APPRAOCH TO THE EVALUATION OF CLINICAL AND METABOLIC FACTORS INTERACTION IN CHILDREN WITH NEPHROTIC SYNDROME

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Nephrotic syndrome (NS) is the most common glomerular kidney disease in childhood. The limited success of existing therapeutic strategies in slowing the progression of chronic kidney disease requires the study of new ways of assessing and interpreting levels of chronic intracellular hypoxia relative to basic clinical data and indicators of kidney function in children with NS.

The purpose of the work – to investigate the main clinical and laboratory indicators, the state of the transcription factor and the marker of intracellular hypoxia HIF-1 alpha as possible factors of cluster division and stratification according to the severity of damage in children with NS, to evaluate the peculiarities of the relationship of the above-mentioned markers in the selected cluster groups of children.

Materials and methods. 37 children with hormone-sensitive NS were examined. The intracellular hypoxia marker HIF-1 alpha, creatinine, cholesterol in blood plasma samples, general blood analysis data, glomerular filtration rate (GFR), proteinuria level were determined. For multiple comparisons and testing the significance of differences, the ANOVA method was used followed by the Kruskal-Wallis post-hoc test. The correlation between the studied factors was determined by the Pearson test. GraphPad Prism 9.0 Software was used. Two-stage cluster analysis was performed using Statistica 10.0 software. P values <0.05 were considered statistically significant.

The results. Four cluster groups of children with NS were formed, which differed in the level of HIF-1 alpha. It was established that the increase of HIF-1 alpha to 189.2±1.37 units. associated with a decrease in GFR, an increase in inflammatory phenotype, and a decrease in cholesterol. HIF-1 alpha at the level of 214.4±1.81 units. associated with a decrease in GFR, an increase in inflammatory status, and a decrease in HIF-1 alpha.

Conclusion. The increase of HIF-1 alpha to 189.2±1.37 units and more in children with NS can be used as a starting point for specific antihypoxic therapeutic measures.

Key words: hypoxia, nephrotic syndrome, kidney function, risk group.

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ПІДХІД ДО ОЦІНКИ ВЗАЄМНОЇ КЛІНІЧНОЇ І МЕТАБОЛІЧНОЇ ФАКТОРІВ У ДІТЕЙ ІЗ НЕФРОТИЧНИМ СИНДРОМОМ

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Нефротичний синдром (НС) є найбільш поширенім гломерулярним захворюванням нирок у дитячому віці. Обмежений успіх існуючих терапевтичних стратегій у сповільненні прогресування хронічної хвороби нирок вимагає вивчення нових шляхів оцінки і трактування рівня хронічної внутрішньоклітинної гіпоклії відносно основних клінічних даних та показників функції нирок у дітей із НС.

Мета – дослідити основні клінічні, лабораторні показники, стан транскрипційного фактора і маркера внутрішньоклітинної гіпоклії HIF-1 alpha як можливих факторів кластерного поділу та стратифікації за тяжкістю пошкоджень у дітей із НС, оцінити особливості взаємозв’язку зазначених вище маркерів у виділенних клінічних групах дітей.

Матеріали та методи. Обстежено 37 дітей із гормон-чутливим НС. Визначали маркер внутрішньоклітинної гіпоклії HIF-1 alpha, креатинин, холестерин у зразках плазми крові, показники загального аналізу крові, швидкість клубочкової фільтрації (ШКФ), рівень протеїну. Для вивчення порівняння та перевірки значущості відмінностей використано метод ANOVA з подальшим пост-нос тестом Краскала-Уолліса. Кореляції між досліджуваними факторами визначали за критерієм Пірсона. Використано пограничне забезпечення GraphPad Prism 9.0 Software. Двостадійний кластерний аналіз виконано за допомогою програмного забезпечення Statistica 10.0. Значення P <0.05 вважали статистично значущими.

Результати. Сформовано чотири кластерні групи дітей із НС, які відрізнялися за рівнем HIF-1 alpha. Встановлено, що підвищення HIF-1 альфа до 189,2±1,37 усл.д.
Introduction

Functional impairment in chronic kidney disease is correlated with tubulointerstitial fibrosis, which is characterized by inflammation, extracellular matrix accumulation, tubular atrophy, and alterations in the peritubular capillaries. The loss of microvasculature suggests that hypoxia is present and may play a significant impact. The most frequent glomerular condition in children is nephrotic syndrome (NS) [1]. In Western Europe, the reported incidence in children ranges from 1.2 to 3.5 per 100,000 per year, while globally it is 4.7 per 100,000 per year [2]. Various aspects, such as the pathomorphological type of disease (focussed segmental glomerulosclerosis, or FSGS) as the most common lesion, genetic markers, and metabolic factors, have been studied in relation to the course of NS.

While studies in rodent models more directly support a causative involvement, recent data show lower renal oxygenation in chronic kidney disease. Hypoxia has been also linked to the maintenance of the inflammatory response, the recruitment, retention, and differentiation of circulating progenitor cells towards a pro-fibrotic phenotype, and the modification of intrinsic stem cell populations’ functions in chronic kidney disease [5]. Chronic kidney diseases (CKDs) of various etiologies are characterized by renal fibrosis, a hallmark of which is the deposition of extracellular matrix (ECM) that disturbs normal tissue architecture and eventually leads to organ failure and renal dysfunction [6].

The pathogenesis of CKD is common to both glomerulosclerosis and tubulointerstitial fibrosis, regardless of the initial insult. It is well known that tubulointerstitial fibrosis is the strongest predictor of the development of end-stage illness. Numerous distinctive characteristics are observed in tubulointerstitial fibrosis [6, 7].

The growing body of evidence points to persistent hypoxia as the last common mechanism leading to end-stage of renal disease. These new pathways of chronic hypoxia in relation to fundamental clinical data and kidney function (GFR level) need to be investigated because the efficacy of current therapies to delay chronic kidney disease is limited [8].

In order to determine potential markets for staging and stratification in children with NS and varying levels of kidney function impairment, this study looked into basic clinical factors such as age and disease duration, as well as basic clinical factors such as serum creatinine, serum cholesterol, complete blood count data, GFR, and proteinuria. Additionally, it evaluated the unique relationships between the aforementioned markers in clustering groups of children with nephrotic syndrome.

The purpose of the work

To investigate the main clinical and laboratory indicators, the state of the transcription factor and the marker of intracellular hypoxia HIF-1alpha as possible factors of cluster division and stratification according to the severity of damage in children with NS; to evaluate the peculiarities of the relationship of the above-mentioned markers in selected cluster groups of children.

Material and methods

The study was conducted on 37 selected patients with NS. A written consent was obtained from the parents of all participants. The study was approved by the local ethical committee of Bogomolets National Medical University (Protocol № 142) and the research is complied with Helsinki Declaration.

Plasma samples were used to measure marker intracellular hypoxia HIF-1alpha by means of western blotting method. In addition, creatinine, cholesterol in blood plasma samples, indicators of general blood analysis, glomerular filtration rate (GFR), proteinuria level were determined by generally accepted methods.

Patients inclusion criteria were those fulfilled the NS triad of, i.e. heavy proteinuria (>3 gm/ day), hypoalbuminemia, and edema. Other than nephrotic causes of proteinuria were excluded in all patients recruited to the study.

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 used to test significance of differences. Pearson correlation was run to study the correlation between factors. Data were processed using GraphPad Prism 9.0 Software for Windows (USA, San Diego, CA). Two-step clustering was done using Statistica 10.0 software. P values < 0.05 were considered statistically significant.

Results and their discussion

The study was carried out during the period over 24 months. 37 patients with steroid-sensitive NS were included in to the study. Basic clinical data of patients are given in Table 1.

The clustered results based on nine variables: HIF-1alpha, disease course, age, proteinuria level, glomerular filtration rate (GFR), serum creatinine (S-Cr), serum cholesterol, white blood cells count (WBC), erythrocytes sedimentation rate (ESR) were shown as 4 subgroups.

Cluster groups named – Group 1, Group 2, Group 3, Group 4. Cluster groups are described in Table 2.
Pearson correlation ran to correlate principal factors found to be those defining clustering groups in children with NS. In Group 1 significant positive correlation was observed between HIF-1alfa and S-Cr levels ($r = 0.9242$, 95 % CI 0.6295 to 0.9864, $R^2 = 0.8541$, $p = 0.001$) (Fig. 1A). In Group 2 significant positive correlation was observed between HIF-1alfa and WBC count levels ($r = 0.7211$, 95 % CI 0.1093 to 0.9366, $R^2 = 0.0284$, $p = 0.0284$) (Fig. 1B). In Group 3 significant positive correlation was observed between HIF-1alfa and GFR levels ($r = -0.9691$, 95 % CI -0.9956 to -0.7996, $R^2 = 0.9392$, 0.0003) and significant positive correlation between HIF-1alfa and S-Cr levels ($r = 0.9140$, 95 % CI 0.5165 to 0.9874, $R^2 = 0.8355$, 0.004) (Fig. 1C). In Group 4 significant positive correlation was observed between HIF-1alfa and Proteinuria ($r = 0.6023$, 95 % CI 0.04346 to 0.8741, $R^2 = 0.3628$, $p = 0.0382$) (Fig. 1D).

Given that hypoxia plays a major role in renal fibrosis, pharmacological control of the hypoxic response may be helpful in halting or reducing the progression of the condition. 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 are some strategies to lessen the effects of hypoxia-mediated profibrotic changes [8,9].

The adaptive response to hypoxia is largely regulated by HIF [10]. Prolyl hydroxylase 2 and factor inhibiting HIF-1, respectively, hydroxylate proline and asparagine residues in an oxygen-dependent manner that controls the stability and activity of the α-subunit in normoxia. HIFα proteins stable under hypoxia, dimerize with HIFβ, and attach to hypoxia-responsive elements found in target genes’ regulatory areas [10,11].

HIF-1α builds up in tubules and papillary interstitial cells in the hypoxic kidney, while HIF-2α is activated in fibroblasts and peritubular endothelial cells. By switching to the synthesis of intestinal collagen and suppressing matrix breakdown, hypoxia modifies PTE matrix metabolism and promotes the formation of extracellular matrix. Fibrosis is increasingly linked to EMT. While prolonged exposure to hypoxia causes mitochondrial damage and death associated with the loss of tubular cells in vivo, exposure of PTE to hypoxia induces a myofibroblastic phenotype [12-14].

In order to determine potential markets for staging and stratification in children with NS and varying levels of kidney function impairment, this study looked into basic clinical factors such as age and disease duration, as well as basic clinical factors such as serum creatinine, serum cholesterol, complete blood count data, GFR, and proteinuria. It also looked into transcriptional factors and the marker of intracellular hypoxia, HIF-1alfa.

Our results show that children from Group 2 have increase in HIF-1 alpha expression and GFR decline absent in Group 1. Meaning the increase of HIF-1 alpha up to 189.2±1.37 a.u reflects decline of GFR that possibly due to microvascular disorders and partially due to increase of inflammatory cells infiltration. In Group 2 WBC count was somewhat higher as compared to Group 1. Moreover, in Group 2 significant positive correlation was observed between HIF-1alfa and WBC count levels.

In like with Group 2 results Groups 3 data show that these patients have average value of HIF-1 alpha 200.2±3.02 a.u. Increase in HIF-1alpha level associated with GFR decline, more prominent inflammatory status. In Group 3 significant negative correlation is observed between HIF-1alfa and GFR levels and significant positive correlation between HIF-1alfa and S-Cr levels meaning that hypoxic injury have direct effect on kidney function impairment.

### Clinical characteristics of the patients with NS

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>NS patients (n=37) M±SEM or %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys</td>
<td>21 (55.3 %)</td>
</tr>
<tr>
<td>Girls</td>
<td>16 (44.7 %)</td>
</tr>
<tr>
<td>Age, years</td>
<td>12.25±0.85</td>
</tr>
<tr>
<td>Disease duration, years</td>
<td>7.65±0.33</td>
</tr>
<tr>
<td>BMI</td>
<td>21.8±0.73</td>
</tr>
<tr>
<td>Hypertension</td>
<td>17 (45.9 %)</td>
</tr>
<tr>
<td>Edema</td>
<td>37 (100 %)</td>
</tr>
<tr>
<td>WBC count, 10³/L</td>
<td>7.45, 10³L</td>
</tr>
<tr>
<td>ESR, mm/h</td>
<td>9.14 mm/h</td>
</tr>
</tbody>
</table>

Note: WBC – white blood cells; ESR – erythrocytes blood cells.

### Clustering groups characteristics

<table>
<thead>
<tr>
<th>Parameters, Mean</th>
<th>HIF-1alfa, a.u.</th>
<th>Age, years</th>
<th>Disease course, years</th>
<th>Proteinuria, mg/24h</th>
<th>GFR, mL/min/1.73 m²</th>
<th>S-Cr, mMol/L</th>
<th>Serum cholesterol, mMol/L</th>
<th>WBC count, 10³/L</th>
<th>ESR, mm/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>187.4</td>
<td>12.25</td>
<td>6,750</td>
<td>9,213</td>
<td>120.4</td>
<td>46.13</td>
<td>11.49</td>
<td>4.975</td>
<td>7.50</td>
</tr>
<tr>
<td>Group 2</td>
<td>189.2</td>
<td>11.44</td>
<td>6,667</td>
<td>8,633</td>
<td>75.62</td>
<td>70.89</td>
<td>10.69</td>
<td>6.282</td>
<td>6.667</td>
</tr>
<tr>
<td>Group 3</td>
<td>200.2</td>
<td>9,000</td>
<td>8,143</td>
<td>6,929</td>
<td>87.75</td>
<td>57.14</td>
<td>8.824</td>
<td>8.273</td>
<td>10.00</td>
</tr>
<tr>
<td>Group 4</td>
<td>214.4</td>
<td>12.00</td>
<td>7,667</td>
<td>9,700</td>
<td>66.95</td>
<td>92.33</td>
<td>10.24</td>
<td>11.44</td>
<td>10.17</td>
</tr>
</tbody>
</table>

Note: WBC – white blood cells; ESR – erythrocytes sedimentation rate; GFR – glomerular filtration rate; S-Cr – serum creatinine.
A. 

HIF-1 alfa, a.u. vs. S-Cr, mcMol/L

\[ r = 0.9242 \]
95% CI = 0.6295 to 0.9864
\[ R^2 = 0.8541 \]
\[ p = 0.0010 \]

B. 

HIF-1 alfa, a.u. vs. WBC count, 10.9/L

\[ r = 0.7211 \]
95% CI = 0.1093 to 0.9366
\[ R^2 = 0.5199 \]
\[ p = 0.0284 \]

C. 

HIF-1 alfa, a.u. vs. GFR, mL/min/1.73 m2

\[ r = -0.9691 \]
95% CI = -0.9956 to -0.7996
\[ R^2 = 0.9392 \]
\[ p = 0.0003 \]

HIF-1 alfa, a.u. vs. S-Cr, mcMol/L

\[ r = 0.9140 \]
95% CI = 0.5165 to 0.9874
\[ R^2 = 0.8355 \]
\[ p = 0.0040 \]

D. 

HIF-1 alfa, a.u. vs. Proteinuria, g/24 h

\[ r = 0.6023 \]
95% CI = 0.04346 to 0.8741
\[ R^2 = 0.0382 \]
\[ p = 0.0382 \]

Fig. 1. Pearson correlation between principal factors found to be those defining clustering groups in children with NS and factors co-incidence. Group 1 (A), Group 2 (B), Group 3 (C), Group 4 (D).

The most prominent changes of the mentioned above parameters were found in Group 4. Also in Group 4 significant positive correlation is observed between HIF-1 alfa and WBC count levels which is a possible sign that the source of HIF-1 alfa in this group of patients might be WBC in parallel with other sources.

Interestingly, the highest level of serum was measured in Group 1, the lowest in Group 3. It is known that NS
is accompanied by disordered of lipid metabolism. The increased synthesis of lipopolysaccharides that coincides with increased hepatic albumin production as a result of hypoalbuminemia was the classic explanation for hyperlipidemia in NS. It has been demonstrated, meanwhile, that albumin production rates have little bearing on serum cholesterol levels. Increased hepatic lipoprotein production could be related to decreased plasma oncotic pressure. Anomalies in regulatory enzymes, including lipoprotein lipase, cholesterol ester transfer protein, and lecithin-cholesterol acyltransferase, also contribute to the dyslipidemia of NS. Its occurrence is caused by a complicated mechanism that combines increased hepatic production of lipoproteins with decreased lipoprotein clearance from the circulation \cite{15, 16}.

We speculate that lower serum levels of cholesterol accompanied with higher HIF-1α levels in Group 2 and Group 3 may be a result of lipids oxidation.

In summary, inflammatory cells that gather at damage sites use hypoxia as a homing signal. In the context of chronic hypoxia-ischemic kidney disease (CKD), it may also excite local immune cells and, as such, be a significant inflammatory stimulus. In particular, in the absence of vascular regeneration, chronic hypoxia may intensify a continuing pro-inflammatory response or obstruct resolution and promote fibrosis. Furthermore, some inflammatory cells may be able to develop into fibroblasts and contribute to the abnormal build-up of extracellular matrix (ECM). It is an exciting notion that needs to be investigated if hypoxia might initiate this process.

A significant amount of data has been gathered from in vivo models, in vitro research, and, more recently, human investigations, placing hypoxia at the core of theories regarding the mechanisms behind the development of chronic kidney disease (CKD). A thorough grasp of how hypoxia functions in fibrosis and how it interacts with other elements that affect the disease’s growth can lead to a number of innovative therapeutic approaches that can be used to stop or slow the advancement of a wide spectrum of incurable kidney disorders.

**Conclusions**

Three groups of patients with nephrotic syndrome with different degrees of risk of GFR reduction were distinguished according to the following characteristics: low-risk group: moderate increase in HIF-1 alpha level, age – teenagers, average duration of the disease – 6 years, proteinuria – 8-9 g/24 h, moderate increase of S-Cr, low inflammatory status; group with a moderate degree of risk: moderate-high increase in the level of HIF-1 alpha, average childhood age, average duration of the disease – 9 years, proteinuria about 7 g/24 h, moderate increase in S-Cr, subhigh inflammatory status; high-risk group: significant increase in the level of HIF-1 alpha, age – teenagers, average duration of the disease – 7.6 years, proteinuria about 9 g/24 h, significant increase in S-Cr, high inflammatory status.

**Список літератури**


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References

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