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Cytokine status and oxidative stress in children with chronic glomerulonephritis

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Abstract
The key place in the development of glomerulonephritis is caused by immune reactions of the body to infections and allergens. There are data on the role of cytokines in the pathogenesis of various forms of glomerulonephritis. It was discovered that cytokines participate in the regulation of the work of immune competent cells in the infectious inflammatory process, regulating the nature and duration of the inflammation, immune response and determining the extent of the disease.

Keywords: glomerulonephritis, kidney, cytokines, CGN activity, oxidative stress

Introduction
The Glomerulonephritis disease in children is one of the most problematic aspect in modern clinical nephrology. According to statistical data, this disease is often the cause of the development of chronic kidney failure and patients with glomerulonephritis consist of the main contingent of chronic hemodialysis and kidney transplantation [6, 9].
The key place in the development of glomerulonephritis is caused by immune reactions of the body to infections and allergens. There are data on the role of cytokines in the pathogenesis of various forms of glomerulonephritis [1, 2]. It was discovered that cytokines participate in the regulation of the work of immunocompetent cells in the infectious inflammatory process, regulating the nature and duration of the inflammation, immune response and determining the extent of the disease [1, 5, 8].
Oxidative stress also participates in the pathogenesis of chronic glomerulonephritis (CGN), which has been shown in a number of studies [4, 10]. Oxidation-reduction mechanisms play an important role in strengthening the chronic process in kidneys. The literature presents data on pronounced changes in oxidative stress in various kidney diseases, including glomerulonephritis [11, 12].
The study of the interconnectedness of cytokines with indicators of oxidative stress in children with CGN will provide an opportunity to understand the pathogenesis of this disease better which, in turn, will help to improve existing treatment of regimens. All of the above indicates the need for further research.
The aim of the study was to evaluate the cytokine profile and indices of oxidative stress and their relationship in children with different CGN variants.

Material and methods of investigation
The study included 288 children with CGN. Boys - 186 (64.6%), girls - 102 (35.4%). The age of the children ranged from 5 to 16 years (mean age - 10.63 ± 3.88 years). The stage of remission was noted in 130 (45.1%), exacerbations in 158 (54.9%) patients. In the form of CGN, sick children were divided into 3 groups: Group I - 104 (36.1%) children with nephrotic form. Group II - 96 (33.3%) children with hematuric form. Group III - 88 (30.6%) children with mixed form. The control group consisted of 30 practically healthy children of the same age (mean age - 10.7 ± 5.11 years).
The classification of glomerulonephritis GN was used. Speranskii *et al*. (1966), with the additions of M.S. Ignatova and Yu.E. Veltischeva (1989).
The children survey approved the existence of an anamnesis of the present disease. The timing of the onset of the disease, the initial symptoms of the kidney pathology and the data on the transferred diseases were taken into account.
Clinical laboratory and instrumental methods of investigation (ultrasound) have been applied.
The concentration of cytokines (IL-1β, IL-8, TNF-α, IFN-α, IL-10, IL-4) was determined by enzyme immunoassay using the appropriate enzyme-linked immunoassay test systems manufactured by Vector-Best CJSC (Russia, Novosibirsk).
The intensity of lipid peroxidation (LPO) is estimated by the level of diene conjugates (DC) by spectrophotometry (Kagan V.E. *et al*., 1986) and malonic dialdehyde (MDA) in a test with thiobarbituric acid (Andreeva LI, Kozhemyakin L. A., 1988). The state of the antioxidant system (AOC) was determined by catalase activity (Korolyuk MA *et al*., 1988), glutathione reductase (Ellman GL, 1959), glutathione peroxidase (Gavrilova AR, Khmara NF, 1986), glutathione reductase (Paglia B., Walentine W., 1967). Blood plasma was used as material for study. Studies were conducted in the stage of exacerbation and remission.
Statistical processing of the results was carried out using standard packages of the program Statistica version 6.0 (USA). The mean square deviation, the confidence interval by the Student's criterion, the correlation coefficients between the main parameters were determined.
The correlation relation was calculated using the Spearman rank correlation coefficient. Statistically significant differences were considered $p < 0.05$.

Results and discussion
According to the history of the disease, 29 (27.9%) children

had hereditary complications of kidney disease, parents suffered from arterial hypertension in 15 (14.4%) children, and mothers (42.3%) underwent acute respiratory viral infection during pregnancy, 11 (10.5%) - pregnancy was complicated by preeclampsia, in 10 (9.6%) - anemia. 7 (6.7%) of children were born prematurely, 5 (4.8%) with asphyxia. Before the diagnosis of CGN in most children (78.8%), changes were observed in the general analysis of urine in the form of microproteinuria - in 11.5%, in microhematuria - in 16.3%, in oxaluria in 28.8%, in uraturia in 22.1% % of patients. In 5 (4.8%) of the patients examined previously, pyeloectasia was detected, in 3 (2.9%) - vesicorenal reflux. Chronic infectious foci were present in 70 (67.3%) children, of which 33 (31.7%) had chronic tonsillitis, 18 (17.3%) had caries, 11 (10.6%) had adenoids and in 8 (7, 7%) children - rhinitis. 49 (47.1%) patients had frequent SARS, 6 (5.8%) had bronchitis. 51 (49.0%) the child of 104 had an accompanying pathology. The diseases of the gastrointestinal tract suffered 19 (18.3%), allergic diseases - 20 (19.2%). In 7,7% of cases (8 children) mitral valve prolapse was detected, in 3.8% of cases (4 patients) - bronchial asthma. On examination, 44 (42.3%) children had swelling, and 50 (48.1%) had high blood pressure. According to ultrasound, 53.8% of the cases showed a consolidation of the renal parenchyma, in 46.2% of cases - an increase in the volume of the kidneys.

At the stage of exacerbation proteinuria in patients of groups I and III reached 9.9 ± 2.77 g / l, during the remission period - 1.65 ± 0.40 g / l.

When assessing the cytokine status changes in immunological reactivity were observed during periods of remission and exacerbation of the disease (Table 1).

Table 1: Level of cytokines in different stages of CGN activity in children

Groups	Stages of activity	Cytokines, pg / ml					
		il-1β	il-8	TNF-α	γ-IFN	il-4	il-10
I group (n=104)	remission (n=48)	7,92± 1,27*	71,88± 3,14*	31,44±1,88*	16,78± 1,05*	12,20± 2,33	38,52±2,06
	exacerbation (n=56)	33,14± 1,89*,**	79,46± 2,88*	39,17± 3,56*	17,72± 3,04*	13,77± 2,16*	40,11±2,77*
II group (n=96)	remission (n=53)	6,31± 2,02*	70,11± 2,67*	30,62± 3,48*	16,44± 1,37*	13,02± 1,34	36,28±2,49
	exacerbation (n=43)	22,17± 3,15*,**	73,38± 2,54*	35,06± 1,52*	17,21± 2,10*	14,20± 1,07*	37,20±2,22
III group (n=88)	remission (n=29)	7,53± 1,08*	72,17± 1,82*	33,18± 1,40*	17,0± 1,48*	12,11± 1,14	38,40±1,77
	exacerbation (n=59)	34,07± 3,16*,**	79,88± 2,17*	40,29± 2,16*	18,11± 3,26*	13,90± 2,18*	41,13±2,46*
Control group (n=30)		3,67±0,94	50,24± 2,13	7,93± 1,06	9,58± 0,78	10,0± 0,67	28,83±2,15

Note: * - statistical reliability of differences with the control group;
** - between stages of CGN (p <0, 05-0,001)

During the exacerbation period, patients with different variants of CGN showed more pronounced changes in the level of cytokines. In this case, particularly pronounced shifts were shown in the content of IL-1β. In children with nephrotic CGN in the stage of exacerbation, the level of IL-1β was 4.2 times higher in the remission period (p <0.01), in children with hematuric CGN - by 3.5 times (p <0.01), with mixed CGN - 4.5 times (p <0.01). At the same time, the level of IL-1β both in the acute stage and in the remission period was significantly higher than in the control group in group I, in 2.1 (p <0.05) and 9.0 times (p <0.001), in group II - in 3.0 (p <0.01) and 6.0 times (p <0.001), in group III - in 2.0 (p <0.05) and 9.3 times (p <0.001). In the patients examined, the level of IL-8, TNF-α and IFN-γ during the remission and exacerbation was also statistically different from the control ones. The number of indicators increased at the stage of exacerbation, but there were no significant differences with the magnitude in the stage of clinical remission. In children with a nephrotic and mixed variant of CGN, in comparison with the control index, the increase in the level of IL-8 during the exacerbation and outside it was 1.6 and 1.4 times (p <0.05), with a hematuric form of 1.5 and 1, 4 times (p <0.05), respectively. The content of TNF-α in the blood of children of the I group in the acute stage exceeded the control ones by 4.9 times (p <0.01), in the stage of remission - by 4.0 times (p <0.01), in group II patients - in 4.4 and 3.9 times (p <0.01), in group III - 5.1 and 4.2 times (p <0.001), respectively. Comparative analysis of the level of γ-IFN with a control value in the I and II groups examined in the stage of exacerbation and remission was higher in 1.8 and 1.7 times (p <0.05), group III in 1.9 and 1.8 times (p <0.05), respectively.

The content of IL-4 in children's bloods with different CGN variants during the exacerbation was significantly higher than the control (p <0.05), and the level of IL-10 was higher than the control level in patients with nephrotic and mixed CGN variant (p <0.05). The analysis showed that the difference between the control value of the level of IL-4 and IL-10 was 1.4 times in patients of all the examined groups (p <0.05). A parallel increase in anti-inflammatory IL-4 and IL-10 cytokines may have resisted inflammation, and we do not rule out its adaptive nature.

In general, the most significant differences were found in the content of IL-1β and TNF-α.

One of the main factors in the development of pathological conditions is oxidative stress. Studies have shown an increase in free radical oxidation products and a decrease in antioxidant

protection in the examined patients (Table 2)

Table 2: Concentrations of indices of oxidative stress in blood plasma in children with CGN

Groups	Stages of activity	indicators					
		DC, µmol / ml	MDA, µmol / l	Catalase U / ml	GSH, nmol / ml	GPO, nmol / min per 1 mg of protein	GR, nmol / min per 1 mg of protein
I group (n=104)	Remission (n=48)	0,90±0,54	2,06±0,92	47,0±2,12	17,0±2,08	1,74±0,09	0,36±0,08
	exacerbation (n=56)	1,01±0,33	2,41±0,69*	45,6±1,91	16,3±1,40	1,65±0,05	0,30±0,04*
II group (n=96)	Remission (n=53)	0,82±0,38	2,56±0,48*	53,0±2,04	13,8±1,46*	1,31±0,04*	0,25±0,04*
	exacerbation (n=43)	0,86±0,42	2,9±0,77*	51,26±1,07	12,8±1,38*	1,30±0,07*	0,22±0,06*
III group (n=88)	Remission (n=29)	0,87±0,34	2,55±0,18*	45,65±0,88	15,60±1,14	1,55±0,03	0,26±0,04*
	exacerbation (n=59)	0,94±0,62	2,70±0,44*	44,74±1,48	15,18±1,26	1,54±0,06	0,25±0,07*
Control group (n=30)		0,80±0,11	1,60±0,34	55,6±1,90	18,1±1,84	1,94±0,04	0,42±0,03

Note: * - statistical reliability of differences with the control group;
** - between stages of CGN ($p < 0.05$)

As can be seen from Table 2, in children with CGN in comparison with the control value there is an increased content of DC, but no significant difference was found. With exacerbation, the concentration of DC in the blood increased, but there was no significant difference between the magnitude outside the exacerbation. The concentration of MDA in children with CGN was significantly increased ($p < 0.05$) in comparison with the control value. With exacerbation, the MDA level increased, but there was no major difference with respect to the magnitude during the remission period.

While studying the indicators of antioxidant protection, their imbalance was revealed. In children with CGN, catalase activity decreased, both in the acute stage and in the period of remission. At the same time, there was no statistically significant difference between the indices in these stages and the control ones. The content of other parameters of the antioxidant system in children with different degrees of activity decreased as the disease got worse. A significant decrease in reduced glutathione (GSH) and glutathione peroxidase (GPO) relative to the control value ($p < 0.05$) was noted in patients with a hematuric variant of CGN in an acute and non-acute stage. The concentration of glutathione reductase (GR) in patients with nephrotic form of CGN was reduced 1.4 times ($p < 0.05$) in comparison with the control one. In children with a hematuric and mixed variant of the disease, the level of GH with respect to the control group was significantly lower ($p < 0.05$) in both the remission and the exacerbation.

As a result, for children with different variants of CGN, rise in the level of cytokines was characteristic, both in acute and remission cases, as well as in the plasma MDA concentrations and a decrease in the antioxidant system, i.e. violation of prooxidant equilibrium.

Having evaluated the relationship between cytokines and oxidative stress indices during remission, a number of statistically significant correlations were revealed. In the nephrotic variant of CGN in children, the most significant interrelationship were found between 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 / GH ($r = -0.488$, $p < 0.01$). In patients with a hematuric variant of CGN, the correlation coefficient for IL-1β / MDA was $r = + 0.238$ ($p < 0.05$), for TNF-α / MDA-r = + 0,316 ($p < 0$) between cytokine levels and oxidative stress indices, 05), for TNF-α / GSH-r = -0.205 ($p < 0.05$), for TNF-α / GPO-r = -0.197 ($p < 0.05$), for TNF-α / GR-r = -0.326 ($p < 0.05$), for IFN-□ / MDA-r = 0.244 ($p < 0.05$). In children with mixed CGN, reliable correlation between IL-1β and MDA levels ($r = 0.401$, $p < 0.05$), IL-1β and GH ($r = -0.241$, $p < 0.05$), TNF-α / MDA ($r = 0.312$, $p < 0.05$), TNF-α / GH-r = -0.340 ($p < 0.05$). Apparently, the correlation dependence was observed between proinflammatory cytokines - IL-1β and TNF-α and MDA, as well as antioxidant system indices.

Consequently the revealed correlation relationships in children with a different variant of CGN between cytokine levels and oxidative stress indices suggest the effect of the cytokine system on the state of cellular mitochondria.

According to the literature, oxidative stress can improve the activation of the system of proinflammatory cytokines [3, 7]. According to the results of several studies, the activity of free radical oxidation process and the development of oxidative stress are the links in the pathogenesis diseases of the urinary system. It has been established that the main process leading to damage and destruction of membranes is a significant activation of lipid peroxidation and a decrease in the level of antioxidants in the blood [4].

It is now established that the plasma membrane of cells includes a receptor-dependent enzyme system for generation of hydrogen peroxide which is stimulated by cytokines [7]. Through the fault of free radicals, many cells appear in the body that have undergone mutations and damage which leads to increased functioning of the immune system, often it can not cope with such a number of damaged cells and as a result, an inflammatory process that leads to chronic diseases occurs [3]. Products of lipid peroxidation have a cytotoxic effect, promote activation of immune mechanisms that are mediated by pro-inflammatory cytokines [3, 4].

Our results support the view that oxidative stress and changes in the immune-inflammatory character are linked in the pathogenesis of chronic glomerulonephritis and there is a relationship between them, they can activate each other [7, 8]. It can be assumed that the revealed shifts in the cytokine profile and the free radical process, as well as the imbalance in the antioxidant system, aggravate the inflammation of the renal glomeruli.

Conclusions

An increase in circulating cytokines and MDA, as well as a

decrease in the parameters of the antioxidant system, confirm the participation of immunoinflammatory mechanisms and oxidative stress in the pathogenesis of CGN.

The revealed correlation links in children with a different variant of CGN between cytokine levels and oxidative stress indicators suggest that the mutual influence of the cytokine system and oxidative stress on CGN flow.

Determination of circulating cytokines and parameters of oxidative stress is necessary for a commensurate assessment of the compensatory capabilities of the organism.

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