

Expression and clinical significance of aquaporin-1 and ET-1 in urine of children with congenital hydronephrosis

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Abstract. – **OBJECTIVE:** Congenital hydronephrosis is induced by congenital obstruction of uretero pelvic junction, bladder vesicoureteral reflux, posterior urethral valve, stricture of ureter end and ureterocyst, which is extremely apt to cause end-stage renal failure in children. It becomes significant to explore the expression profile and clinical significance of aquaporin-1 (AQP-1) and ET-1 (endothelin-1) in the urine of children with congenital hydronephrosis.

PATIENTS AND METHODS: 80 cases of children with congenital hydronephrosis were selected to be the observation group and another 40 cases of children with other diseases were served as control group. Pre-operative morning urine, intra-operative renal pelvis urine and morning urine at the 7th day after the operation of all the children were collected for the detection of the level of ET-1, Cr level and AQP1 in the urine. Urine various indexes of different diseases stages in children of both groups were compared.

RESULTS: There was no significant difference between children with mild and children in control group ($p > 0.05$). In the observation group, the AQP-1 level during the operation was significantly lower than that before operation, but it was significantly higher in post-operation than that during the operation, which was still lower than that in control group ($p < 0.05$). Urine ET-1 level in observation group and its positive rate were significantly higher than that in control group ($p < 0.05$). Serum stress indexes in each stage of the observation group were significantly higher than that in control group ($p < 0.05$).

CONCLUSIONS: The expression levels of urine AQP-1 and ET-1 of children with congenital hydronephrosis were obviously increased. The AQP-1 level during the operation was lower than that before operation. This post-operation level was significantly higher than before the operation. The expression of AQP-1 and ET-1 could be used as important indexes for clinical diagnosis.

Key Words:

Congenital hydronephrosis, AQP-1, ET-1.

Introduction

Congenital hydronephrosis is a common pediatric deformity disease in clinic with the incidence rate of about 1-2%. Studies show that this disease is mainly induced by congenital ureteropelvic junction obstruction, bladder vesicoureteral reflux, the posterior urethral valve, stricture of ureter end and ureterocyst, which is extremely apt to cause end-stage renal failure in children^{1,2}. On account of this, our hospital attempted to explore the expression and clinical significance of aquaporin-1 (AQP-1) and ET-1 (endothelin-1) in the urine of children with congenital hydronephrosis and has achieved good results.

Patients and Methods

Patients

80 cases that were suffered from congenital hydronephrosis and were treated in Qilu Children's Hospital of Shandong University from January 2013 to January 2015, were selected as observation group. Examinations such as ultrasound, ECT, CT, etc. were carried out in all children. All of them were diagnosed according to the diagnosis standard of congenital hydronephrosis³. Another 40 in-hospital cases with other diseases were selected as the control group. These children did not have diseases of urinary system. Exclusion criteria were: serious illness such as heart and liver, abnormal renal function, urinary tract infection, other urinary system diseases, autoimmune disease and cancer, etc.

General Material

In the observation group, there were 16 boys, 24 girls, with an average age of 37.34 ± 9.04 months. There were 24 cases having their diseased regions on the left side of the body, 16 cases on the right side. Patients' symptoms were classified according to the shape variation of renal calyces affected by obstruction: renal calyces nipple flattening was considered as mild hydrups, globular was considered as moderate and serious expansion was considered as severe hydrups. Giant hydronephrosis indicated that the kidney had lost the renal structure. According to the classification, there were 11 cases with mild hydrups, 15 with moderate hydrups and 14 with severe hydrups. The reasons were: 29 cases of obstruction at the pelvis-ureter junction and 11 cases of obstruction at the bladder ureter junction. According to the condition of children, patients shall accept corresponding interventions (dismembered pyeloplasty and nephrostomy for obstruction at the pelvis-ureter junction; bladder ureter re-implantation for obstruction at bladder ureter junction; no operative treatments for children with mild hydrups). In the control group, there were 17 boys and 23 girls, with an average age of 37.14 ± 9.27 months.

Methods

Specimen Collection

Pre-operative morning urine, intra-operative renal pelvis urine and morning urine on the 7th day after operation of all children in the observation group were collected. Two groups of children were under water replenishment, with a dose of 100 ml/kg at daytime, without water replenishing from 22 p.m. to 7 a.m. neat morning. Then, the collected urine specimen was centrifuged, sub-packaged and detected. The centrifuge was performed at 3000 r/min for 20 min. Sub-packaging was used for osmotic pressure measurement and stored at 80°C after 20:1 concentration for urine AQP-1 detection.

Detecting Urine ET-1 and Cr Level

Instrument: human AQP-1 ELISA kit purchased from RB Company (Shanghai Kaibo Biochemical Reagent Co., Ltd, Shanghai, China). AQP-1 level was detected in accordance with the product specifications.

Observed Indexes

Firstly, the urine AQP-1 level of different disease stages in both groups, including: AQP-1 level of different disease stages. Secondly, compare the AQP-1 level of different disease time in both groups, including: pre-operation, during operation and post-operation. Thirdly, compare the urine ET-1 level and positive rate in both groups. The dividing value was 845 pg/mmol Cr, the positive was defined as ≥ 845 pg/mmol Cr and the negative as < 845 pg/mmol Cr. Lastly, compare the serum stress indexes at all stages before and after the operation in both groups including: the serum stress indexes at all stages before and after operation (serum epinephrine, blood sugar, cortisol, interleukin-6 and C-reactive protein).

Statistical Analysis

SPSS 17.0 statistical software (SPSS Inc., Chicago, IL, USA) was adopted for data analysis. The average age, AQP-1 level and ET-1 level were showed as average value \pm standard deviation. *t*-test was used for measurement data. The positive and negative rates were showed as a percentage. χ^2 test was used for enumeration data. $p < 0.05$ was used to indicate the significant difference.

Results

Urine AQP-1 Level at Different Stages in Both Groups

There were no significant differences on gender and age of children in both groups ($p > 0.05$). The urine AQP-1 levels in children with mild, moderate and severe problems in observation group were respectively 40.0 ± 2.9 mOsm/kg, 36.1 ± 4.9 mOsm/kg and 31.3 ± 3.4 mOsm/kg, while the AQP-1 level in control group was 41.1 ± 3.5 mOsm/kg. There was no significant difference between the mild and the control group ($p > 0.05$). AQP-1 levels in the moderate and severe group were decreased ($p < 0.05$). There was significantly difference compared with that of the control group. The specific was shown in Table I.

Comparison of Urine AQP-1 Level of Different Time in Both Groups

Through statistical analysis, the AQP-1 level in observation group was 37.5 ± 4.7 mOsm/kg

Table I. Urine AQP-1 level at different stages in both groups (mOsm/kg).

Groups	No.	Mild	Moderate	Severe
Observation group	40	40.0 ± 2.9	36.1 ± 4.9	31.3 ± 3.4
Control group	40	41.1 ± 3.5		
<i>t</i>		2.75	10.91	21.19
<i>p</i>		<i>p</i> > 0.05	<i>p</i> < 0.05	<i>p</i> < 0.05

before operation, 24.9 ± 4.0 mOsm/kg during operation and 33.2 ± 4.5 mOsm/kg post-operation. This data in the control group was 41.1 ± 3.5 mOsm/kg. There was significant change before and after operation in the observation group. The AQP-1 level during the operation was lower than that after, and the AQP-1 level post operation was higher than that during operation but still lower than that in control group, with statistical significance (*p* < 0.05). The specific was shown in Table II.

Comparison of the Urine ET-1 Level and Positive Rate in Both Groups

After statistical analysis, the urine ET-1 level in observation group was 2034.41 ± 892.14 pg/mmol Cr, with the positive rate of 77.50% and negative rate of 22.50%. The ET-1 level in control group was 568.20 ± 212.35 pg/mmol Cr, with the positive rate of 10.00% and negative rate of 90.00%. The urine ET-1 level and positive detection rates in observation group were significantly higher than that in control group with statistical significance (*p* < 0.05). The specific was shown in the Table III.

Comparison of the Serum Stress Indexes at all Stages Before and After the Operation in Both Groups

After treatment, the epinephrine level in observation group 1 day after operation was raised to 0.27 ± 0.05 ng/mL. The stress indexes in observation group 1 day after operation was different from that before the operation. The epinephrine level in observation group 3 days after operation was declined to 0.21 ± 0.04 ng/mL, which indicated that the stress indexes of patients 3 days after operation was lower than that 1 day after operation. All of the serum stress indexes at each period in observation group were significantly better than that in control group. The data was statistically significant (*p* < 0.05). The specific was shown in Table IV.

Discussion

AQPs are a kind of specific channel, widely distributed in the body, and have the function of water transporting³. Studies show that kidney tubules at different parts of the kidney distribute

Table II. Comparison of urine AQP-1 level of different time in both groups.

Groups	No.	Before operation	During operation	7 d after operation
Observation group	40	37.5 ± 4.7	24.9 ± 4.0	33.2 ± 4.5
Control group	40	41.1 ± 3.5	41.1 ± 3.5	41.1 ± 3.5
<i>t</i>		7.95	37.41	17.67
<i>p</i>		<i>p</i> < 0.05	<i>p</i> < 0.05	<i>p</i> < 0.05

Table III. Comparison of the urine ET-1 level and positive rate in both groups (%).

Groups	No.	Dividing value 845 pg/mmol Cr	Positive	Negative	Urine ET-1 level
Observation group	40	0	31 (77.50)	9 (22.50)	2034.41 ± 892.14
Control group	40	0	4 (10.00)	36 (90.00)	568.20 ± 212.35
<i>t/χ²</i>			37.03	37.03	279.03
<i>p</i>		<i>p</i> > 0.05	<i>p</i> < 0.05	<i>p</i> < 0.05	<i>p</i> < 0.05

Table IV. Comparison of the serum stress indexes before and after the operation in both groups.

Groups	No.	Epinephrine (ng/mL)	Blood sugar (mmol/L)	Cortisol (ng/mL)	IL-6 (pg/mL)	CRP (mg/L)
Control group	40					
Pre-operation		0.17 ± 0.05	4.59 ± 0.35	230.95 ± 18.55	12.43 ± 2.44	12.19 ± 2.38
Post-operation		0.27 ± 0.05 ^Δ	5.90 ± 0.41 ^Δ	256.35 ± 20.08 ^Δ	16.80 ± 2.52 ^Δ	15.47 ± 2.61 ^Δ
Observation group	40					
Pre-operation		0.17 ± 0.04	4.60 ± 0.35	231.06 ± 18.65	12.44 ± 2.40	12.18 ± 2.40
Post-operation		0.36 ± 0.08	7.19 ± 0.39	277.18 ± 20.29	20.35 ± 2.61	18.80 ± 2.48

Note: At the same time point, ^Δ*p* < 0.05 vs. control group; *p* < 0.05 vs. self-group before operation; **p* < 0.05 vs. 1 d after operation.

different AQPs, which play an important role in water reabsorption by kidney. AQP-1 is an efficient channel in water transporting. There are many research findings indicating that AQP-1 can be accurately located in the proximal tubules and descending thin limbs in kidneys. It is of great significance on the urine concentration mechanism and is especially of great important clinical significance on the descending thin limbs and counter-current multiplication mechanism^{4,5}. In this research, the urine AQP-1 level in children with mild, moderate and severe problems in observation group was respectively 40.0 ± 2.9 mOsm/kg, 36.1 ± 4.9 mOsm/kg and 31.3 ± 3.4 mOsm/kg, while the data in control group was 41.1 ± 3.5 mOsm/kg. There was no significant difference between the mild and the control group (*p* > 0.05). AQP-1 levels in the moderate and severe were obviously decreased (*p* < 0.05). There was a significant difference compared with that of the control group. When comparing the urine AQP-1 levels at different time in both groups, the AQP-1 levels were 37.5 ± 4.7 mOsm/kg (pre-operation), 24.9 ± 4.0 mOsm/kg (during operation), 33.2 ± 4.5 mOsm/kg (post-operation) in observation group. This data in control group was 41.1 ± 3.5 mOsm/kg. There was significant change before and after operation in observation group. The AQP-1 level during operation was lower than that after, and the AQP-1 level post-operation was higher than that during operation, while still lower than that in control group with statistical significance (*p* < 0.05). The AQP-1 expression was closely related to the urine concentration dysfunction in obstructive hydronephrosis and the decrease of the AQP-1 expression was the important reason that induced this problem above⁶. Existing related research has put forward that the AQP-1 can be used as a new marker of renal cell carcinoma (RCC) for the screening and diagnosis

of early stage RCC, which is mainly according to the AQP-1 detection findings. The AQP-1 exists in the healthy human urine. Once someone suffers from RCC, the AQP-1 expression in urine will be increased obviously. By establishing renal ischemia reperfusion and kidney transplant model, the urine AQP-1 can be detected in a mouse experiment, indicating that the AQP-1 level can indirectly reflect the functional change of renal cells, as well as the progress of kidney-related diseases⁷⁻¹¹. Wang et al¹² detected the AQP-1 level in children with hydronephrosis by the method of ELISA and showed that the AQP-1 level in children with mild was slightly lower than that in control group. Moreover, they showed that AQP-1 level in children with moderate was significantly lower than that in control group and children with severe was further decreased. The urine AQP-1 level before the operation was slightly lower than that in control group, further decreased during operation and much lowered after operation than that in control group. The AQP-1 level was significantly increased 7d after the operation, but still lower than that in control group. Therefore, it comes to a conclusion that the more severe of the hydronephrosis, the lower level of the AQP1. Before and after operation, the AQP1 levels undergo a process of decreasing to increasing dramatically. That is to say the decreasing urine AQP1 levels may have a close relation with hydronephrosis^{6,13}. Regarding serum stress indexes, after treatment, the epinephrine level in observation group 1d after operation has risen to 0.27 ± 0.05 ng/mL, which had significant difference compared with that before the operation. The epinephrine in observation group 3d after the operation has declined to 0.21 ± 0.04 ng/mL, which indicated that the stress indexes of patients 3 d after the operation was lower than that 1d after. Serum stress indexes in each stage of

observation group were significantly higher than that in control group ($p < 0.05$). Animal studies have shown that AQP1-4 has certain actions in the process of the kidney urine concentration and dilution. AQP1-4 takes part in the trans-membrane water transporting process in the proximal tubules, descending thin limbs and collecting tubes in kidneys. The complete expression of AQP1-4 is significant to maintain the normal urine concentrating function of kidneys¹⁴. Research findings of Li et al¹⁵ showed that AQP1-4 mRNA and protein expressions in kidneys of patients with congenital hydronephrosis were declined and AQP1-4mRNA and protein levels were changed with the severity of the change of renal hydrops pathological changes. Therefore, it is concluded that the change of AQP1-4 expression level may take part in the pathological process of congenital hydronephrosis^{16,17}. Urine ET-1 refers to the endothelin-1 (ET-1), which has not been applied to clinical diagnosis now. Although ET-1 showed no significant involvement in the advanced stage of proliferative diabetic retinopathy (PDR), of note, existing research has verified that ET-1 participated in the progress of glomerulus arteriole arteriosclerosis and renal interstitial fibrosis induced by upper urinary tract obstruction¹⁸. Meanwhile, ET-1 has a close relation with the tissue damage and dysfunction induced by unilateral ureter obstruction¹⁹. Animal experimental results of unilateral ureter obstruction showed that the expression level of ET-1 in the renal veins blood flow was higher than that in the renal artery, which indicates that the ET-1 level is related to the kidneys²⁰. ET-A blockade effectively impeded the progression of renal injury and maintained renal hemodynamics, function, and microvascular density in the stenotic kidney, supporting ET-1/ET-A as a potential therapeutic target in chronic renovascular disease²¹. Experiments in rats show that the renal ET-1 level of rats with unilateral ureteral obstruction was increased, but the ET-1 expression level of another kidney of rats was decreased²². Also, the animal model with obstruction receiving bosentan displayed that the inhibition of rats ET-1 receptor was closely related to the recovery and apoptosis rate of obstructive renal blood flow²³. When compared with that in control group, the cell apoptosis rate had no significant difference. It can be speculated that ET-1 is the important mediator of blood vessel and cellular damage in unilateral ureteral obstruction. Previous studies found that the pelvis and ET-1 gene

expression at the ureteropelvic junction of rats with unilateral congenital ureteropelvic junction obstruction were significantly increased when compared with that in control group²². The expression of ET-1 in rats with ureteropelvic junction obstruction at the stenotic segment tissue was higher than that in control group, which indicated that the ET-1 expression increasing in ureteropelvic junction obstructive tissue may be closely related to the development and progression of diseases. Taha et al²⁴ measured ET-1 in the urine samples collected from children with unilateral ureteropelvic junction obstruction who underwent pyeloplasty. The result showed that the cutoff value of 3 fmol/mg creatinine gave a sensitivity of 74.3%, a specificity of 90%, and an overall accuracy of 81.5%. ET-1 significantly decreased at 12 months after pyeloplasty, compared with the preoperative value, indicating that ET-1 is a noninvasive marker for the diagnosis and long-term follow-up of children with ureteropelvic junction obstruction.

Conclusions

In this study, with the deterioration of the disease of the children in the observation group, the urine AQP1-1 level is decreased, while increased after the operation. Meanwhile, the ET-1 expression is significantly increased. Therefore, the urine AQP1-1 and ET-1 expressions can be used as important indexes in the clinical diagnosis.

Conflict of Interest

The authors declare no competing financial or commercial interests in this manuscript.

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