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Original Article

Analysis of expression and correlation of fibroblast growth factor 23 and smooth muscle actin alpha in patients with uremia in internal drteriovenous fistula

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Abstract: Objective: The goal of this study was to investigate the expression and correlation of fibroblast growth factor 23 (FGF-23) and smooth muscle actin alpha (α-SMA) in patients with uremia during arteriovenous fistula (AVF). Methods: A total of 82 patients with uremia were in the research group, 67 males and 15 females, aged 46 to 75, with an average age of (61.57±8.43). A further 80 patients were in the control group, including 60 males and 20 females, aged 42 to 76, with an average age of (62.33±9.27). Venous blood (4 mL) was taken from each patient before treatment (T0), 3 days after treatment (T1) and 7 days after treatment (T2). FGF-23 and α-SMA were detected by enzyme-linked immunosorbent assay (ELISA). The concentrations of FGF-23 and α-SMA in diagnosis of uremia, the prediction for curative effect and the correlation with the treatment time between the study group and control group were analyzed. Results: Serum of FGF-23 and α-SMA in the study group were significantly higher than those in the control group (P<0.001). The ROC curve indicated that FGF-23 and α-SMA were effective in diagnosis of uremia (P<0.001). FGF-23 and α-SMA were negatively correlated with treatment time (r=-0.49, -0.79, P<0.001). Patients who were cured and effective in the study group were divided into group A while those who were effective and ineffective were divided into group B. The serum of FGF-23 and α-SMA in group A after treatment were significantly lower than those in the control group (P<0.001). The ROC curve indicated that FGF-23 and α-SMA were effective in predicting treatment (P<0.001). Conclusion: The concentrations of FGF-23 and α-SMA significantly increased in patients with uremia, were negatively correlated with the time of AVF, which may be targeted therapy for uremia in the future.

Keywords: Uremia, arteriovenous fistula, FGF-23, α-SMA

Introduction

The complex of irreversible kidney damage syndromes in the human body to the end stage leads to uremia. Patients with uremia cannot produce urine, metabolic wastes, glucose, amino acid, sodium through the kidneys [1, 2]. Uremia is not an independent disease but a comprehensive manifestation of renal failure [3]. Uremia is common in middle-aged and elderly people, but studies have showed that many young people suffer from it [4, 5]. Clinically, uremia is a common chronic disease, with an increase of 130,000 patients each day in the world [6]. Accompanied by complications from the respiratory system and the blood system, uremia has great harm to the human body [7]. Without specific symptoms in the early stage, patients often delay the treatment [8]. A study revealed that the 5-year survival rates of patients with late-stage uremi range from 30.0% to 60.0% [9]. Accumulated from various diseases, patients with uremia is difficult to treat and has long rehabilitation cycle [9]. Efforts have been made to improve the treatment efficiency and prognosis of patients with uremia. At present, the most common therapy for uremia is hemodialysis [10]. With the aging population and an increase of dialysis in recent years, patients with fistula or internal fistula dysfunction are commonly seen [11]. Therefore, patients with uremia were treated with hemodialysis combined with arteriovenous fistula surgery to improve vascular anastomosis and provide sufficient blood supply [12].

At present, the pathogenesis of uremia has not been clarified. Some study suggested that the occurrence of uremia was related to calcium inhibitory factor in blood [13]. FGF-23 and α-SMA, the main factors leading to vascular calcification, was closely related to the occurrence and development of uremia [14]. There are few studies on the relationship of FGF-23, α-SMA and uremia at home and abroad. Therefore, the study aimed at the effect of FGF-23 and α-SMA during the treatment, so as to provide a reference for diagnosis and treatment of uremia.

Materials and methods

A total of 82 patients with uremia in Hebei University of Engineering Affiliated Hospital were treated as the study group, 67 males and 15 females, aged 46 to 75, with an average age of (61.57±8.43). An additional 80 patients were treated as the control group, with 60 males and 20 females, aged 42 to 76, with an average age of (62.33±9.27). The experiments were approved by the Ethics Committee of our hospital and patients signed informed consent.

Inclusion and exclusion criteria

Inclusion criteria were as follows: patients in the research group met the clinical performance of uremia [15]. Patients who were diagnosed as uremia received arteriovenous fistula. Patients with complete clinical data cooperated with the medical staff. Exclusion criteria were as follows: presence of tumors, infectious diseases, connective tissue diseases; genetic disorders, severe organ failure, physical disability, mental disorders.

Methods

Patients in the study group were treated with hemodialysis combined with arteriovenous fistula in Hebei University of Engineering Affiliated Hospital. Blood flow of ulnar artery, radial artery and cephalic vein was evaluated by color Doppler flow imaging (CDFI). The skin and subcutaneous tissues were cut after anesthesia and the radial artery, cephalic vein and subcutaneous tissue were dissected and ligated. The ligation end near the heart was cut off with hemostatic forceps and clamped with surgical forceps. Heparin physiological water was injected for 2 minutes. Then, the instruments were removed and the diameter of artery-vein shunt was observed. After arteriovenous anastomosis, gauze with 0.9% sodium chloride solution was used to treat the anastomosis.

Detection method

A volume of 4 mL of venous blood was taken from each patient before treatment (T0), 3 days after treatment (T1) and 7 days after treatment (T2), respectively and centrifuged for 10 minutes (4000 rpm/min) after placing at room temperature for 30 minutes to obtain upper serum. FGF-23 and α-SMA were tested by enzyme-linked immunosorbent assay (ELISA), of which FGF-23 kit was purchased from Shanghai Hengfei Biotechnology Co., Ltd. SEA746Hu-1, α-SMA kit was purchased from Shanghai Fushen Biological Technology Co., Ltd., FSEA1144. The operation was in accordance with the kit instructions.

Observation indexes

The treatment effect of the study group was as follows: patients with no obvious clinical symptoms but normal urination were cured; patients significantly improved with creatinine (Scr) decreased 30% or below, blood urea nitrogen (BUN) decreased 30% or below were effective. There were still some clinical symptoms after treatment with Scr decreased 20% or below, BUN decreased 20% or below were effective. Symptoms that were unchanged or deteriorated were invalid. The concentrations of FGF-23 and α-SMA before treatment and the changes of FGF-23 and α-SMA during the treatment were observed in the two groups.

Statistical methods

SPSS 24.0 statistical software (Beijing StrongVinda Information Technology Co., Ltd.) was used to analyze the experimental results. All the graphs were drawn using Graphpad8 (Shenzhen Soft Head Technology Co., Ltd.) software, and the results were repeated twice. Count data, such as curative effect of the study group, are expressed by [n (%)] and compared by the chi-square tests between the two groups. Measurement data such as concentration of FGF-23 and α-SMA are expressed by (mean ± standard deviation) and compared by t-tests. The comparison at different time points was analyzed by repeated measurement data. The

Table 1. Comparison of clinical data of patients [n (%)]

	Research group (n=82)	Control group (n=80)	t or χ^2	P
Age	61.57±8.43	62.33±9.27	0.546	0.586
BMI	27.15±3.84	26.96±4.12	0.304	0.762
Platelet ($\times10^9$/L)	228.63±42.86	216.96±50.15	1.594	0.113
Red blood cell ($\times10^{12}$/L)	3.86±1.06	3.60±1.15	1.49	0.136
White blood cell ($\times10^9$/L)	7.63±2.56	7.05±3.84	1.134	0.259
Gender			1.076	0.300
Male	67 (81.71)	60 (75.0)		
Female	15 (18.29)	20 (25.00)		
Place of residence			0.800	0.371
Town	75 (91.46)	76 (95.00)		
Rural	7 (8.54)	4 (5.00)		
Smoking			0.193	0.660
Yes	59 (71.95)	60 (75.00)		
No	23 (28.05)	20 (25.00)		
Drinking			0.018	0.894
Yes	48 (58.54)	46 (57.50)		
No	34 (41.46)	34 (42.50)		
Sports habit			0.494	0.482
Yes	12 (14.63)	15 (18.75)		
No	70 (85.37)	65 (81.25)		
Primary underlying disease				
Glomerulus nephritis	9 (10.98)			
Hypertensive nephropathy	31 (37.80)			
Diabetic nephropathy	25 (30.49)			
Nephritis	15 (18.29)			
Other	2 (2.44)			

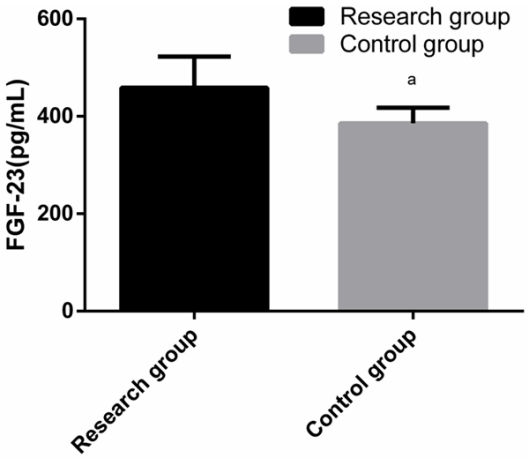

Figure 1. Comparison of FGF-23 in serum of patients between the research group and the control group. [a]indicates a comparison with the research group, P<0.001.

differences of FGF-23 and α-SMA with different effects was analyzed by the ROC curve and the correlation was analyzed by a Spearman test.

Results

No significant difference in general data

There were no significant differences in age, BMI, platelet, red blood cells, white blood cells, gender, place of residence, smoking, drinking, sports habit between the two groups (P>0.050). In the study group, primary diseases were 10.98% glomerulonephritis (9 cases), 37.80% hypertensive nephropathy (31 cases), 30.49% diabetic nephropathy (25 cases), 18.29% nephritis (15 cases) and 2.44% other diseases (2 cases; **Table 1**).

Serum levels of FGF-23 and α-SMA are higher than that in the control group

The serum level of FGF-23 in the study group was (458.82±63.85) pg/mL, higher than that (385.82±31.85 pg/mL) of the control group (P<0.001). The serum level of α-SMA in the study group was (3.29±0.32) ng/mL, higher than that (2.49±0.62) ng/mL) in the control group (**Figures 1** and **2**).

Predictive value of FGF-23 and α-SMA are lower in uremia

According to the ROC curve, when the cut-off value of FGF-23 was 37.80, the sensitivity and specificity of uremia diagnosis were 98.75% and 60.95%, respectively. When the cut-off value of α-SMA was 13.41, the sensitivity and specificity were 73.75% and 60.34%, respectively (**Figures 3** and **4**, **Table 2**).

Correlation between FGF-23, α-SMA are negatively correlated with treatment time

FGF-23 at T0, T1 and T2 in the study group were (458.82±63.85) pg/mL, (424.63±51.04) pg/

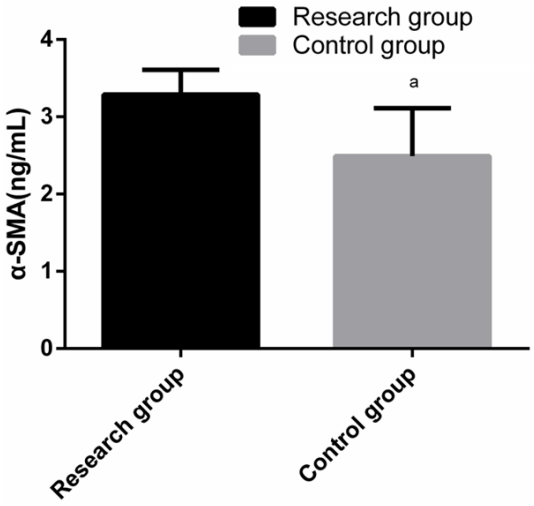

Figure 2. Comparison of α-SMA in serum of patients between the research group and the control group. [a]indicates a comparison with the research group, P<0.001.

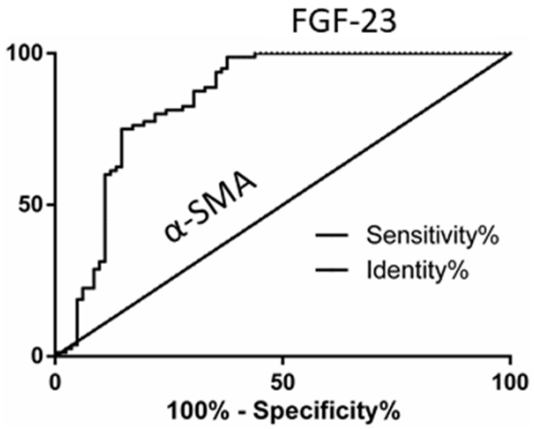

Figure 3. Analysis of FGF-23 in diagnosis of uremia. According to the ROC curve, when the cut-off value of FGF-23 was 37.80, the sensitivity and specificity were 98.75% and 60.95%, respectively.

mL and (405.96±36.87) pg/mL, respectively; While α-SMA were (3.29±0.32) ng/mL, (2.92±0.26) ng/mL and (2.51±0.12) ng/mL, respectively. Spearman test indicated that FGF-23 was negatively correlated with treatment time (r=-0.49, P<0.001), and α-SMA also negatively correlated with treatment time (r=-0.79, P<0.001; **Figures 5** and **6**).

Serum levels of FGF-23 and α-SMA are lower than that in the control group

In the study group, 31.71% (26 cases) were cured, 26.83% (22 cases) were effective, 35.37%

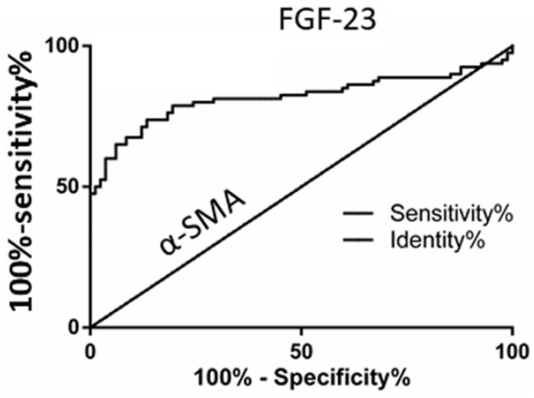

Figure 4. Analysis of α-SMA in diagnosis of uremia. According to the ROC curve, when cut-off value of α-SMA was 13.41, the sensitivity and specificity were 73.75% and 60.34%, respectively.

Table 2. Predictive value of FGF-23 and α-SMA for uremia

	FGF-23	α-SMA
Cut-off	37.80	13.41
Area	0.85	0.82
Std. Error	0.03	0.04
95% CI	0.79~0.91	0.74~0.89
P	<0.001	<0.001
Sensitivity (%)	98.75	73.75
Specificity (%)	60.95	60.34

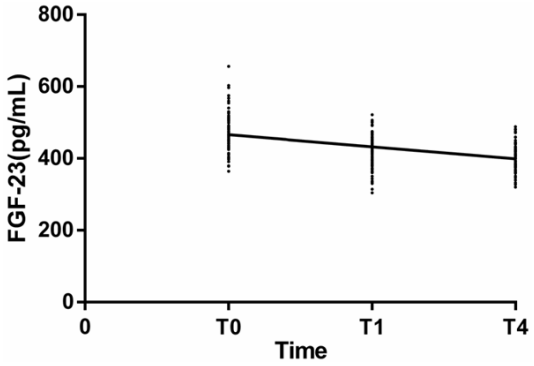

Figure 5. Analysis of the correlation between FGF-23 and treatment time. According to Spearman, FGF-23 was negatively correlated with treatment time (r=-0.49, *P*<0.001).

(29 cases) were effective, and 6.10% (5 cases) were invalid. Patients who were cured and effective in the study group were divided into group A (48 cases) while those who were effective and ineffective were divided into group B (34 cases). The serum level of FGF-23 in group

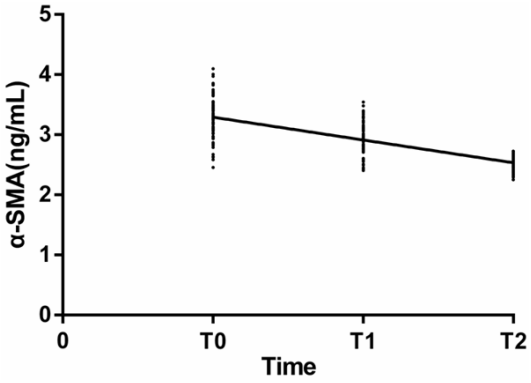

Figure 6. Analysis of the correlation between α-SMA and treatment time. According to Spearman, α-SMA was negatively correlated with treatment time (r=-0.79, P<0.001).

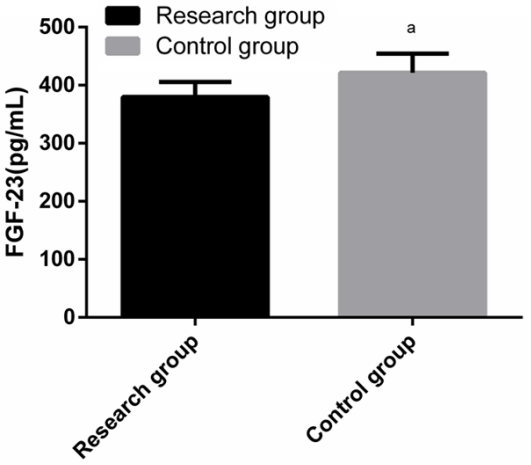

Figure 7. Comparison of FGF-23 in serum between group A and group B after treatment. [a]indicates a comparison with group A after treatment, P<0.001.

A after treatment was (379.78±25.80) pg/mL, lower than that (421.37±33.00 pg/mL) in group B (P<0.001). After treatment, the serum level of α-SMA in group A was (2.65±0.30) ng/mL, lower than that (3.08±0.36 ng/mL) in the control group (**Figures 7** and **8**).

Predictive value of FGF-23 and α-SMA are lower in the treatment of uremia

According to the ROC curve, the concentrations of FGF-23 and α-SMA in group A and group B were analyzed. When the cut-off value of FGF-23 was 33.33, the sensitivity and specificity for predicting treatment effect were 90.63% and 57.29%, respectively. When the cut-off value of α-SMA was 22.92, the sensitivity and specificity were 76.32% and 53.40%, respectively (**Table 3**, **Figures 9** and **10**).

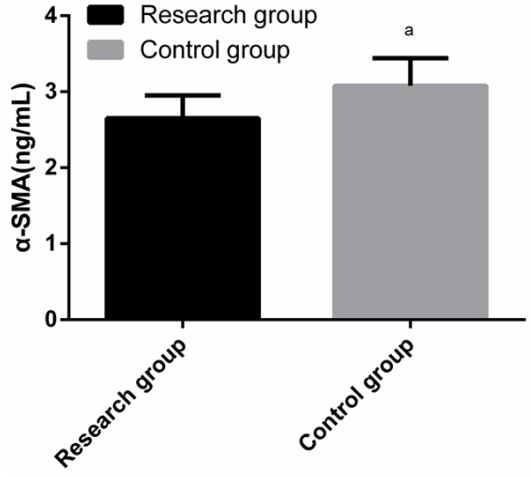

Figure 8. Comparison of α-SMA in serum between group A and group B after treatment. [a]indicates a comparison with group A after treatment, P<0.001.

Table 3. Predictive value of FGF-23 and α-SMA in the treatment of uremia

	FGF-23	α-SMA
Cut-off	33.33	22.92
Area	0.84	0.83
Std. Error	0.04	0.04
95% CI	0.75~0.93	0.75~0.92
P	<0.001	<0.001
Sensitivity (%)	90.63	76.32
Specificity (%)	57.29	53.40

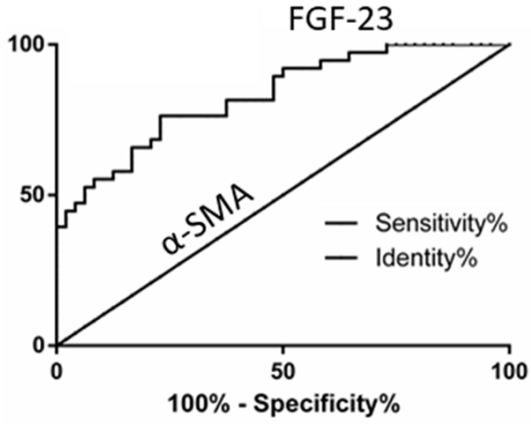

Figure 9. Analysis of FGF-23 for therapeutic effect according to ROC curve, when the cut-off value of FGF-23 was 33.33, the sensitivity and specificity were 90.63% and 57.29%, respectively.

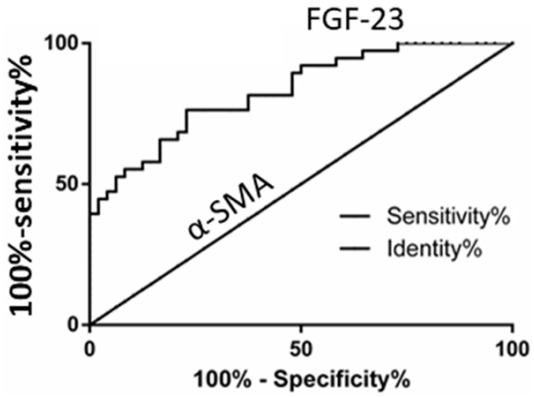

Figure 10. Analysis of α-SMA for therapeutic effect according to the ROC curve, when the cut-off value of α-SMA was 22.92, the sensitivity and specificity were 76.32% and 53.40%, respectively.

Discussion

Uremia is one of the most harmful chronic disease syndromes for middle-aged and elderly people and has a high mortality rate and difficulty in treatment [16, 17]. Without timely treatment, uremia is likely to cause pathological changes [18]. Therefore, uremia has been a hot topic in clinical research [19]. Currently, the pathogenesis of uremia has not been clarified. Studies have revealed that uremia is closely related with previous diseases [20]. Uremia can be diagnosed by means of urine routine, blood routine, renal function examination, imaging and biopsy [21]. The study aimed at the changes of FGF-23 and α-SMA in the treatment, which is of great significance for targeted therapy and diagnosis of uremia. Research on uremia has been limited to the clinical treatment at home and abroad. The research objects is selected based on the inclusion and exclusion criteria and calculated with advanced statistical software. The roles of the FGF-23 and α-SMA in uremia are analyzed in detail. The results are as follows.

According to the experiment, data show that FGF-23 and α-SMA in the research group were significantly higher than those in the control group, indicating that FGF-23 and α-SMA were involved in the development of uremia. FGF-23, a serum phosphorus regulatory protein secreted by bone cells, has a great influence on inorganic phosphorus metabolism of the kidney [22]. Serum phosphorus is the main cause of vascular calcification [23]. FGF-23 promotes urine phosphorus excretion by inhibiting phosphorus reabsorption in proximal renal tubules, thus lowering the activity of 1a-hydroxylase in the kidneys, reducing absorption and metabolism of nutrients in renal function. The dynamic balance was destroyed, causing kidney disease and some metabolic diseases, or even uremia. α-SMA, a marker protein that differentiated into myofibroblasts, not only promotes the mesenchymal-mesenchymal transition in epithelial cells, but also regulates the synthesis and degradation of extracellular matrix [24, 25]. The increase of α-SMA in patients with uremia may be peritoneal damage of organ epithelial cells, resulting in mesenchymal-mesenchymal transition of epithelial cells, during which the intercellular junction, skeleton arrangement and the original structure of basement membrane are destroyed. Substances exhibited fluidity form and deposited rapidly, resulting in a wide range of organ peritoneal fibrosis. The diagnostic value of FGF-23 and α-SMA for uremia was analyzed by the ROC curve. When the cut-off value of FGF-23 was 37.80, the sensitivity and specificity of uremia diagnosis were 98.75% and 60.95%, respectively. When the cut-off value of α-SMA was 13.41, the sensitivity and specificity were 73.75% and 60.34%, respectively. FGF-23 and α-SMA with diagnostic value for uremia, are likely to be a new diagnostic marker for uremia. According to the correlation analysis of Spearman, FGF-23 and α-SMA, negatively correlated with the treatment time of uremia, could be predictors of deterioration and rehabilitation of patients with uremia, which is conducive to the evaluation of patients. Furthermore, patients with uremia were divided into group A with better efficacy and group B with poor efficacy and the ROC curve were used to analyze the differences in FGF-23 and α-SMA between group A and group B. Data showed that the cut-off value of FGF-23 was 33.33, the sensitivity and specificity for predicting therapeutic effect were 90.63% and 57.29%, respectively. When the cut-off value of α-SMA was 22.92, the sensitivity and specificity were 76.32% and 53.40%, respectively. Thus, FGF-23 and α-SMA may be potential therapeutic targets for uremia in the future, which is of clinical significance.

In this study, concentrations of FGF-23 and α-SMA in the serum of patients were analyzed. With limited experimental conditions, the me-

chanism for diagnosis of FGF-23 and α-SMA need to be proved. Moreover, blood samples from patients, instead of FGF-23 and α-SMA in the tissue, were selected as the research object. ELISA was also used due to limited funds. Furthermore, short experimental periods cannot deliver the prognosis of FGF-23 and α-SMA for patients with uremia. A follow-up survey on the subjects will be conducted for the relationship between FGF-23, α-SMA and uremia.

In summary, the concentrations of FGF-23 and α-SMA in patients increased significantly and were negatively correlated with arteriovenous fistula in hemodialysis, which may be potential therapeutic targets for uremia in the future.

Disclosure of conflict of interest

None.

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