-urethritis, cholangitis, infective endocarditis before the development of PIGN or during PIGN. Laboratory markers of infections were detected by bacteriological and/or molecular genetic research methods in 90% of patients: Streptococcus pyogenes - in 18.3%, Staphylococcus aureus - in 21.6%, Staphylococcus haemoliticus - in 5%, Escherichia coli - in 7%, Klebsiella pneumoniae - 5%, Epstein-Barr virus - 10%, Human betaherpesvirus 6A - 8%, Hepatitis B virus (HBV) - 3%, Hepatitis C virus (HCV) - 2%, etc. In 4 (6.6%) patients, infection (mainly acute upper respiratory tract infections) manifested clinically, but was not detected by laboratory testing. 2 (3%) patients had no clinical symptoms of infection, but a high titer of antistreptolysin-O was detected - more than 330 U/l.

In 72% of patients with PIGN, the serum level of the complement C3 component was below 0.9 g/l (in healthy people: 0.94 - 1.88 g/l), in 14% the serum level of C4 was reduced below 0.1 g/l (in healthy people: 0.10 - 0.52 g/l).

Diagnostic nephrobiopsy with morphological examination of the biopsy using light and immunofluorescence microscopy was required to verify the diagnosis in 36 patients due to the low-symptomatic nature of the disease. According to the results of a morphological study, diffuse endocapillary GN was identified in 34 patients, and extracapillary GN with the formation of crescents in 2 patients.

An immunofluorescent study of biopsy using mAbs to C3, IgG, IgA, IgM revealed isolated C3 deposition in 11 (30%) of the examined patients, and combined C3 and IgG deposition in 33 (92%) patients. In 1 (2.7%) patient, IgA deposits were observed in the absence of C3 and other classes of immunoglobulins.

The control group included 9 women and 21 men, whose average age was 35.8±8.2 years. The values of gender and age indicators of healthy individuals making up the control group did not differ from the corresponding indicators of the sick groups ($p>0.05$ for all indicators).

Immuno-laboratory blood profile in patients with PIG. In the group of patients with PIGN, an increase in the absolute content of B(CD20+) cells, levels of IgM and IgA was found in the peripheral blood (Table 1). The results of studying the expression of differentiation markers on T-lymphocytes of patients indicated a decrease in the relative content of T(CD3+) cells and the absolute number of Treg cells - T-helper cells with high expression of the CD25 marker. In addition, the number of cells (CD14+) expressing CD282 (TLR2) was increased in patients. The levels of complement components C3 and C4 in PIGN were significantly reduced, and the concentration of CEC was increased.

Immuno-laboratory profile of urine in patients with PIG. In order to level out the influence of the filtration function of the kidneys on the content of immunoglobulins, C3, C4 and cytokines in the urine, normalized indicators of these biosubstrates were calculated as the ratio of their absolute indicators to the level of creatinine in the urine.

Analysis of the obtained indicators revealed a significant increase in the group of patients with PIGN in normalized indicators of C3 and immunoglobulins - IgA and IgG (Table 2). IgM was not detected in the urine of either patients or healthy individuals.

Cytokines in blood serum and urine in patients with PIGN. Serum levels of all studied cytokines, with the exception of Ig-4, in patients with PIGN exceeded the corresponding values in healthy people (Table 3). Normalized urine levels of all studied pro-inflammatory cytokines (GL-1p, IL-2, IL-8, IL-17A) and the anti-inflammatory cytokine IL-10 in patients were increased, while the levels of IL-4 and RaIL-lp did not differ from similar indicators in a group of healthy individuals.

The results of the correlation analysis demonstrated the presence of a connection between serum levels of cytokines mainly with such indicators as the relative number of CD14+ TLR2+ cells and the content of C3 in the blood serum. In particular, the percentage of CDl4+ TLR2+ cells correlated with levels (r = 0.36, pra = 0.022), IL-10 (r = - 0.46, = 0.022). The C3 concentration had negative relationships with the levels (r = - 0.34, p = 0.031) and Ig-4 (r = - 0.71, = 0.021).

The study of correlations between serum and urinary levels of cytokines revealed the existence of a direct connection between the serum level of IL-1P and the normalized value of the content of this cytokine in urine (r = 0.59; r = 0.001). For other cytokines, serum levels did not correlate with either absolute or normalized urinary levels.

Discussion. In recent decades, there have been significant changes in the epidemiological and etiological characteristics of PIGN. Streptococcus has ceased to be the predominant etiological agent. According to the results of our study, the association of PIGN with Streptococcus pyogenes was detected in 18.3%. In 23% of cases, a connection between PIGN and viral infections was found. The expansion of the spectrum of infectious agents that cause the development of PIGN is evidenced by data from many authors. It has been shown that PIGN can be caused by influenza viruses, Epstein-Barr, Coxsackie, hepatitis B and C, adenoviruses, echovirus type 9, as well as parasites (malarial plasmodia) [2, 17]. In countries with a high social standard of living, the development of PIGN is often preceded by premorbid renal damage of varying severity associated with diabetic nephropathy, chronic alcoholism, infective endocarditis, and intravenous drug use. In these cases, the disease is more often triggered by Staphylococcus aureus infection against the background of immunological disorders present in patients [3, 9]. У 21,6% обследуемых нами пациентов была обнаружена ассоциация заболевания с Staphylococcus aureus. In 21.6% of the patients we examined, an association of the disease with Staphylococcus aureus was found. If earlier PIGN, which has a streptococcal etiology, was considered a disease with a favorable course and a favorable outcome, then in modern conditions, PIGN

often manifests itself as rapidly progressive GN and often has a chronic course. In the present observation, in 5.5% of patients the disease developed as extracapillary GN with the formation of crescents and acquired a rapidly progressive course, complicated by end-stage renal failure.

The results of our study indicate that PIGN develops against the background of activation of the innate immune system, which is confirmed by the increased expression of TLRs related to pathogen recognition receptors on monocytes. Ten different types of TLRs have been described in humans [1, 10]. Among them, the most significant in the development of a protective antibacterial immune response are two types of TLRs - TLR2 and TLR4, the first of which ensures recognition of patterns of gram-positive bacteria (peptidoglycans, membrane lipoteichoic acids), and the second - membrane lipopolysaccharides of gram-negative microorganisms [1, 12]. In patients with PIGN, the expression of ThR2 on monocytes is increased, which can be explained by the connection between the development of the disease and infections caused predominantly by gram-positive coccal bacteria (staphylococci, streptococci). TLR-mediated recognition of pathogens by innate immune cells plays an important role in inducing the proinflammatory immune response necessary to clear infection. However, inflammatory activation under certain circumstances can be uncontrolled and exaggerated, which causes the development of an autoimmune process.

Thus, the debut of PIGN is associated with the activation of innate immune cells expressing pathogen recognition receptors - TLR2 in response to infection caused predominantly by gram-positive microorganisms. We do not exclude the possibility of increased expression of other types of TLRs in PIGN. Thus, the results of experimental studies indicate a connection between the development of GN and the overexpression of TLR3 and TLR8 [2, 13]. Following the activation of innate immune cells, adaptive immunity is activated, in particular its B-cell component, against the background of depression of the T-cell component and impaired immunoregulation due to a reduction in the number of Treg cells.

The initiation of the activity of innate immune cells is accompanied by the launch of a cascade of cytokine production [2, 11], which explains the increase in serum and urinary levels of pro- and anti-inflammatory cytokines (IL-1P, IL-2, IL-8, IL-10, IL) that we found in PIGN. -17A). The exceptions were the anti-inflammatory cytokines IL-4 and RaIL-1p. The content of the first of them did not change either in the blood or in the urine, while the content of the second was elevated in the blood, but remained at healthy levels in the urine. The results of other researchers also confirm the existence of a general trend toward increased levels of proinflammatory cytokines in various forms of GN [1, 14]. Correlation analysis revealed a close relationship between cytokine levels and various components of innate and acquired immunity. The greatest number of correlations was found in the serum and urinary levels of IL-1P, an early

proinflammatory cytokine produced in the kidneys by intrarenal macrophages, mesangial cells and podocytes [2, 12]. It is known that the receptor of IL-1 family members has a cytoplasmic segment homologous to the intracellular region of TLR - the TIR domain (from Toll-Il-1 Receptor) and can use the TLR activation pathway, activating innate immune cells [2, 7]. IL-1P initiates the recruitment of inflammatory cells - macrophages and neutrophils in the kidneys [25], shows a correlation with innate immunity indicators - the number of CD14+ TLR2+ cells, the level of C3, changes in which, in particular an increase in TLR2+ monocytes and a decrease in the level of C3 due to activation of the complement system along the alternative pathway, have a pathogenetic role in PIGN. IL-1P induces, through a chain reaction mechanism, the formation of other late proinflammatory cytokines (IL-6, IL-8 and IFN-γ) [2, 6].

Activation of innate immunity and the system of pro-inflammatory cytokines causes stimulation of adaptive immunity and a number of associated cytokines - IL-2, IL-4, IL-10. It is known that the last two cytokines have an anti-inflammatory effect, causing the activation of repair processes and the completion of inflammation [2, 9]. In accordance with the results of the present study, at the onset of PIGN, stimulation of IL-10 production is detected, but IL-4 production remains at healthy levels.

Conclusions. Summarizing the data obtained, we can conclude that the identified changes in the immunological parameters of blood and urine in patients with PIGN are associated with the pathogenetic mechanisms of the disease. A limitation of the study is the small sample of patients with PIGN included in the study. Further multicenter studies using a larger cohort of patients are required to explore the possibility of using immunological parameters of blood and urine for the early diagnosis of PIGN in cases of subclinical disease or absence of clinical symptoms.

LITERATURE

1. Zhiznevskaya I.I., Khmelevskaya I.G., Razinkova N.S., Minenkova T.A., Yakovleva A.V. Features of the immune status in children with acute and chronic glomerulopathy. Kurskiy nauchno-prakticheskiy vestnik «Chelovek i ego zdorov'e». 2017; 4: 42-5. Doi: 10.21626/ vestnik/2017-4/08. (in Russian)
2. Hunt E.A.K., Somers M.J.G. Infection-Related Glomerulonephritis. Pediatr. Clin. North. Am. 2019; 66(1): 59-72. Doi:10.1016/j. pcl.2018.08.005.
3. Kimmel M. Infekt-assoziierte Glomerulonephritiden [Infections-associated Glomerulonephritis]. Dtsch. Med. Wochenschr. 2020; 145(4): 240-7. Doi:10.1055/a-0974-9420.
4. Даминов Ф. А. и др. Особенности лечебного питания для ранней профилактики желудочнокишечных осложнений у обожженных //Журнал Неотложная хирургия им. ИИ Джанелидзе. – 2021. – №. S1. – С. 21-21.
5. Kudratova Zebo Erkinovna, Karimova Linara Alixanovna Age-related features of the respiratory system // ReFocus. 2023. №1. URL: https://cyberleninka.ru/article/n/age-related-features-of-the-respiratory-system.
6. Dushanova G. A., Nabiyeva F. S., Rahimova G. O. FEATURES OF THE DISTRIBUTION OF HLA-ANTIGENS AMONG PEOPLE OF THE UZBEK

NATIONALITY IN THE SAMARKAND REGION //Open Access Repository. – 2023. – Т. 10. – №. 10. – С. 14-25.
7. Sabirovna I. N., Shekhrozovna B. F. PRIMARY IMMUNODEFICIENCY CONDITIONS //Web of Medicine: Journal of Medicine, Practice and Nursing. – 2024. – Т. 2. – №. 1. – С. 59-61.
8. Ибрагимова Н. С., Рашидов А. ДИАГНОСТИКА ПЕРВИЧНЫХ ИММУНОДЕФИЦИТОВ //TADQIQOTLAR. UZ. – 2024. – Т. 30. – №. 3. – С. 153-158.
9. Sabirovna I. N. et al. ETIOPATHOGENETIC AND DIAGNOSTIC CRITERIA FOR GILBERT'S SYNDROME //Web of Medicine: Journal of Medicine, Practice and Nursing. – 2024. – Т. 2. – №. 1. – С. 34-37.
10. Sadriddinovna N. F., Ugli A. S. S., Kizi O. B. K. BIOLOGICAL PROPERTIES OF THE YEAST SACCHAROMYCES CEREVISIAE //Research Focus. – 2022. – Т. 1. – №. 4. – С. 18-22.
11. Kudratova Z. E.Isomadinova L. K.Sirojeddinova S. F. Tursunova M. E.Current modern etiology of anemia. novateur publications international journal of innovations in engineering research and technology. № 10. 2023, P. 1-4.
12. Isomadinova L.K. Qudratova Z.E. Shamsiddinova D.K.Samarqand viloyatida urotiliaz kasalligi klinik-kechishining o'ziga xos xususiyatlari. Central asian journal of education and innovation №10. 2023 , P. 51-53
13. Даминов Ф. А. и др. Хирургическая тактика лечения диффузно-токсического зоба //Академический журнал Западной Сибири. – 2013. – Т. 9. – №. 1. – С. 21-21.
14. Mansurov T. T., Daminov F. A. Laparoscopic adhesiolysis in treatment of acute adhesive intestinal obstruction //Conference Zone. – 2021. – С. 141-142.
15. Berdiyarova Sh.Sh., Ahadova M.M., Ochilov S.A. COMPLICATIONS OF TREATMENT OF ACUTE HEMATOGENOUS OSTEOMYELITIS, LITERATURE REVIEW, Galaxy International Interdisciplinary Research Journal 293-298
16. Бердиярова Ш.Ш., Юсупова Н.А., Ширинов Х.И. Клинико-лабораторная диагностика внебольничных пневмоний у детей, Вестник науки и образования, 80-83
17. Бердиярова Ш.Ш., Юсупова Н.А. Особенности иммунометаболических нарушений иммунологической реактивности при гематогенных остеомиелитах, Вестник науки и образования, 29-32