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ABSTRACT

of the dissertation for the degree of Doctor of Science

ETHIOLOGY AND PATHOGENETIC MECHANISMS OF CHRONIC GLOMERULONEPHRITIS IN CHILDREN

Speciality: 3220.01 – Pediatrics

Field of science: Medicine

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GENERAL DESCRIPTION OF THE WORK

Relevance of the theme. With eachpassing year the interest in the problem of glomerulonephritis (GN) in children is growing, as the prevalence and progression of this disease is becoming more frequent. According to various sources, the incidence of GN in children ranges from 5 to 50%, moreover, in 90% of cases a chronic course of the disease is noted[1,2,3].

Currently, studies are being conducted on the processes of free radical oxidation in pediatric nephrology, in particular inthe presence of GN. According to some existing opinions, an increase in the concentration of lipid peroxidation parameters can be considered as a predictor of the progression of kidney diseases[4]. The study of immuno-inflammatory and molecular mechanisms remains an urgent and promising direction. It was revealed that in children with the formation of CGN, there is an imbalance of pro- and anti-inflammatory cytokines[5]. However, the role of various immune

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disorders in the formation of GN, especially in children, remains poorly studied. There is nounanimous opinion on the differentiated mechanisms of immunopathogenesis of acute and chronic GN. Therefore, it is extremely important to study the etiology and pathogenesis of CGN in early childhood, both to understand the essence of the immune reactions that occur in the body and in the kidney tissue, and to elucidate the mechanism of development of this disease. Considering the importance of immune disorders in the pathogenesis of GN, a further prognosisat the early stages of the disease can be determined by immunodiagnosis. However, to the presentdate, the role of circulating cytokines in the blood, in the formation of various clinical forms of GN, has not been clarified.

Alongside the immune mechanism in GN, much attention is paid to the role of antimicrobial peptides (AMPs) in the pathogenesis of this disease, which are considered to be secondary pathogenetic factors[6]. Until now,thereare only a few reports on the level of AMP in children with CGN, indicating a certain role of disorders in the AMP system, in particular trypsin-like and chymotrypsin-like, in the pathogenesis of GN, in thefunctionally compensated stage[7].

In order to understand the pathogenetic mechanisms of the formation of GN and to improve preventive measures, it is important to study the clinical and genetic features of CGN[8].Genetic studies can become the basis for personalizing kidney diseases.

We were not able findstudies on the molecular genetic aspects of CGN in Azerbaijan, while abroad, there are worksin whichthe role of polymorphisms of candidate genes in the onset and the course of this diseaseissufficiently described in detail. Wherein, it is possible to transfer the results of foreign researchers to the population of Azerbaijan only in an indicative or comparative aspect, since each

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ethnos, including Azerbaijani, has its own gene pool structure, particularly, candidate genes for multifactorial diseases that differ from genetic characteristics of other nations.

Consideringthe role of chronic inflammation and autoimmune reactions in the formation and the progression of glomerular pathology, it can be assumed that the identification of immunogenetic predictors during the chronic course of the disease will be important for timely diagnostics.

In the comprehensive diagnostics of GN, the morphological study of the kidneys plays a key role in the improvement of diagnostic criteria.

Thus, at the present stage, an integrated approach in assessing the clinical and the immunogenetic status of the state of lipid peroxidation and AOS, is likely to be relevant not only from the point of view of a timely diagnosis, but also the possibility of predicting the nature of the course of GN.

The aim of the study: to study the state of lipid peroxidation and the antioxidant system, the immune and cytokine profiles, antimicrobial peptides, polymorphism of the nephrin and podocin genes, morphological features and toevaluate their pathogenetic significance for chronic glomerulonephritis in children and adolescents.

The objectives of the study:

1. To determine the clinical features of the course and the progression of various clinical forms of chronic glomerulonephritis in children and adolescents at the present stage.
2. To study the state of lipid peroxidation and the antioxidant system in children and adolescents with chronic glomerulonephritis.
3. To evaluate the cellular andhumoral immunity links and the cytokine status in children and adolescents with chronic glomerulonephritis.
4. To determine the role of antimicrobial peptides in the pathogenesis of chronic glomerulonephritis in children.
5. To study the polymorphism of the nephrin and podocin genes in Azerbaijani children and adolescents with CGN.

6. To evaluate the results of histological analysis of nephrobiopathies in children and adolescents with CGN and draw parallels with indicators of oxidative stress.

7. Develop diagnostic criteria for chronic glomerulonephritis and a screening program that takes into account oxidative stress and the role of immune and molecular mechanisms.

Research methods.

During the study, the anamnesis of the examined children and their mothers was collected. Clinical, laboratory, instrumental (ultrasound, computed tomography, excretory urography, nephroscintigraphy with 99Tc DMSA), biochemical, optical (spectrophotometry), immune (ELISA), genetic (PCR), histological, and statistical methods were used.The classification of glomerulonephritis by G.N. Speransky et al. (1966) was applied, with additions by M.S. Ignatova and Yu.Ye. Veltischev (1989). The glomerular filtration rate is calculated using the Schwartz formula.

Key theses tobe defended

– The prognostic parameters of the further development of CGN in children and adolescents were determined - glomerular filtration rate, proteinuria, hypercholesterolemia and hypercreatininemia;

– CGN in children and adolescents is accompanied by changes in the pro-oxidant system and antioxidant defense, manifested by a significant decrease in the enzymes of the glutathione redox system;

– Immune hyperreactivity and the predominance of pro-inflammatory cytokines are associated with components of the pro-oxidant and antioxidant systems;

– A frequent genotype of the nephrine gene (NPHS1) associated with the nephrotic syndrome in children and adolescents in Azerbaijan, is the heterozygous genotype GA, the podocin gene (NPHS2) - genotypes A> G and C> T;

– Identification of a specific histopathological picture of glomerular damage during kidney biopsy is important for determining the cause of glomerulonephritis;

– An optimal examination program for children with CGN has been developed.

Scientific novelty of research

For the first time in Azerbaijan, in children with various forms and at various stages of CGN activity:

– the role of oxidative stress in pathogenesis was determined;

– changes in immunological reactivity in children with various types of the nephrotic syndrome were revealed;

– cytokine indices and the proportion of each pro-inflammatory cytokine in the total number of cytokineswere calculated and estimated; sensitivity (Se), specificity (Sp) and cytokine efficacywere determined;

– the level of antimicrobial proteins in blood wasinvestigated;

– the relationship between inflammatory mediators and oxidative stresswas studied;

– polymorphism of the nephrin and podocin genes in children with various types of the nephroticsyndromewas studied;

– morphological characteristicswere studied;

– interactions of T-lymphocytes, cytokines, OS markers were revealed, and differences in the cytokine profile and glutathione cycle enzymes, as well as morphological variants in children with different forms of CGN were determined.

– significant factors in the pathogenesis of CGN were identified.

Theoretical and practical significance of the research

The clinical course of various forms of CGN was analyzed and indicators of the progression of the disease were identified. Based on a comprehensive assessment of the state of lipid peroxidation and AOS, as well as indicators of cellular and humoral immunity and cytokine status, statistically significant predictors, that allow for the predicting of the nature of the course of CGN in children, were determined.

The results of this work will contribute to the decodingof the mechanisms of the CGN pathogenesis and the formation of ideas about the molecular and genetic basis of the disease in Azerbaijani children. Genetic markers of the types of thenephrotic syndrome have been identified. Conducting molecular and genetic testing of patients with CGN created the prerequisites for the formation of risk groups for the adverse course of the disease.

Approbation and implementation

Approbation of the work was carried out on June 24th, 2019 at a meeting of the Academic Council.

The main provisions of the dissertation were reported on:

- XX Congress of Russian Pediatricians, Moscow, February 16th-18th, 2018;
- III-IV International Conference of the Caspian states in Astrakhan, 2018-2019;
-51st International Congress of the European Society of Pediatric Nephrology Turkey, October 3-6, 2018;
-XVII International Congress of Avrasia, Baku, September 2019;
-Republican Society of Nephrologists, Baku, 2017-2019;
-I International Conference of Allergists-Immunologists and Immunodeficiency States in Baku, May 2019;
-I International Scientific and Practical Conference on Human Genetics and Genetic Diseases Baku May 30-31, 2020.

The results of the research wereimplementedin the educational process.

The dissertation was completed at the Azerbaijan Medical University.

Publications

Fragments of the dissertation were reflected inprinted works (21 articles, 7 theses).

Volume and structure of the dissertation. The dissertation consists in text – of 372.190 characters, contains 43 tables, 53 graphs, 7 figures and 16 microphotos; it includes an introduction (11.294 characters), a literature review (66.974 characters), a chapter of material and methods (27.458 characters), 5 chapters of own research work (199.594 characters), discussion (63.513 characters), conclusions (3.357 characters), practical recommendations, a list of references containing 349 sources and a list of abbreviations and conventions.

RESEARCH MATERIAL AND METHODS

The work was performed at the Azerbaijan Medical University as part of a scientific work plan. Clinical and laboratory studies were conducted at the Department of Biochemistry at the Azerbaijan Medical University (Baku), Hospital No. 3, Republican Children's Hospital and Children's Polyclinic No. 1. Molecular genetic studies were performed at the "AFGEN" Genetic Diagnostic Center (Biological Medicine, Baku), at the Medical Faculty of the Aegean Medical University (Izmir, Turkey).

Researches were conducted in accordance with the principles of the Helsinki Declaration. The study protocol was approved by the ethics committee of all participating institutions. The informed consent of parents as well as older children was obtained to participate in the study.

The study included 288 children with CGN. For those included in the study, the following criteria were followed: age less than 16 years, morphologically confirmed CGN. Children with genetic syndromes, chromosomal aberrations, connective tissue diseases, and vasculitis were not included in the study. A total of 186 (64.6%) boys and 102 (35.4%) girls were examined, with an average age of 10.63 ± 3.88 years. The control group consisted of 30 practically healthy children of the same age - the average age was 10.7 ± 5.11 years, there were 19 boys (63.3%) and 11 girls (36.7%).

Patients were divided into groups according to the form of CGN. Group I included 104 children with a nephrotic form of CGN, that is characterized by the violation of glomerular filtration, as a result of which, the protein passes through the filtration barrier, that leads to severe proteinuria. Group II consisted of 96 patients with a hematuric form of CGN, which is characterized by the recurrent isolated (without edema and AH) macro- or microhematuria in combination with proteinuria of less than 1 g / day. Group III included 88 patients with a mixed form of CGN, which is characterized by a combination of NS with hematuria and / or AH. The control group consisted of 30 healthy children of comparable age.

Clinical, laboratory, and instrumental methods of investigation were used (ultrasound, computed tomography, excretory urography, nephroscintigraphy with 99Tc DMSA).

The classification of glomerulonephritis by Speransky G.N. et al. (1966), with the additions of M.S. Ignatova and Yu.E. Veltishchev (1989) was applied. According to the degree of activity of NS, children were distributed according to a modified classification of activity (Ehrich J.H.H., Drukker A., 2000).

The severity of hematuria was determined by testing according to Nechiporenko. Glomerular filtration rate was calculated according to the Schwartz formula. The determination of CD was carried out in hexane extracts of blood serum using spectrophotometry, and MDA- in the test with thiobarbituric acid.

In plasma and red blood cells, catalase activity was determined by the formation of hydrogen peroxide (H2O2) with ammonium molybdate; reduced glutathione (GSH) - according to the method of Ellman (1989) in the modification of Anderson M.E.; glutathione peroxidase (GPO) according to the method of A.R. Gavrilova, and glutathione reductase - according to the method of I. Carlberg and B. Maimervik.

The analysis of cellular immunity indices ($CD3^+$, $CD4^+$, $CD8^+$, $CD16/56^+$, $CD19^+$, $CD95^+$) was carried out by flow cytometry using a FACSCalibur apparatus from Becton Dickinson (USA) using monoclonal antibodies from Becton Dickinson, Beckman Coulter (France).

The concentration of immunoglobulins (IgA, M, G) and cytokines (IL-1β, IL-8, TNF-α, IFN-γ, IL-10, IL-4) was determined by an enzyme-linked immunosorbent assay using the appropriate enzyme-linked immunosorbent assay systems manufactured by ZAO "Vector-Best "(Russia). The cytokine index (CI) and the interleukin index (IL), as well as the ratio of pro- and anti-inflammatory cytokines were calculated. The determination of the functional activity of neutrophils was carried out during the formulation of the NST test (spontaneous and stimulated by lipopolysaccharide) with the use of latex. The phagocytic index (PI) was calculated as the average number of latex particles absorbed by one phagocyte, and

the phagocytic activity of neutrophils (FAN) was determined by the method of S.G.Potapova. etc (1977) using latex particles as a phagocytized object.

The concentration of lactoferrin in the blood was determined by the method of enzyme-linked immunosorbent assay using the diagnostic kits "Lactoferrin-IFA-Best", D-4156, RU No. FS 012-2005 / 2960-06, ZAO "Vector-Best" (Russia), whiledefensins and 3-nitrotyrosine –by the method of IFA using the test system "Hycultbiotechnology" (Netherlands).

Polymorphism of the NPHS1 and NPHS2 genes was evaluated by using the amplification of a refractory mutation system - PTSR. DNA extraction from leukocytes was performed using the DNA Prep 200 DIAtom ™ reagent kit after taking 200 μl of blood from a vein.

Renal biopsy, obtained by percutane ous puncture biopsy after histological analysis, was examined morphologically using light, electron and immunofluorescence microscopy.

Statistical processing of the results was carried out using standard software packages – Statistica version 6.0 (USA) in Excel. The mean value (M), standard error (m), and standard deviation (δ) were calculated. Average values are presented as M ± m. The parametric criterion of Student t attributes and the nonparametric U-criterion, Mann-Whitney, are used. The critical level of significance in testing statistical hypotheses was taken to be $p < 0.05$. Sensitivity (Se) and specificity (Sp) were determined. Pearson correlation (r) and determination coefficient (R2) were calculated.

RESEARCH RESULTS

Clinical characteristics of patients with various forms of chronic glomerulonephritis and the analysis of disease progression in children

104 patients with nephrotic CGN were examined (group I). There were 67 boys (64.4%), and 37 girls (35.6%). The age of the children varied from 5 to 16 years (average age - 10.18 ± 2.92 years). CGN with nephrotic syndrome is often found between the ages of 7 and 11 years, in children of both genders. Amongforms of NS, NS with

minimal changes dominates, as evidenced by its frequency, which is 1.5 times higher ($p < 0.05$) than that of steroid-resistant NS and 12.0 times ($p < 0.001$)than that of congenital NS. Of the renal diseases in parents and close relatives, the most common were urolithiasis and kidney stones - 62.1% (18), as well as pyelonephritis - 31.0%, in isolated cases, hydronephrosis (3.4%) and nephroptosis (3.4%) were noted. 15 (14.4%) children had parents, and 67 (64.4%) children had close relatives suffering from arterial hypertension. In addition, 35 (33.6%) patients had a burdened allergic history in the line of close relatives. The onset of the disease was seen more often at the age of 4 years. In most cases, children were troubled by edema, poor health and anxiety. The typical course of the disease prevailed. Along-side this, it is important to note, hypoproteinemia (100%), hypoalbuminemia (84.6%), hypercholesterolemia (55.8%), lymphopenia (74.0%), neutrophilia (54.8%), proteinuria (89.4%), leukocyturia (83.6%), erythrocyturia (78.8%). Children with a nephrotic form of CGN were distinguished by thickened renal parenchyma. The glomerular filtration rate (GFR) averaged 72.6 ± 9.69 (55.7; 91.4) ml / min / 1.73 m^2, (in the control group - 94.2 (82.6; 101.7) ml / min / 1.73 m^2).Analyzing the signs of progression of the nephrotic form of CGN in the examined patients, it is possible to note more pronounced changes in children aged 5-6 years. At the same time, patients of all age groups had severe hypoproteinemia (100%). In the age group of children 5-6 years old, cases with hypercreatinemia in comparison with the age group of 7-11 years old and 12-15 years old, were observed more often, respectively, 1.5 times ($p < 0.05$) and 1.3 times. Also, in this group, the frequency of cases with a low level of GFR was 5.2 times higher than that in the age group of 7-11 years ($p < 0.01$). Proteinuria in children aged 5-6 years was observed in all, which was 1.6 times ($p < 0.05$) more often, than in the 12-15 age group. In the group of children that were 7-11 years old, cases of hypoalbunemia (90.5%), proteinuria (95.2%), hypercholesterolemia (66.7%) were most often observed. In the adolescent group, the frequency of hypercholesterolemia (91.7%), high creatinine levels (66.7%), proteinuria (62.5%), hypoalbuminemia (58.3%) should be noted. In the group of patients

aged 5-6 years, a combination of 4 predictors of progression of the course (hypoproteinemia, hypoalbuminemia, hypercreatinemia, proteinuria) was found in 78.9% of cases (30 patients). In the group of children that were 7-11 years old, a combination of 5 predictors (hypoproteinemia, hypoalbuminemia, hypercreatinemia, proteinuria, hypercholesterolemia) was found in 57.1% of cases (24 patients), and in the group of children that were 12-15 years old - in 58.3% of cases (14 patients).

A total of 96 children with hematuric CGN (group II) were examined. Of the examined children with CGN, there were 58 boys (60.4%), girls - 38 (39.6%). The age of the children ranged from 7 to 15 years, the average age - 12.06 ± 2.36 years. The duration of the disease averaged 4.12 ± 1.77 years. The study of the genealogical history revealed a hereditary burden of kidney disease in almost 52 (54.2%) children. Hereditary burden with the same frequency was detected on the paternal and maternal lines. Urolithiasis (15.6%), inflammatory diseases of the urinary system (17.7%), pyelonephritis (9.4%) were common, arterial hypertension (5.2%), diabetes mellitus (6.2%) were less common. Among inflammatory diseases, it is worth noting cystitis - 10.4%, prostatitis - 5.2%, urethritis - 2.1%. The study of the antenatal period showed that in most children this period was accompanied by complications of pregnancy. According to the anamnesis, children often suffered from ARI (60.4%), angina (28.1%), influenza (11.5%). In 34 (35.4%) patients, CGN was accompanied by allergic diseases, in 18 (18.8%) - diseases of the gastrointestinal tract, in 10 (10.4%) - helminthic invasions.

Children with exacerbation of the hematuric form of CGN at the time of examination noted pain in the abdomen (20.8%) and in the lower back (19.8%), gross hematuria (32.3%), asthenia (36.4%). At the time of the study, hypertension was observed in 26 (27.1%) patients, edema syndrome - in 7 (7.3%) patients.

In the clinical blood test of children with a hematuric form of CGN, statistically significant changes were defined by leukocytosis, neutrophilia, accelerated ESR, in the clinical analysis of urine by – proteinuria, leukocyturia, erythrocyturia, cylindruria and crystalluria. In patients with hematuric CGN, a normal echographic picture was

more often observed.

According to the anamnesis, the parents and other relatives of children with CGN in 23.9% of cases had kidney diseases (pyelonephritis, kidney stones, hydronephrosis), in 25.0% of cases - AH and in 17.0% of cases (15) - other cardiovascular diseases (rheumatic heart disease, angina pectoris, ischemic heart disease).

Hereditary complications were often detected on the part of the mother. At the time of the study, the general condition in most of the patients was of moderate severity, the skin and visible mucous membranes were pale in 53.4% of patients, no rashes were observed, edema of varying severity was noted, decreasing in the evening.

63 children had chronic infectious foci. Patients had leukocytosis, relative neutrophilia, absolute lymphocytosis, high ESR, blood pressure, statistically significantly high concentration of total cholesterol, elevated levels of creatinine and urea in the blood, proteinuria, leukocyturia, erythrocyturia, cylindruria.

88 children with a mixed form of CGN (group III) were examined, of which 61 (69.3%) were boys, 27 (30.7%) were girls. The age of the children ranged from 11 to 15 years, the average age was 13.84 ± 0.88 years. When assessing GFR, a wide variation was revealed - from 42.4 to 88.7 ml / min, which averaged 64.2± 7.90 ml / min (control 94.2 ml / min - 82.6; 101.7). The echogram with the same frequency showed an uneven contour of the kidneys, hyperechogenicity and thinning of the parenchyma.

Indicators of intensity of the pro- and antioxidant system in children and adolescents with CGN

In children of group I, an increase in the content of CD and MDA was detected, both in plasma and in erythrocytes. Compared with the control parameters, the average CD level in blood plasma was higher by 17.5%, in erythrocytes - by 48.9% ($p < 0.01$). The concentration of MDA in children with CGN relative to the control values was significantly higher, both in plasma and in erythrocytes, by 34.4% ($p < 0.05$) and 42.8% ($p < 0.05$), respectively. In group II,the concentration MDA, both in plasma and in erythrocytes, exceeded the control level by an average of 43.5% ($p < 0.01$) and 36.8% (p

<0.05), respectively. In children of group III, a significant increase in CD in erythrocytes was revealed by 46.9% (p <0.01), as well as MDA in blood plasma and erythrocytes, respectively, by 40.3% (p <0.05) and by 45.5% (p <0.01). In group III, the maximum values of CD in plasma and erythrocytes were observed in children with high SBP (152 mm Hg) and DBP (98 mm Hg) - 0.97 μmol / ml and 1.37 μmol / ml, respectively.

The value of CD in patients of groups I and III was higher than in group II - in plasma, respectively, by 14.4% and 9.8%; in erythrocytes - by 45.9% (p <0.01) and 43.8% (p <0.05).There was no statistically significant difference in the content of MDA in plasma and in blood erythrocytes in patients with different forms of CGN. The content of CD in erythrocytes at all degrees of NS activity was significantly higher than the control one (p <0.05) and, as in plasma, increased with increasing NS activity. The concentration of MDA in the blood plasma of children with a mixed form of CGN in remission and during exacerbation exceeded the control value by 37.3% (p <0.05) and 40.8% (p <0.05), in erythrocytes - by 42, 2% (p <0.05) and 46.5% (p <0.05), respectively.

The activity of catalase in blood plasma in children of group I compared with the control value was reduced by 19.8%, in erythrocytes - by 34.9% (p <0.05). The amount of GSH in the blood plasma of sick children was reduced (by 10.4%), in erythrocytes the difference was 66.7% (p <0.01). The difference in the level of GPO in children with CGN and healthy children was 19.8% in blood plasma, and 80.2% in erythrocytes (p <0.01).

In patients of group II, the activity of catalase in comparison with the control value was slightly reduced in plasma (by 8.6%) and significantly decreased in erythrocytes (by 67.8%, p <0.01). The activity of GR enzyme in plasma was on average reduced by 75.0% (p <0.01), in erythrocytes - by 92.7% (p <0.01). The activity of GPO, in comparison with the control parameter, was reduced in plasma and in erythrocytes almost equally - by an average of 48.1% (p <0.05) and 47.2% (p <0.05) respectively. The GSH level in comparison with the control indicator was lower by 36.1% (p <0.05) and 78.2% (p <0.01), in plasma and erythrocytes,respectively.

In group III patients, all AOS indices were lower than the control ones. The activity of catalase in plasma and erythrocytes was lower than the control one by 24.1% and 35.4%, respectively ($p < 0.05$). A significant decrease was detected in the activity of reduced GSH in plasma - by 20.7% in erythrocytes - by 72.2% ($p < 0.01$). Low GPO andGR intensity was determined both in blood plasma by 26.0%, ($p < 0.05$) and 55.6% ($p < 0.01$), andin erythrocytes by 81.5%, ($p < 0.01$) and 100.0% ($p < 0.01$), respectively.

Comparative analysis between the groups showed that the minimum activity of catalase in the blood plasma was observed in children with a mixed form of CGN (group III), which was lower than in groups I and II by 3.6% and 14.3%, respectively. In blood erythrocytes, the minimum activity of catalase, averaging 1217.4 ± 22.44 U / ml, was observed in patients of group II, which was 24.4% and 23.9% lower than in groups I and III, respectively.The minimum GSH activity was observed in the hematuric form (group II), both in plasma and in erythrocytes. In comparison with the group of patients with nephrotic and mixed forms, the difference in blood plasma was 23.3% and 12.8%, respectively, in erythrocytes - 6.9% and 3.4%, and in group II, in comparison with the indicator of groups I and III was lower, by 23.7% and 17.6%, respectively.

A significant decrease in the GR / GPO ratio was observed in patients with hematuric CGN (group II). In plasma, the GR / GPO ratio in patients of this group was 0.03 ± 0.01, which was 5.7 times ($p < 0.001$), less than in groups I and III.

In plasma and erythrocytes, the catalase activity in the remission stage was 18.3% and 11.9% lower than in the control, in patients with grade 1 NS - by 18.8% and 18.9%, with grade 2 by 22, 7 and 29.5% ($p < 0.05$), with grade 3 - by 23.3% and 31.9% ($p < 0.05$), respectively, in plasma and erythrocytes.

In patients in remission relative to the control value in plasma, there was a decrease in GSH activity by 6.5%, in patients with grade 1 - by 7.7%, with grade 2 - by 11.7%, with grade 3 - by 13.1 %.At the same time, the average level of this indicator in erythrocytes in patients in remission was lower than in the control value by 17.4%, in children with 1, 2 and 3 degrees of NS activity - by 32.5% (p

<0.05), by 40.9% (p <0.05) and 59.8% (p <0.01), respectively. Comparison of GSH activity in erythrocytes with grade 1 NS with the indicator in remission revealed a decrease in the enzyme by 12.8%, and the difference in plasma was not determined. In patients with grade 2 NS, the GSH activity in plasma and erythrocytes was decreased by 4.9% and 20.0%, respectively, compared to the activity in the remission stage. The GSH activity in plasma and erythrocytes in children with grade 3 NS compared with the value in remission was 5.9 and 36.1% lower (p <0.05), respectively.

Significantly reduced GPO activity in erythrocytes was found in patients with 2nd and 3rd degrees of NS activity - by 61.6% (p <0.01) and 84.2% (p <0.01). In the remission stage there was a statistically significant decrease in erythrocytes in children with 2 and 3 degrees of NS activity - by 34.3% (p <0.05) and 53.2% (p <0.01).

Analysis of the GR activity showed a statistically significant decrease in the activity in the blood plasma in children with degrees 2 and 3 of the process activity, respectively by 40.0% (p <0.05) and 55.6% (p<0.01).). The decrease in the plasma GR activity in NS of 1st degree of activity compared with the control was 16.7%. The activity of GR in erythrocytes in children with 1st, 2nd and 3rd degrees of NS, compared with the value in the remission stage, decreased by 5.4%, 23.4% and 38.4% (p <0.05).

In patients of group I in remission, the value of the GR / GPO ratio was lower than the control by 21.4% (p < 0.05). In patients with grade 1 NS, the plasma GR / GPO ratio was lower than in the control group and in the group of patients with remission by 13.6% and 9.5%, respectively. The ratio of GR / GPO in erythrocytes in children with grade 1 NS lower than the control by 21.4% (p <0.05).

In patients with grade 2 NS, the ratio of GR / GPO in plasma in relation to the control and the stage of remission decreased by 18.2% and 14.3%, respectively. In erythrocytes, the value of the GR / GPO ratio was lower than the control one by 14.3% and higher than in the period of remission by 9.1%. A similar change in the GR / GPO ratio was determined in patients with grade 3 NS.

In group II, the activity of catalase in erythrocytes in the examined children with clinical remission in comparison with patients in the

acute stage was higher by 13.8%, but lower than the control value by 45.2% ($p < 0.05$). The average activity of the enzyme in erythrocytes in patients with exacerbation of CGN was also lower than the control indicator by 68.5% ($p < 0.01$). The maximum content of GSH in plasma and erythrocytes was observed in children with clinical remission and minimum - with exacerbation of CGN, and in both groups the GSH level was significantly lower than in the control group. The activity of plasma and erythrocyte GPO in the active course of CGN and remission did not practically differ, but were significantly lower than the control ($p < 0.05$). The activity of GR in plasma and in erythrocytes during clinical remission and exacerbation of CGN did not differ significantly between themselves, but were significantly reduced ($p < 0.05$) relative to the control values. The activity of the GPO and GR enzymes, compared with the control value, was reduced by 49.2% ($p < 0.05$) and 90.9% ($p < 0.01$) in plasma, and by 48.4 ($p < 0$, 05) and 96.3% ($p < 0.01$) in erythrocytes. At the same time, the erythrocyte activity of GPO and GR was higher than the plasma one by 74.2% ($p < 0.01$) and 79.6% ($p < 0.01$). Plasma GSH content was higher than in erythrocytes during remission (15.7 times, $p < 0.001$), and with exacerbation of the disease (15.2 times, $p < 0.001$). The GR / GPO ratio in patients with hematuric CGN in plasma during remission was lower than the control in plasma by 15.8%, in erythrocytes - by 27.3% ($p < 0.05$). With an exacerbation of the process, the GR / GPO ratio in plasma was lower than the control one by 29.4% ($p < 0.05$) and lower than the indicator in the remission stage by 11.8%, in erythrocytes, respectively, by 33.3% ($p < 0.05$) and 4.8%.

In group III, the level of catalase in erythrocytes in the acute stage was 13.5% lower than in remission and lower than the control - by 29.9% ($p < 0.05$). The difference in the concentration of catalase in erythrocytes in children in remission relative to the control was 14.5%. The content of reduced glutathione in plasma and erythrocytes in patients in remission and exacerbation did not differ among themselves, but in comparison with the control value in plasma and erythrocytes in remission, it decreased by 16.0% and 72.2% ($p < 0.01$) respectively.

Comparison with the control level in blood plasma showed a decrease in enzyme activity by an average of 25.2% ($p < 0.05$), in erythrocytes - by an average of 78.1% ($p < 0.01$).The concentration of glutathione reductase in blood plasma and in erythrocytes did not differ at different stages, but in comparison with the control, there was a significant decrease by 61.5% ($p < 0.01$) and 92.7% ($p < 0.01$), respectively. Consequently, the greatest changes were observed in the erythrocytes.

The ratio of GR / GPO in patients with a mixed form of CGN in plasma during remission was lower than the control in plasma by 29.4% ($p < 0.05$), in erythrocytes - by 3.7%. With an exacerbation of the process, the ratio of GR / GPO in plasma was lower than the control one by 37.5% ($p < 0.05$) and lower than the indicator in the remission stage by 6.2%, in erythrocytes by 12.0% and 8.0%, respectively.

A weak multidirectional linear correlation between LPO and AOS was found. In group II, there was an inverse correlation between CD and GR in blood plasma - $r = -0.17$ ($p = 0.16$), in group III: in erythrocytes, a direct correlation with catalase $r = 0.19$ ($p = 0, 15$) and inverse relationship with GSHr = -0.18 ($p = 0.15$).

When determining the structure of correlations between MDA and AOS indicators, comparatively significant relationships, in contrast to the control group, were revealed in patients with CGN (fig. 1).

Immunological reactivity in children with CGN.

Decreased levels of T-lymphocytes were noted in 38 (36.5%) patients with nephrotic CGN, increased rates in 62 (64.6%) patients with hematuric and 59 (67.0%) patients with mixed CGN.

Compared to the control values, a statistically significant increase in $CD4^+$, $CD16/56^+$($p < 0.05$) was determined. The study of the apoptosis marker $CD95^+$ revealed an increase of 1.5 times ($p < 0.05$), in the relative level in group I compared to the control, in the absolute level- 1.8 times ($p < 0.05$), in group II, respectively, 1,5 ($p < 0.05$) and 1.6 times ($p < 0.05$), in group III - 1.7 ($p < 0.05$) and 2.1 times ($p < 0.05$), respectively. Wherein, no statistically significant differences were observed among the groups.

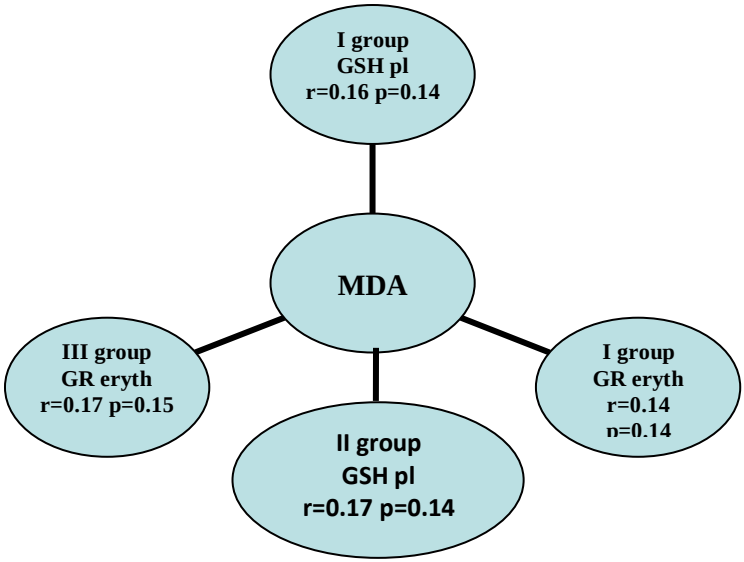


Fig. 1.The structure of the correlation relationships between MDA and AOS parameters in patients with CGN.

Compared to the control group, the relative and absolute number of B-lymphocytes in group I was reduced by 47.4% (p <0.05) and 6.7%, in group II - by 51.4% (p <0, 01) and 6.7%, in group III - by 50.2% (p <0.05) and by 14.3%.

A significant increase in the amount of IgA was observed relevant to the control value in patients of group I and III, 2.1 and 2.0 times respectively, (p <0.05) and IgM - 2.2 and 1.8 times (p <0 , 05). In patients of group II, the average concentration of IgA was 1.4 times (p <0.05) and the level of IgM was 1.1 times higher than in the control. The concentration of IgG in the blood, relative to the control, was increased by 1.7 and 1.6 times respectively, in nephrotic and mixed forms (p <0.05). The level of CICs in patients with a nephrotic form was increased by 2.4 times (p<0.05), in children with a hematuric form by 2.2 times (p <0.05) and by 2.6 times (p <0.05) in children with a mixed form of CGN.

A decrease in the neutrophilic activity was noted.

Under our observation, there were 60 children with minimal

changes in the NS, 39 with steroid-resistant NS and 5 children with congenital (inborn NS), which showed the decrease of B-lymphocytes, the increase in the levels of immunoglobulins A, M and G , CICs and in the decrease of neutrophils reserve activity (Fig. 2).

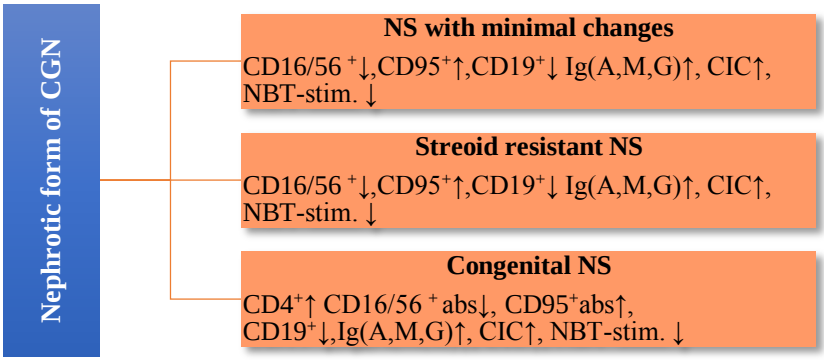


Fig. 2.Statistically significant changes in the immunological reactivity in children with different types of NS.

A significant increase in TNF-α was detected in all groups of children with CGN. In patients with a hematuric form of CGN, the content of this cytokine in the blood exceeded the control by 3.9 times ($p < 0.01$), with a nephrotic and mixed form – by 4.2 ($p < 0.01$) and 4.3 times ($p < 0.01$), respectively. The level of IL-1β and IL-8 relative to the control values was higher in the nephrotic form by 2.0 and 1.5 times ($p < 0.05$), in the hematuric form - by 1.8 and 1.4 times ($p < 0, 05$), in the mixed – by 2.0 and 1.5 times ($p < 0.05$), respectively. A comparative assessment of the level of IFN-γ showed that in children with CGN, regardless of its clinical form, the value of this cytokine exceeded the control by 1.8 times ($p < 0.05$).

The cytokine index (IC) in patients with a nephrotic and mixed form of CGN was 2.6 conventional units, in children with a hematuric form of the disease - 2.5 conventional units.

In children with CGN, regardless of form, there was a remarkable

increase in the index of the ratio of IL-1β / IL-4 ($p < 0.05$), TNF-α / IL-4 and TNF-α / IL-10 ($p < 0.01$). In addition, a statistically significant increase in the IFN-γ/ IL-4 coefficient was detected in patients with the nephrotic and mixed CGN form ($p < 0.05$), as well as the index of the ratio of IFN-γ / IL-10 in children with nephrotic and hematuric CGN form.

In the study of the relationship between cytokines, on the basis of absolute values, we determined the propotion of each cytokine in a percentage form (%) in the total amount of children with different types of CGN. Indicators for TNF-α were reliable, in children of groups I, II and III they were 25.5, 24.9, 25.9%, respectively, which were 2.3, 2.2 and 2.3 times ($p < 0.05$) higher than the control indicator (11.1%). For IL-4 in children with a mixed form of CGN, the indicator was 6.7%, which was 1.4 times lower ($p < 0.05$) compared to the control (9.1%).

When assessing the proportion of each pro-inflammatory cytokine in the total number of cytokines in CGN, a high percentage of TNF-α (25.5%) and a low percentage of IL-8 (13.0%) was revealed. The highest sensitivity of IL-1β and TNF-α was noted in all clinical forms of CGN. The greatest efficiency was observed with an increase in TNF-α.

The results of the analysis of the ratio of pro-inflammatory and anti-inflammatory cytokines in different variants of NS showed that in comparison with the indicators of the control group in children with minimal changes in NS there was an increase in the index of IL-1β / IL-4 ratio by 1.6 times ($p < 0.05$) , IL-1β / IL-10 1.5 times ($p < 0.05$), TNF-α / IL-4 - 3.3 times ($p < 0.01$), TNF-α / IL-10 - 3.1 times ($p < 0.01$), IFN-γ/ IL-4 - 1.5 times ($p < 0.05$) and IFN-γ/ IL-10 - 1.4 times ($p < 0.05$). Patients with steroid-resistant NS had significantly increased IL-1β / IL-4 indices 1.6 times ($p < 0.05$), IL-1β / IL-10 indices 1.5 times ($p < 0.05$), TNF -α / IL-4 - 3.6 times ($p < 0.01$), TNF-α / IL-10 - 3.3 times ($p < 0.01$), IFN-γ/ IL-4 - 1.5 times ($p < 0.05$) and IFN-γ/ IL-10 - 1.5 times ($p < 0.05$). In congenital NS, the following statistically significant indices were observed: IL-1β / IL-4 by 1.7 times ($p < 0.05$), IL-1β / IL-10 by 1.5 times ($p < 0.05$), TNF-α / IL-4 - 3.6 times ($p < 0.01$), TNF-α / IL-10 - 3.3 times ($p < 0.01$), IFN-

γ/ IL-4 - 1.6 times (p <0.05) and IFN-γ/ IL-10 - 1.5 times (p <0.05).

As can be seen, in all NS variants, similar differences in the ratio of pro- and anti-inflammatory cytokines were observed relative to the control data.

The proportion of IL-1β in patients with minimal changes was 4.18% (control - 3.44%), in patients with steroid-resistant NS variant - 4.22% and congenital NS - 4.3%. The proportion of IL-8 in children with minimal changes in NS was 20.8% lower than the control, and by an average of 20.0% with steroid-resistant and congenital NS, respectively. The proportion of anti-inflammatory IL-4 in the blood of children with minimal changes was 7.5%, which was lower than the control (10.0%) by 25.0%, in patients with steroid-resistant NS - by 28.1% (p <0.05) and in the congenital variant - by 29.0% (p <0.05). When compared with the control indicator, a reduced proportion was also determined in relation to IL-10. With minimal changes in NS, the proportion of this anti-inflammatory cytokine was reduced by 24.9% compared to the control group, in steroid-resistant by 24,9% and in congenital NS by 28,0 (p<0,05), respectively.

In patients with minimal changes in NS, the share of TNF-α was 22.4% (control - 7.7%), exceeding the control by 2.9 times (p <0.01), in patients with steroid-resistant and congenital NS, the share of this cytokine practically did not differ and amounted to 23.3% and 23.1%, respectively, which was 3.0 times higher than the control data (p <0.01). The percentage of IFN-γ with minimal changes in NS was 10.9%, with steroid-resistant variant - 11.0% and congenital NS - 11.2%, which was 1.1 and 1.2 times higher than the control indicator, respectively.

We observed 89 children with IgA nephropathy. According to the study, the average level of all cytokines in patients with IgA nephropathy exceeded the control. The average level of IL-1ß in the blood was 7.05 ± 1.04 pg / ml (control - 3.67 ± 0.68 pg / ml), IL-8 - 71.29 ± 3.37 pg / ml (control - 50.24 ± 2.77 pg / ml), TNF-α - 32.12 ± 1.66 pg / ml (control - 7.93 ± 1.0 pg / ml). IFN-γ - 17.0 ± 2.55 pg / ml (control - 9.58 ± 1.47 pg / ml), IL-4 - 12.92 ± 2.80 pg / ml (control - 10.0 ± 1.33 pg / ml) and IL-10 - 37.54 ± 2.92 pg / ml

(control - 28.83 ± 2.44 pg / ml). When compared to the control group, the statistically significant difference in relation to IL-1β was 1.9 times ($p < 0.05$), IL-8 - 1.4 times ($p < 0.05$), TNF-α - 4.0 times ($p < 0.01$), IFNγ -1,8 - 1.8 times ($p < 0.05$), IL-4 and IL-10 - 1.3 times ($p < 0.05$), respectively.

The concentration of defensin in group I patients ranged from 80.0 ng / ml - 86.6 ng / ml and averaged 83.0 ± 1.51 ng / ml, in group II patients - in the range 82.2 - 91. 4 ng / ml, which averaged 88.7 ± 8.14 ng / ml; in group III patients, the defensin level varied from 82.2 to 93.3 ng / ml and averaged 89.2 ± 6.57 ng / ml. The concentration of defensin in the blood exceeded the control value by 1.6 times ($p < 0.05$), with hematuric and mixed forms, respectively, by 1.7 times ($p < 0.05$).

In patients with the nephrotic form (group I), the lactoferrin level was 811.3 ± 47.7 ng / ml (fluctuation range 682.5 - 871.6 ng / ml), with hematuric form (group II) - 811.3 ± 100 , 4 ng / ml (range of fluctuations 733.0 - 864.7 ng / ml), in children with a mixed form (group III) - 887.2 ± 109.6 ng / ml (range of fluctuations 781.8 - 954.8 ng / ml). The level of lactoferrin in comparison with the control value in groups I and II was 1.6 times higher ($p < 0.05$), respectively, in group III - 1.7 times ($p < 0.05$). In children of group I, the average level of nitrotyrosine in the blood was 12.0% higher than the control indicator, in patients of group II - by 37.1% ($p < 0.05$), in group III - by 42.1% ($p < 0.05$).

The lactoferrin level in patients with minimal changes was 724.0 ± 20.2 [682.5; 765.4] ng / ml, with the steroid-resistant variant - 800.9 ± 31.6 [743.6; 856.2] ng / ml ml and congenital NS - 852.4 ± 13.2 [831.0; 871.6] ng / ml. In contrast to defensin, the level of lactoferrin in the blood in patients with congenital NS was higher than its value in the group with minimal changes and steroid-resistant NS, respectively, by 17.7% and 6.4%. When compared with the data of the control group, the concentration of lactoferrin in patients with minimal changes was 1.4 times higher, with steroid-resistant and congenital NS - 1.6 times ($p < 0.05$), respectively.

In children of group I, the average level of nitrotyrosine in the blood was 2.5 ± 0.17 [2.2; 3.4] nmol / L, which was 12.0% higher

than in the control group (2.2 ± 0.6 nmol / L), in patients of group II, the concentration of nitrotyrosine fluctuated in the range of 2.3 - 3.7 nmol / l, averaging 3.5 ± 1.0 nmol / l and exceeding the control indicator by 37.1% ($p < 0.05$), in group III the average level of nitrotyrosine was 3.8 ± 1.1 [2.3; 4.0], which was 42.1% higher than the control value ($p < 0.05$). Comparative analysis of the concentration of 3-nitrotyrosine between groups of patients with CGN revealed its maximally increased level in children with a mixed form. Thus, the content of nitrotyrosine in patients of group III was higher than that in groups I and II, respectively, by 52.0% ($p < 0.05$) and 8.6%. At the same time, in the group of children with a mixed form of CGN, the level of nitrotyrosine, within the control limits, was determined only in 12.5% of the cases, in the group with nephrotic and hematuric forms – in 56.7% and 26.0% of the cases, respectively.

The concentration of 3-nitrotyrosine in the blood in children with minimal changes averaged 2.6 ± 0.13 [2.2; 2.9] nmol / l, with the steroid-resistant variant - 3.3 ± 0.1 [3.1; 3.4] nmol / l and congenital HC - 3.4 ± 0.04 [3.3; 3.4] nmol / l. In the group of children with steroid-resistant and congenital NS, the value of 3-nitrotyrosine exceeded the control value by an average of 1.5 times ($p < 0.05$) and 1.3 times that in the group with minimal changes in NS.

In patients with IgAH, the content of 3-nitrotyrosine in the blood ranged from 2.9 to 3.4 nmol / l, which averaged 3.2 ± 0.87 nmol / l and exceeded the control indicator by 1.5 times ($p < 0.05$).

Correlation analysis revealed significant direct and inverse relationships between the parameters of the immune system, MDA and antioxidant activity. The most significant relationships were found between CD95 and MDA ($r = + 0.582$, $p < 0.05$), CEC / MDA ($r = + 0.462$, $p < 0.05$), IL-1β / MDA ($r = + 0.417$, $p < 0.05$), IL-8 / MDA ($r = + 0.422$, $p < 0.05$), TNF-α / MDA ($r = +0.417$, $p < 0.05$), TNF-α / GR ($r = -0.488$, $p < 0.01$), i.e. the results indicate the relationship between immunity and LPO and antioxidant activity.

In the nephrotic variant of CGN in children, an average direct correlation was found between IL-1β/ MDA ($p < 0.05$), IL-8 / MDA ($p < 0.05$), TNF-α / MDA ($p < 0.05$) and the average feedback

between TNF-α / GR (p <0.01). In patients with hematuric CGN, weak direct correlations were found between the levels of cytokines and indicators of oxidative stress for IL-1β / MDA (p <0.05), TNF-α / MDA (p <0.05) and IFN-γ/ MDA (p <0.05) and weak feedbacks for TNF-α / GSH (p <0.05), TNF-α / GPO (p <0.05), for TNF-α / GR (p <0 , 05). In children with a mixed form of CGN, direct correlations were found between the levels of IL-1β and MDA (p <0.05), TNF-α / MDA (p <0.05), as well as inverse correlations between IL-1β and GR (p <0.05) and TNF-α / GR (p <0.05). In all forms of CGN, a direct correlation was observed between pro-inflammatory cytokines - IL-1β and TNF-α with MDA, as well as an inverse relationship between pro-inflammatory TNF-α and GH, an indicator of the antioxidant system. Moderate correlations were found between AMP and cytokines and components of oxidative stress.

Frequency and spectrum of mutations of polymorphism of nephrin (NPHS1) and podocin (NPHS2) genes in children of the Azerbaijani population with nephrotic syndrome due to CGN

To determine the frequency of polymorphism of the NPHS1 and NPHS2 genes in Azerbaijani children with different forms of NS caused by CGN, 36 children were examined. In 25 (69.4%) of the patients, NS with minimal changes was observed, in 6 (15.7%) - steroid-resistant NS, and in 5 (13.9%) - congenital (inborn) NS.

A molecular genetic study of the NPHS1 gene polymorphism in 6 patients with NS revealed a heterogeneous mutation of the splicing site c.1223G> A (p.Arg408Gln) in exon 10 in 5 (83.3%) patients; splicing site mutation c.3315G> A (p. Ser1105Ser) in exon 26 - in 2 (33.3%) patients; splicing site mutation with c 3447C> T (p.Arg1160X) in exon 27 - in 2 (33.3%) patients; splicing mutation c.1320C> T (p.Pro440Pro) in exon 11 - in 1 (16.7%) patient; site mutation c.2289C> T (p. Val763Val) in exon 17 - in 1 (16.7%) patient; mutation of the splicing site c.3230A> G (p.Asn1077Ser) in exon 24 - in 1 (16.7%) patient; c.349G> A (p.E117K) in exon 3 in 1 (16.7%) patient and a splicing site mutation in c.IVS27+ 45C> T in the intron region of exon 27 in 1 (16.7%) patient (table 1).

Table 1

Nucleotide substitutions in the NPHS1 gene detected in children with NS (n = 6)

exon	Polymorphism	heterozygous	Nucleotides	Patient, n (%)
3	c.349G>A	**G**AG/**A**AG	E117K	1 (16.7)
10	c.1223G>A	C**G**G/C**A**G	p.Arg408Gln	5 (83.3)
11	c.1320C>T	CC**C**/CC**T**	p.Pro440Pro	1 (16.7)
17	c.2289 C>T	GT**C**/GT**T**	p.Val763Val	1 (16.7)
24	c.3230A>G	A**A**T/A**G**T	p.Asn1077Ser	1 (16.7)
26	c.3315G>A	TC**G**/TC**A**	p.Ser1105Ser	2 (33.3)
27	c.3478C>T	**C**AG/TA**G**	p.Arg1160X	2 (33.3)

According to the results of the study, three mutant combinations were determined in two (33.3%) of the 6 patients examined: 1) c. 1320C> T (p.Pro440Pro) in exon 11, c. 2289C> T (p. Val763Val) in exon 17 and c. 3230A> G (p.Asn1077Ser) in exon 24; 2) c.1223G> A (p.Arg408Gln) in exon 10, c.3315G> A (p. Ser1105Ser) in exon 26 and c. IVS27 + 45C> T in the intron region of exon 27. In the remaining 4 patients, two heterozygous mutant combinations were noted. Moreover, the combination c.1223G> A (p.Arg408Gln) in exon 10 and s.3478C> T (p.Arg1160X) in exon 27 was found in 2 (33.3%) patients, the combination c.1223G> A (p. Arg408Gln) in exon 10 and c. 3315G> A (p. Ser1105Ser) in exon 26, as well as c. 349G> A (p. E117K) in exon 3 and c. 1223G> A (p.Arg408Gln) in exon 10 were determined in 1 (16.7%) patient, respectively.

In NS, the GA genotype and the G allele of the NPHS1 gene dominated in children from the Azerbaijani population (Fig. 3).

Of 6 patients, congenital NS was diagnosed in 4 (66.7%), steroid-resistant NS in 1 (16.7%) and NS with minimal changes in 1 (16.7%) patient. In patients with congenital NS, a heterogeneous mutation of the splice site c.1223G> A (p.Arg408Gln) in exon 10 was more often observed. In a patient with steroid-resistant NS, three heterogeneous mutant combinations were determined, c.1320C> T (R.Pro440Pro) in exon 11, s. 2289C> T (p.Val763Val) in exon 17 and p.3230A> G (p.Asn1077Ser) in exon 24.

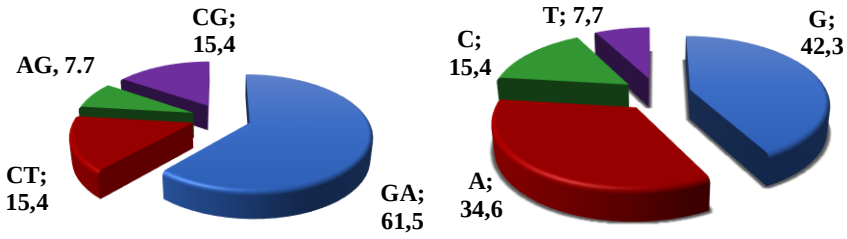


Fig. 3. The frequency of occurrence rate of heterozygous genotypes and alleles (%) of the NPHS1 gene in children with NS (n = 6).

Also, three heterogeneous mutant combinations were determined in the patient with minimal NS changes - c.1223G> A (p.Arg408Gln) in exon 10, s. 3315G> A (p. Ser1105Ser) in exon 26 and s. IVS27 + 45C> T in the intron region. In all 4 patients with congenital NS there were two combinations of a heterogeneous mutation of the nephrin gene: c.1223G> A (p.Arg408Gln) in exon 10 and c.3315G> A (p. Ser1105Ser) in exon 26; c.349G> A (p.E117K) in exon 3 and c.3315G> A (p. Ser1105Ser) in exon 26; c.1223G> A (p. Arg408Gln) in exon 10 and c. 3478C> T (p. Arg1160X) in exon 27; c.1223G> A (p.Arg408Gln) in exon 10 and c.3478C> T (p.Arg1160X) in exon 27.

Polymorphic markers of the podocin gene (NPHS2) were identified in 30 children, of which 24 (80.0%) had NS with minimal changes, 5 (16.7%) had steroid-resistant NS and 1 (3.3%) - congenital NS.

A study of the podocin gene polymorphism (NPHS2) in children with NS revealed a high homozygous mutation rate (Table 2).

The 5' untranslated region (5'-UTR) was determined in 10.0% of the cases in the heterozygous form.

The analysis of the podocin gene polymorphism (NPHS2) revealed two mutant combinations in 2 (6.7%) boys: a homozygous mutation c.102A> G (p.Gly34Gly) in exon 1, a homozygous mutation c.686G> A (p.Arg229Gln) in exon 5, a heterozygous

mutation c.954C> T (p.Ala318Ala) in exon 8 and a heterozygous mutation in the uncoded region 5'UTR (5'UTR-51G> T) in the first boy, and in the second boy, a homozygous mutation c.102A> G (p.Gly34Gly) in exon 1, homozygous mutation c.954C> T (p.Ala318Ala) in exon 8, heterozygous mutation in the uncoded 5'UTR region (5'UTR-51G> T) and in the intron region adjacent to exon 3, heterozygous displacement of nucleotides IVS3-46 C> T and IVS3-21C> T.

Table 2

Nucleotide substitutions in the NPHS2 gene in children with NS (n = 30)

Exon	Polymorphic markers	Nucleotide	Homozygous/ heterozygous	Patients n (%)
1	c.102A>G	p.Gly34Gly	GC**A**>GG**G** Homozygous	20 (66,7)
1	c.59C>T	p.Pro20Ley	C**C**G>C**T**G Homozygous	2 (6,7)
4	c.506T>C	p.Leu169Pro	C**T**C>C**C**C Homozygous	2 (6,7)
4	c.503G>A	p.Arg168His	C**G**T>C**A**T Homozygous	1 (3,3)
5	c.686G>A	p.Arg229Gln p.Arg229Gln	heterozygous C**G**A>C**A**A Homozygous	1 (3,3) 1 (3,3)
5	c.538G>A	p.Val180Met	**G**TG>**A**TG Homozygous	1 (3,3)
7	c.868G>A	p.Val290Met	**G**TG>**A**TG Homozygous	2 (6,7)
8	c.954C>T	p.Ala318Ala p.Ala318Ala	heterozygous GC**C**>GC Homozygous	2 (6,7) 12 (40,0)
5'UTR	5'UTR-51G>T		heterozygous	3 (10,0)
Intron				
	IVS3-46C>T		heterozygous	1
	IVS3-21C>T		heterozygous	2

In 1 (3.3%) girl, three mutant combinations were identified: homozygous mutation c.102A> G (p.Gly34Gly) in exon 1, heterozygous mutation c.954C> T (p.Ala318Ala) in exon 8 and heterozygous mutation in the unencoded 5'UTR region (5'UTR-51G> T). In 11 (36.6%) patients, two mutant combinations were determined. Moreover, in 10 (33.3%) patients, a combination that included a homozygous mutation c. 102A> G (p.Gly34Gly) in exon 1 and a heterozygous mutation c. 954C> T (p.Ala318Ala) in exon 8 was observed ,and in 1 (3.3%) boy, a homozygous mutation

c.954C> T (p.Ala318Ala) in exon 8 and heterozygous IVS3-21C> T mutation in the intron region adjacent to exon 3 was determined. 16 patients (53.3%) had one type of mutation. Homozygous mutation c.102A> G (p.Gly3Gly) in exon 1 occurred in 7 (23.3%) patients; homozygous mutation c.59C> T (r. Pro20Ley) in exon 1 - in 2 (6.7%) patients; homozygous mutation s.506T> C (p.Leu169Pro) in exon 4 - in 2 (6.7%) patients; homozygous mutation s.868G> A (p.Val290Met) in exon 7 - in 2 (6.7%) patients; homozygous mutation c.503G> A (p.Arg168His) in exon 4, homozygous mutation c.538G> A (p.Val180Met) in exon 5 and homozygous mutation c.686G> A (p.Arg229Gln) in exon 5 were noted in 1 (3.3%) of the patients, respectively.

The analysis of the frequency of genotypes and alleles of the podocin gene showed that the genotype A> G and the alleles A and G were more common (Fig. 4).

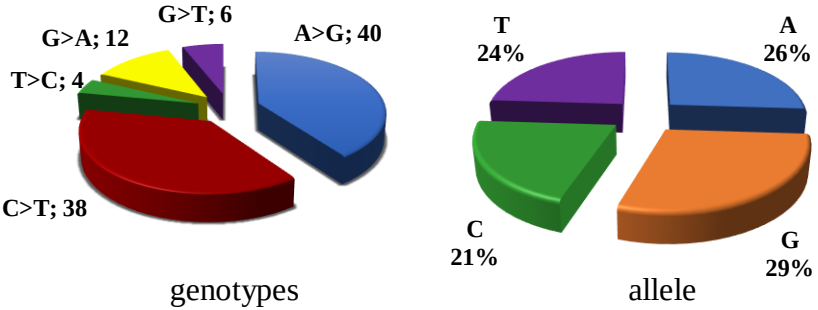


Fig. 4. The frequency of genotypes and alleles (%) of the NPHS2 gene in children with NS (n = 30).

In children with NS from the Azerbaijani population, the genotypes AG and CT of the podocin gene (NPHS2) were found with almost the same frequency, the GT and TC genotypes were less common. There was no significant difference in the frequency of alleles.

Among 30 examined children with NS with a podocin gene polymorphism, 24 (80.0%) patients had NS with minimal changes, 5 (16.7%) were steroid-resistant, and 1 (3.3%) patient had congenital NS (Table 3). In children with minimal changes in the NS, mutations

in the intron and the uncoded region were not determined, whereassuch mutations occurred in children with a steroid-resistant NS.

In a patient with congenital NS, four mutant combinations were determined: homozygous mutation c.102A> G (p.Gly34Gly) in exon 1, homozygous mutation c.686G> A (p.Arg229Gln) in exon 5, heterozygous mutation c.954C> T (p.Ala318Ala) in exon 8 and a heterozygous mutation in the uncoded region of 5'UTR (5'UTR-51G> T). Four mutant combinations were also observed in 1 patient with steroid-resistant NS: c.102A> G (p.Gly34Gly) in exon 1, heterozygous mutation c.954C> T (p.Ala318Ala) in exon 8, heterozygous mutation in the uncoded 5'UTR region (5'UTR-51G> T) and heterozygous nucleotide displacement IVS3-46 C> T and IVS3-21C> T (table 3).

Table 3

Nucleotide substitutions in the NPHS2 gene in children with various types of NS (n = 30)

Exon	polymorphic markers	Nucleotides	With minimal changes in NS (n = 24), abs	With steroid resistant NS. (n=5), abs	Congenital NS, (n=1), abs
1	c.102A>G	p.Gly34Gly	15	4	1
1	c.59C>T	p.Pro20Ley	2	-	-
4	c.506T>C	p.Leu169Pro	2	-	-
4	c.503G>A	p.Arg168His	1	-	-
5	c.686G>A	p.Arg229Gln hete p.Arg229Gln homo	- 1	-	1
5	c.538G>A	p.Val180Met	1	-	-
7	c.868G>A	p.Val290Met	2	-	
8	c.954C>T	p.Ala318Ala hete p.Ala318Ala homo	2 6	3 2	1
5'UTR	5'UTR-51G>T	heterozygous	-	2	1
	IVS3-46C>T	heterozygous	-	1	-
	IVS3-21C>T	heterozygous	-	2	-

In 1 girl with steroid-resistant NS, three mutant combinations were noted: homozygous mutation c.102A> G (p.Gly34Gly) in exon 1, heterozygous mutation c.954C> T (p.Ala318Ala) in exon 8, and

heterozygous mutation in the uncoded 5 'region UTR (5'UTR-51G> T).

Two mutant combinations: a homozygous mutation c.102A> G (p.Gly34Gly) in exon 1 and a heterozygous mutation c.954C> T (p.Ala318Ala) in exon 8 were observed in 8 patients with minimal changes. Similar two mutant combinations, as well as the mutant combination, heterozygous c.954C> T mutation (p.Ala318Ala) in exon 8 and heterozygous IVS3-21C> T nucleotide displacement were observed in 2 and 1 patients, respectively. 16 patients in whom one type of mutation was noted belonged to the group with minimal changes in NS. In 3 patients, polymorphism in the nephrine and the podocin gene was determined.

Therefore, in one boy with a congenital NS in the nephrine gene (NPHS1), a heterozygous combination of c.1223G> A (p.Arg408Gln) in exon 10 and c.3315G> A (p. Ser1105Ser) in exon 26 was observed, and in the podocin gene (NPHS2) there were four mutant combinations - homozygous mutation c.102A> G (p.Gly34Gly) in exon 1, homozygous mutation c.686G> A (p.Arg229Gln) in exon 5, heterozygous mutation c.954C> T (p.Ala318Ala) in exon 8 and a heterozygous mutation in the uncoded region of 5'UTR (5'UTR-51G> T). A heterozygous mutation in the NPHS1 gene c.1223G> A (p.Arg408Gln) in exon 10 and a homozygous mutation in the NPHS2 gene (c.102A> Gp.Gly34Gly in exon 1 and c.954C> Tp.Ala318Ala in exon 8 were revealed in a girl with minimal changes. In a girl with steroid-resistant NS, three mutant combinations of the NPHS1 gene (c. 1320C> T p. Pro440Pro in exon 11, c. 2289C> T p. Val763Val in exon 17 and c. 3230A> G p. Asn1077Ser in exon 24) and three mutant combinations in the NPHS2 gene (homozygous mutation c.102A> G (p.Gly34Gly) in exon 1, heterozygous mutation c.954C> T (p.Ala318Ala) in exon 8 and heterozygous mutation in the uncoded 5'UTR (5'UTR -51G> T)) were revealed. In 2 of the 3 cases, the same heterozygous mutation was noted in the NPHS1 gene - c.1223G> A (p.Arg408Gln) in exon 10. In all three patients, regardless of the type of NS, in the gene NPHS2, homozygous mutation p.102A> G (p.Gl y34Gly) in exon 1 and the heterozygous mutation c.954C> T (p.Ala318Ala) in exon 8

were revealed.

In 2 patients with minimal changes in NS, in 1 patient with steroid-resistant NS and in 1 patient with congenital NS, a mutation of the TRPC6 gene was revealed. Moreover, in patients with minimal changes in NS, there was a homozygous mutation in the NPHS2 gene (c.102A> Gp.Gly34Gly in exon 1 and c.954C> Tp.Ala318Ala in exon 8), and a homozygous mutation c.102A> G (p.Gly34Gly) in exon 1. In a patient with steroid-resistant NS, a homozygous mutation c.954C> T (p.Ala318Ala) in exon 8 and in a patient with congenital NS who had mutations innephrine and podocin, heterozygous displacement of nucleotides IVS3-21C> T were detected.

Thus, in children with NS from the Azerbaijani population, the genotypes AG and CT of the podocin gene (NPHS2) were found with a frequency of 40.0% and 38.0%, respectively. In 3 patients, polymorphism in the nephrine and the podocin gene was determined, and in a patient with congenital NS two mutant combinations in the NPHS1 gene and four mutant combinations in the NPHS2 gene; in a patient with minimal changes, two mutant combinations in the NPHS1 and NPHS2 genes, respectively; in a patient with SRNS, three mutant combinations of the NPHS1 gene and three mutant combinations in the NPHS2 gene were identified. In all three patients, regardless of the type of NS, the homozygous mutation c.102A> G (p.Gly34Gly) in exon 1 and the heterozygous mutation c.954C> T (p.Ala318Ala) in exon 8 were detected in the NPHS2 gene.

The results of a molecular genetic study in children with NS caused by CGN did not reveal homozygous mutations in the NPHS1 gene.

Morphological characteristics of CGN in children.

A transdcutaneous kidney biopsy was performed in 79 patients, 4.77 ± 1.23 months after the onset of the disease. The most common histopathological diagnosis was a form of mesangioproliferativeGN - IgA nephropathy. Of the 51 patients with a nephrotic form, who had undergonenephrobiopsy, 17 were determined FSGS, 16 - MCD, 15 -

IgA-nephropathy and 3 patients - membranous GN. In 14 patients with a hematuric form of CGN, IgA nephropathy was determined, and out of 14 examined children with a mixed form of CGN, 7 were determined by membranous GN and 7 by MPGN, respectively. Therefore, IgA nephropathy was detected in all nephrobiopathies in patients with a hematuric form (100%) and in 29.4% with a nephrotic form. In the mixed form of CGN, morphological variants — MPGN and membranous GN — were equally determined.

IgA nephropathy was detected in 19 (65.5%) boys and 10 (34.5%) girls. The age of the children ranged from 7 to 15 years, the average age was 11.82 ± 1.67 years. At the age of 7-10 there were 7 (41.6%) children, 11-15 years old - 22 (58.4%) children. The duration of the disease averaged 4.12 ± 1.77 years.

Mesangialhypercellularity was observed in 8 (27.6%) kidney biopsy specimens of children with IgAN, fibrous semilunasin 7 (24.1%), and sclerotic glomeruli in 14 (48.3%).

Considering that oxidative stress causes damage to cells and tissues, we analyzed the levels of MDA and AOS parameters in IgAN. Patients, depending on the signs of IgAN progression, were divided into 3 groups: group 1 - 8 patients whose mesangialhypercellularity predominated in nephrobiopathies, group 2 - 7 patients with a predominance of fibrous semilunas in nephrobiopsyspecimens, and group 3 - 14 patients with a predominance of sclerotic glomeruli. In patients with IgAN, with all signs of progression, the MDA level in red blood cells was statistically significantly ($p < 0.05$) higher than the control value. Moreover, in children with glomerular sclerosis, a more noticable difference was noted. In group 1 (with mesangial hypercellularity), the level of MDA was higher than in the control group by an average of 35.5% ($p < 0.05$), in group 2 (with fibrous semilunas) - by 39.1% ($p < 0.05$)) and in group 3 (with glomerular segmental sclerosis) - by 41.7% ($p < 0.05$). The maximum decrease in AOS indicators was also noted in group 3. The concentration of catalase in red blood cells in comparison with the control in groups 1, 2 and 3 was reduced by 19.0, 27.1 ($p < 0.05$) and 36.4% ($p < 0.05$), respectively. There was a decrease of 74.2% in the amount of reduced glutathione compared to

the control group ($p < 0.01$) in group 1, 72.2% ($p < 0.01$) in group 2 and 80.2% ($p < 0.01$) in group 3. The level of glutathione peroxidase in comparison with the control indicator in group 1 was reduced on average by 42.5% ($p < 0.05$), in groups 2 and 3 by 53.3% ($p < 0.01$) and 72.3% ($p < 0.01$), respectively. The content of another enzyme, glutathione reductase, decreasedalmost equally in patients of all three groups in comparison with the control group: in groups 1 and 2 by 98.1% ($p < 0.01$), respectively, in group 3 - by 92.7% ($p < 0.01$).

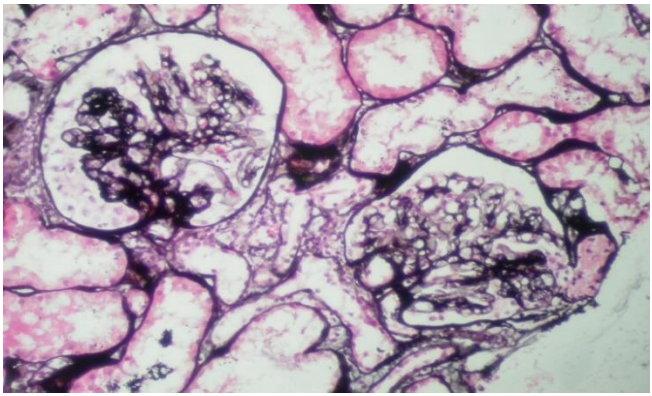
Thus, children suffering from IgAN experience increased oxidative stress, especially pronounced in the presence of glomerular segmental sclerosis.

The study of nephrobioptates revealed theMinimal Change Disease (MCD) in 16 patients with a nephroticform of GN. In patients with MCD, the level of MDA in red blood cells exceeded the control value by 34.9% ($p < 0.05$). The AOS parameters were lower than the values of the control group. The average catalase level compared to the control was 12.7%, reduced glutathione - 19.2%, glutathione peroxidase - 18.0% and glutathione reductase - 35.9% ($p < 0.05$) lower. A statistically significant difference was noted with respect to MDA and GR.

FSGS was detected in 17 patients. All children had a nephrotic form of CGN. Sclerosis of certain glomeruli was observed in 56.5%of (13) cases, sclerosis of 2nd and 3nd lobes of the glomerulus of the kidney in 43.5% (10) and complete glomerular damage in 26.1% of (6) cases. Morphological analysis of nephrobiopathies revealed 4 forms of FSGS. In 7 (41.2%) micropreparations, the classic version was determined. In these cases, focal and segmental consolidation of the tuft was revealed in the histograms, due to an increase in the extracellular matrix, obliterating the glomerular capillary lumen. Mild hypertrophy or hyperplasia of podocytes was also observed (microphoto 1).

The peripheral form of FSGS was determined in 5 (29.4%) of the patients. In this form, segmental lesions can be characterized either by endocapillaryhypercellularity or sclerosis. Foam cells are common. Hyalinosis is variable. Often podocyte hypertrophy / hyperplasia overlaps the involved segment. Mesangial

hypercellularity, glomerulomegaly, and arteriolar hyalinosis are variable results.



Microphoto 1. Segmental lesions of sclerosis in the depicted glomeruli. Sclerosis lesions are characterized by an increased matrix, causing obliteration of the capillary lumen. The distribution of lesions within the tuft is variable, affecting both peripheral and perichyllar segments (methenamine silver stain).

In the perihilarform of FSGS detected in 3 (17.6%) patients, often glomeruli with segmental lesions had perihilarhyalinosis with sclerosis. Glomerulomegaly and adhesions were also found, arteriolar hyalinosis was often determined, sometimes in combination with hyalinosis in the perihilar segment. Foam cells also had sclerotic lesions.

The content of MDA in erythrocytes in patients with different forms of FSGS did not practically differ from each other. Moreover, in comparison with the control value, there was a statistically significant difference. In patients with classical FSGS, the level of MDA in erythrocytes exceeded the control by 38.1% ($p < 0.05$), with the peripheral form- by 38.4% ($p < 0.05$), with perihilar and cellular by 39.1% ($p < 0.05$) and 39.8% ($p < 0.05$), respectively. The erythrocyte catalase level in patients with the classic FSGS form was lower than in the control group by 21.0% on average, with peripheral FSGS by 23.9%, and with the perihilar and cellular forms by 24.5%,

respectively. The amount of GSH in erythrocytes of patients with different morphological categories of FSGS was practically the same and lower than the value in the control group by, onaverageof 70.3% ($p < 0.01$), respectively. A comparative analysis revealed a statistically significant decrease in erythrocytes of GPO and GR in the classic version by 80.7% and 65.6% ($p < 0.01$), with peripheral, perihilar and cellular forms, respectively, by 81.1% and 66.9% ($p < 0.01$).

Thus, in examined patients with FSGS, sclerosis of certain glomeruli was observed in 47.1% of cases, sclerosis of 2nd and 3ndglomerular lobes in 35.3% and total glomerular damage in 17.6% of the cases. Within the morphological categories of FSGS, classical (41.2%) and peripheral (29.4%) ones were more frequent. Oxidative stress with different morphological forms of FSGS was equally observed.

Membrane proliferative GN was determined in 7 children with a mixed form of CGN within the ages of 11 - 15 years. A morphological study of nephrobiopathies revealed the proliferation of endothelial and mesangial cells, expansion of the mesangial matrix, thickening of the capillary walls due to subendothelial immune deposits and / or dense deposits directly from the membrane, the infiltration of mesangium into the capillary wall, resulting in double contours.

According to the results of the study, type I was determined (subendothelial arrangement of deposits), in 71.4% of cases (5 patients), while type II of MPGN (intmembrane arrangement),was determined in 28.6% of cases (2 patients).

The content of MDA in red blood cells in patients with type I and type II exceeded the control indicator by 41.9% ($p < 0.05$). The levels of catalase, reduced glutathione, GPO and GR were reduced lower than in the control group in patients with type I and type II MPGN, respectively, by 34.6 ($p < 0.05$) and 33.4% ($p < 0.05$) by; 72.2 ($p < 0.01$) and 70.3% ($p < 0.01$); by 78.1% ($p < 0.01$) and 77.2% ($p < 0.01$); 89.3 ($p < 0.01$) and 100% ($p < 0.01$).

Thus, in children with CGN, IgA - nephropathy occurred in 36.7% of the cases, FSGS in 21.5%, MCD in 20.2%, MPGN in 8.9%

and membranous glomerulopathyin 12.6% of the cases. Moreover, IgA - nephropathy was observed with the nephrotic form (19.0%)and hematuric form (17.7%), MCD - with the nephrotic form of CGN (20.2%), FSGS - with nephrotic form (21.5%), MPGN and membranous glomerulopathy - in patients with mixed CGN (8.9%, respectively).

In the morphological form of IgAN, mesangialhypercellularity was determined in 27.6% of the nephrobioptates, fibrous semilunas in 24.1%, and sclerotic glomeruli in 48.3. In FSGS, sclerosis of certain glomeruli was determined in 56.5% of the patients, sclerosis of 2nd and 3nd glomerular lobes in 43.5% of the patients, and glomeruli were completely affected in 26.1% of the patients. Within morphological forms of FSGS, the classical form was noted in 41.2%, the peripheral form - in 29.4% of cases, the perihilarform - in 17.6%, and the cellular form - in 11.7% of the cases. According to the analysis of nephrobiopathies with MPGN, type I was determined in 71.4% of the patients, and type II MPGN in 28.6% of the patients. The level of OS markers between the types of MPGN did not differ significantly. According to our data, stage 1 of membranous GN was determined in 70.0% of the patients, stage 2 - in 30.0% of the children.

The results of the study shown in Fig. 5.

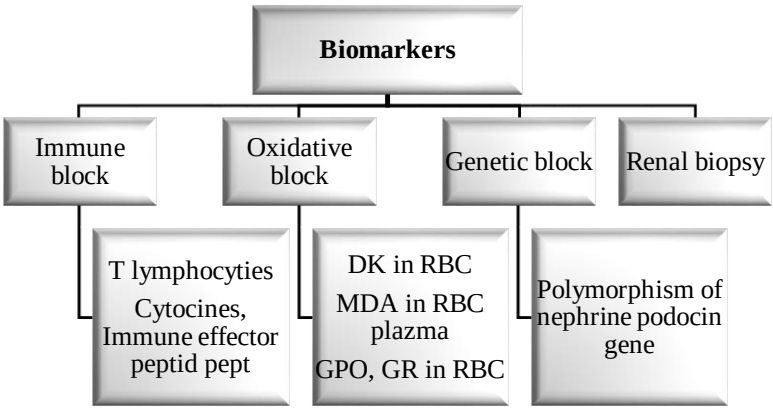


Fig. 5. Biomarkers recommended for the determination of chronic glomerulonephritis in children.

Thus, in the pathogenesis of CGN in children, the following are significant:

Immune factors. Inflammation, which is often immuno-mediated, is central in the pathogenesis of GN. Glomeruli have three properties that make them vulnerable to an immunological attack:

Glomeruli :

-“filter” and retain immune complexes

- contain immunologically competent cells capable of processing theantigen

- have structures such as a glomerular basement membrane and mesangial cells that can act as “targets” for the antibody.

The circulating immune complex. Deposition of immune complexes in the glomeruli, which leads to inflammation.

Cell-mediated immune GN. Cell-mediated immune mechanisms. The direct role of T-cells in mediating proteinuria andinthe formation of semilunar.

Immune damage Mediators. Chronic damage. The dominant cells are monocytes / macrophages and T-cells. The main mechanism for attracting these cells is the secretion of cytokines and the expression of leukocyte adhesion molecules by local endothelial and resident cells. Cytokines contribute to the activation of inflammatory cells, directly causing tissue damage.

Oxidative stress.

Genetic factors. Nephrotic syndrome.Mutations in the nephrine and the podocin.

As a result of the study, the interactions of T-lymphocytes, cytokines, and OS markers were revealed, and the differences in the cytokine profile and the enzymes of the glutathione cycle, as well as morphological variants in children with different forms of CGN were determined.

FINDINGS

1. Significant predictors of the CGN progression in the nephrotic form are reduced GFR, hypoproteinemia, hypoalbuminemia, hypercholesterolemia, hypercreatinemia, proteinuria; inthehematuric

form - proteinuria, hematuria; in the mixed form - reduced GFR, hypertension, hypercholesterolemia and hypercreatininemia [6].

2. In children with CGN there is an imbalance in the pro- and antioxidant systems, which contribute to the development of oxidative stress, leading to the destruction of cell structures. A change in the performance of the glutathione system is expressed in a decrease in the level of reduced glutathione, glutathione peroxidase and glutathione reductase in plasma and thered blood cells. The mostremarkable decreases are found in the concentration of glutathione peroxidase and glutathione reductase [1,4,5].

3. Biomarkers that can be easily monitored and used for non-invasive detection of redox disorders in children with CGN are malondialdehyde, glutathione peroxidase and glutathione reductase in the red blood cells [8, 12, 13].

4. A statistically significantandadirect relationship was established between the activity of catalase and glutathione peroxidase ($r = 0.611$, $p = 0.53$; $R2 = 0.373$) in the red blood cells in patients with a 3rd degree nephrotic syndrome activity. A moderate and noticeable relationship was found between MDA-GPO ($R2 = 0.347$), MDA-GR ($R2 = 0.509$) [13].

5. In children with CGN, immunehyperreactivity is manifested by a statistically significant increase in T-helpers, the apoptosis marker, parameters of humoral immunity, the level of CICs, pro-inflammatory cytokines and a decrease in T-killers, B-cells and the phagocytic activity. Innephrotic and mixed forms of CGN, a more pronounced degree of changes in immunoglobulins takes place [9, 16].

6. In all clinical forms of CGN, proinflammatory cytokines and a Th1 immune response predominate. A change in cytokine regulation is manifested by an imbalance of pro- and anti-inflammatory cytokines, activation of lipid peroxidation and a decrease in the antioxidant activity [10].

7. The most significant relationships were found between CD95 and MDA, CIC / MDA, IL-1β / MDA, IL-8 / MDA, TNF-α / MDA, TNF-α / GR, which indicate a change in the activity of immune reactions, LPO reactions and antioxidant defense and the close

relationship between them [2, 11].

8. In patients with a hematuric and a mixed form of CGN, IgAN, the level of antimicrobial proteins increased with exacerbation [15, 21, 25, 26].

9. The hypersensitivity, specificity and effectiveness of the investigated cytokines was observed with increasing levels of TNF-α and IL-1β. The revealed imbalance of cytokines, correlating with a moderate feedback with indicators of phagocytic activity, may affect the pathogenetic mechanism of CGN. A statistically significant increase in IL-1β, TNF-α and IFN-γ in the blood and a decrease in phagocytic activity contributes to the chronic course of the inflammatory process and autoimmune processes [20,28].

10. In children with the nephrotic syndrome from the Azerbaijani population, the GA genotype (61.5%) and the G allele (42.3%) of the nephrine gene (NPHS1) and the genotypes A> G (40%) and C> T (38%) of podocin gene (NPHS2) dominated [14, 17, 18, 19, 22, 23, 24, 27].

11. In children with CGN, IgA - nephropathy occurred in 36.7% of the cases, focal segmental glomerulosclerosis in 21.5% of the cases, minimal changes disease in 20.2% of the cases, membranous disease in 12.6% and membrane proliferative glomerulonephritis in 8.9% of the cases. IgA - nephropathy was observed in the nephrotic form (19.0%) and the hematuric form (17.7%), minimal changes disease - in the nephrotic form of CGN (20.2%), focal segmental glomerulosclerosis - in the nephrotic form (21.5%)), membrane proliferative (8.9%) and membranous glomerulonephritis (8.9%) - in patients with mixed CGN [3, 12].

12. The diagnostic algorithm and the examination program for children with all forms of CGN, as well as the generally accepted approach, includes a comprehensive determination of cytokines, markers of oxidative stress, enzymes of the glutathione cycle, antimicrobial proteins, polymorphism of nephrin and podocin genes [13].

PRACTICAL RECOMMENDATIONS

1. Monitoring OS biomarker levels seems to be a promising way to improve modern diagnostic methods for CGN.

2. To assess the course of CGN in children, along with the determination of the level of cytokines in the blood, the determination of the index of the ratio of pro- and anti-inflammatory cytokines is also recommended.

3. Erythrocytes CD and MDA and glutathione cycle enzymes evaluation, as well as the determination of lactoferrin and defensin is appropriate in assessing the degree of the pathological process for a comprehensive examination of children with CGN.

4. For the differential diagnosis of various forms of CGN in children it is appropriate to determine antimicrobial proteins and 3-nitrotyrosine.

5. A genetic study of the mutations of podocyte genes in Azerbaijani children is advisable.

6. The use of a complex algorithm of morphometric, functional and immunological parameters as diagnostic parameters contributes to the improvement of CGN diagnostics.

LIST OF PAPERS PUBLISHED ON THE TOPIC OF THE DISSERTATION

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LIST OF ABBREVIATIONS

AMP	antimicrobial peptides
AOS	antioxidant system
BP	blood pressure
CD	conjugated dienes
CIC	circulating immune complexes
CGN	chronic glomerulonephritis
CI	cytokine index
ELISA	enzyme-linked immunosorbent assay
FSGS	focal segmental glomerulosclerosis
GFR	glomerular filtration rate
GN	glomerulonephritis
GPO	glutathione peroxidase
GR	glutathione reductase
GSH	glutathione - reduced glutathione
Ig	immunoglobulins
IgA-H	immunoglobulin A nephropathy
IL	interleukin
LPO	lipid peroxidation
MCD	minimal change disease
MDA	malondialdehyde
MPGN	membrane proliferative glomerulonephritis
NBT	nitro blue tetrazolium
NS	nephrotic syndrome
NPHS1	nephrine
NPHS2	podocin
OS	oxidative stress
TNF-α	tumor necrosis factor-α.

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