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Molecular factors predicting steroid resistance in pediatric nephrotic syndrome

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Abstract. *Objectives: the objective of this paper was to study the levels of cellular hypoxia, apoptosis controlling factors in children with steroid-sensitive and steroid-resistant nephrotic syndrome.*

Background: patients with steroid-resistant nephrotic syndrome (SRNS) represent a challenging subset of patients with nephrotic syndrome who often fail standard immunosuppression and have a higher likelihood of progressing to end-stage renal disease. The search of the biochemical markers undergoing the steroid-resistance is under urgent need.

Methods: an examination of kidney biopsies and blood of 56 patients (aged 10 to 15 years) with nephrotic syndrome was done. Conventional clinical investigations, immunohistochemistry, immunoblotting were used in this study.

Results: patients with steroid-resistant nephrotic syndrome show an increased level of HIF-1 alfa (a marker of cellular hypoxia) as compared to the control group and children with steroid-sensitive nephrotic syndrome. Patients with steroid-resistant nephrotic syndrome show a down-regulation of anti-apoptotic marker BcL-xL as compared to the control group and children with steroid-sensitive nephrotic syndrome.

Conclusion: hypoxia-induces disorders and apoptosis activation markers are considered to be included in the complex scheme predicting steroid-resistance in nephrotic children.

Keywords: *treatment, nephrotic syndrome, HIF-1α, BcL-xL, steroid-resistance.*

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Молекулярні маркери, що є предикторами стероїд-резистентності у дітей з нефротичним синдромом

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Резюме. *Мета: метою даної роботи було вивчення рівнів клітинної гіпоксії, факторів контролю апоптозу у дітей із стероїд-чутливими та стероїд-резистентним нефротичним синдромом.*

Актуальність: пацієнти зі стероїд-резистентним нефротичним синдромом (СРНС) представляють складну підгрупу пацієнтів, які часто не мають адекватної терапевтичної відповіді на стандартну імуносупресивну терапію і мають більшу ймовірність прогресування до термінальної стадії хронічного захворювання нирок. Пошук біохімічних маркерів, що є предикторами стійкості до стероїдів, є нагальною необхідністю.

Методи: проведено дослідження біоптатів нирок та крові 56 пацієнтів (у віці від 10 до 15 років) з нефротичним синдромом. У цьому дослідженні використовувались звичайні клінічні дослідження, імуногістохімія, імуноблотинг.

Результати: у пацієнтів зі стероїд-резистентним нефротичним синдромом спостерігається підвищений рівень HIF-1 альфа (маркер клітинної гіпоксії) порівняно з контрольною групою та дітьми з чутливим до стероїдів нефротичним синдромом. У пацієнтів зі стероїд-резистентним нефротичним синдромом спостерігається зниження рівнів експресії антиапоптозного маркера BcL-xL порівняно з контрольною групою та дітьми із стероїд-чутливим нефротичним синдромом.

Висновок: порушення, що виникають на фоні клітинної гіпоксії, та маркери активації апоптозу, доцільно включати в комплексну схему оцінки прогнозування стійкості до стероїдів у дітей з нефротичним синдромом.

Ключові слова: *лікування, нефротичний синдром, HIF-1α, BcL-xL, стероїд-резистентність*

Introduction. Nephrotic syndrome is a clinical condition characterized by proteinuria, hypoalbuminemia, edema, and hyperlipidemia. Nephrotic syndrome represents the most common primary glomerular disease in children and affects ~2 per 100,000 children aged <16 years in Europe and the USA [1, 2].

There are many specific causes of nephrotic syndrome. These include kidney diseases such as minimal-change nephropathy, focal glomerulosclerosis, and membranous nephropathy. Nephrotic syndrome can also result from systemic diseases that affect other organs in addition to the kidneys, such as diabetes, amyloidosis, and lupus erythematosus [3].

At the time of the first presentation, ~80% of children achieve complete remission within 4 weeks of corticosteroid therapy and are classified as having steroid-sensitive nephrotic syndrome (SSNS). However, 60%–70% of the patients initially classified as steroid sensitive have >1 relapse. Among children who relapse, 30% will be further classified as having frequently relapsing nephrotic syndrome or steroid-dependent nephrotic syndrome [4].

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Steroid resistance, defined as the absence of remission despite 4 weeks of therapy with daily prednisone at a dose of 2 mg/kg/d, encompasses an even smaller proportion of patients. Despite representing a smaller proportion of nephrotic syndrome cases, patients with steroid-resistant nephrotic syndrome (SRNS) have proven more difficult to treat, with 36%–50% progressing to end-stage renal disease within 10 years [5].

Main pathomorphological outcomes that apply to kidney damage in nephrotic syndrome are glomerulosclerosis, vascular sclerosis, tubule-interstitial fibrosis [6]. Inflammation plays an important role in the development and progression of chronic proteinuric kidney pathologies and is the primary and persistent violation, which underlies the pathogenesis of others. Renal histology in chronic renal pathologies including nephrotic syndrome is characterized by typical signs of inflammation i.e. infiltration with white blood cells, hyperemia, fibrosis etc. In addition to inflammation, fibrosis has a role in nephrotic syndrome. These disorders are accompanied by activation of the renin-angiotensin-aldosterone system, oxidative stress, endothelial dysfunction and others [6, 7, 8]. All mentioned above pathophysiological violations might be accompanied by apoptosis.

Patients with steroid-resistant nephrotic syndrome (SRNS) represent a challenging subset of patients with nephrotic syndrome who often fail standard immunosuppression and have a higher likelihood of progressing to end-stage renal disease. Appropriate treatment of SRNS requires an adequate understand-

ing of the historical treatment, renal histopathology, and genetics associated with the disease.

The aim of this study is to analyze biochemical markers related to cellular hypoxia and apoptosis activation in children with SRNS.

Materials and methods. Patients. An examination of renal biopsies of 56 patients (aged 10 to 15 years) with nephrotic syndrome hospitalized in the Pediatric Nephrology Unit of the Children Clinical Hospital №7 (Kyiv, Ukraine) was done. All patients were treated accordingly to the national protocols. Among all patients 26 (46,4%) were with steroid-sensitive nephrotic syndrome, others – 30 (54,7%) showed steroid-resistant nephrotic syndrome. Complex examination other than conventional methods (inspection, monitoring blood pressure, general and biochemical blood tests, determination of daily proteinuria, urinary sediment study and concentration ability of the kidneys, ultrasound of the abdomen etc.) done. The level of kidney function impairment (stage of Chronic Kidney Disease, CKD) was assessed by the value of glomerular filtration rate (GFR). GFR was calculated by the Schwartz formula. Patients with GFR<30 mL/min/1,73 m^2 were excluded from the study.

Immunoblotting for detection of HIF-1α, BcL-xL. Proteins solubilized in Laemmli sample buffer were resolved in polyacrylamide gels by SDS-PAGE and transferred to a polyvinylidene difluoride membrane. Membranes were then blocked in 5% non-fat milk in TBS-T (136 mM NaCl, 10 mM Tris, 0.05% Tween 20) and immunoblotted using the Mouse anti-Human HIF-1alfa Ab (BD Transduction Laboratories, USA), та Mouse anti-Bcl-xL Ab (Cell Signaling, USA) and actin mouse mAb (BD, Lexington KY, USA) for 1 hour at room temperature. The actin mouse mAb was used as a loading control. After three washes with TBS-T, the membranes were incubated with secondary anti-rabbit or anti-mouse antibodies labeled with horseradish peroxidase for 1 hour at room temperature. Membranes were washed three times with TBS-T. The protein bands were visualized by chemiluminescent substrate ECL. Quantification of the protein content was done by densitometric analysis.

Control values were obtained at the survey of 20 relatively healthy children ("Control group" later). In this group, immunoreactivity to HIF-1 alfa and Bcl-xL levels was taken as 100%.

Statistics. Statistical analysis was done using the method of variation statistics (STATISTICA 6.0), Excel and nonparametric statistical approaches (Mann-Whitney test). Results are presented as Mean ± SEM. P<0.05 was considered statistically significant.

Results. A total of 56 patients (aged 8 to 15 years) with nephrotic syndrome were hospitalized in the Pediatric Nephrology Unit of the Pediatric Clinical Hospital №7 (Kyiv, Ukraine) done. All patients were treated accordingly with the national protocols. Characteristics of patients are included into the given in Table 1.

Table 1

Group characteristics for participating patients

Characteristics	Value
Age. years	12.29 ±3.06
Body mass index	22.5 ± 1.3
Sex (male/female)	29/27
Normal BP/AH	24/32
Steroid-sensitive type of nephrotic syndrome/ steroid-resistant type of nephrotic syndrome	26/30
Average disease course, years	8.09 ± 2.26

BP – blood pressure, AH - arterial hypertension.

Previously, we reported the level of cellular hypoxia in children with nephrotic syndrome. HIF-1 alfa is used as a marker of cellular hypoxia. We found increased HIF-1 alfa in blood serum of all patients with nephrotic syndrome as compared to the control group. Moreover, dependence between the levels of renal insufficiency (assessed by GFR) and cellular hypoxia has been documented. The levels of HIF-1α in the group with CKD I st. was detected at a level over excided the control group value by 28.6% (p<0.01 compared to the control group) and by 41,3% (p<0.01 compared to the control group) in patients with CKD II-III st. [**9, 10**].

In the current study, we analyzed HIF-1 alfa in subgroups of patients with steroid-sensitive and steroid-resistant nephrotic syndrome. The general level of HIF-1 alfa exceeded the HIF-1 level of the control group by 36,02% (<0.01). Control group value set as 100. Comparison of steroid-sensitive and steroid-resistant groups show that HIF-1 alfa is higher in the steroid-sensitive group as compared to control by 25.7 % (<0.01). In the steroid-resistant group HIF-1 alfa was detected at a level that is higher than the control group value by 40.1% (<0.01) (Fig. 1, A-B).

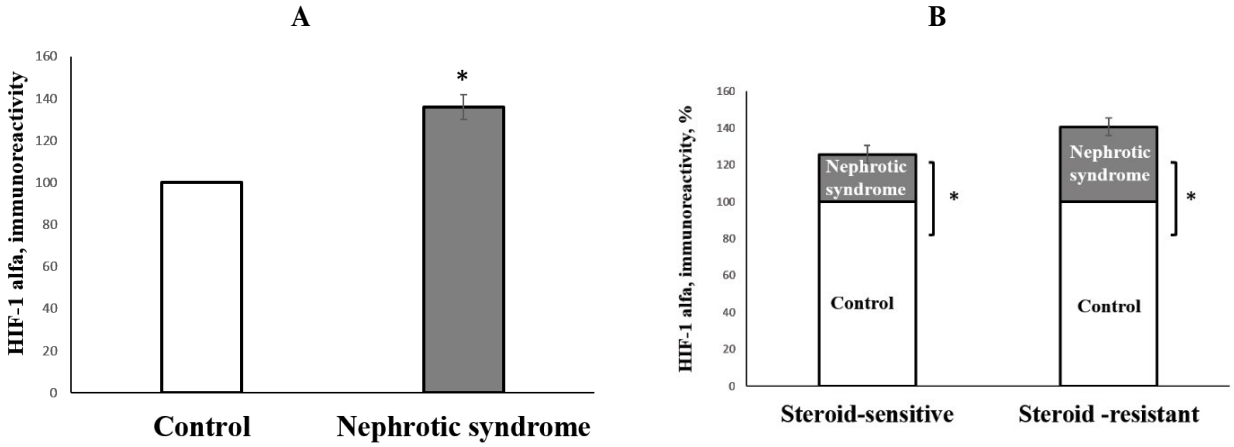

Fig. 1. Level of HIF-1α in plasma of patients with nephrotic syndrome and control group patients (A).
Level of HIF-1α in plasma of patients with steroid-sensitive and steroid-resistant nephrotic syndrome (B).

In our previous study, we evaluated the condition of the apoptosis controlling system in children with nephrotic syndrome. BcL-xL level as a marker of anti-apoptotic defense used. We found a reduction of anti-apoptotic protection in all children with nephrotic syndrome and dependence on CKD level evaluated. In nephrotic children with CKD, st I. Bcl-xL expression was reduced to 75.1% as compared to the control group, and to 60.1% in the group with CKD st II-III ($p<0.01$ and $p<0.001$, respectively) [9, 10].

In the current study, we analyzed BcL-xL in subgroups of patients with steroid-sensitive and steroid-resistant nephrotic syndrome. The total level of BcL-xL was down-regulated in nephrotic children as compared to the control group by 30.9% ($p<0.01$). Control group value set as 100. Comparison of steroid-sensitive and steroid-resistant groups show that BcL-xL expression was lower in the steroid-sensitive group as compared to control by 27.8 % ($p<0.01$). In the steroid-resistant group, BcL-xL was detected at a level that is lower than the control group value by 41.1% ($p<0.01$) (Fig. 2, A-B).

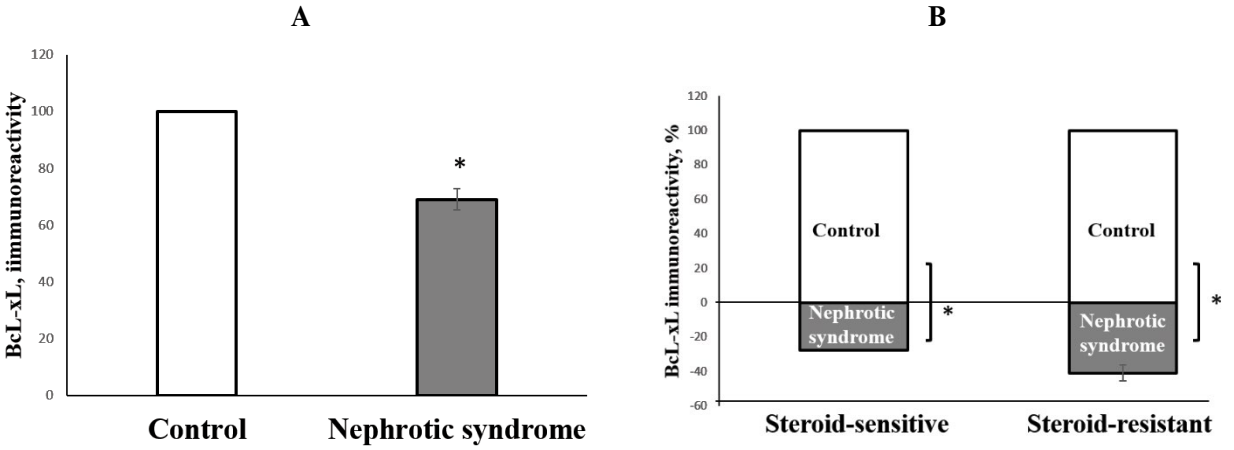

Fig. 2. Level of BcL-xL in plasma of patients with nephrotic syndrome and control group patients (A).
Level of BcL-xL in plasma of patients with steroid-sensitive and steroid-resistant nephrotic syndrome (B).

Discussion. While comprising a small minority of the patients presenting with nephrotic syndrome (8%–15%), patients with SRNS prove a particularly challenging set of patients to manage and treat. Currently, there are data regarding many of the candidates predisposing to the phenomenon of the steroid-resistance in nephrotic children.

The presence of a circulating serum factor associated with SRNS has long been sought. In a seminal paper by Savin et al [11], the association between a circulating factor and disease activity was evaluated in patients with recurrent FSGS. Serum samples were analyzed in patients with FSGS pre-and postrenal transplant to evaluate if the serum samples of patients with recurrent FSGS increased glomerular-capillary permeability to albumin. A permeability value was assigned to each sample, with the lower permeability values indicating poor glomerular-capillary permeability to albumin and higher permeability values indicating increased glomerular-capillary permeability to albumin [12].

More recently, many factors, i.e., Vitamin D–binding protein (VDBP), alpha-1 acid glycoprotein 1 (AGP1), alpha-1 acid glycoprotein 2 (AGP2), alpha-1-B glycoprotein (A1BG), fetuin-A, prealbumin, thyroxine-binding globulin and hemopexin, and alpha-2 macroglobulin were measured and combined with urine neutrophil gelatinase–associated lipocalin (NGAL), were discussed as a players in development of steroid-resistant typy of nephrotic syndrome [13]. However, too few data about the quantification of the apoptosis and apoptosis-related disorders in children with steroid-resistat type on nephrotic syndrome.

It is accepted that proteinuria is a hallmark of glomerular disease, and the magnitude of proteinuria is an adverse prognostic factor in varied nephropathies including nephrotic syndrome. There is evidence that proteinuria is a mechanism of kidney disease progression. A reduction in proteinuria is associated with a slower decline in GFR. Enhanced albuminuria leads to the secondary pathologycal prosecces – inflammation, hypoxia, fibrosis. Ischemia as a result of peritubular capillary loss or hypoperfusion is also considered a major factor for the progression of tubulointerstitial damage, which is closely associated with impairment of renal function. Renal tissue hypoxia induces profibrogenic responses and tubulointerstitial injury, which includes degeneration, dedifferentiation, cell death [14-16].

Moreover, hypoxia has been shown to induce apoptosis, where HIF-1 plays a complex role. It has also been demonstrated that the expression of HIF-1α significantly correlated with apoptosis. This finding has been shown in in vitro models [16].

Conclusions. Here we show that HIF-1α is up-regulated and anti-apoptotic factor BcL-xL is down-regulated in children with nephrotic syndrome meaning that chronic hypoxia is a factor predisposing disturbances in the system controlling apoptosis in this cohort of patients. Our current data are in line with previous results showing a dependence between the level of hypoxia, pro-apoptotic factor Bax and anti-apoptotic factor BcL-xL and stage of CKD.

Further study of the molecular markers, predicting the steroid-resistance in nephrotic children, in particular markers of the apoptosis controlling system has proved critical in unraveling the pathophysiologic mechanisms of disease and in guiding therapeutic options. With further research in the field, it is likely that new mechanisms will be implicated in the pathophysiology of SRNS and further treatment options can be elucidated to optimize treatment of this disease.

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Author's contribution to work:

Burlaka Ie.A.: literature search, study design planning, samples collection, experimental work, data analysis, manuscript writing and submission.

Bagdasarova I.V.: literature search, study design planning, data analysis, manuscript writing and submission.

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References:

1. *El Bakkali L, Pereira RR, Kuik DJ, Ket JCF, van Wijk JAE.* Nephrotic syndrome in the Netherlands: a population-based cohort study and a review of the literature. Pediatr Nephrol. 2011;26(8):1241–1246. doi: 10.1007/s00467-011-1851-8.
2. Report of the International Study of Kidney Disease in Children Early identification of frequent relapses among children with minimal change nephrotic syndrome. A report of the International Study of Kidney Disease in Children. J Pediatr. 1982;101(4):514–518. https://doi.org/10.1016/S0022-3476(82)80692-6
3. *Eddy AA, Symons JM.* Nephrotic syndrome in childhood. Lancet. 2003; 362(9384):629-639. doi: 10.1016/S0140-6736(03)14184-0.
4. *Nakanishi K, Iijima K, Ishikura K, Hataya H, Nakazato H, Sasaki S, Honda M, Yoshikawa N.* Japanese Study Group of Renal Disease in Children. Two-year outcome of the ISKDC regimen and frequent-relapsing risk in children with idiopathic nephrotic syndrome. Clin J Am Soc Nephrol. 2013; 8(5):756–762. doi: 10.2215/CJN.09010912.
5. *Trautmann A, Schnaidt S, Lipska-Ziętkiewicz BS, Bodria M, Ozaltin F, Emma F, Anarat A, Melk A, Azocar M, Oh J, Saeed B, Gheisari A, Caliskan S. et al.* Long-Term Outcome of Steroid-Resistant Nephrotic Syndrome in Children. J Am Soc Nephrol. 2017; 28(10):3055-3065. doi: 10.1681/ASN.2016101121.
6. *Hemmelgarn BR, Manns BJ, Lloyd A, James MT, Klarenbach S, Quinn RR. et al.* Relation between kidney function, proteinuria, and adverse outcomes. JAMA. 2010; 303(5):423-9. doi: 10.1001/jama.2010.39.
7. *Abbate M, Zoja C, Remuzzi G.* How does proteinuria cause progressive renal damage? J Am Soc Nephrol. 2006;7:2974–2984. doi: https://doi.org/10.1681/ASN.2006040377.
8. *Gordillo R, Spitzer A.* The Nephrotic Syndrome. Pediatrics in Review. 2009;30: 94-105. doi: https://doi.org/10.1542/pir.30-3-94.
9. *Burlaka IeA, Bagdasarova IV.* Apoptosis as a mechanism of the albumin-induced kidney damage in childhood nephrotic syndrome. Ukr J Nephr Dial. 2018; 3(59): 25-30. doi: 10.31450/ukrjnd.3(59).2018.04.

10. *Maidannyk VG, Burlaka EA, Bagdasarova IV, et al.* Role of hypoxia-induced apoptosis in chronic glomerulonrphritis progression in children. Ukr J Nephr Dial. 2014; 4 (44): 41-45. Available from: https://ukrjnd.com.ua/index.php/journal/article/view/52/42.
11. *Savin VJ, Sharma R, Sharma M, McCarthy ET, Swan SK, Ellis E, et al.* Circulating factor associated with increased permeability to albumin in recurrent focal segmental glomerulosclerosis. N Engl J Med. 1996;334(14):878–883. doi: 10.1056/NEJM199604043341402.
12. *Nourbakhsh N, Mak RH.* Steroid-resistant nephrotic syndrome: past and current perspectives. Pediatric Health Med Ther. 2017;8:29-37. doi: 10.2147/PHMT.S100803.
13. *Bennett MR, Pleasant L, Haffner C, Ma Q, Haffey WD, Ying J, Wagner M, Greis KD, Devarajan P.* A Novel Biomarker Panel to Identify Steroid Resistance in Childhood Idiopathic Nephrotic Syndrome. Biomarker Insights. 2017;12: 1–11. doi: 10.1177/1177271917695832.
14. *Eltzschig HK, Carmeliet P.* Hypoxia and inflammation. N Engl J Med. 2011; 364(7):656–665. doi: 10.1056/NEJMra0910283.
15. *Du R, Xia L, Ning X, Liu L, Sun W, Huang C, Wang H, Sun S.* Hypoxia-induced Bmi1 promotes renal tubular epithelial cell-mesenchymal transition and renal fibrosis via PI3K/Akt signal. Mol Biol Cell. 2014 Sep 1;25(17):2650-9. doi: 10.1091/mbc.E14-01-0044.
16. *Volm M, Koomägi R.* Hypoxia-inducible factor (HIF-1) and its relationship to apoptosis and proliferation in lung cancer. Anticancer Res. 2000;20(3A):1527-1533. Available from: https://pubmed.ncbi.nlm.nih.gov/10928066/.