Endothelial dysfunction in children with pyelonephritis

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Aim. To study the characteristics of vascular endothelium system functioning as a marker of the balance between endothelium-dependent vasodilation and endothelium-dependent vasoconstriction in children with acute and chronic pyelonephritis.

Materials and methods. 39 children with acute and 37 with chronic pyelonephritis aged 11–17 years, without exacerbation of the inflammatory process were examined. The control group consisted of 30 apparently healthy children. Endothelium-dependent vasodilation test adapted to children was used.

Results. In children with acute pyelonephritis, the initial index of the brachial artery diameter did not differ significantly from the norm (3.40 ± 0.19 mm, P > 0.05), while it was significantly less in patients with chronic pyelonephritis (2.20 ± 0.12 mm, P < 0.05). In the phase of maximal vasodilation, the brachial artery diameter index was significantly higher (4.10 ± 0.15 mm, P < 0.05) in patients with acute pyelonephritis, and in patients with chronic pyelonephritis – significantly less than the norm (2.60 ± 0.17 mm, P < 0.05). As a result, the brachial artery diameter increase was significantly less in children with chronic pyelonephritis than in healthy children (8.2 ± 0.2 %, P < 0.05), while it was significantly higher (24.10 ± 0.53 %, P < 0.05) in children with acute pyelonephritis.

Conclusion. Endothelial dysfunction is registered in 17.9 % of acute pyelonephritis and in 64.9 % of chronic pyelonephritis cases in children. Diverging paths are observed: in patients with acute pyelonephritis, activity of vasodilatory agents predominates, and in chronic pyelonephritis – vasoconstrictor agents. Endothelial dysfunction associated with chronic pyelonephritis has the risk of unfavorable course of the disease and requires differential management.
Introduction

Currently, there is a common understanding that the system of vascular endothelium (SVE) is a powerful autocrine-paracrine organ that provides homeostasis of the vessel wall and prevents the development of pathology [2,7,9]. As a multifunctional organ, SVE is simultaneously the effector and target of many pathological processes [2,16,20]. Endothelium synthesizes and releases vasoactive substances in response to constant influence of endovascular factors. [2,5,10,17]. Due to the prolonged exposure to adverse factors (hemodynamic overload, hypoxia, intoxication, inflammation), a dysfunction of endothelial lining develops with vasoconstriction, hypercoagulation, proliferation of the vascular elements and thrombosis with intravascular fibrin/fibrinogen deposition as the response to regular stimuli, resulting in disorders of blood microcirculation, capillary-trophic insufficiency and ischemia [5,8]. That is, endothelial dysfunction (ED) is formed. ED in the modern sense is a complex system of disorders that starts on the molecular-genetic level, imbalance between vasodilator production, angio- and antiproliferative factors, on the one hand, and vasococontractors, prothrombotic, pro- and antiproliferative factors, on the other hand [5,14,18].

Recent studies have confirmed that ED plays one of the main roles in renal disease development, it is already present in early stages of chronic kidney disease (CKD) in children, being one of the early markers and an important pathogenetic link in the progression of CKD [6,7,10,14]. Increasing structural changes lead to disturbance of SVE morphogenetic equilibrium, therewith changed SVE tends to maintain its pathological functioning [8].

To date, the concept of ED as a key link in CKD pathogenesis has been formulated, and the role of the endothelium, the impaired function of which has been demonstrated to be long before the structural changes development in the kidneys [9,12,15].

However, until now it remains unclear which markers of ED are the most significant in reducing renal functions in adults and children, and which concentrations in the body can serve as predictors of CKD progression in childhood [3,15]. The modern concept of CKD which reflects the nature and rate of renal disease progression to the end stage of renal failure requires careful study of ED role, as a possible prognostically significant factor in the development of nephrosclerosis [1,5,7,10,15].

Aim

To study the peculiarities of vascular endothelium system functioning as a marker of the balance between endothelium-dependent vasodilation and endothelium-dependent vasoconstriction in children with acute and chronic pyelonephritis.

Materials and methods

We examined 76 children aged 11–17 years (31 boys and 45 girls) with pyelonephritis (PN), 39 of whom were with acute PN (APN) and 37 – with chronic PN (CPN). All the children received an inpatient treatment in the Nephrology Department of CI “Dnipropetrovsk Regional Children’s Clinical Hospital”, DRC. The control group consisted of 30 relatively healthy children undergoing examination for functional diseases of the gastrointestinal tract. The criteria for inclusion in the study were: the presence of a voluntary informed consent of the child and his/her parents for study participation; age of patients 11–17 years 11 months 29 days; presence of verified diagnoses of acute and chronic pyelonephritis; a period of 1 month after the last acute pyelonephritis; absence of clinical and laboratory signs of exacerbation of chronic pyelonephritis during the last 3 months. The criteria for exclusion of patients from the study were: refusal of the child or his/her parents to participate in the study; the presence of clinical and laboratory signs of chronic pyelonephritis exacerbation. The children were grouped according to the nosological principle.

Clinical-laboratory and instrumental examinations were carried out in the Clinical-Diagnostic Laboratory and the Department of Ultrasound Diagnostics of the CI “Dnipropetrovsk Region Children’s Clinical Hospital” DRC.

To assess the functional state of the endothelium, we used a reactive hyperemia test adapted to children (Patent No. 32359 “Method for diagnosing endothelium-dependent vasodilation in children”, IPC (8):A61V8/00 of May 12, 2008, authors O. P. Volosovets, S. P. Kryvopustov, T. S. Moroz).

Measurement of flow-dependent dilatation of the brachial artery according to ultrasound data is currently considered as the «gold standard» for the endothelial function of vessels study [4,17,18,20]. The study method is high-tech and non-invasive, which greatly simplifies and accelerates the diagnosis of ED in children with various pathology [2,11,17,19].
Endothelium-dependent vasodilatation (EDVD) test was performed as follows: patients were examined in the supine position after a 10–15-minute rest, at least a 10-hour fast and abstinence from coffee, tea, bad habits and exercise. All participants were requested to refrain from taking any vasoactive medications no less than 48 hours before the study.

A scan of the right brachial artery was performed on a Toshiba Xario (Japan) device, in areas 2 to 15 cm above the bend of elbow using a linear 7.5 MHz sensor, which allowed to estimate the diameter of the brachial artery (DBA) to within 0.1 mm to 0.2 mm. After obtaining the initial data of arterial pressure (AP), diameter of the brachial artery (DBA), and linear blood flow rate (LBFR), the cuff was inflated for 1.5–2.0 min to 50 mm Hg plus systolic blood pressure of the patient. The time of occlusion depended on the appearance of pain, tingling, numbness of fingers. The diameter of the brachial artery (DBA) was measured again 30 seconds before deflation. 15 seconds following release of the occlusion cuff (in the phase of reactive hyperemia), the linear blood flow (LBFR) was recorded, and in 60 seconds, in the phase of maximal vasodilation – the diameter of the brachial artery (DBA).

EDVD was calculated as percentage change in brachial artery diameter from baseline by the formula:

$$\Delta d, \% = 100 \times \frac{DBA_3 - DBA_2}{DBA_1},$$

Depending on DBA dynamics during the test, four types of reaction were identified: normoergic type – with the increase in DBA from 10 % to 20 %; hyperergic type – with the increase in DBA by 20–40 %; hypoergic type – with the increase in DBA less than 10 % or absence of reaction; paradoxical type – with the decrease in DBA in comparison with the baseline. ED was diagnosed in case of hypoergic or paradoxical types of reaction.

In addition, the quantitative angle-dependent indices were determined: the maximum blood flow rate in systole (Vmax, m/s), the minimum blood flow rate in diastole (Vmin, m/s). Parameters of the peripheral vascular resistance were estimated – resistance index, or Pourcelot index (RI). The RI was determined by the formula: $RI = (Vps - Ved) / Vps$, where $RI$ – peripheral resistance index, $Vps$ – peak systolic blood flow velocity, $Ved$ – end-diastolic blood flow velocity.

The statistical processing of the obtained data was performed using the Microsoft Office 2010 applications integrated in Windows 7. The significant differences between average values were determined by Student’s parametric criterion, chi-squared distribution and Fisher’s criterion. Differences were considered significant in a P value < 0.05.

**Results**

In children with APN, DBA index had a tendency to increase, statistically insignificantly differing from the norm – 3.40 ± 0.19 mm, $P > 0.05$ (Table 1).

By contrast, DBA index was significantly lower ($2.20 \pm 0.12$ mm, $P < 0.001$) in CPN patients as compared to both APN and the control group patients. The most pronounced changes were recorded in children with chronic renal inflammatory process due to congenital anomalies of the urinary tract system. Patients had AP level exceeding 95 percentile.

The dynamics of DBA by the DBA index recorded during EDVD test 30 seconds before deflation significantly differed in APN children from the values in the control group: 2.30 ± 0.13 mm, $P < 0.001$. But herein analyzing the dynamics of this index within the group, one can say that DBA narrowing in CPN patients was significant and DBA index did not differ statistically from DBA index.

In CPN patients, the dynamics of DBA index was similar: there was a tendency to decrease as compared to baseline data in patients within the group, the value was significantly lower in comparison with both groups ($P < 0.001$).

During the test, after deflation, in the phase of maximal vasodilation, DPA index was statistically significantly higher in APN patients than the norm (4.10 ± 0.15 mm, $P < 0.05$). That is, vasodilation prevailed.

As a result, the increase in DBA by Δd value was 2.2 times less in children with CPN than in healthy subjects (8.2 ± 0.2 %; $P < 0.001$). At the same time, in children with APN, the increase in DBA testified to the predominance of vasodilation (24.10 ± 0.53 %; $P < 0.001$) as compared to those with CPN. Distribution of the DBA response variants to EDVD in children with pyelonephritis was as follows (Table 2).

The overwhelming majority of children with APN had favorable (normal and hyperergic) types of response to test – a total of 82.1 %, which did not differ significantly from the control group. However, in APN children, in contrast to the control group, hyperergic variant dominated (53.9 %, $P < 0.05$), and normal one was registered less frequently (28.2 %, $P < 0.05$). Pathological types of reaction – hypoergic and paradoxical were recorded as a whole in 17.9 %, without a significant difference from those in the control group (12.8 and 5.1 %, respectively, $P > 0.05$).

In the group of children with CPN, only 37.8 % of patients had norm- and hyperergic types of reaction to the test. Thus, normoergic type was registered only in 13.5% of patients ($P < 0.001$), hyperergic type – in 24.3 % ($P < 0.05$). The majority of patients had pathological variants of the reaction: hypoergic type was registered in the overwhelming majority – in 40.6 % of cases, which was significantly greater as compared to healthy children and patients with APN, paradoxical type – in 21.6 %, which was statistically significant in comparison with the same two groups. This confirmed serious disturbances of the SVE balancing vasoregulatory mechanisms in these patients, which had been detected earlier in the DBA findings registration.

LBFR index was significantly lower in APN children in comparison with those of both the control and CPN groups (Table 3).

In children with CPN, the value of this index was statistically higher as compared to those of the other two groups (148.70 ± 1.18 cm/sec). LBFR index in group of CPN patients was reduced remaining statistically increased in comparison with those of both the control and APN groups (142.60 ± 1.18 cm/sec, $P < 0.001$). At the same time, in children with APN, LBFR index did not differ significantly from the norm but it was significantly lower than that in CPN patients.

The RI dynamics in patients with pyelonephritis in response to DBA and LBFR changes is shown in Table 4.

Thus, in APN patients, the value of RI at rest was evidently less than the physiologic values (0.70 ± 0.02 c.u.), while in CPN group – statistically higher in comparison with that of both the control and APN groups (0.92 ± 0.03 c.u.).
After deflation, the value of RI was reduced and statistically differed between the all groups (0.64 ± 0.04 c.u. for APN patients and 0.88 ± 0.04 c.u. for CPN patients). Both groups of patients with PN in the process of EDVD test had a diverging LBFR dynamics compared with the control group, which confirmed the presence of vasomotor manifestations of ED in this category of patients.

**Discussion**

In APN patients, the dynamics of EDVD test, especially after deflation, in the phase of maximal vasodilation, demonstrated the vasodilation prevalence. This may be due to the increase in the synthesis of nitric oxide in this group of patients. Such an imbalance in the SVE can be considered as a result of the compensatory reaction – the maximum level of nitric oxide contributes to the inhibition of endothelial dysfunction caused by endothelin-1 hyperproduction, being a favorable factor in these conditions [7].

In contrast, in CPN patients, as compared to APN and the control group patients, the dynamics of the EDVD test, beginning with DBA\textsubscript{1} index, was significantly lower. This shows an imbalance in the SVE towards predominance of factors resulting in endothelium-dependent vasoconstriction in CPN patients, and vice versa, – they suppress endothelium-dependent vasodilatation.

**Table 1.** Dynamics of brachial artery diameter during endothelium-dependent vasodilation test in children with pyelonephritis

<table>
<thead>
<tr>
<th>Index</th>
<th>Control group, n = 30</th>
<th>APN group, n = 39</th>
<th>CPN group, n = 37</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter of brachial artery, mm At rest, DBA\textsubscript{1}</td>
<td>3.10 ± 0.16</td>
<td>3.40 ± 0.19</td>
<td>2.20 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>(P &gt; 0.05)</td>
<td>(P &lt; 0.05)</td>
<td>(P &lt; 0.001)</td>
</tr>
<tr>
<td>30 sec. before deflation, DBA\textsubscript{2}</td>
<td>2.30 ± 0.13</td>
<td>3.10 ± 0.11</td>
<td>1.80 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
</tr>
<tr>
<td>60 sec. after deflation, DBA\textsubscript{3}</td>
<td>3.70 ± 0.11</td>
<td>4.10 ± 0.15</td>
<td>2.60 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>(P &lt; 0.05)</td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
</tr>
<tr>
<td>Increase in DBA, ∆d,%</td>
<td>18.40 ± 0.46</td>
<td>24.10 ± 0.53</td>
<td>8.20 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
</tr>
</tbody>
</table>

\(P_1\): significant difference in comparison with the control group; \(P_2\): significant difference in comparison with APN group.

**Table 2.** Distribution of response variants to endothelium-dependent vasodilatation test in children with pyelonephritis

<table>
<thead>
<tr>
<th>Variant of reaction</th>
<th>Control group, n = 30</th>
<th>Children with APN, n = 39</th>
<th>Children with CPN, n = 37</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoergic</td>
<td>78.7 %</td>
<td>23</td>
<td>28.2 %</td>
</tr>
<tr>
<td></td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
</tr>
<tr>
<td>Hyperergic</td>
<td>23.3 %</td>
<td>7</td>
<td>53.9 %</td>
</tr>
<tr>
<td></td>
<td>(P &lt; 0.05)</td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
</tr>
<tr>
<td>Hypoergic</td>
<td>–</td>
<td>–</td>
<td>12.8 %</td>
</tr>
<tr>
<td></td>
<td>(P &lt; 0.05)</td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
</tr>
<tr>
<td>Paradoxical</td>
<td>–</td>
<td>–</td>
<td>5.1 %</td>
</tr>
<tr>
<td></td>
<td>(P &lt; 0.05)</td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
</tr>
</tbody>
</table>

\(P_1\): significant difference in comparison with the control group; \(P_2\): significant difference in comparison with APN group.

**Table 3.** Dynamics of linear blood flow rate during endothelium-dependent vasodilatation test in children with pyelonephritis

<table>
<thead>
<tr>
<th>Rate</th>
<th>Control group, n = 30</th>
<th>APN group, n = 39</th>
<th>CPN group, n = 37</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear blood flow rate cm/sec At rest, LBFR\textsubscript{1}</td>
<td>137.40 ± 2.12</td>
<td>111.80 ± 0.17</td>
<td>148.70 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
</tr>
<tr>
<td>In 15 sec after deflation, LBFR\textsubscript{2}</td>
<td>105.20 ± 1.19</td>
<td>107.10 ± 0.26</td>
<td>142.60 ± 1.18</td>
</tr>
<tr>
<td></td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
</tr>
</tbody>
</table>

\(P_1\): significant difference in comparison with the control group; \(P_2\): significant difference in comparison with APN group.

**Table 4.** Dynamics of the resistance index during endothelium-dependent vasodilatation test in children with pyelonephritis

<table>
<thead>
<tr>
<th>Rate</th>
<th>Control group, n = 30</th>
<th>APN group, n = 39</th>
<th>CPN group, n = 37</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistance index(c.u.) At rest, RI\textsubscript{1}</td>
<td>0.83 ± 0.03</td>
<td>0.70 ± 0.02</td>
<td>0.92 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
</tr>
<tr>
<td>After deflation, RI\textsubscript{2}</td>
<td>0.78 ± 0.01</td>
<td>0.64 ± 0.04</td>
<td>0.88 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>(P &lt; 0.05)</td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
</tr>
</tbody>
</table>

\(P_1\): significant difference in comparison with the control group; \(P_2\): significant difference in comparison with APN group.
Such disorders can be explained by a combination of several mechanisms: in particular, sympathetic hyperactivity development from an early CKD stage directly or indirectly via stimulation of renin-angiotensin-aldosterone system (RAAS) leads to activation of endothelin-1 synthesis, a powerful vasoconstrictor. This, in turn, causes reduced vascular wall relaxation in response to vasodilating stimuli and accounts for systemic changes in microcirculation and perfusion violation as one of the key non-immune mechanisms of CKD progression [7]. In addition, the vascular endothelium has its own renin-angiotensin system, which supports vasoconstriction [8]. Another factor that may contribute to ED with vasoconstriction predominance is the reduction of nitric oxide (NO) production, which is closely associated with a decreased number of functioning nephrons due to kidney damage as one of the pathogenetic links of ED in CKD patients [7,8,10]. According to M. Carlstrom and M. F. Montenegro [12], a decrease in the bioavailability of NO is considered to be the main adverse consequence of free radical reactions in oxidative stress in CKD patients. All these factors could contribute to the vasomotor form of ED development in CPN patients.

The most pronounced changes were recorded in children with chronic renal inflammatory process due to congenital anomalies of the urinary tract system. Patients had AP values exceeding 95 percentile. From literary reviews published by N. D. Inozemtseva et al. [5], it is known that structural changes that disturb normal renal blood flow lead to similar ischemic damage that may occur in vasoconstriction of the vessels in the parenchyma caused by increased content of angiotensin II, endothelin-1 or reduced concentration of nitric oxide.

In the phase of maximal vasodilation in patients with CPN, DPA index was significantly increased in comparison with the control group. This indicated a reduction in arterial compliance and viscoelastic properties of vessel walls deterioration due to activation of endothelial vasoconstrictors synthesis on the one hand and the violation of endothelium dependent vasodilation, on the other.

Inozemtseva N. D. et al. [5] also reported that a shift in balance between vasodilators and vasoconstrictors towards the latter results in vasospasm and makes a weighty contribution to CKD progression.

The obtained results can be explained by the elevated level of vascular endothelial growth factor, which increases with CPN progression in children earlier than decrease in glomerular filtration rate (GFR), which makes it possible to consider it as a prognostic indicator. It is known that this growth factor regulates the vascular wall tone supporting spastic processes, and with the increase in its concentration pathological vasospasm develops, promoting sclerotic process development and kidney disease progression [1]. In general, this can be considered as one of the mechanisms of kidney pathological process progression and chronization.

From literary reviews published by T. P. Makarova and others [7] it is known that in children with CKD, ED is observed from the first stage of the disease development, as the increase in endothelin-1 content, a powerful vasoconstrictor, was registered in patients, which was accompanied by the decrease in concentration of vasodilator NO as well as the decrease in NO / endothelin-1 ratio. The association between biomarkers and multiple ED indicates the presence of several endothelial function disorders in CKD patients [13].

So, the dynamics of indices in the course of EDVD test in APN patients we considered as a compensatory reaction and in CKD children – as a variant of the SVE functioning decompensation.

Conclusions

1. Endothelial dysfunction is an important pathogenetic link in the development of acute and chronic pyelonephritis in children. The convalescence period in 17.9 % of acute pyelonephritis cases and remission period in 64.9 % of chronic pyelonephritis cases are accompanied by signs of endothelial dysfunction.

2. The risk factors for developing endothelial dysfunction are the presence of congenital anomalies of the urinary tract system and arterial hypertension.

3. Acute and chronic pyelonephritis in children differs by diverging paths of endothelial dysfunction: in case of acute pyelonephritis, endothelial dysfunction during convalescence period is characterized by predominant activity of vasodilatory agents and remission of chronic pyelonephritis – vasoconstrictor agents.

4. Endothelial dysfunction associated with chronic pyelonephritis has a risk of developing an unfavorable course of the disease, probably due to violations of renal tissue perfusion, which requires differential medicamentous therapy.

Prospects for further research. It is advisable to study further ED in CPN children with various management regimens.

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Conflicts of interest: authors have no conflict of interest to declare.

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