

Original Article

Significance of TRPV5 and OPN biomarker levels in clinical diagnosis of patients with early urinary calculi

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Abstract: Objective: To investigate the clinical significance of the expression levels of transient receptor potential vanilloid 5 (TRPV5) and Osteopontin (OPN) biomarkers for the diagnosis of early-stage urinary stones. Methods: A total of 48 calcium-containing kidney stone patients admitted to our hospital between February 2018 and February 2019 were selected as the experimental group in this study, and another 48 age-matched stone-free healthy individuals were selected as the control group, and the expression levels of TRPV5 and OPN biomarkers in the two groups were examined and compared with respect to related indicators. Results: The urine oxalic acid content of the experimental group was found to be notably higher than the control group, while the citric acid content was lower; we observed higher levels of the 24-hour urine calcium content in the experimental group, while the citric acid/calcium ratio was remarkably lower; 24-hour urine magnesium and phosphorus levels of the two groups showed no marked difference; the mRNA expression levels of TRPV5 and OPN in the kidney tissues of the two groups were statistically different. Conclusion: The level of TRPV5 in urinary calculi patients was found to be significantly lower, whereas the level of OPN was significantly higher than the normal control, thus TRPV5 and OPN biomarker levels can be used as diagnostic markers for early urinary calculi.

Keywords: TRPV5 marker, OPN marker, early urinary stones, citrate

Introduction

Urinary calculi are common urological diseases. They can grow in any part of the bladder, kidney, ureter and urethra. Among them, ureter and kidney stones are frequently occurred. According to relevant reports, the global incidence of the disease reaches 15%, while in China it falls to around 1.2% to 6%, but exhibits an increasing trend [1-4]. Multiple options were applied for clinical treatment of urinary calculi, such as laser, ultrasound, and pneumatic ballistic techniques to remove the stone by surgery or extracorporeal lithotripsy. Despite the effectiveness, the adverse reactions are prone to occur after surgery and the recurrence rate is rather high. Therefore understanding the mechanism of stone formation is extremely urgent for the prevention and early diagnosis of urinary stones. Transient receptor potential vanilloid 5 (TRPV5) is an epithelial calcium channel protein that is reabsorbed by urinary calcium. It mainly binds to Ca⁺ in the urine to adjust the level of Ca⁺ in the urine, and the reabsorbed Ca⁺ serves to maintain the body's calcium balance. Clinical studies reported that a decrease

in the expression level of TRPV5 protein or a decrease in protein activity will lead to an increase in the level of Ca⁺ in the urine [5-8]. Osteopontin (OPN) is a secreted phosphorylated multifunctional glycoprotein. OPN can effectively inhibit the formation of urinary calculi by binding to Ca⁺. What's more, OPN can disturb the crystal lattice of crystals during cell migration, thereby inhibiting the formation of calcium oxalate crystals [9, 10]. This study aimed to investigate the clinical value of TRPV5 and OPN biomarker expression levels in the diagnosis of early urinary calculi.

Materials and methods

General information

The 48 patients with calcium-containing kidney stones presenting to our hospital between February 2018 and February 2019 were enrolled as the experimental group, and another 48 healthy people without stones were included as the control group. Midstream urine was taken from all the study subjects early in the morning as a specimen.

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The enrollment criteria of the experimental group

The inclusion are as followings: ① comply with the clinical diagnostic criteria for urolithiasis in the *Guidelines for the Diagnosis and Treatment of Urological Diseases in China*; ② complete clinical medical records; ③ all were diagnosed and confirmed by medical history, B-ultrasound and intravenous urography. While patients who met the followings were excluded ① primary or secondary renal tubular, glomerular and renal interstitial diseases, hereditary urinary stones, hyperuricemia, hyperparathyroidism, renal tubular acidosis, acute and chronic diseases, gastrointestinal diseases, urinary tract malformations, chronic renal failure, etc.; ② mental or other cognitive impairment or refuse to cooperate with the experiment; ③ incomplete clinical data.

The enrollment criteria of the control group

Patients were included meeting the followings ① no medical history, no urinary calculi by B-ultrasound, intravenous urography or CT examination; ② no history of taking related drugs; ③ no factors affecting the expression of related detection indicators. And those fitting these criteria were excluded ① patients and their immediate family members have a history of urinary calculi; ② brain, heart, kidney, liver and other organ tissue diseases; This study was reviewed and garnered the approval from the hospital ethics committee, and the patients and their families were informed of the purpose and process of the study and voluntarily signed an informed consent form.

Methods

10 ml of the first midstream urine in the morning was taken as a specimen, then centrifuged at 3000 r/min for 20 min, and placed it in a refrigerator at 2.8°C for later use; 24 hours later, all subjects' urine was taken (from 8 o'clock in the morning of the day to 8 o'clock in the morning the next day), added 1% boric acid for preservative, accurately measured and recorded. After mixing, 10 ml specimens were taken for inspection [11, 12]. The content of oxalic acid and citric acid in urine was detected by ultraviolet spectrophotometry.

Real-time PCR: 30 mg specimens were placed in a mortar, a small amount of liquid nitrogen was added, and they were quickly ground; after homogenization, the total RNA was extracted, and the OD value was measured with a spectro-

photometer. RT-PCR kit was used to synthesize cDNA (42°C, 1 h; 70°C, 10 min; 4°C, place for 10 min), and stored at -80°C. 2 ng cDNA was taken for Real-time PCR reaction (94°C for 5 min; 94°C, 30 s; 58°C, 40 s; 72°C, 20 s, 35 cycles; 72°C, 5 min). The relative mRNA expression of TRPV5 and OPN was detected, and the average value was recorded after 3 independent repeated experiments.

TRPV5 primer sequences: F 5'-TGCATACACAA-GTCATACAGG-3'; R 5'-AGGTGGGTCAGAGGGTCT-GAGG-3. OPN primer sequences: F: 5'CCACATG GC-TAAACCCTGACC3'; R: 5'CATGGCTTTCGTTG-GACTTACTTG3'.

Western blot: 50 mg specimens were taken, connective tissue and adipose tissue were removed, and cut on ice; 1 mL Lysis Bullbr (50 mmol/Ltris-HCl pH=7.5, 150 mmol/LNaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) was added and resuspended, sonicated, 12000 r/min, and centrifuged at 4°C for 10 min to determine the total protein content. 30 µg sample was taken for SDS-PAGE, transferred to PVDF membrane, blocked with 5% skimmed milk powder at room temperature for 1 h, and incubated the primary antibodies TRPV5 (1:2000) and OPN (1:1000) [13-15]. The membrane was washed with TBST and the secondary antibody was incubated for 1 h at room temperature.

Statistical analyses

We carried out the analysis using SPSS20.0 (Inc.; Chicago, IL, USA) software, and mapped the graphics using GraphPad Prism 7 (GraphPad Software, San Diego, USA). The count data and measurement data in the study were performed using X^2 test, t test and normality test, respectively. The difference was defined as statistically significant as a *P* value of less than 0.05.

Results

Baseline information

They were of comparability regarding the general data such as the gender, age and educational background. See **Table 1**.

The content of oxalic acid and citric acid in urine samples

In regard to the content of oxalic acid in the urine, higher level was identified in the experimental group as compared to the control group,

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Table 1. Comparison of general information of the two groups of patients

Factors	Control group (n=48)	Experimental group (n=48)	X ² /t	P
Gender			1.1875	0.665
Male	31 (64.58)	33 (68.78)		
Female	17 (35.42)	15 (31.25)		
Average age (years)	46.13±6.17	44.64±6.19	1.1811	0.2405
body mass index (kg/m ²)	23.65±2.36	24.12±2.47	1.6357	0.3624
History of smoking			0.1778	0.673
No	19 (39.58)	17 (35.42)		
Yes	29 (60.42)	31 (64.58)		
History of drinking			0.2110	0.646
No	14 (29.17)	12 (25)		
Yes	34 (70.83)	36 (75)		
Hypertension			0.2110	0.646
No	45 (93.75)	46 (95.83)		
Yes	3 (6.25)	2 (4.17)		
Marriage status			0.7111	0.399
Single	46 (95.83)	44 (91.67)		
Married	2 (4.17)	4 (8.33)		
Educational background			1.3689	0.563
High school or above	38 (79.17)	39 (79.59)		
Middle school	10 (20.83)	9 (18.37)		

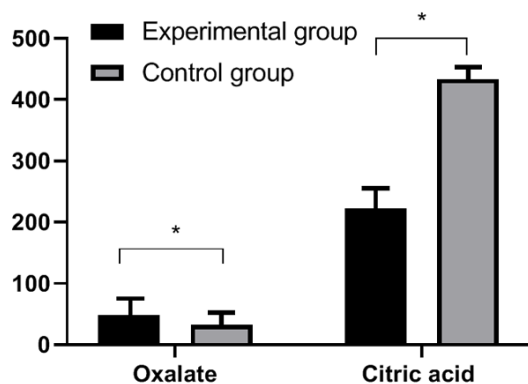


Figure 1. The content of oxalic acid and citric acid in urine samples ($\bar{x} \pm s$). Note: The abscissa indicates oxalic acid and citric acid, and the ordinate indicates the content of the specimen, mg; The contents of oxalic acid and citric acid in the experimental group were 48.14 ± 10.37 mg and 222.84 ± 102.43 mg respectively; The contents of oxalic acid and citric acid in the control group were 32.43 ± 5.23 mg and 433.85 ± 176.82 mg respectively; Significant differences exist in urine oxalic acid content between the experimental group and the control group ($t=9.3715$, $*P<0.05$); The urine citric acid content of the experimental group and the control group is significantly different ($t=7.1541$, $*P<0.05$).

while the content of citric acid was observed in a lower level than the control group, and the differences were of statistical significance, as shown in **Figure 1**.

24-hour urine electrolyte content and citrate/calcium ratio

Regarding the 24-hour urine calcium content, a higher level was detected in the experimental group, while the ratio of citric acid/calcium was lower than in comparison with the control group, with statistical differences ($P<0.05$, **Table 2**). The urinary magnesium and phosphorus content in the two groups were not statistically different, as shown in **Figure 2**.

mRNA expression levels of TRPV5, OPN and the expression levels of TRPV5 and OPN in kidney tissue

The mRNA expression levels of TRPV5 and OPN in the kidney tissues of the two groups were significantly different, with statistical significance, as shown in **Figure 3A**. The expression levels of TRPV5 and OPN in the experimental group were different from those in the control group, and the differences were statistically significant, as shown in **Figure 3B**.

Discussion

The main components of urinary calculi are calcium oxalate, calcium phosphate, uric acid and cystinite, of which the calcium oxalate amounts may take up to 85%. Clinical studies have found

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Table 2. 24-hour urine calcium content and citrate/calcium ratio of the two groups of patients ($\bar{x}\pm s$)

Groups	n	24 h urine calcium (mg/24 h)	citrate/calcium ratio
Experimental group	48	154.7±60.1	1.71±0.84
Control group	48	123.1±52.1	4.89±2.87
t		2.7525	7.3675
P		0.0071	0.001

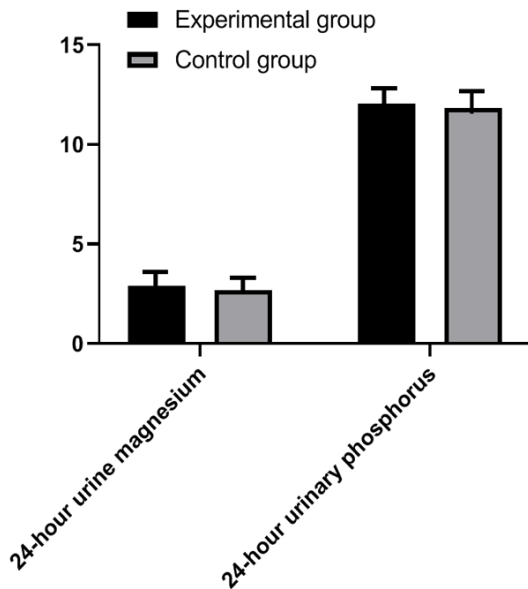


Figure 2. 24-hour urine magnesium and phosphorus content ($\bar{x}\pm s$). Note: The abscissa represents the 24-hour urine magnesium and phosphorus, the ordinate represents the content, mmol/24 h; The 24-hour urine magnesium and phosphorus of the experimental group were (2.89±1.35) and (12.07±5.51), respectively; The 24-hour urine magnesium and phosphorus of the control group were (2.68±1.13) and (11.84±5.73), respectively; There was no statistical difference in the 24-hour urine magnesium content between the experimental group and the control group (t=0.8264, P=0.4107); There was no statistical difference in the 24-hour urine phosphorus content between the experimental group and the control group (t=0.2005, P=0.8416).

that abnormal calcium metabolism in the body constitutes the major cause for the formation of stones, and the exact molecular mechanism forming the stones is still unclear. Good living habits, reasonable diet, and regular inspections are the favorable ways to prevent the formation of urinary stones [16-19]. This study reported that the experimental group exhibited higher urine oxalic acid content, while the citric

acid content was significantly lower in comparison with the control group. The 24-hour urine calcium content of the experimental group was detected in a higher level than the control group, while the ratio of calcium/calcium was relatively low. Meanwhile, we detected no difference between the two groups in the 24-hour urine magnesium and phosphorus content. The mRNA expression levels

of TRPV5 and OPN in the kidney tissues of the two groups and the expression levels of TRPV5 and OPN reported statistical differences between the groups.

It suggested that TRPV5 can serve as a marker for the diagnosis of early urinary calculi, while OPN was abnormally highly expressed, so the two can be used as a clinical diagnostic marker of the disease. Similar conclusions were drawn from a study by JOHN HIBMA [20] and others which proposed that the concentration of TRPV5 in the urine of patients with urinary calculi is drastically lower than that of ordinary people, and OPN is highly expressed, and both are related to the disease; TRPV5 expression is negatively correlated with disease, OPN expression is in a positive correlation with disease. This fully demonstrates that TRPV5 and OPN biomarker levels have high clinical value in the diagnosis of early urinary calculi.

However, some limitations exist in this study. The efficacy of serum TRPV5 and OPN levels was not tested. It is still not clear which one is better (alone or in combination). Therefore, in the future, the research will be expanded to increase the diagnostic performance test to provide rigorous clinical data.

To sum up, in the kidney tissue of patients with urinary calculi, the TRPV5 is abnormally lowly expressed, while OPN is highly expressed, and both are of statistical differences. The levels of TRPV5 and OPN can be served as diagnostic indicators for early urinary calculi.

Disclosure of conflict of interest

None.

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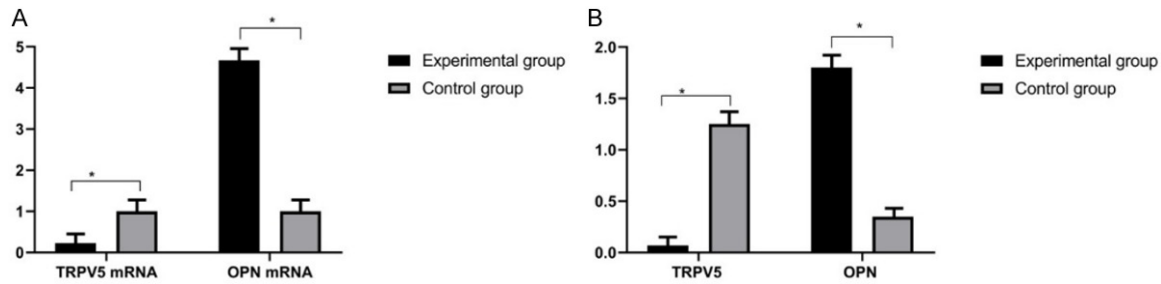


Figure 3. mRNA expression levels of TRPV5, OPN and the expression level of TRPV5 and OPN in kidney tissue. Note: A. The abscissa represents TRPV5 mRNA and OPN mRNA, and the ordinate represents the expression level, g/L; The expression levels of TRPV5 mRNA and OPN mRNA in the experimental group were (0.23±0.02) g/L and (4.67±0.18) g/L respectively; The expression levels of TRPV5 mRNA and OPN mRNA in the control group were (1.00±0.03) g/L and (1.00±0.02) g/L respectively; The expression level of TRPV5 mRNA in the experimental group was significantly lower than that in the control group (t=147.9584, *P<0.05); The OPN mRNA expression level of the experimental group was significantly higher than that of the control group (t=140.3944, *P<0.05). B. The abscissa represents TRPV5 and OPN, the ordinate represents the expression level, g/L; The expression levels of TRPV5 and OPN in the experimental group were (0.07±0.02) g/L and (1.80±0.11) g/L, respectively; The expression levels of TRPV5 and OPN in the control group were (1.25±0.04) g/L and (0.35±0.02) g/L, respectively; The expression level of TRPV5 in the experimental group was significantly lower than that in the control group (t=182.8048, *P<0.05); That the OPN expression level of the experimental group was significantly higher than that of the control group (t=89.8532, *P<0.05).

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