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Chronic intracellular hypoxia as a clustering and stratifying factor for clinical severity grade in nephrotic syndrome in children

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Abstract. *Nephrotic syndrome is the most common glomerular kidney disease in childhood. It is known that chronic hypoxia is a severe disorder and potent factor of kidney damage. The limited success of existing therapeutic strategies in slowing the progression of chronic kidney disease requires the study of new ways to assess and interpret the levels of chronic intracellular hypoxia concerning basic clinical data, grades of NS activity in children, type of therapeutic response.*

The study aimed to investigate the state of transcription factor and marker of intracellular hypoxia HIF-1alfa in children with different degrees of change in basic clinical and laboratory parameters; to evaluate HIF-1alfa as a possible factor of stratification of activity grade of nephrotic syndrome.

Methods. This case-control study was carried out in the duration from June 2018 to August 2020. The study was conducted on 35 selected patients with NS collected from the nephrology department, Pediatric Clinical Hospital №7 (Kyiv, Ukraine). Plasma samples were used to measure marker intracellular hypoxia HIF-1alfa. ANOVA followed by the post hoc Kruskal-Wallis test for multiple comparisons was used to test the significance of differences. GraphPad Prism 9.0 Software for Windows and Statistica 10.0 software used. P values <0,05 considered statistically significant.

Results. Three groups of children with different activity grades were stratified on basis of indicators of proteinuria levels, total blood protein, blood alpha2-globulin levels, serum cholesterol levels, and edema. 1st-grade group found to have a mild increase of HIF-1alfa up to 185-195 a.u. proteinuria 3,5-5,5 g/24 h, total blood protein 47-53 g/L, alfa2-globulins level in blood 20-23 g/L, serum cholesterol level 6-8,5 mMol/L, edema - 1-1.6 points. 2nd grade group found to have moderate increase of HIF-1alfa up to 195,1-205 a.u., proteinuria 5,51-8,5 g/24 h, total blood protein 46,9-40 g/L, alfa2-globulins level in blood 23,1-27 g/L, serum cholesterol level 8.51-10,5 mMol/L, edema 1.61-2.2 points. 3rd-grade group found to have pronounced increase of HIF-1alfa up to 205,1-220 a.u., proteinuria 8,51-14 g/24 h, total blood protein 39,9-32 g/L, alfa2-globulins level in blood 27,1-30 g/L, serum cholesterol level 10.51-13.5 mMol/L, edema 2.21-3 points. Higher HIF-1alfa level appears in children with NS and frequent relapses as compared to the group with rare relapses.

Conclusion. Thus, the increase of HIF-1 alpha to the level of 185-205 a.u., which corresponds to the I-II degree of activity in children with NS can be used as a starting point and therapeutic window for specific anti-hypoxic and antioxidant interventions. Determination of HIF-1 alpha levels in children with NS can be used as a factor for stratification of the activity grade.

Key words: *hypoxia, nephrotic syndrome, clustering of groups, activity grade, therapeutic response.*

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Хронічна внутрішньоклітинна гіпоксія як фактор стратифікації ступенів активності нефротичного синдрому у дітей

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Резюме. *Нефротичний синдром є найбільш поширеним гломерулярним захворюванням нирок у дитячому віці. Відомо, що хронічна гіпоксія є потужним порушенням і фактором пошкодження нирок. Обмежений успіх існуючих терапевтичних стратегій у сповільненні прогресування пошкодження нирок вимагає вивчення нових шляхів оцінки і трактування рівнів хронічної внутрішньоклітинної гіпоксії у відношенні до основних клінічних даних, ступенів активності НС у дітей, характеру терапевтичної відповіді.*

Метою дослідження було дослідити стан транскрипційого фактора і маркера внутрішньоклітинної гіпоксії HIF-1alfa у дітей з різними ступенями зміни основних клінічних та лабораторних показників у дітей з нефротичним синдромом (НС); оцінити HIF-1alfa як можливий фактор стратифікації ступенів активності нефротичного синдрому.

Методи. Дане дослідження дизайну «випадок-контроль» проводилось у період з червня 2018 року по серпень 2020 року. Обстежено 35 дітей з НС, які спостерігались у відділенні дитячої нефрології дитячої клінічної лікарні №7 (Київ, Україна). Зразки плазми використовували для вимірювання маркера внутрішньоклітинної гіпоксії HIF-1alfa. ANOVA з подальшим post-hoc тестом Краскала-Уолліса використовувалась для множинних порівнянь та перевірки значущості відмінностей. Використано програмне забезпечення GraphPad Prism 9.0 Software. Двоетапний кластерний аналіз виконано за допомогою програмного забезпечення Statistica 10.0. Значення P <0,05 вважаються статистично значущими.

Результати. Стратифіковано три групи дітей із НС за ступенем активності на основі показників рівнів протеїнурії, загального білка крові, рівня альфа2-глобулінів у крові, рівня холестерину в сироватці крові, набряків.

У групі з I ст активності виявлено незначне підвищення HIF-1alfa до 185-195 у.о., протеїнурія на рівні 3,5-5,5 г/24 год, помірна гіпопротеїнемія 47-53 г/л, помірне підвищення рівня альфа2-глобулінів - 20-23 г/л, помірна гіперхолестеринемія - 6-8,5 ммоль/л, показник набряків - 1-1.6 бала.

II ст активності НС характеризується наступними даними: виражене підвищення HIF-1alfa до 195,1-205 у.о., протеїнурія 5,51-8,5 г/24 год, виражена гіпопротеїнемія - 46,9-40 г/л, виражена гіпер-альфа2-глобулінемія - 23,1-27 г/л, виражена гіперхолестеринемія - 8.51-10,5 ммоль/л, набряки - 1.61-2.2 бала.

У дітей з III ст активності НС виявлено: високий рівень HIF-1alfa - 205,1-220 у.о., значна протеїнурія - 8,51-14 г/24 год, значна гіпопротеїнемія - 39,9-32 г/л, значне підвищення рівня альфа2-глобулінів - 27,1-30 г/л, значна гіперхолестеринемія - 10.51-13.5 ммоль/л, показник набряків - 2.21-3 бала. Вищий рівень HIF-1alfa спостерігається у дітей з НС і частими рецидивами порівняно з групою з рідкими рецидивами.

Висновки. Таким чином, підвищення HIF-1 альфа до рівня 185-205 у.о., що відповідає I-II ст активності у дітей з НС може використовуватися як відправна точка та терапевтичне вікно для специфічних антигіпоксичних та антиоксидантних впливів. Визначення рівня HIF-1 alfa у дітей з НС може бути використане в якості фактора стратифікації за ступенем активності.

Ключові слова: *гіпоксія, нефротичний синдром, кластеризація груп, ступінь активності, терапевтична відповідь.*

Introduction. In chronic kidney disease, functional impairment correlates with tubulointerstitial fibrosis characterised by inflammation, accumulation of extracellular matrix, tubular atrophy and peritubular capillaries changes. Loss of the microvasculature implies a hypoxic condition and suggested an important role for hypoxia. Nephrotic syndrome (NS) is the most common glomerular disease in childhood [1]. The reported incidence in children varies between 1.2 and 3.5 per 100,000 per year in Western Europe, 4.7 per 100,000 per year worldwide [2]. Different factors discussed in terms of NS progression, i.e. pathomorphological type of disease (the most dominant lesion is focal segmental glomerulosclerosis (FSGS), genetic markers [3], metabolic factors [3, 4].

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Recent data provide evidence of decreased renal oxygenation in chronic kidney disease while more direct support for a causal role comes from data in rodent models. Additional postulated roles for hypoxia in chronic kidney disease are the sustaining of the inflam-

matory response, the recruitment, retention and differentiation towards a pro-fibrotic phenotype of circulating progenitor cells and the alteration of the function of intrinsic stem cell populations [5]. Renal fibrosis is the hallmark of chronic kidney diseases (CKDs) of diverse etiologies in which accumulation of extracellular matrix (ECM) disrupts normal tissue architecture leading to progressive renal dysfunction and organ failure [6]. Regardless of the initiating insult, CKD presents a common pathology of glomerulosclerosis and tubulointerstitial fibrosis and it is well established that tubulointerstitial fibrosis provides the best predictive indicator of progression to end-stage disease. Tubulointerstitial fibrosis presents a number of characteristic features [6, 7].

That accumulating data suggests that chronic hypoxia is a final common pathway to end-stage renal disease. The limited success of existing strategies in retarding chronic kidney disease mandates that these new avenues of chronic hypoxia in relation to basic clinical data and kidney function (GFR level) be explored [8].

This study aimed to investigate basic clinical (age, disease duration), basic clinical (serum creatinine, serum cholesterol, complete blood count data, GFR, proteinuria, total blood albumin level, alfa2-globulins, edema), transcriptional factor and marker of intracellular hypoxia HIF-1alfa as possible markets of staging and stratification in children with NS and different severity grade; to evaluate the peculiarities of the mentioned above markers into-relation in clustering groups of children with nephrotic syndrome.

Material and methods. ***Patients.*** This case-control study was carried out in the duration from June 2018 to August 2020. The study was conducted on 35 selected patients with NS collected from the nephrology department, Pediatric Clinical Hospital №7 (Kyiv, Ukraine) which is a clinical base of the Bogomolets National Medical University. Informed written consent was obtained from the parents of all participants. The study was approved by the local ethical committee of Bogomolets National Medical University and the research has complied with Helsinki Declaration.

NS was diagnosed by the triad of heavy proteinuria (>3 gm/ day), hypoalbuminemia, and edema. The steroid-sensitive nephrotic syndrome (SSNS) group included 35 patients who responded to steroid treatment within 8 weeks.

Patients were also subjected to physical examination to document fever, edema, ascites, nutritional status, volume status, and hypertension. The laboratory data including; complete blood count, serum creatinine (Scr), serum urea, serum cholesterol, eGFR using Schwartz formula, 24 h urine protein, urine analysis.

Immunoblotting for detection of HIF-1alfa. Plasma samples were used to measure marker intracellular hypoxia HIF-1alfa. 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 HIF-1α Ab (Cell Signaling Technology, Danvers, MA 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.

Statistics. The data expressed as means ± SEM and as frequencies and percentages when appropriate. ANOVA followed by the post hoc Kruskal-Wallis test for multiple comparisons was used to test the significance of differences. Pearson correlation was run to study the correlation between factors. Data processed using GraphPad Prism 9.0 Software for Windows (USA, San Diego, CA).

Two-step clustering was done using Statistica 10.0 software. An intelligent clustering method in which the optimal clustering number is automatically determined was done. It identifies clusters by two processes: first, clustering, followed by hierarchical clustering. Hierarchical algorithms were used to estimate the optimal clustering number based on the silhouette width, the calculation of the distance using the log-likelihood and clustering following Schwarz's Bayesian criterion. P values <0,05 considered statistically significant.

Results. ***Patients.*** The study was carried out over a period of 24 months. 35 patients with steroid-sensitive NS were included in the study. The mean of patients ages 12.25±0.85 years, mean disease duration – 7.65±0.33 years. 55.3% of patients included in the study were males and 44,7% were females. The average BMI value was 21.8±0.73. All of them have edema, and 17 patients (48.6%) were hypertensive (Table 1).

Table 1

Clinical characteristics of the patients with NS

Characteristics	NS patients (n=35) M±SEM or %
Boys	20 (55,3%)
Girls	15 (44,7%)
Age, years	12.25±0.85
Disease duration, years	7.65±0.33
BMI	21.8±0.73
Hypertension	17 (48.6%)
Edema	35 (100%)
WBC count, 109/L	8.1±0.58
ESR, mm/h	8.67±0.4

Identification of four clusters by remodeling the cluster analysis based on nine variables. The clustered results were based on nine variables, - HIF-1alfa, disease course, age, proteinuria level, GFR, S-Cr, serum cholesterol, total blood protein, alfa2-globulins shown as 3 subgroups (Fig. 1).

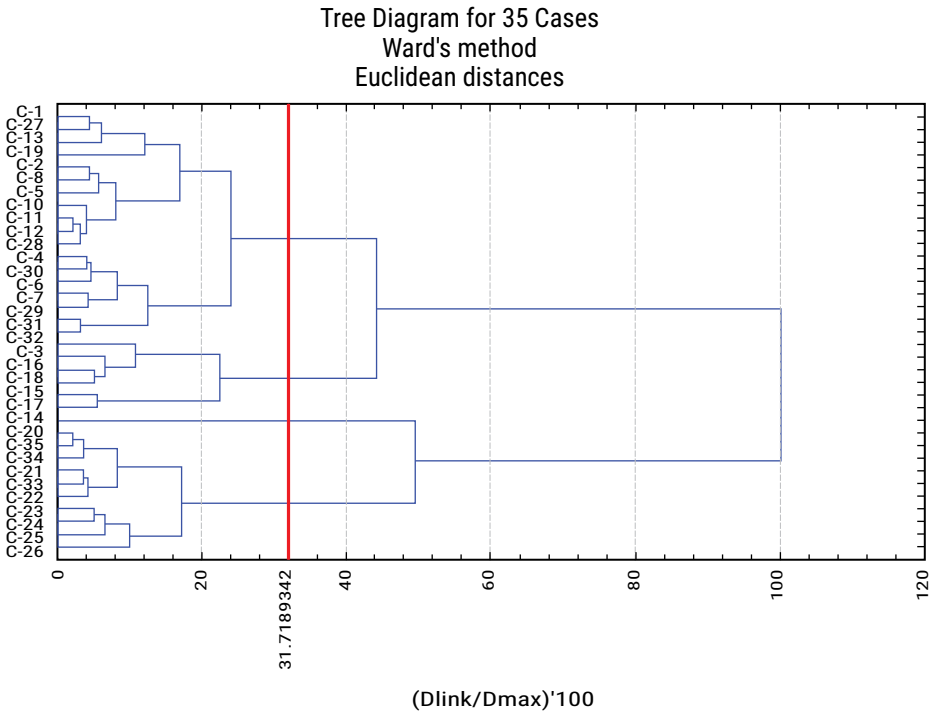

Fig. 1. Identification of four clusters by remodeling the cluster analysis based on nine variables in children with NS.

Cluster groups were named as 1st grade, 2nd grade, 3rd grade. Here we give characteristics of the examined patients. An average age did not show any difference between cluster groups – 11.06±0.55 years, 10.17±1,22 years, 11.1±1.06 years for Group 1, Group 2, Group 3, respectively (p>0,05). No difference in disease course was found for each of the groups – 7.11±0.32 years, 7.0±0.89 years, 8.0±0.26 years, for Group 1, Group 2, Group 3, respectively (p>0.05) (Fig. 2).

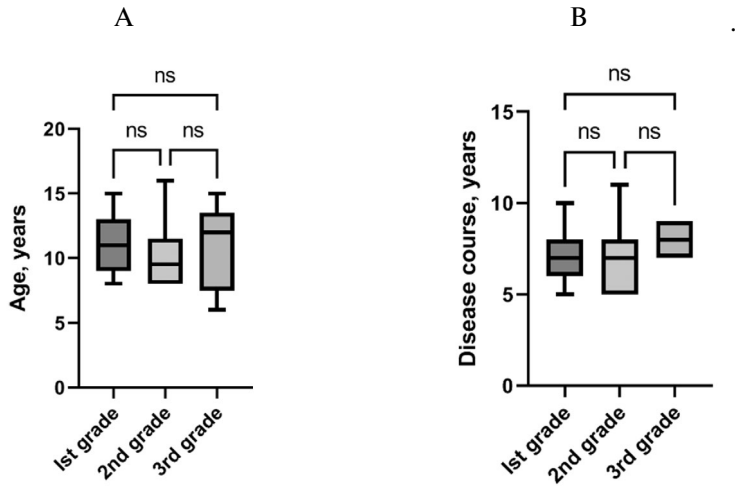

Fig. 2. Age (A) and disease course (B) in clustering groups of children with NS. Ns – not significantly different. Histograms represent means ± SEM. Statistical analysis was performed using the post hoc Kruskal-Wallis test.

Proteinuria level, Glomerular filtration rate (GFR) and S-Cr level were selected as markers of kidney function evaluation. An average proteinuria level in the 1st-grade group was 4.58±0.2 g/24 h, in groups 2nd grade - 7.98±0.27 g/24 h (p<0.01) and 3rd grade – 14.42±0.8 g/24 h (p<0.001 as compared to group 2nd grade and p<0.0001 as compared to group 1st grade) (Fig. 3A).

No difference in GFR level found in all three groups – 112.2±2.02 mL/min/1.73 m^2, 100.4±4.94 mL/min/1.73 m^2 and 105.6±1.65 mL/min/1.73 m^2, re-

spectively (p>0,05). (Fig. 3B). S-Cr level documented at the levels which did not show difference between groups – 69.33±2.26 mcMol/L, 71.83±4.88 mcMol/L and 69.33±2.26 mcMol/L, respectively (p>0.05) (Fig. 3C).

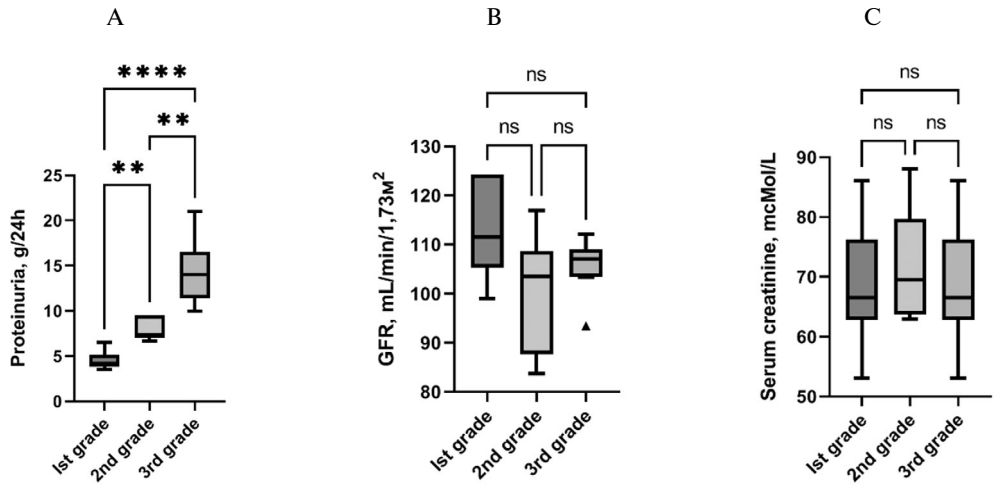

Fig. 3. Proteinuria (A) GFR (B) and S-Cr (C) levels in clustering groups of children with NS. Ns – not significantly different. * - p<0.05, **** p<0.0001. Histograms represent means ± SEM. Statistical analysis was performed using the post hoc Kruskal-Wallis test.

Serum cholesterol level as a marker of lipids metabolism disorders and risk factor for the further vascular damages analyzed in all children. Serum cholesterol level was higher in group 3rd grade 13.31±0.36 mMol/L as compared to group 1st grade 6.12±0.22 mMol/L (p<0.0001) and group 2nd grade 10.74±0.29 mMol/L (p>0,05). Group 1st grade and group 2nd grade values were statistically different (p<0.001) (Fig. 4).

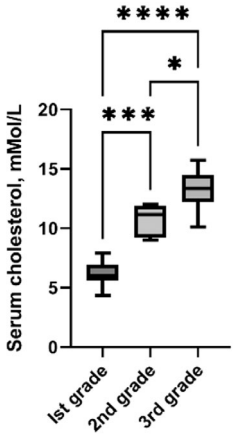

Fig. 4. Serum cholesterol levels in clustering groups of children with NS. Ns – not significantly different. * - p<0.05, **** p<0.001, **** p<0.0001. Histograms represent means ± SEM. Statistical analysis was performed using the post hoc Kruskal-Wallis test.

Total blood protein, alfa2-globulins and edema grade levels were selected as markers of NS severity grade. The average total blood protein level in 1st-grade group was 50.28±0.78 g/L, in groups 2nd grade - 45.44±0.54 g/L (p<0.05) and 3rd grade – 36.39±0.98 g/L (p<0.001 as compared to group 2nd grade and p<0.0001 as compared to group 1st grade). (Fig. 5A).

The average blood alfa2-globulins level in the 1st-grade group was 21.56±0.32 g/L, in groups 2nd grade - 26.17±0.49 g/L (p<0.01) and 3rd grade – 29.78±0.32 g/L (p<0.01 as compared to group 2nd grade and p<0.0001 as compared to group 1st-grade) (Fig. 5B).

Finally, edema grade was based on the number of points evaluated in all children. 1 point refers to local edema in 1-2 areas. 2 points refer to local edema in 3 and more areas, 3 points – to signs of general edema. An average edema grade did not show any difference between cluster groups 1st grade and 2nd grade – 1.11±0.08 points, 1.5±0.12 points (p>0.05). The highest edema grade appeared in the 3rd-grade group – 2.8±0.1 points (p<0.0001, as compared to group 2nd grade) and (p<0.0001, as compared to group 1st grade) (Fig. 5C).

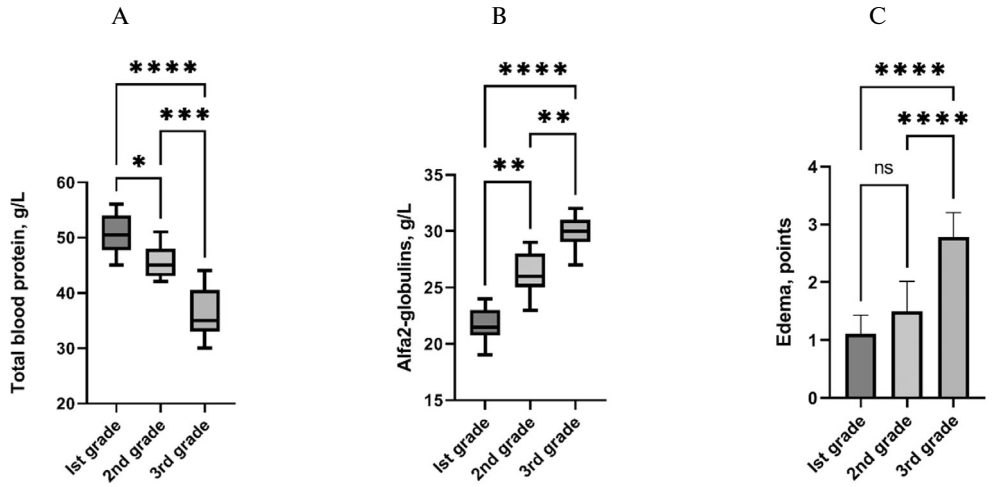

Fig. 5. Total blood albumin (A), alfa2-globulins (B) and edema (C) levels in clustering groups of children with NS. Ns – not significantly different. * - p<0.05, ** - p<0.01, *** - p<0.001, **** p<0.0001. Histograms represent means ± SEM. Statistical analysis was performed using the post hoc Kruskal-Wallis test.

The patients' distribution between the clustering groups was the following – Group 1st grade – 51.4%, Group 2nd grade – 17.1%, Group 3rd grade – 31.5% (Fig. 6).

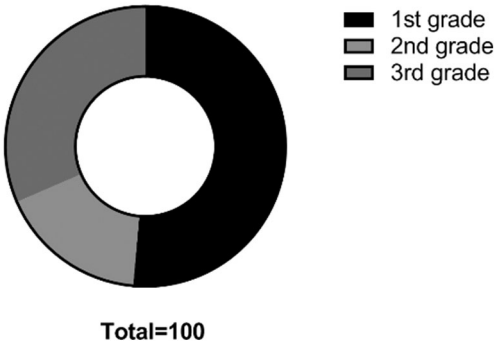

Fig. 6. Patients distribution between clustering groups with different activity grade of NS.

HIF-1alfa in nephrotic children with different activity grades, in groups based on therapeutic response. Obtained results allowed us to verify three groups of NS activity. Based on these stratifications we analyzed levels of HIF-1alfa as a marker of intracellular hypoxia and inflammation. No difference in HIF-1alfa was found between the 3rd-grade group and 2nd-grade group: 222.8±2.22 a.u., 202.4±1.93 a.u., respectively (p>0.05). The lowest level of HIF-1alfa found in the 1st-grade group – 184.4±1.27 a.u. as compared to the 2nd-grade group (p>0.05) 3rd-grade group (p<0.01) and control group (p<0.001). Control group value 144.4±3.64 a.u. significantly lower in comparison to all groups of patients with NS - 1st-grade group (p<0.01), 2nd-grade group (p<0.001), 3rd-grade group (p<0.001) (Fig. 7A).

Finally, we analyzed HIF-1alfa levels in children with NS and different relapses frequency. Nephrotic children with 1-2 relapses/yeas assumed as a group with rare relapses. Nephrotic children with 3-4 relapses/yeas assumed as a group with frequent relapses. The group with rare relapses showed lower HIF-1alfa levels as compared to the group with frequent relapses – 188.2±2.03 a.u. and 222.1±2.13 a.u., respectively (p<0.01). Both groups' HIF-1alfa values show a statistical difference in comparison to the control group p<0.001 and p<0.0001, respectively (Fig. 7B).

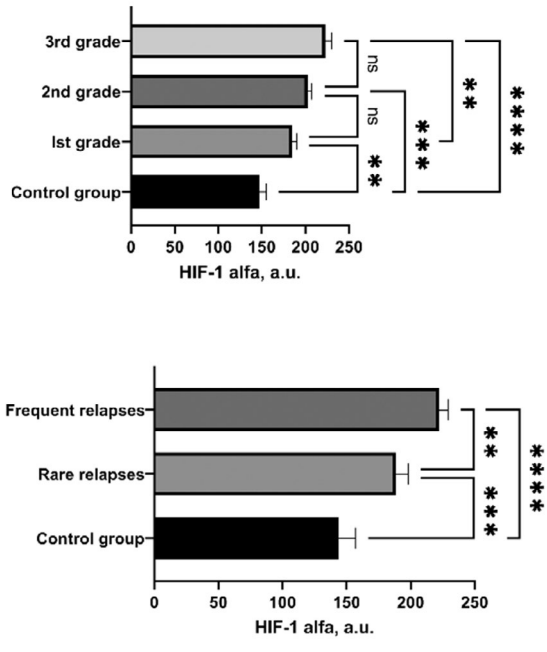

Fig. 7. HIF-1alfa in nephrotic children with different activity grades, in groups based on therapeutic response. ** p<0,01, *** p<0,001, **** p<0,0001. Histograms represent means ± SEM. Statistical analysis was performed using the post hoc Kruskal-Wallis test.

Discussion. The central role of hypoxia in renal fibrosis suggests that therapeutic manipulation of the hypoxic response may be of benefit in preventing or

retarding disease. Approaches to reduce hypoxia-mediated profibrotic changes induced include correction of anemia; normalization of vascular tone and intrarenal microvascular perfusion; preservation, repair, and stabilization of the tubulointerstitial microvasculature; stabilization of HIF in the tubulointerstitium; and, manipulation of hypoxia-induced cell homing [8, 9].

HIF is a major regulator of the adaptive response to hypoxia. HIFs are heterodimeric transcription factors comprising a constitutively expressed β-subunit and an oxygen-regulated α-subunit [10]. In normoxia, stability, and activity of the α-subunit is regulated by oxygen-dependent hydroxylation of proline and asparagine residues by prolyl hydroxylase 2 and factor inhibiting HIF-1, respectively. In hypoxia, HIFα proteins are stabilized, dimerized with HIFβ and bind to hypoxia-response elements in the regulatory regions of target genes [10, 11].

In the hypoxic kidney, HIF-1α accumulates in tubules and papillary interstitial cells whereas HIF-2α is induced in peritubular endothelial cells and fibroblasts (reviewed in Nangaku and Ekardt. Hypoxia alters PTE matrix metabolism, promoting ECM accumulation with a switch to production of interstitial collagen and suppression of matrix degradation. EMT is increasingly implicated in fibrosis. Exposure of PTE to hypoxia induces a myofibroblastic phenotype whereas more prolonged exposure leads to mitochondrial injury and apoptosis consistent with the loss of tubular cells in vivo [12].

Data from non-renal fibroblasts suggest additional profibrotic effects of hypoxia in renal fibroblasts in suppressing apoptosis, inducing the production of proinflammatory factors and upregulating components of the renin-angiotensin system [13, 14]. However, this issue has not been studied in patients with NS.

This study aimed to investigate basic clinical (age, disease duration), basic clinical (serum creatinine, serum cholesterol, complete blood count data, GFR, proteinuria, total blood albumin level, alfa2-globulins, edema), transcriptional factor and marker of intracellular hypoxia HIF-1alfa as possible markets of staging and stratification in children with NS and different severity grade; to evaluate the peculiarities of the mentioned above markers into-relation in clustering groups of children with nephrotic syndrome based on treatment response.

Our results show that all examined children with NS may be divided into three groups based on the severity grade of NS. Stratification based on levels of proteinuria, total blood protein, alfa2-globulins level in blood, serum cholesterol level, edema.

The 1st-grade group was found to have a mild increase of HIF-1alfa up to 185-195 a.u. proteinuria 3,5-5,5 g/24 h, total blood protein 47-53 g/L, alfa2-globulins level in blood 20-23 g/L, serum cholesterol level 6-8,5 mMol/L, edema – 1-1.6 points. The 2nd-grade group found to have moderate increase of HIF-1alfa up to 195,1-205 a.u., proteinuria 5,51-8,5 g/24 h, total blood protein 46,9-40 gL, alfa2-globulins level in blood 23,1-27 g/L, serum cholesterol level 8.51-10,5 mMol/L, edema 1.61-2.2 points. The 3rd-grade group found to have pronounced increase of HIF-1alfa up to 205,1-220 a.u., proteinuria 8,51-14 g/24 h, total blood protein 39,9-32 g/L, alfa2-globulins level in blood 27,1-30 gL, serum cholesterol level 10.51-13.5 mMol/L, edema 2.21-3 points.

Our results show a gradual increase of HIF-1alfa in children with NS and frequent relapses as compared to the group with rare relapses. Interestingly, the highest level of serum cholesterol was in the 3rd-grade group, the lowest in the 1st- grade group. It is known that NS is accompanied by disordered lipid metabolism. The traditional explanation for hyperlipidemia in NS was the increased synthesis of lipoproteins that accompany increased hepatic albumin synthesis due to hypoalbuminemia. However, serum cholesterol levels have been shown to be independent of albumin synthesis rates. Decreased plasma oncotic pressure may play a role in increased hepatic lipoprotein synthesis. Also contributing to the dyslipidemia of NS are abnormalities in regulatory enzymes, such as lecithin-cholesterol acyltransferase, lipoprotein lipase, and cholesterol ester transfer protein. The mechanism for its occurrence is complex and involves a combination of reduced clearance of lipoproteins from the circulations and increased hepatic synthesis of lipoproteins [15, 16].

To summarize, hypoxia provides a homing signal for inflammatory cells that accumulate at sites of injury. It may also activate resident immune cells and as such may be an important inflammatory stimulus in the setting of CKD particularly where, in the absence of vascular regeneration, chronic hypoxia may potentiate an ongoing pro-inflammatory response or impede resolution and stimulate fibrosis. In addition, it has been suggested that some inflammatory cells have the potential to differentiate into fibroblasts and to contribute to pathological ECM accumulation, whether hypoxia can drive this process is an intriguing possibility that remains to be tested. Finally, our results allowed us to determine groups of patients with NS with grades of severity and pattern of the treatment response (Fig. 8).

A substantial body of evidence has accumulated from in vitro studies, in vivo models, and, more recently, from studies in humans to place hypoxia at the center of ideas on mechanisms of progression of CKD. A detailed understanding of the role of hypoxia in fibrosis and the interaction of hypoxia with other factors influencing progression opens the door to a variety of novel therapeutic strategies aimed at preventing or retarding a wide range of intractable kidney diseases.

Conclusions:

- Three groups of children with NS may be stratified based on the severity grade of NS. Stratification based on levels of proteinuria, total blood protein, alfa2-globulins level in blood, serum cholesterol level, edema.

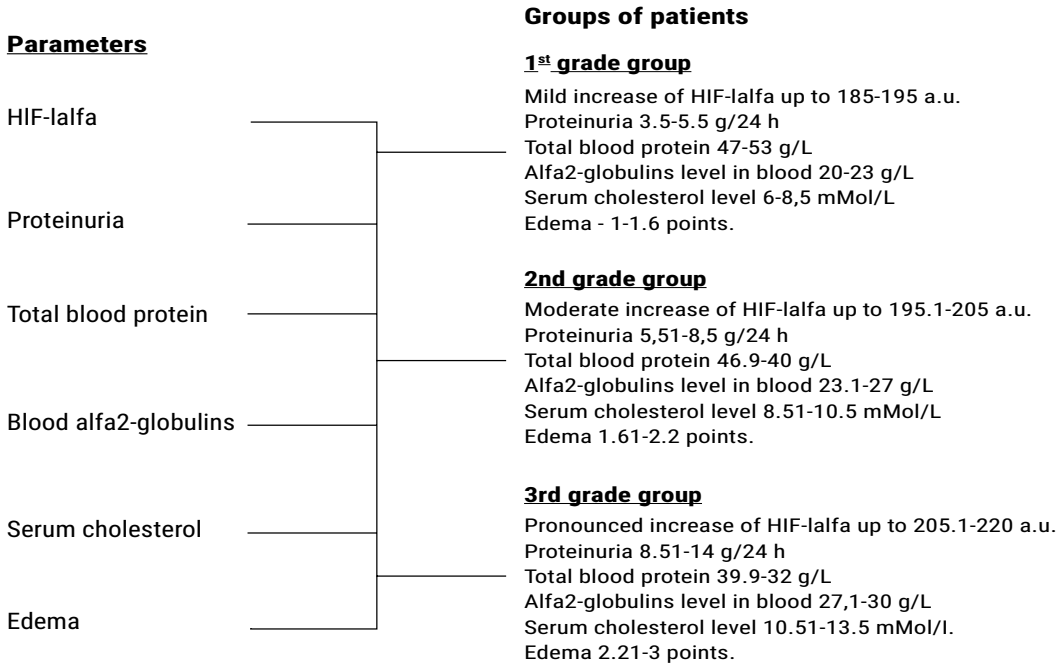

Fig. 8. Groups of patients with NS with different activity grades and patterns of the treatment response.

- 1st-grade group found to have a mild increase of HIF-1alfa up to 185-195 a.u. proteinuria 3,5-5,5 g/24 h, total blood protein 47-53 g/L, alfa2-globulins level in blood 20-23 g/L, serum cholesterol level 6-8,5 mMol/L, edema - 1-1.6 points.
- 2nd grade group found to have moderate increase of HIF-1alfa up to 195,1-205 a.u., proteinuria 5,51-8,5 g/24 h, total blood protein 46,9-40 g/L, alfa2-globulins level in blood 23,1-27 g/L, serum cholesterol level 8.51-10,5 mMol/L, edema 1.61-2.2 points.
- 3rd-grade group found to have pronounced increase of HIF-1alfa up to 205,1-220 a.u., proteinuria 8,51-14 g/24 h, total blood protein 39,9-32 g/L, alfa2-globulins level in blood 27,1-30 g/L, serum cholesterol level 10.51-13.5 mMol/L, edema 2.21-3 points.
- Higher HIF-1alfa level appears in children with NS and frequent relapses as compared to the group with rare relapses.
- Increased level of HIF-1alfa in the range 185-205 a.u. which corresponds to the 1st-2nd grade of NS severity groups can be used as a starting point in the implementation of specific anti-hypoxia and anti-oxidant therapeutic interventions in children with NS.
- A detailed understanding of the role of hypoxia in fibrosis and other kidney cells and the interaction of hypoxia with other factors influencing progression opens the door to a variety of novel therapeutic strategies aimed at preventing or retarding a wide range of intractable kidney diseases.

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Authors contribution.

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

Mityuryayeva I.O.: literature search, study design, planning, data analysis, manuscript writing and submission.

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

References

1. *Li S, Wang F, Sun D.* The renal microcirculation in chronic kidney disease: novel diagnostic methods and therapeutic perspectives. Cell Biosci. 2021; 11, 90. doi: 10.1186/s13578-021-00606-4.
2. *Lee JM, Kronbichler A, Shin JI, et al.* Current understandings in treating children with steroid-resistant nephrotic syndrome. Pediatr Nephrol. 2021; 36: 747–761. doi: 10.1007/s00467-020-04476-9.
3. *Hjorten R, Anwar Z, Reidy KJ.* Long-term Outcomes of Childhood Onset Nephrotic Syndrome. Front Pediatr. 2016; 4:53. doi:10.3389/fped.2016.00053.
4. *Burlaka IeA, Bagdasarova IV.* Molecular factors predicting steroid resistance in pediatric nephrotic syndrome. Ukr J Nephrol Dialys. 2021; 2 (70):32-37. doi:10.31450/ukrjnd.2(70).2021.04.

5. *Mihai S, Codrici E, Popescu ID, Enciu A-M, Albulescu L, Necula LG, Mambet C, Anton G, Tanase C.* Inflammation-Related Mechanisms in Chronic Kidney Disease Prediction, Progression, and Outcome. Journal of Immunology. 2018; 16. doi: 10.1155/2018/2180373.
6. *Eddy AA.* Progression in chronic kidney disease. Adv Chronic Kidney Dis. 2005; 12: 353-365. doi: 10.1053/j.ackd.2005.07.011.
7. *Zoja C, Abbate M, Remuzzi G.* Progression of renal injury toward interstitial inflammation and glomerular sclerosis is dependent on abnormal protein filtration. Nephrol Dial Transplant. 2015; 30: 706-712. doi: 10.1093/ndt/gfu261.
8. *Tanaka S, Tanaka T, Nangaku M.* Hypoxia and hypoxia-inducible factors in chronic kidney disease. Ren Replace Ther. 2016; 2:25. doi.: 10.1186/s41100-016-0038-y.
9. *Ziello JE, Jovin IS, Huang Y.* Hypoxia-Inducible Factor (HIF)-1 regulatory pathway and its potential for therapeutic intervention in malignancy and ischemia. Yale J Biol Med. 2007; 80(2):51-60. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2140184/.
10. *Kling L, Schreiber A, Eckardt K-U, Kettritz R.* Hypoxia-inducible factors not only regulate but also are myeloid-cell treatment targets. Journal of leukocyte biology. 2021; 110 (1), 61-75. doi: 10.1002/JLB.4RI0820-535R.
11. *Schofield C, Ratcliffe P.* Oxygen sensing by HIF hydroxylases. Nat Rev Mol Cell Biol. 2004; 5:343–354. doi: 10.1038/nrm1366.
12. *Haase VH.* Hypoxia-inducible factors in the kidney. Am J Physiol Renal Physiol. 2006; 291(2): 271-281. doi:10.1152/ajprenal.00071.2006.
13. *Adamcova M, Kawano I, Simko F.* The Impact of microRNAs in Renin-Angiotensin-System-Induced Cardiac Remodelling. Int J Mol Sci. 2021; 22(9): 4762. doi: 10.3390/ijms22094762.
14. *Liu Y, Su YY, Yang Q, et al.* Stem cells in the treatment of renal fibrosis: a review of preclinical and clinical studies of renal fibrosis pathogenesis. Stem Cell Res Ther . 2021; 12: 333. doi: 10.1186/s13287-021-02391-w.
15. *Agrawal S, Zaritsky JJ, Fornoni A, Smoyer WE.* Dyslipidaemia in nephrotic syndrome: mechanisms and treatment. Nat Rev Nephrol. 2018; 14(1): 57-70. doi: 10.1038/nrneph.2017.155.
16. *Tao M, Wang HP, Sun J, Tian J.* Progress of research on dyslipidemia accompanied by nephrotic syndrome. Chronic Dis Transl Med. 2020; 6(3): 182-187. doi: 10.1016/j.cdtm.2020.03.002.