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Expression of mir-29a-5p, sclerostin and fetuin-A in patients with chronic kidney disease and their correlation with vascular calcification

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ABSTRACT

The objective of the current study was to analyze the expression of mir-29a-5p, osteosclerotin and fetuin-A in patients with chronic kidney disease and their correlation with vascular calcification. For this purpose, 162 patients with chronic kidney disease treated in our hospital from January 2020 to January 2022 were selected retrospectively, and then 162 healthy people who underwent physical examination with our hospital in the same period were selected. The expressions of serum mir-29a-5p, sclerostin and fetuin-A were analyzed after fasting venous blood was drawn from the two groups. According to the coronary artery calcification score (CACS), patients with chronic kidney disease were divided into the calcification group (69 cases) and the non-calcification group (93 cases). The expressions of mir-29a-5p, sclerostin and fetuin-A in the two groups were analyzed, and the correlation between the three in chronic kidney disease and vascular calcification was analyzed. Results showed that compared with the control group, the expression of mir-29a-5p and sclerostin in the study group was higher, and the expression of fetuin-A was lower, the difference was statistically significant ($P < 0.05$); The expression of mir-29a-5p, sclerostin and fetuin-A in calcified group was higher than that in non-calcified group, and the expression of fetuin-A was lower ($P < 0.05$); Mir-29a-5p and sclerostin showed positive correlation ($r=6.776$, $P=0.011$); The expression of mir-29a-5p and fetuin-A showed negative correlation ($r=-5.326$, $P=0.001$); The expression of mir-29a-5p and sclerostin showed negative correlation ($r=-9.677$, $P=0.001$); Mir-29a-5p and sclerostin were positively correlated with vascular calcification ($r=0.695$, $P=0.001$; $r=0.715$, $P=0.001$), and fetuin-A was positively correlated with vascular calcification ($r = -0.953$, $P = 0.001$). Then, Mir-29a-5p, sclerostin and fetuin-A are abnormally expressed in chronic kidney disease. There is an abnormal correlation among them in chronic kidney disease, and they are correlated with vascular calcification.

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Introduction

Chronic kidney diseases (CKD) are a common clinical condition with high prevalence and mortality rates that severely injures patients' quality of life and threatens their safety, which has developed into one of the public health problems (1-2). As an endogenous non-coding small single-stranded RNA of 18-25 nucleotides in length, microRNAs are capable of regulating the transcription of gene expression and thus the progression of the disease (3). And as a negative regulator of bone formation, sclerostin plays an important role in regulating bone reconstruction (4). FetuinA is involved in the disruption of calcium and phosphorus metabolism in the body, which is the key to calcification in coronary arteries, together with excessive deposition of calcium and phosphorus in the intercellular matrix. Vascular calcification, a common complication of end-stage renal disease, is the critical clinical cause of coronary artery calcification. Clinical investigations have shown that vascular calcification is closely associated with a number of coronary atherosclerosis and myocardial infarction as well as the occurrence of cardiovascular events such as cardiac arrest (5). Some scholars have pointed out in their studies that vascular calcification is one of the geographical risk factors for the development of clinical cardiovascular disease, and it, therefore, is particularly important to early diagnose CKD patients (6). In this paper, the expression of miR-29a-5p, sclerostin and fetuin-A in CKD patients was analyzed to find their correlation with vascular calcification.

Materials and Methods

Subjects

A total of 162 CKD patients admitted to our hospital from January 2020 to January 2022 were enrolled in the study group by retrospectively reviewing their electronic health records before 162 healthy people who underwent health check-ups at the hospital during the same period formed the control group. The control group consisted of 86 males and 76 females aged 40-72 years with an average value of (56.1±12.7) years, BMI 21-32 kg/m^2 with an ave-

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rage value of (26.5±4.4) kg/m^2. The study group consisted of 85 males and 77 females aged 41-73 years with an average value of (57.1±12.72) years, BMI 21-32 kg/m^2 with an average value of (26.5±4.4) kg/m^2. Patients with CKD were divided by their coronary artery calcification scores (CACs) into a calcification group (n=69) and a no-calcification group (n=93). The former included 36 males and 33 females aged 41-70 years with a mean value of (55.6±11.5) years, BMI 20-31 kg/m^2 with a mean value of (25.8±4.2) kg/m^2; the no-calcification group included 54 males and 39 females, aged 40-71 years with a mean value of (55.5±12.0) years, BMI 21-30 kg/m^2 with a mean value of (25.6±3.5) kg/m^2. No statistical difference in the clinical data was discovered between the control group and the study group and between the calcification group and the non-calcification group (P > 0.05), hence comparable.

Inclusion criteria: CKD patients who met the diagnostic criteria for CKD under the K/DOQI guidelines (7); and those on haemodialysis, peritoneal dialysis and other treatments.

Exclusion criteria: Those complicated by systemic diseases such as allergic purpura and rheumatoid arthritis; complicated by cardiopulmonary disease, sudden progressive deterioration of renal function; or complicated by malignant bone tumours.

Assessment of vascular calcification

Vascular calcification was measured using CACs. All patients underwent a CT scan of the chest and were scored for calcification of the left main coronary artery, left anterior descending artery, left circumflex artery and right coronary artery by the Agatston method, followed by evaluation of total calcification. It was recorded as no calcification when CACs = 0 and as calcification when CACs > 0.

Serum specimen collection

First, 5ml of fasting venous blood was collected from all participants and centrifuged at 2000r/min for 15min at 4°C. The supernatant was then extracted and placed in a 1.5ml EP tube and stored at -80°C for testing.

miR-29a-5p assay

Real-time fluorescent RT-PCR was performed. First, total RNA was extracted from miR-145 cells with a miRNA Isolation Kit, microRNA reverse transcription with a TaqMan microRNA reverse transcription kit, and quantitative PCR with SYBR Premix ExTaq II kit. The reaction conditions were set as follows: pre-fire at 95°C, pre-denaturation for 30 min, removing and cooling down to 90°C, denaturation for 5s, rapid cooling to 58°C, annealing for the 20s, temperature maintained at 60°C, extension for 45s, 40 consecutive cycles, and repeating 3 times. The relative expression of miR-145 was calculated using the $2^{-\Delta\Delta Ct}$ method with U6 as the internal reference. U6: upstream primer: 5'-CGGGTTTGTTTTCATTTGT-3'; downstream primer: 5'-AGTCCCAGCATGAACAGCTT-3'. miR-29a-5p: upstream primer: 5'-TGTTTCCTCGTCCCGTA-3'; downstream primer: 5'-TCACCGTTTCACAC-3'.

Sclerostin and fetuin-A assays

Cell lysates were prepared by detecting sclerostin and fetuin-A through a Western blotting assay and were measured for protein concentrations with BCA protein assay reagents. Equal amounts of protein (10-30μg) were electrophoresed on 8% or 12% SDS-polyacrylamide gels and transferred to PVDF membranes. The transferred proteins were then closed for 2 hours at room temperature in 5% fat-free milk powder in phosphate-buffered saline (PBS) containing 0.1% Tween 20 (PBST). Subsequently, the membranes were incubated overnight at 4°C with primary antibodies containing 3% non-fat milk powder in PBS. The membranes were washed and then incubated with a 1:3000 dilution of each HRP-conjugated secondary antibody for 2 hours before being rinsed again with PBST. Protein expression was finally displayed using the ECL kit.

Statistical analysis

The data were analyzed using SPSS 25.0. The measurement data were tested for chi-square by the Levene method, proved to show normal distribution by Shapiro-Wilk and expressed as mean ± standard deviation ($\bar{x} \pm s$) using repeated-measures ANOVA. The Independent Samples t-test was conducted to compare two groups at the same time point. By the Pearson correlation method, an analysis was conducted on the correlation of miR-29a-5p, sclerostin, and fetuin-A with CKD. The count data, expressed as a rate (%), were subject to χ^2-test, and differences were considered statistically significant when P<0.05.

Results

Expression of miR-29a-5p, sclerostin, and fetuin-A in both groups

As shown in Table 1, miR-29a-5p and sclerostin showed higher expression, but the fetuin-A expression was lower in the study group than in the control group, indicating

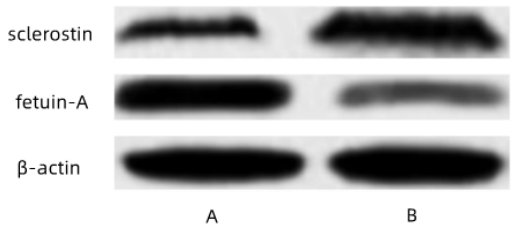

Figure 1. Expression of sclerostin and fetuin-A in both groups. Note: A: control group; B: study group.

Table 1. Analysis of miR-29a-5p, sclerostin and fetuin-A expression in both groups ($\bar{x} \pm s$).

Group	Cases (n)	miR-29a-5p	sclerostin	fetuin-A
Control	162	0.94±0.25	3.68±0.49	268.91±34.16
Study	162	3.67±0.65	6.87±1.24	200.45±31.06
t		6.354	9.526	5.748
P		0.001	0.001	0.001

Table 2. Expression of miR-29a-5p, sclerostin, and fetuin-A in the presence or absence of calcification.

Group	Cases (n)	miR-29a-5p	sclerostin	fetuin-A
Non-calcification	93	2.95±0.33	5.98±1.34	210.43±29.54
Calcification	69	4.05±1.04	7.55±1.69	197.53±26.11
t		4.623	5.110	9.842
P		0.001	0.001	0.001

Table 3. Correlation of miR-29a-5p, sclerostin, and fetuin-A expression with vascular calcification.

Indicators	Vascular calcification	
	r	*P*
miR-29a-5p	0.695	0.001
sclerostin	0.715	0.001
fetuin-A	-0.953	0.001

statistically significant differences (P < 0.05).

Expression of miR-29a-5p, sclerostin, and fetuin-A in calcification/non-calcification groups

As shown in Table 2, the expression of miR-29a-5p, sclerostin and fetuin-A was higher in the calcification group than in the non-calcification group. In contrast, fetuin-A expression was lower, suggesting statistically significant differences (P < 0.05).

Correlation of miR-29a-5p, sclerostin, and fetuin-A expression in CKD

As illustrated in Figure 1, miR-29a-5p and sclerostin showed a positive correlation (r=6.776, P=0.011); miR-29a-5p and fetuin-A a negative correlation (r=-5.326, P=0.001); miR-29a-5p and sclerostin a negative correlation (r = -9.677, P=0.001).

Correlation of miR-29a-5p, sclerostin, and fetuin-A expression with vascular calcification

As shown in Table 3, miR-29a-5p and sclerostin showed a positive correlation with vascular calcification (r=0.695, P=0.001; r=0.715, P=0.001), whilst fetuin-A presented a degree correlation with this condition (r=-0.953, P=0.001).

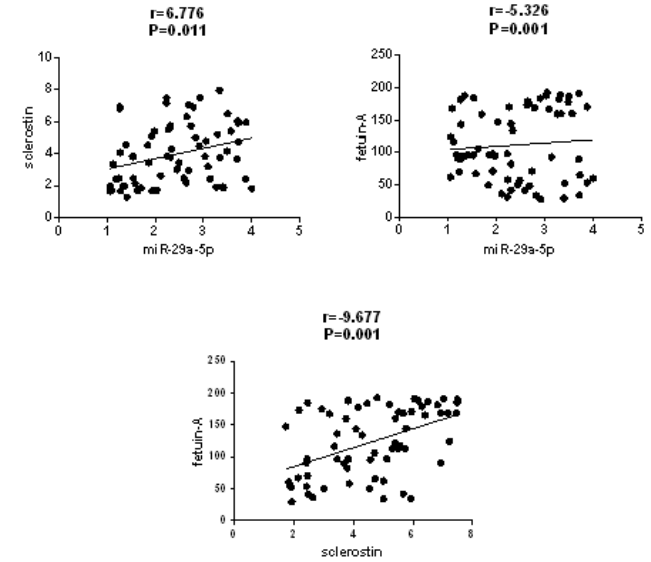

Figure 1. Correlation of miR-29a-5p, sclerostin and fetuin-A expression in CKD.

Discussion

It has been reported that vascular calcification, a clinically regulated process, is triggered by an imbalance in the regulation of various inducing factors and inhibiting factors (8-9). It is an important cause of CKD deterioration, so its study is expected to provide a practical indicator for the clinical assessment of vascular calcification in patients with CKD.

In patients with CKD, miR-29a-5p was found to be highly expressed in CKD, and in this paper, it was also found that and showed a positive correlation with the presence of vascular calcification (r=0.695, p=0.001). As small anticoding regulatory RNAs are widely present in human tissues and cells, miRNAs play an important role in the development of tumors. In an organismal malignancy, circulating miRNAs have shown potential as novel markers for early diagnosis of tumors (10) and are specifically expressed in lung, prostate and ovarian cancers. miR-29a-5p, a member of the miR-29 family, is highly expressed in tumour tissues and aberrantly expressed in chronic diseases, as reported in several studies (11). In this study, miR-29a-5p was further found to be highly expressed in CKD patients and positively correlated with vascular calcification (r=0.695, p=0.001) as its expression increased when patients developed vascular calcification.

Sclerostin, an acidic glycoprotein, is a newly discovered inhibitor of calcification that inhibits the differentiation and proliferation of osteoblasts through its own secretion. It has been shown that sclerostin is involved in the process of vascular calcification, mainly through interactions between the kidney, the vascular system and the bone (12). It is primarily secreted by the liver and then enters into the blood, with different levels of expression in different tissues and organs (13-14). It has also been suggested that sclerostin is a clinically negative acute response protein that blocks cytokine-induced calcification by inhibiting inflammatory levels (15-16). In this study, miR-29a-5p was found to be highly expressed in CKD patients and positively correlated with vascular calcification (r=0.715, p=0.001) as its expression rose when vascular calcification was present.

As a 59kD glycoprotein synthesized by the liver, fetuin-A is widely present in the extracellular fluid. Responsible for half of the inhibition of calcium and phos-

phorus deposition, it is indicated to be a key factor behind the development of vascular calcification (17). Clinical investigations have revealed that fetuin-A inhibits the pro-calcification effects of bone formation proteins and transforming growth factor-β, in addition to binding to matrix GLA proteins and calcium-phosphate minerals to prevent the progression of vascular calcification (18-19). According to an animal study, severe multi-organ calcification occurs in rats with targeted deletion of the Fetuin gene, suggesting that fetuin-A has an important role in inhibiting vascular calcification (20-21). In this study, fetuin-A was found to be lowly expressed in CKD patients and positively correlated with vascular calcification (r=-0.953, P=0.001) as its expression rose when vascular calcification was present. Furthermore, miR-29a-5p and sclerostin showed a positive correlation in CKD (r=6.776, P=0.011); miR-29a-5p and fetuin-A a negative correlation (r=-5.326, P=0.001); miR-29a-5p and sclerostin a negative correlation (r=-9.677, P=0.001).

In conclusion, miR-29a-5p, sclerostin and fetuin-A were aberrantly expressed in CKD patients, abnormally associated with each other in CKD, and associated with the presence of vascular calcification.

Acknowledgments
Not applicable.

Interest conflict
The authors declare that they have no conflict of interest.

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