

Diagnostic value of urinary RBP, ALB and AQP2 in neonatal hydronephrosis and the relationship with expression of MCP-1 in the prenatal maternal peripheral blood

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Abstract. Diagnostic value of urinary retinol binding protein (RBP), albumin (ALB) and aquaporin-2 (AQP2) in neonatal hydronephrosis and their relationship with the expression of monocyte chemoattractant protein 1 (MCP-1) in the prenatal maternal peripheral blood was investigated. Forty-six child patients with hydronephrosis admitted to Hongqi Hospital Affiliated to Mudanjiang Medical College from December 2016 to November 2017 were selected as the observation group and the control included 46 normal newborn infants. The urinary RBP, ALB, AQP2 and the expression of MCP-1 in the prenatal maternal peripheral blood in the two groups were compared. The diagnostic value of the combination of urinary RBP, ALB and AQP2 for the neonatal hydronephrosis was assessed through the area under curve (AUC). The changes of urinary RBP, ALB and AQP2 of child patients were observed and the correlations between RBP, ALB, AQP2 and MCP-1 were analyzed. The concentrations of RBP and ALB in the observation group were obviously increased compared to those in the control group. The AQP2 concentration in the observation group was lower than that in the control group. In the observation group, the MCP-1 level in the prenatal maternal blood was significantly higher than that in the control group ($P < 0.05$). After treatment, the concentration of RBP and ALB in the child patients were significantly decreased and AQP2 concentration was increased compared with that before treatment ($P < 0.05$). The AUC of the diagnosis combining with RBP, ALB and AQP2 was 0.913. RBP and ALB were positively correlated to MCP-1 in the prenatal maternal peripheral blood and there was a negative correlation between AQP2 and MCP-1 ($P < 0.05$). In conclusion, urinary RBP, ALB and AQP2

can be regarded as markers for the diagnosis of the neonatal hydronephrosis and they are also closely related to the MCP-1 level in the prenatal maternal peripheral blood.

Introduction

Congenital hydronephrosis is one of the common childhood malformations in clinic which is usually caused by the interaction of glomerular hemodynamics with the changes in renal tubular function, with a morbidity of 1-2% in newborn infants (1). Hydronephrosis can eventually lead to renal failure in children, so it is very important to find the early congenital hydronephrosis and treat it timely (2). Clinically, the neonatal hydronephrosis is often diagnosed through imaging examination and a variety of assisting biochemical indicators. However, the biochemical indicators such as serum creatinine and blood urea nitrogen are susceptible to various factors. Therefore, the diagnosis often lags and cannot accurately reflect the extent of neonatal renal injury. As a result, looking for indicators reflecting the degree of renal injury of children with hydronephrosis at early stage is necessary (3). Retinol binding protein (RBP) is currently considered to be the most sensitive and stable transporter that reflects the renal function changes in urine and can be monitored at any time for the diagnosis of renal biopsy morphology (4). Urinary micro-albumin (ALB) is one of the early diagnosis markers for the degree of glomerular injury (5). Aquaporin-2 (AQP2) is a kind of aquaporin that widely exists in the special channel for water transporting on the membrane and it is the marker for the diagnosis of hydronephrosis (6). Monocyte chemoattractant protein 1 (MCP-1) is an inflammatory marker whose overexpression would result in the shallow implantation of placenta in pregnant women and the impaired endothelial function, thus bringing an adverse effect on the normal growth of fetuses (7). In the study, the expression levels of urinary RBP, ALB, AQP2 and MCP-1 in the prenatal maternal blood were detected and analyzed to provide the diagnostic proof for neonatal hydronephrosis.

Patients and methods

Patients. A total of 46 newborn infants with hydronephrosis admitted to Hongqi Hospital Affiliated to Mudanjiang

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Medical College (Mudanjiang, China) from December 2016 to November 2017 were selected as the observation group. Inclusion criteria: i) child patients complying with the hydronephrosis diagnostic criteria (8), ii) child patients who were born within 28 days and iii) approval obtained from the Ethics Committee in Hongqi Hospital Affiliated to Mudanjiang Medical College and informed consent signed by the family members. Exclusion criteria: i) patients with bilateral hydronephrosis and ii) child patients with urinary tract infection and febrile diseases. Compared with the 46 normal newborn infants at the same period in the control group, there were no significant differences in general materials of the child patients in the two groups ($P > 0.05$; Table I).

Methods. The midstream urine samples were collected from two groups of newborn infants in the morning (taken at 6:00 a.m.), and were stored in a refrigerator at -80°C for detection. After removal from the refrigerator during the detection, the urine samples were thawed in water at 4°C and centrifuged at $8,100 \times g$ for 5 min. Neonatal urinary RBP and ALB were detected via the immunity transmission turbidity method. The related kits were provided by Shanghai Beijia Biochemical Reagent Co., Ltd. (Shanghai, China) and the operation was conducted in strict accordance with the instructions. Neonatal urinary AQP2 concentration was detected through the enzyme-linked immunosorbent assay (ELISA). The related kits were provided by R&D systems (Minneapolis, MN, USA) and the determination was in strict accordance with the product manual.

A total of 3 ml of peripheral blood was collected from each pregnant woman in the two groups before the infants were born, placed at 20°C for 30 min and centrifuged at $1,800 \times g$ for 20 min at 4°C . The separated serum was poured into an Eppendorf (EP) tube and stored in a refrigerator at -80°C . The serum MCP-1 level was examined via ELISA. The operation was performed strictly according to the protocol of the related kit (provided by Beyotime, Shanghai, China), followed by sample adding, incubation, washing with enzyme, incubation and washing and color development. After the addition of terminative solution, the optical density (OD) value in each well was measured sequentially through a microplate reader (Bio-Rad, Hercules, CA, USA) at the wavelength of 450 nm within 15 min, and the MCP-1 level was calculated.

Evaluation indexes

Judgement criteria for the hydronephrosis degree (9): i) mild (renal pelvis and renal calices were full and visualized during 10-30 min, but the cup became shallow), ii) moderate (renal pelvis and renal calices developed during 31-60 min, there were enlargement of renal pelvis and renal calices as well as ureter expansion, but the calices cup disappeared) and iii) severe (renal pelvis and renal calices developed over 60 min and there was clumpy deformation of renal pelvis and renal calices).

Neonatal urinary RBP and ALB concentrations were detected through immunity transmission turbidity method. Neonatal urinary AQP2 and MCP-1 concentrations in peripheral blood of prenatal pregnant women were detected via ELISA.

Statistical analysis. Statistical Product and Service Solutions (SPSS) 19.0 (SPSS, Inc., Chicago, IL, USA) software was

Table I. Baseline data of child patients in the two groups.

Items	Control group (n=46)	Observation group (n=46)	t/ χ^2	P-value
Sex (male/female)	27/19	25/21	0.044	0.833
Time of birth (days)	6-21	6-23		
Average day-age (days)	13.58 \pm 5.43	13.83 \pm 5.52	0.219	0.827
Body mass (kg)	2.63 \pm 0.76	2.69 \pm 0.75	0.381	0.704
Degree of hydronephrosis (n, %)				
Mild	29 (63.04)	30 (65.21)	0.326	0.849
Moderate	9 (19.57)	7 (15.22)		
Severe	8 (17.39)	9 (19.57)		

Table II. Comparison of RBP, ALB AQP2 and maternal MCP-1 levels in the two groups of newborn infants.

Groups	n	RBP (mg/l)	ALB (mg/ml)	AQP2 (ng/ml)	Maternal MCP-1 (ng/ml)
Observation group	46	7.64 \pm 2.63	1.59 \pm 0.78	7.63 \pm 1.24	208.63 \pm 16.43
Control group	46	1.96 \pm 0.75	0.37 \pm 0.15	12.79 \pm 2.35	141.22 \pm 13.28
t value		14.086	10.417	13.171	21.642
P-value		<0.001	<0.001	<0.001	<0.001

RBP, retinol binding protein; ALB, albumin; AQP2, aquaporin-2; MCP-1, monocyte chemoattractant protein 1.

used for data processing. Measurement data were presented as mean \pm standard deviation, and t-test was used for the examination. Measurement data were shown as ratio, and χ^2 test was used for the examination. Diagnostic value was analyzed via receiver operating characteristic (ROC) curve and the correlation was analyzed via Pearson's correlation coefficient. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

RBP, ALB and maternal MCP-1 levels. As for the conditions of RBP, ALB and maternal MCP-1 in the two groups of newborn infants, the levels of RBP and ALB in the observation group were significantly higher than those in the control group and the concentration of AQP2 in the observation group was lower than that in the control group. The maternal MCP-1 level in the observation group was significantly higher than that in the control group ($P < 0.05$; Table II).

ROC curve for RBP, ALB and AQP2. ROC curve of the combined diagnosis of RBP, ALB and AQP2 is shown in Fig. 1, with an area under curve (AUC) of 0.913, sensitivity of 91.5% and specificity of 89.8%.

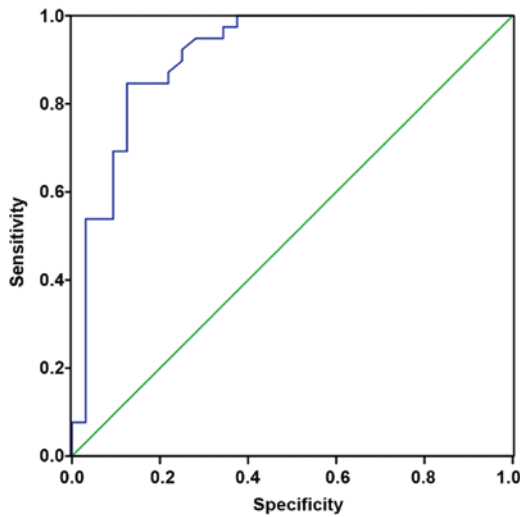


Figure 1. Neonatal hydronephrosis diagnosed through the combination of RBP, ALB and AQP2. The AUC of ROC curve is 0.913 and the sensitivity and specificity are high. RBP, retinol binding protein; ALB, albumin; AQP2, aquaporin-2; AUC, area under curve; ROC, receiver operating characteristic.

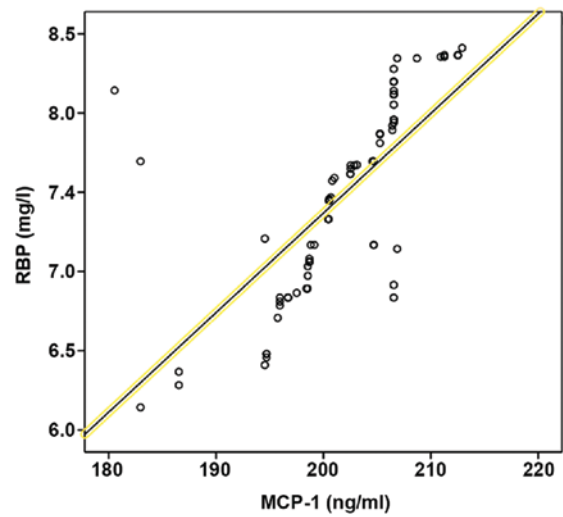


Figure 2. Correlation analysis of RBP with prenatal MCP-1 level in pregnant women. RBP, retinol binding protein; MCP-1, monocyte chemoattractant protein 1.

Table III. RBP, ALB and AQP2 of child patients before and after treatment.

Time	n	RBP	ALB	AQP2
Before treatment	46	7.64±2.63	1.59±0.78	7.63±1.24
After treatment	46	2.56±1.25	0.68±0.15	11.89±1.35
t value		11.832	7.770	15.762
P-value		<0.001	<0.001	<0.001

RBP, retinol binding protein; ALB, albumin; AQP2, aquaporin-2.

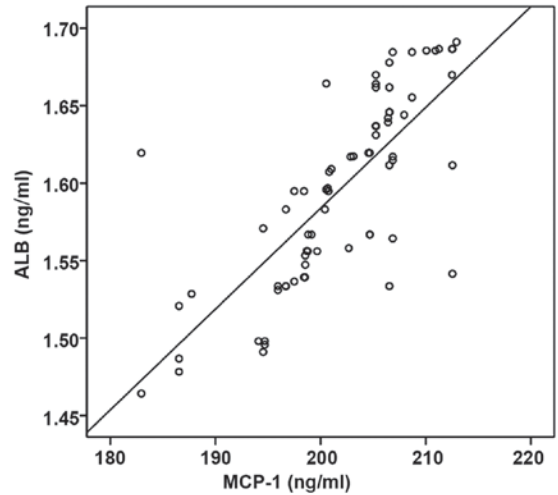


Figure 3. Correlation analysis of ALB with prenatal MCP-1 level in pregnant women. ALB, albumin; MCP-1, monocyte chemoattractant protein 1.

Table IV. Correlation analysis of RBP, ALB and AQP2 with MCP-1.

Items	r	P-value
RBP	0.568	0.003
ALB	0.501	0.014
AQP2	-0.445	0.017

RBP, retinol binding protein; ALB, albumin; AQP2, aquaporin-2; MCP-1, monocyte chemoattractant protein 1.

Changes of RBP, ALB and AQP2 in child patients before and after treatment. In terms of the changes of RBP, ALB and AQP2 in child patients before and after treatment, the levels of RBP and ALB in child patients after treatment were significantly lower than those before treatment and AQP2 concentration was increased compared with that before treatment ($P < 0.05$; Table III).

The correlation between RBP, ALB, AQP2 and prenatal MCP-1 level. The correlation between RBP, ALB, AQP2 and prenatal MCP-1 level in pregnant women were analyzed

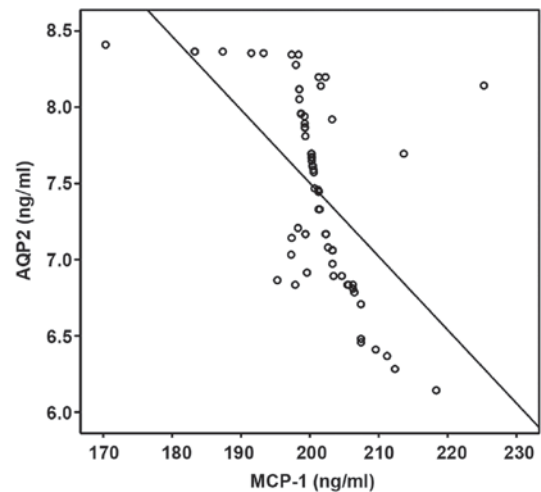


Figure 4. Correlation analysis of AQP2 with prenatal MCP-1 level in pregnant women. AQP2, aquaporin-2; MCP-1, monocyte chemoattractant protein 1.

via Pearson's correlation coefficient. RBP and ALB were positively correlated with MCP-1, but AQP2 and MCP-1 were negatively correlated ($P < 0.05$; Table IV, Figs. 2-4).

Discussion

Neonatal hydronephrosis can account for >50% of prenatal defects, which can be caused by the urinary catheter end stenosis, vesicoureteral reflux, posterior urethral valve, ureteral stenosis and ureterocele. Most of the neonatal hydronephrosis results from the congenital ureteral renal pelvis junction obstruction, accounting for approximately 85% (10). The urinary tract obstruction leads to increasing pressure in the ureter and renal tubular and brings direct or indirect damage to the renal tubular and renal parenchyma. The manifestations are weakened reabsorption function in the renal tubular, the damaged secretion function of hydrogen ion and potassium ion, decreased urine concentration and abnormal renal function in the distal segment of the kidney (11). With the progress of the disease, the progressive decline of renal function eventually leads to renal failure (12). At present, the diagnosis of hydronephrosis mainly relies on imaging and technology is very advanced. However, the routine detection such as serum creatinine and blood urea nitrogen test for neonates, especially for the early diagnosis of renal damage, is often lagging. Surgery should be performed when children's renal function begins to deteriorate. Therefore, timely and dynamic diagnosis of changes in renal function is very important.

RBP is a low molecular weight protein secreted by hepatocytes and belongs to the retinol transporters. It is widely present in body fluids such as blood, urine and cerebrospinal fluid and plays an important role in the storage, transportation and metabolism of vitamin A (13). In clinic, RBP is often used as a marker of renal tubular injury. It is simple to be detected, ready to be monitored and difficult to be decomposed in urine. With strong stability, it is not affected by the PH value of sphygmomanometer and other factors. Therefore, it can provide effective supplement for the renal biopsy diagnosis (14). Results of this study showed that RBP level in children with hydronephrosis was significantly higher than that in normal children, and it was significantly decreased after treatment ($P < 0.05$). This is because the glomerular filtration in neonates with hydronephrosis decreases while RBP continues to accumulate. When the renal tubular reabsorption function is impaired, RBP cannot be reabsorbed and degraded, thus it would be excreted with the urine. Then its concentration in urine may increase. After effective treatment, the renal function can recover effectively, RBP is absorbed and degraded again, and therefore, the concentration is naturally decreased in urine.

ALB is a highly sensitive middle-molecule protein that is a marker of glomerular injury (15). Results of this study showed that the level of ALB in children with hydronephrosis was significantly higher than that in normal children, and the level of ALB was significantly decreased after treatment ($P < 0.05$). This is because ALB is reabsorbed by proximal convoluted tubule in normal people, while in neonates with hydronephrosis, the glomerular filtration rate is decreased, so that the filtration of ALB far exceeds the maximum absorption amount of renal tubular. As a result, ALB enters into the urine and its

content increases, indicating that the early glomerular lesions occur where there is some damage. Through the intervention treatment, ALB concentration in the urine can be reduced.

AQP2 was first discovered in the early 1990s, it is a member of the aquaporin family (16). AQP2 mainly exists in the cytoplasm of principal cell of renal collecting duct and the cell membrane towards the luminal side, which can increase the ability of water reabsorption and urine concentration (17). It was indicated in the results of this study that ALB level in children with hydronephrosis was significantly lower than that in normal children and it was significantly increased after treatment ($P < 0.05$). This is due to the fact that when hydronephrosis occurs in the neonates, the concentration of AQP2 which carries on the trans-membrane transportation of water is decreased, so that the water permeability of collecting ductis also decreased. As a result, the transmembrane transportation of water is hindered and the urine concentration is decreased (18). After effective treatment, the amount of AQP2 on the cell membrane is increased, leading to an increase in water reabsorption.

MCP-1, a chemokine most closely associated with pregnancy, plays a key role in maintaining mononuclear phagocyte localization and the invasion of vascular endothelial trophoblasts (19). Results of this study revealed that the level of MCP-1 in maternal peripheral blood in the observation group was significantly higher than that in the control group ($P < 0.05$). The reason may be that pregnant women with overexpression of MCP-1 may have shallow implantation of placenta, thus affecting the normal healthy growth of fetuses and increasing the risk of hydronephrosis after birth of the fetuses. In this study, Pearson's correlation coefficient analysis indicated that VRBP and ALB had a positive correlation with MCP-1 while AQP2 and MCP-1 were negatively correlated ($P < 0.05$). This may be related to the fact that MCP-1 endows chemotaxis on a large number of inflammatory cells and expand the inflammatory response. When the level of MCP-1 content is changed, especially in the case of its constantly increasing concentration, the trophoblast invasion function is affected, and the microvessel density changes occur and lead to placental atherosclerosis, thus causing a series of pathological reactions such as elevated blood pressure (20). Substance exchange between mother and baby is decreased and fetal hypoxia appears, which changes the growth and development. This leads to kidney damage and hydronephrosis, increased RBP and ALB levels and decreased AQP2 level, indicating that there is a positively and negatively dependent relationship between the three indexes and MCP-1.

In conclusion, urinary RBP, ALB and AQP2 can be used as diagnostic indicators for the degree of renal dysfunction, which are closely related to the expression of MCP-1 in the peripheral blood of pregnant women, and can be combined with imaging to guide the diagnosis and treatment of neonatal hydronephrosis.

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Availability of data and material

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

MZ wrote the manuscript and collected the midstream urine samples. YZ detected and analyzed neonatal urinary RBP and ALB concentrations. LL performed ELISA. HW analyzed the changes of RBP. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Hongqi Hospital Affiliated to Mudanjiang Medical College (Mudanjiang, China) and informed consents were signed by the parents of the child patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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