D.A. Morozov, O.L. Morozova, N.B. Zakharova, D.Y. Lakomova
Scientific Research Institute of Fundamental and Clinical Uronephrology, Saratov State Medical University

Early diagnosing and predicting the course of nephrosclerosis in children with a vesicoureteral reflux

Urgency. In the setting of vesicoureteral reflux (VUR), nephrosclerosis forms in 30-60% of patients and leads to the final stage chronic renal disease development in 25-60% of them. Nephrosclerosis initiation and progression mechanisms at VUR depend not only on the degree of uro- and hemodynamics malfunction, but also on the level of a considerable number of biologically active substances: cytokines, adhesion molecules, fibrogenic and vasoformative factors. However, objective sensitive diagnostic and prognostic criteria of early renal parenchyma damage in children in the setting of this pathology have not yet been designed.

Aim: to design early diagnostic and prognostic criteria of nephrosclerosis in children with VUR on the basis of study of biological inflammation markers (monocytic chemoattractant protein-1 (MCP-1)); fibrogenesis (transforming growth factor (TGF-β1)); damage of nephron structures (type IV collagen; α-glutathione S-transferase (α-GST); π-glutathione S-transferase (π-GST)); angiogenesis (vascular endothelial growth factor (VEGF)) in urine in the pathology course dynamics (6 months before and after treatment).

Patients and methods. The study was conducted owing to the RF President grant MD-303.2010.7. To assess the degree of activity of inflammatory process, angio- and fibrogenesis in urinary tracts the quantitative determination of MCP-1, TGF-β1, α-GST, π-GST, type IV collagen and VEGF in urine was conducted in 80 children with VUR by the enzyme-linked immunosorbent assay method with the help of test-systems “Vector-Best” (Russia), “Invitrogen”, “Argutus Medical” (Ireland, Dublin) and “Bender Medsystems” (Austria) in the disease dynamics (initially and 6 months after treatment). All patients were divided into 3 groups depending on the VUR degree and methods of

its correction: 1 (n=25) – grade II-III reflux and conservative treatment; 2 (n=39) – grade III-IV reflux and endoscopic correction; 3 (n=16) – grade III-IV reflux and ureters' reimplantation. Control group was made up of 20 children with mild surgical pathology in preoperational period, stratified by age and sex.

Results. Study results' data are given in the form of a Bayesian *p*-confidence interval – median (m), lower (LQ) and upper (UQ) quartiles (LQ-UQ). Study results' analysis showed that the TGF-β1 level in urine increased in all group 1 children 6 months after conservative treatment (m=657 ng/ml; LQ=606 ng/ml; UQ=798 ng/ml; $p<0.0009$) in comparison with the control group (m=371.4 ng/ml; 328.2-418.8) and initial data (m=406.8 ng/ml; 378-438; $p<0.0001$). MCP-1 content in urine was higher (m=55.4 pg/ml; 50.9-78.1; $p<0.01$) than in the control group (m=43.75 pg/ml; 23.75-63.35) and before treatment (m=36.4 pg/ml; 14.4-56.5; $p<0.002$). VEGF concentration reduced by 8 times (m=49.7 pg/ml; 34.1-67.9; $p<0.001$) in comparison with the initial values (m=411.3 pg/ml; 310.1-573.5) and did not differ from the norm significantly (m=56.1 pg/ml; 42.6-80.9; $p=0.6$). π-GST level reduced (m=1.1 mcg/ml; 0.9-1.7; $p<0.0001$) in comparison with the level before treatment (m=2.1 mcg/ml; 1.3-2.76) and did not exceed ordinary values (m=0.48 mcg/ml; 0.3-0.68; $p=0.184$). Type IV collagen content was lower (m=40.2 mcg/ml; 32.2-55.2; $p<0.0001$) than before treatment (m=78.9 ng/ml; 68.0-88.9) and did not differ from the control group values (m=50.25 ng/ml; 59.3-38.6; $p=0.15$).

In group 2 patients 6 months after the endoscopic treatment the TGF-β1 level in urine increased twice (m=729 ng/ml; 606–795; $p<0.0009$) in comparison with the control group (m=371.4 ng/ml; 328.2-418.8) and initial values (m=407 ng/ml; 379.2-423; $p<0.0001$). MCP-1 content was higher (m=173.6 pg/ml; 137.8-228; $p<0.0001$) than in the control group (m=43.75 pg/ml; 23.75-63.35) and before treatment (m=47.85 pg/ml; 22.7-93.08; $p<0.000001$). VEGF concentration reduced (m=164 pg/ml; 129.7-225.4; $p<0.000001$) 3 times in relation to the initial values (m=573.8 pg/ml; 335.4-1368), but remained significantly higher than the norm (m=56.1 pg/ml; 42.6-80.9; $p<0.0001$). π-GST level reduced 3 times (m=1.22 mcg/ml; 0.62-2.7; $p<0.002$) in comparison with the values before treatment (m=4.24 mcg/ml; 2.76-11.62), although

remained high in relation to ordinary values (m=0.48 mcg/ml; 0.3-0.68; p<0.0008). Type IV collagen reduced twice (m=99.4 ng/ml; 88.3-134.7; p<0.0008) in relation to the initial data (m=160.8 ng/ml; 110.6-210.6) and control group (m=50.25 ng/ml; 59.3-38.6; p<0.0008).

In group 3 patients 6 months after ureters' reimplantation the TGF-β1 level in urine increased twice (m=801 ng/ml; 711-894; p<0.0009) in relation to the control group values (m=371.4 ng/ml; 328.2-418.8) and initial data (m=423.6 ng/ml; 392.4-435; p<0.0001). MCP-1 content increased considerably (m=394.7 pg/ml; 362.5-469.8; p<0.0004) in relation to the norm (m=43.75 pg/ml; 23.75-63.35) and values before treatment (m=215.6 pg/ml; 165.4-373; p<0.007). VEGF concentration reduced 6 times (m=801 pg/ml; 711-894; p<0.002) in comparison with the initial values (m=2660 pg/ml; 2003-5017), although remained higher than in the control group (m=56.1 pg/ml; 42.6-80.9; p<0.0004). π-GST level reduced 15 times (m=2.33 mcg/ml; 0.67-4.28; p<0.002) in relation to values before treatment (m=35.47 mcg/ml; 21.7-49.69), although was higher than the ordinary values (m=0.48 mcg/ml; 0.3-0.68; p<0.0008). Type IV collagen level reduced twice (m=185.2 ng/ml; 173.6-202; p<0.002) in comparison with the initial values (m=252.5 ng/ml; 234.9-293.7) and ordinary values (m=50.25 ng/ml; 59.3-38.6; p<0.0004). Significant α-GST content variations in patients of all groups were net recorded throughout the pathology course dynamics.

ROC-analysis (receiver operator characteristics) showed that VEGF, renal glomeruli basic membrane injury factors (type IV collagen and epithelial cells of distal tubules (π-GST)) in urine had the highest sensitivity and specificity to record early renal parenchyma damage in children with VUR.

Conclusion. Increase in levels of biomarkers in urine allows diagnosing early stages of nephrosclerosis forming and latent phase of chronic inflammatory process in ureters. Prognostically unfavorable criteria characteristic of early renal damage in the setting of VUR are pronounced increase of VEGF, type IV collagen and π-GST content in urine.