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THE ROLE OF INTERLEUKIN-1β AND INTERLEUKIN-6 ON CLINICAL EVOLUTION IN CHILDREN WITH GLOMERULONEPHRITIS

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SUMMARY

Key words: IL-1β, IL-6, glomerulonephritis, children.
The aim of the study is to evaluate the urinary concentration ofinterleukins (IL)-1β and IL-6 in children with glomerulonephritis in different stages of the disease. The prospective study of them data on 75 patients with glomerulonephritis, who were examined during the exacerbation and remission. The control group consisted of 20 healthy children. Determination of urinary levels of IL-1β and IL-6 is a non-invasive predictive method for assessing disease activity and monitoring disease progression in children with different glomerulonephritis forms.

РЕЗЮМЕ

РОЛЬ ИНТЕРЛЕЙКИНА-1β И ИНТЕРЛЕЙКИНА-6 В КЛИНИЧЕСКОЙ ЭВОЛЮЦИИ У ДЕТЕЙ С ГЛОМЕРУЛОНЕФРИТОМ

Ключевые слова: IL-1β, IL-6, гломерулонефрит, дети.
Целью исследования является оценка концентрации интерлейкина (IL)-1β и IL-6 в моче у детей с гломерулонефритом в различные клинические стадиях заболевания. В проспективное исследование были включены данные о 75 пациентах с гломерулонефритом, которые были обследованы в период обострения и ремиссии. Определение уровня IL-1β и IL-6 в моче является неинвазивным прогностическим методом для оценки активности заболевания и мониторинга прогрессирования заболевания у детей с различными формами гломерулонефрита.

REZUMAT

ROLUL INTERLEUKINEI-1β ȘI INTERLEUKINEI-6 ÎN EVOLUȚIA CLINICĂ LA COPIII CU GLOMERULONEFRITĂ

Cuvinte-cheie: IL-1β, IL-6, glomerulonefrită, copii.
Scopul studiului a fost evaluarea concentrației urinare a interleukinelor (IL) -1β și IL-6 la copiii cu glomerulonefrită la diferite stadii clinico-evolutive ale bolii. Studiul prospectiv a inclus 75 de pacienţi cu glomerulonefrită, care au fost examinați în perioada acutizării și a remisiunii bolii. Grupul de control l-au constituit 20 de copii practic sănătoși. Determinarea concentrațiilor urinare ale IL-1β și IL-6 reprezintă o metodă non-invazivă predictivă pentru estimarea activității maladiei și monitorizarea evoluţiei bolii la copiii cu diferite variante ale glomerulonefritei.

Introduction.
Nephrotic syndrome (NS) is the commonest glomerular disease in children. The estimated incidence of NS constitutes 1-16 cases per 100000 children, which vary by ethnicity and regional aspects [1]. In recent years, clinical and experimental studies provided clear evidence that pro-inflammatory cytokines IL-1β and IL-6 are involved in pathophysiology of idiopathic nephrotic syndrome INS [2]. IL-1β plays an important role in acute inflammation, it is mainly synthesized by activated macrophages (antigenically stimulated), lymphocytes B and NK cells (natural killer) that act not only on the glomerulonephritis progression by stimulating the synthesis of cell adhesion molecules, but also participates in the formation of renal interstitium fibrosis [3].
During the course of the experimental nephrotoxic nephritis (NTN) animal model, it has been determined that the role of IL-1, tumor necrosis factor (TNF), and IL-1R is the cause of the decline of glomerular function [4]. High expression of key protein genes related to the

formation of inflammasomes, such as renal dendritic cells, IL-1β, was detected in the cells of nephrotoxic nephritis animal model [5]. IL-1β can stimulate the growth of eicosanoid synthesis. The latter can pass through the endothelial cells, affecting negatively charged podocytes that usually prevent the passage of albumin, thus causing their neutralization and proteinuria [6].

Interleukin-6 (IL-6) is a pleiotropic cytokine that not only regulates the immune and inflammatory response but also affects hematopoiesis, metabolism, and organ development. IL-6 can simultaneously elicit distinct or even contradictory physiopathological processes, which is likely discriminated by the signaling pathways, termed classic and trans-signaling [7].

Recent studies have shown that IL-6 plays a major role in acute and chronic inflammation stages. IL-6 is synthesized by endothelial and mesangial cells in response to a wide variety of agonists such as: Ag, mitogens, endotoxins, IL-1, and TNF-α. IL-6 cytokine family members were increased in the renal tissue of patients with kidney diseases, including: glomerulonephritis, diabetic nephropathy and obstructive nephropathy. Kidney cells that express and secrete IL-6 cytokine family members include podocytes, endothelial cells, mesangial cells and tubular epithelial cells. In these cell types, IL-6 cytokine family member can promote cell proliferation, impact differentiation or increase tubulointerstitial fibrosis [7].

The aim of the study was to evaluate the urinary concentration of IL-1β and IL-6 at clinical and evolutionary stages in children with glomerulonephritis.

Material and methods.

The study included 75 children with glomerulonephritis: 20 children with steroid-sensitive nephrotic syndrome (SSNS), 15 with steroid-resistant nephrotic syndrome (SRNS), 20 with chronic glomerulonephritis (CGN) nephrotic form, and 20 with mixed form of glomerulonephritis. At the first visit, all patients were treated with prednisolone, administered dose - 2 mg/kg/24h over a period of 6-8 weeks. Exclusion criteria included patients with partial remission, congenital nephrotic syndrome, secondary nephrotic syndrome, glomerular filtration rate (<60 ml/min/1,73 m^2), acute infections and allergic diseases.

The children were divided into 2 groups in accordance with glucocorticoid response to the therapy: SSNS and SRNS. Patients with SSNS and SRNS were divided into 2 groups according to the disease activity (SSNS relapse, SSNSremission, SRNS relapse, SRNS remission). The control group included 20 practically healthy children.

The research was carried out at Mother and Child Institute and Nicolae Testemitanu SUMPh Biochemistry Laboratory based on the biological samples collected according to the contemporary research principles, approved by Nicolae Testemitanu SUMPh Research Ethics Committee. Nephrotic syndrome was diagnosed in children with edema, massive proteinuria (> 40 mg/m^2/h or urinary protein /creatinine ratio> 2,0 mg/mg) and hypoalbuminemia (<2,5 mg/dl) [8].

Urinary levels of IL-1β and IL-6 was determined by the ELISA method using the PeproTech Company (USA) sandwich mini-ELISA kit, according to the attached instructions.

Statistical methods were used to assess the significant difference between the studied indices of the compared groups, by estimating the average arithmetic mean [X], the mean square deviation, and the average error of the average arithmetic mean [± m]. Also, the non-parametric statistical test "U Mann-Whitney" and the significance threshold p <0,05.

Results.

Clinical manifestations in children with NS at the onset of the disease were generalized edema, oligoanuria, abdominal pain and the lumbar region. The estimation of biochemical markers in plasma revealed in all subgroups of patients with NS such symptoms as hypoproteinemia,

Table 1. Urinary levels of IL-1β and IL-6 in children with glomerulonephritis (pg/mM creatinine)

Study groups	IL-1β		IL-6	
	exacerbation	remission	exacerbation	remission
SSNS	21,9±1,57*** 192,1 %	15,3±0,84** 134,2 %, p_1<0,01	28,7±1,51*** 302,1 %	60,4±2,87*** 635,8%, p_1<0,001
SRNS	31,6±2,94*** 277,2 %, p_2<0,05	-	39,7±2,20*** 417,9%, p_2<0,01	-
CGN nephrotic form	23,1±4,73* 202,6%	18,2±2,53 159,6 %, p_1>0,05	46,6±9,99*** 490,5%	30,3±3,30*** 318,9%, p_1>0,5
CGN mixt form	30,7±3,38*** 269,3 %	12,4±0,64 108,8%, p_1<0,001	51,8±8,19*** 545,3%	24,5±2,11*** 257,9%, p_1<0,01
Controls	11,4±0,45		9,5±0,41	

Note: statistically significant difference compared to the control group values: * p <0.05; ** p <0.01; *** p <0.001. p_1 - the authenticity in comparison with the respective index registered at the acute phase; p_2 - authenticity when comparing SSNS with SRNS.

disturbance of lipid metabolism indices, and proteinuria > 3,5 g/l. The dynamics of IL-1β and IL-6 levels in urine of children with glomerulonephritis have been were assessed. The evaluation results of the concentration of IL-1β and IL-6 in urine of children with glomerulonephritis are shown in table 1.

According to the obtained results, can be concluded, that the presence of alterations in the urinary level of IL-1β is more pronounced in patients with CGN, compared with those with acute glomerulonephritis (AGN). At the same time, there were higher mean values of IL-1β concentration in the urine of patients with SRNS compared to those with SSNS. Urinary IL-1β levels increased 2,8 times (up to 31,6 ± 2,94 μg/mM creatinine) in patients with SRNS at onset, whereas in those with SSNS it increased 1,9 times, (up to 21,9 ± 1,57 μg /mM creatinine), compared to the control values.

In CGN mixed form, during the period of exacerbation, the urine level of IL-1β increased 2,7 times (up to 30,7±3,38 pg/mM creatinine), while in CGN, nephrotic form, increased 2,0 times (up to 23,1±4,73 μg/mM creatinine) versus 11,4±0,45 μg/mM creatinine, in the control group. At the same time, strong negative correlations between IL-1β and IL-6 levels were recorded (r_{xy} = -0,731, p<0,01). During the remission of CGN the mixed form, urine concentration of IL-1β reached the control group level, whereas in CGN nephrotic form, the IL-1β level remained high. Data presented in table1 showed that patients with SRNS had significantly higher mean levels of IL-6 in urine compared to those with SSNS. Thus, the urine level of IL-6 increased 4,2 times (up to 39,7±2,2 μg/mM creatinine) in SRNS, the onset period, whereas in SSNS it increased 3 times (up to 28,7±1,51 pg /mM creatinine), versus 9,5±0,41 pg/mM creatinine in the control group. During remission, the urine level of IL-6 increases 6,4 times in the SSNS, thus indicating the persistence of the pathological process in kidneys. The highest urine level of IL-6 was recorded in CGN, mixed form, the period of exacerbation, during which the concentration of cytokine increased 5,5 times (up to 51,8±8,19 μg/mM creatinine). Whereas, in CGN, nephrotic form, IL-6 level in urine increased 4,9 times (up to 46,6 ± 9,99 pg/mM creatinine), compared to the control group (9,5 ± 0,41 pg/mM creatinine). During remission, urine level of IL-6 in CGN remained high, indicating the persistence of a pathological process in kidneys and the presence of incomplete immunobiochemical rehabilitation.

Discussions.

In the study, significantly increased urinary levels of IL-1β and IL-6 were identified in all patient groups during the period of exacerbation, compared with the period of remission.

The activation of T-cells leads to release of local cytokines that work as soluble circulating factors provoking increased glomerular permeability and podocytes' barrier dysfunction with subsequent proteinuria[9]. Previous reports had suggested that some factors may increase the permeability of the glomerular basement membrane (GBM) in the kidneys [10].

Clinical and experimental studies suggest that IL-6 contributes to renal injury in glomerulonephritis and other forms of renal disease. Elevated IL-6 expression in kidneys and urine of patients with mesangial proliferative glomerulonephritis is often associated with poor outcome. In this context, IL-6 induces mesangial cell proliferation [11].These data are in agreement with studies results that showed increased urine level of IL-6 in 30-50% of patients with IgA nephropathy [12]. IL-6 can also stimulate activation of procoagulant factors that causes vascular thrombogenesis and increases glomerular capillary permeability and urinary protein excretion [13]. These results are reliable to our data. Thus, an important effect of increasing urinary excretion of IL-6 in combination with IL-1β is the increase of leukocytes from two sources: bone marrow and solitary leukocytes attached to endothelial cells. Increased levels of leukocytes indicate that infiltration of monocytes and macrophages constitute a major potential source of inflammatory cytokines, in particular IL-1β, IL-6 and, more rarely, glomerular resident cells.

Evidence from a mouse model of lupus nephritis suggests that the increase in IL-6 is the result of a decrease in expression of a micro-RNA that regulates IL-6 [14]. Preclinical and clinical studies showed that IL-6 could plays both a role of injury and protection in response to kidney diseases [15].

Zhang et al [16] have shown that IL-6 plays an important role in angiotensin II-induced hypertension, proteinuria and renal fibrosis in chronic kidney disease (CKD). IL-6 was increased in the renal biopsies of CKD patients compared to normal control, and its levels were further elevated in CKD patients with hypertension. Zhang's work on IL-6 deficient mice has provided direct evidence of the effect of IL-6 on GBM in the kidneys. The decrease in IL-1β and IL-6 urinary levels in INS patients during remission is expected and probably a response to treatment with steroids.

Recent study indicated that increase in IL-1β, IL-6 in the urine of INS patients during relapse which disappeared during remission of the disease. These findings support the assumption of the important role of these cytokines in the immune process during a relapse [17].

Conclusions.

The study have shown that the determination of the urine levels of IL-1β and IL-6 is a non-invasive predictive method for assessing the disease activity and monitoring the evolution of different variants of glomerulonephritis in children.

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