CORRELATION BETWEEN URINE PODOCALYXIN, NEPHRIN EXPRESSION AND CLINICO-PATHOLOGICAL FEATURES IN PATIENTS WITH PRIMARY NEPHROTIC SYNDROME

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ABSTRACT

Objective*: To investigate the correlation between urine podocalyxin, nephrin expression and clinicopathological features in patients with primary nephrotic syndrome (NS).*

Methods: *From October 2018 to October 2019, 100 patients with NS were divided into (MsPGN) group (n=26), (MCN) group (n=29), (MN) group (n=25) and (FSGS) group (n=20). At the same time, 20 healthy people were selected as control group. Urine and fasting venous blood were collected. Urine podocalyxin, nephrin, urinary protein, serum albumin, blood lipid and creatine levels were measured by enzyme-linked immunosorbent assay (Elisa). The correlation between urine podocalyxin, nephrin expression and urinary protein content, serum albumin, blood lipid and creatine was investigated in patients with primary nephrotic syndrome.*

Results: *The expression levels of PCX and nephrin in urine of patients with PNS were significantly higher than those of healthy people. The expression levels of PCX and nephrin in MsPGN group, MCN group, MN group and FSGS group were significantly higher than those in healthy people. The expression levels of PCX and nephrin in FSGS patients were the highest, the difference was statistically significant (P<0.05). The 24 h urinary protein level in patients with PNS was significantly higher than that in healthy people, MsPGN group and MCN group. The levels of 24-hour urinary protein in MN group and FSGS group were significantly higher than those in healthy population, and the levels of cholesterol, TG and Cre in FSGS group were significantly higher than those in healthy population, and the levels of cholesterol, TG and Cre in MsPGN group, MCN group (P<0.05), MN group and FSGS group were significantly higher than those in healthy population, and the levels of cholesterol, TG and Cre in PNS group were significantly higher than those in healthy group (P<0.05). The level of BSA in patients with PNS was significantly lower than that in healthy people, and the level of BSA in MsPGN group, MCN group, MN group and FSGS group was significantly lower than that in healthy people (P<0.05). Pearsoon linear analysis showed that PCX was positively correlated with 24 h urinary protein, cholesterol, TG and Cre (r=0.468, 0.326, 0.511, 0.432, P<0.05). There was a negative correlation with BSA (r=0.386, P<0.05). Nephrin was positively correlated with 24 h urinary protein, cholesterol, TG and Cre (r=0.369, 0.472, 0.415, 0.434, P<0.05), and negatively correlated with BSA (r=0.512, P<0.05).*

Conclusion: *The expression of PCX, nephrin in urine of patients with PNS is highly expressed, which is related to clinicopathological features.*

Keywords: *Primary nephrotic syndrome, clinicopathological features, podocalyxin, nephrin.*

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Introduction

Nephrotic syndrome (NS) refers to a group of clinical symptoms such as increased basement membrane permeability, large amount of albuminuria, low plasma albumin, hyperlipidemia and edema[(1)]. Massive albuminuria and hypoalbuminemia are necessary conditions for diagnosis, and can also be accompanied by hematuria, hypertension and persistent renal function damage[(2)]. There are many causes of NS, which can be divided into two categories according to etiology: primary nephrotic syndrome (PNS) and secondary nephrotic syndrome[(3)]. PNS is one of the most common types of NS, which has a wide range of onset and can occur at different age stages. It is one of the main causes of chronic renal disease in clinic[(4)]. It has been found that the pathogenesis of PNS is related to podocyte injury[(5)]. Foot calyx glycoprotein (podocalyxin, PCX) is a clinical standard for evaluating podocyte injury. Studies have shown

that PCX can evaluate the pathological grade of renal tissue and podocyte injury in children with Henoch-Schonlein purpura (Henoch-Schonlein purpura). Nephrotic protein (nephri) is a proteolytic enzyme released by peribulbar granulosa cells of the paracral apparatus and is a part of renin-angiotensin system[6]. It has been found that nephri interacts with a variety of proteins to establish a filtration barrier for kidneys[7]. This study further investigated the correlation between urine PCX, nephrin expression and clinicopathological features in patients with PNS, and provided reference for further study of its pathogenesis.

Material and methods

General information

From October 2018 to October 2019, 100 patients with primary nephrotic syndrome treated in the Department of Nephrology in our hospital were collected and included in the criteria:

• All patients met the diagnostic criteria of PNS in the third edition of Nephrology;

• The patients with 24 h urinary protein content greater than 3.5 g;

• The serum albumin of the patient is less than 30g/L;

• The patient did not receive any hormone and immunosuppressive therapy before admission;

• All patients and their families were informed and signed informed consent forms.

Exclusion criteria:

• Patients with severe bacterial infection, systemic lupus erythematosus and autoimmune diseases and other immune diseases;

• Patients with drug poisoning;

• Patients with diabetic nephropathy, hepatitis B virus-associated kidney disease and other secondary nephrotic syndrome.

There are 100 patients in PNS group, 53 males and 47 females, the average age is (26.05±10.36) years, the average BMI value is (20.05±1.02) Kg/m^2.

According to the pathological classification, the patients were divided into Mesangial proliferative glomerulonephritis (MsPGN) group, Micro pathological nephropathy (MCN) group, Membranous nephropathy (MN) group and Focal segmental glomerulosclerosis (FSGS) group. There were 26 patients in MsPGN group, 14 males and 12 females, with an average age of (26.12±10.48) years and an average BMI of (20.13±1.11) Kg/m^2. There were 29 patients in MCN group, 15 males and 14 females, with an average age of (26.23±11.52) years and an average BMI of (20.15±1.06) years. There were 13 males and 12 females, with an average age of (25.98±12.14) years and an average BMI of (20.08±0.98) Kg/m^2. There were 20 patients in FSGS group, 11 males and 9 females, with an average age of (26.04±9.86) years and an average BMI of (20.21±0.76) Kg/m^2. At the same time, the healthy people who were examined in the physical examination center of our hospital were selected as the control group. There were 20 cases in the control group, 10 males and 10 females, with an average age of (25.99±9.52) years and an average BMI of (20.04±1.08) Kg/m^2. There was no significant difference in age, sex and BMI in each group (P>0.05).

Observation indexes

PCX and nephrin

Collected 10 ml urine for the first time in the morning and centrifuged at the speed of 3000r/min for 10 min. the serum was carefully separated and refrigerated in a refrigerator of-80 degrees to avoid repeated freeze-thaw. The levels of PCX in urine and nephri in urine were measured by competitive enzyme-linked immunosorbent assay (Elisa) and double antibody sandwich enzyme-linked immunosorbent assay (Elisa).

Urine protein

Collect the urine of all subjects for 24 hours, record the total urine volume, centrifuge 15min at the rotating speed of 3000r/min, carefully separate the smear, refrigerate the refrigerator at-80°C to be tested, and avoid repeated freeze-thaw. The 24 h urinary protein level of each group was measured by automatic analyzer.

Serum indexes

Fasting venous blood was collected from all subjects in the early morning of 5ml, centrifuged at room temperature for 15 min by 3000r/min, carefully separated and refrigerated with a refrigerator of-80°C to avoid repeated freeze-thaw. Serum serum albumin (BSA) levels were measured by bromcresol green colorimetric assay, and serum cholesterol, glycerol (Glycerin three fat, TG and creatine (Cre) levels were measured by automatic analyzer.

Statistical methods

The data of this study were analyzed by SPSS20.0 software package. All the measurement data were expressed by ($\bar{x}\pm s$), t test was used to compare the data between groups, and the percentage of counting data was expressed by percentage test, and

the comparison between groups was compared with each other. Ridit test was used to compare the grade data. Pearsoon linear analysis was used to analyze the correlation between the expression of PCX, nephrin and urinary protein, BSA, cholesterol, TG and Cre. P<0.05 was considered statistically significant.

Results

Comparison of the expression levels of PCX and nephrin in urine of each group

The expression levels of PCX and nephrin in the urine of PNS patients were significantly higher than that of healthy people, and MsPGN group, MCN group, MN group and FSGS group showed significant difference in the expression levels of PCX and nephrin, and the level gradually increased, among which the expression levels of PCX and nephrin in FSGS patients were the highest, with statistically significant difference (P<0.05). See table 1.

Group	n	PCX (ng/ml)	Nephrin (ng/ml)
Control group	20	4.16±1.03	5.61±1.05
MsPGN group	26	10.89±3.33^{a}	18.75±4.01^{a}
MCN group	29	22.56±2.23^{ab}	25.13±3.12^{ab}
MN group	25	24.78±4.12^{abc}	28.79±5.43^{abc}
FSGS group	20	30.14±3.45^{abcd}	44.56±4.81^{abcd}

Table 1: Comparison of the expression levels of PCX and nephrin in urine of each group ($\bar{x}$±s).

Note: a represents the comparison with the control group, ^{a}P<0.05; b represents the comparison with the MsPGN group, ^{b}P<0.05; c represents the comparison with the MCN group, ^{c}P<0.05; d represents the comparison with the MN group, ^{d}P<0.05.

Comparison of urine protein content in urine of each group at 24h

PNS patients' urine protein level at 24h was significantly higher than that of healthy people, and PSPGN group, MCN group, MN group and FSGS group showed significant differences in urine protein level at 24h, and the level gradually improved, among which FSGS patients had the highest urine protein level at 24h, the difference was statistically significant (P<0.05). Are shown in table 2.

Group	n	24h urine protein (g/24h)
Control group	20	0.05±0.02
MsPGN group	26	3.45±0.56^{a}
MCN group	29	4.03±0.78^{ab}
MN group	25	4.41±1.12^{abc}
FSGS group	20	4.61±1.43^{abcd}

Table 2: Comparison of urine protein content in each group at 24h ($\bar{x}$±s).

Note: a represents the comparison with the control group, ^{a}P<0.05; b represents the comparison with the MsPGN group, ^{b}P<0.05; c represents the comparison with the MCN group, ^{c}P<0.05; d represents the comparison with the MN group, ^{d}P<0.05.

Comparison of serum levels of BSA, cholesterol, TG and Cre in each group

The levels of cholesterol, TG and Cre in PNS patients were significantly higher than those in healthy people. MsPGN group, MCN group, MN group and FSGS group showed significant differences in cholesterol, TG and Cre levels (P<0.05).

The level of BSA in PNS patients was significantly lower than that in healthy people, MsPGN group, MCN group, MN group and FSGS group, and the level of BSA decreased gradually, and the difference was statistically significant (P<0.05). See table 3.

Group	n	BSA (g/L)	Cholesterol (g/L)	TG (mmol/L)	Cre (μmol/L)
Control group	20	38.49±4.81	4.43±0.79	0.76±0.38	71.46±13.44
MsPGN group	26	24.78±3.59^{a}	6.31±1.72^{a}	1.46±1.04^{a}	96.48±47.11^{a}
MCN group	29	21.76±3.41^{ab}	7.42±1.76^{ab}	1.96±1.21^{ab}	128.46±58.43^{ab}
MN group	25	19.76±3.21^{abc}	8.76±2.13^{abc}	2.24±0.88^{abc}	174.81±79.66^{abc}
FSGS group	20	17.86±2.74^{abcd}	9.95±2.16^{abcd}	2.56±1.12^{abcd}	235.16±204.16^{abcd}

Table 3: Comparison of serum BSA, cholesterol, TG and Cre levels in each group ($\bar{x}$±s).

Note: a represents the comparison with the control group, ^{a}P<0.05; b represents the comparison with the MsPGN group, ^{b}P<0.05; c represents the comparison with the MCN group, ^{c}P<0.05; d represents the comparison with the MN group, ^{d}P<0.05.

Correlation analysis between expression levels of PCX and nephrin and clinicopathological features in PNS patients

Pearsoon linear analysis showed that PCX was positively correlated with 24h urine protein, cholesterol, TG and Cre (r=0.468, 0.326, 0.511, 0.432, P<0.05, respectively), and negatively correlated with BSA (r=-0.386, P<0.05).

Nephrin was positively correlated with 24h urine protein, cholesterol, TG and Cre (r=0.369, 0.472, 0.415 and 0.434, respectively, P<0.05), and negatively correlated with BSA (r=-0.512, P<0.05). Shown in table 4.

Index		24h Urine protein	BSA	Cholesterol	TG	Cre
PCX	*r*	0.468	-0.386	0.326	0.511	0.432
	p	0.005	0.015	0.026	0.026	0.02
nephrin	*r*	0.369	-0.512	0.472	0.415	0.434
	p	0.012	0.023	0.048	0.035	0.031

Table 4: Analysis of correlation between expression level and clinicopathological features of pcx, nephrin in PNS patients.

Discussion

PNC is a kind of NS, patients with unclear etiology, which may have different degrees of edema, anxiety, malnutrition and other symptoms, which greatly reduces the quality of life of patients[8]. Podocyte is a kind of epithelial cell that constructs glomerular filtration membrane, and podocyte membrane is the main component of glomerular filtration barrier and the basis of filtration function[9]. Many studies have shown that there is a certain relationship between proteuria and podocyte fusion and podocyte foramen diaphragm injury in patients with PNS[10]. At present, the main way to diagnose NS is renal pathological examination, but pathological examination has certain trauma, there is a risk of infection, and the price is expensive, there are also general requirements for the patient's condition, unable to multiple, timely examination of the patient[11].

Therefore, it has been a difficult problem in medical field to find a simple, convenient, rapid and low risk method for the diagnosis of PNS and the evaluation of PNS.

PCX is the main glycoprotein expressed on the apical surface of bulbar podocytes and one of the markers of podocytes. It has strong negative charge and is the main component of the charge barrier of bulbar basement membrane. It can also maintain the normal physiological structure of podocytes and ensure the normal effect of podocytes[12]. It has been suggested that the change of PCX level may predict the occurrence of glomerular injury and other diseases[13]. In this study, the level of PCX in urine of PNS patients was significantly higher than that of healthy people, MsPGN group, MCN group, MN group and FSGS group, and the level of PCX increased gradually. The expression level of PCX in FSGS patients was the highest, the difference was statistically significant ($P<0.05$). Pearsoon linear analysis showed that PCX was positively correlated with 24 h urinary protein, cholesterol, TG and Cre ($r=0.468$, 0.326, 0.511, 0.432, $P<0.05$), and negatively correlated with BSA ($r=0.386$, $P<0.05$).

It is further suggested that PCX can be used as a test index for the diagnosis of PNS and the evaluation of the condition of PNS patients.

Nephrin is a necessary podocyte membrane protein for the proper operation of glomerular filtration barrier. It is one of the proteins that express the selective effect of glomerular filtration barrier and is mainly involved in the transduction of extracellular signal in podocyte[14]. Some studies have shown that Nephrin can alleviate the apoptosis of podocytes, reduce the damage of protein factors, maintain the function of glomerular filtration, reduce the occurrence of albuminuria to a certain extent, and alleviate the condition of PNS patient[15].

In this study, the level of Nephrin in urine of PNS patients was significantly higher than that of healthy people, MsPGN group, MCN group, MN group and FSGS group, and the level of Nephrin increased gradually. The expression level of nephrin in FSGS patients was the highest, the difference was statistically significant ($P<0.05$). It is suggested that the increased expression of nephrin can improve the damage of renal filtration function and alleviate the symptoms of PNS patients. Pearsoon linear analysis showed that nephrin was positively correlated with 24 h urinary protein, cholesterol, TG and Cre ($r=0.369$, 0.472, 0.415, 0.434, $P<0.05$), and negatively correlated with BSA ($r=0.512$, $P<0.05$). It is further suggested that nephrin can be used as a test index for the diagnosis of PNS and the evaluation of the condition of PNS patients.

In conclusion, the high expression of PCX, nephrin in urine of patients with PNS is positively correlated with 24 h urinary protein, cholesterol, TG and Cre in clinical indexes, and negatively correlated with BSA. It can be used as a test index to diagnose PNS and evaluate the condition of patients with PNS, and can be widely used in clinic. However, the sample of this experiment is small and the research time is short. The mechanism of PCX,nephrin and renal immunity still needs to be further studied.

References

1) Kamei K, Ishikura K, Sako M, Ito S, Nozu K, et al. Rituximab therapy for refractory steroid-resistant nephrotic syndrome in children. Pediatr Nephrol 2020; 35: 17-24.

2) Lei L, Muhammad S, Al-Obaidi M, Sebire N, Cheng IL, et al. Successful use of ofatumumab in two cases of early-onset juvenile SLE with thrombocytopenia caused by a mutation in protein kinase C δ. Pediatr Rheumatol Online J 2018; 16: 61.

3) Selewski DT, Chen A, Shatat IF, Pais P, Greenbaum LA, et al. Vitamin D in incident nephrotic syndrome: a Midwest Pediatric Nephrology Consortium study. Pediatr Nephrol 2016; 31: 465-472.

4) Ghelani H, Razmovski-Naumovski V, Inampudi V, Chang D, Nammi S. Atorvastatin Improves Hepatic Lipid Metabolism and Protects Renal Damage in Adenine-Induced Chronic Kidney Disease in Sprague-Dawley Rats. Biomed Res Int 2019; 2019: 8714363.
5) Kopp JB. Global glomerulosclerosis in primary nephrotic syndrome: including age as a variable to predict renal outcomes. Kidney Int 2018; 93: 1043-1044.
6) Tamayo-Orbegozo E, Amo L, Riñón M, Nieto N, Amutio E, et al. Podocalyxin promotes proliferation and survival in mature B-cell non-Hodgkin lymphoma cells. Oncotarget 2017; 8: 99722-99739.
7) Wang JH, Zhu R. In patients with type 2 diabetic nephropathy glycosylated hemoglobin, urine trace albumin and biochemical indexes of related research. J College Yichun 2017; 12: 70-71.
8) Liu Y, Hou DY. Diagnostic value of tubular markers in primary nephrotic syndrome. J Kunming Med Univ 2016; 37: 105-108.
9) Young C, Lampe R. Primary Epstein-Barr Virus Infection in an Adolescent Female Complicated by Acute Acalculous Cholecystitis. Cureus. 2019; 11: 5044.
10) Pei MH, Zhao C, Gao F, Zhang MF. [Clinical features of glucocorticoid eye drops induced ocular hypertension in pediatric and adult uveitic eyes]. Zhonghua Yan Ke Za Zhi 2018; 54: 839-842.
11) Del Sordo R, Brugnano R, Covarelli C, Fiorucci G, Falzetti F, et al. Nephrotic syndrome in primary myelofibrosis with renal extramedullary hematopoiesis and glomerulopathy in the JAK inhibitor era. Clin Nephrol Case Stud 2017; 5: 70-77.
12) San IV, Prost G, Williams JA, Moss SE, Nuber UA. High Podocalyxin levels promote cell viability partially through up-regulation of Annexin A2. Biochem Biophys Res Commun 2016; 478: 573-579.
13) Yamada S, Itai S, Kaneko MK, Kato Y. Anti-Podocalyxin Monoclonal Antibody 47-mG 2a Detects Lung Cancers by Immunohistochemistry. Monoclon Antib Immunodiagn Immunother 2018; 37: 91-94.
14) Mallela SK, Mitrofanova A, Merscher S, Fornoni A. Regulation of the amount of ceramide-1-phosphate synthesized in differentiated human podocytes. Biochim Biophys Acta Mol Cell Biol Lipids 2019; 1864: 158517.
15) Tahara A, Takasu T. Prevention of progression of diabetic nephropathy by the SGLT2 inhibitor ipragliflozin in uninephrectomized type 2 diabetic mice. Eur J Pharmacol 2018; 830: 68-75.

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