Expression profiles of miR-129 and miR-29a-5p in vascular calcification of patients with end-stage renal disease and the underlying correlation

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ABSTRACT

To analyze the changes and correlation of Mir-129 and Mir-29A-5p in vascular calcification in end-stage renal disease. A total of 97 patients with end-stage renal disease admitted to our hospital from August 2021 to August 2020 were selected as the research objects, and another 97 healthy people who underwent physical examination in our hospital during the same period were selected as the control study. According to X-ray examination, 97 subjects were divided into the vascular calcification group (39 cases) and the non-vascular calcification group (58 cases). Blood samples were extracted from each group, and the expressions of serum Mir-129 and Mir-29A-5p were detected by RT-PCR after centrifugation. The expressions of Mir-129 and Mir-29A-5p in healthy people with end-stage renal disease and vascular calcification were analyzed. To analyze the correlation of Mir-129 and Mir-29A-5p in vascular calcification of end-stage renal disease and its correlation with vascular calcification of end-stage renal disease. Compared with healthy people, the expression of Mir-129 and Mir-29A-5p in patients with the end-stage renal disease was abnormally increased, and there was a difference between the two groups (P<0.05). The expression levels of Mir-129 and Mir-29A-5p in patients with end-stage renal disease without vascular calcification were lower than those in the vascular calcification group, and there were differences between the two groups (P<0.05). Compared with mild vascular calcification, mir-129 and Mir-29A-5p expressions were higher in moderate and severe vascular calcification patients. In addition, compared with moderate patients, the expressions of Mir-129 and Mir-29a-5p were lower in mild patients and higher in severe patients. The expressions of Mir-129 and Mir-29A-5p in patients with mild and moderate vascular calcification were lower than those in patients with severe vascular calcification, and there were differences among the three groups (P < 0.05). Mir-129 and Mir-29A-5p were positively correlated with vascular calcification in end-stage renal disease (R =5.426, P=0.001). Mir-129 was positively correlated with vascular calcification in end-stage renal disease (r=0.649, P=0.001). Mir-29a-5p was positively correlated with vascular calcification in end-stage renal disease (r=0.529, P=0.001). Mir-129 and Mir-29a-5p showed high expression in the patients with end-stage renal disease, and they also increased with the occurrence of vascular calcification, and they showed a positive correlation in the vascular calcification of end-stage renal disease.

Introduction

End-stage renal disease, as a kind of irreversible renal hypofunction, can cause a disorder in the metabolism of calcium and phosphorus, which could further induce cardiovascular calcification or even the death of patients (1). Vascular calcification has been taken as an independent risk factor of death caused by chronic renal function, and, thus, the early diagnosis of vascular calcification in end-stage renal disease is quite necessary (2). Micro RNAs, as a group of endogenous, non-coding, small, single chain RNA, could induce the degradation or block the translation of mRNA via incomplete binding to the untranslated regions (UTR) of mRNA, thereby modulating the progression of the disease (3). Existing clinical data have shown that miRNA is capable of inducing the development of vascular calcification by regulating the activity of vascular smooth muscle cells, and thus, it has been taken as a key marker in peripheral blood for the prediction of vascular calcification (4, 5). As such, in this study, patients with end-stage renal disease carrying vascular calcification were chosen, from which were analyzed the expression profiles of miR-129 and miR-29a-5p and the underlying correlation, aiming to provide the reference for the exploration of the markers for the vascular calcification in end-stage renal disease.

Materials and Methods

Subjects

A total of 97 patients with end-stage renal disease who were admitted to this hospital between August 2020 and August 2021 were enrolled in this study. Simultaneously, 97 healthy subjects undergoing physical examination

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in this hospital during the same period were taken as the control. Of the patients, there were 54 males and 43 females, ages ranging from 56 to 78 years old, with an average of (67.1±8.7) years old, and the BMI of patients ranged from 23 to 31 kg/m², with an average of (27.1±3.1) kg/m². For the healthy subjects, there were 52 males and 45 females, ages ranging from 55 to 79 years old, with an average of (67.3±9.0) years old, and the BMI of patients ranged from 22 to 30 kg/m², with an average of (26.8±2.8) kg/m². A comparison of the general data between the two groups showed no significant difference, suggesting that the baseline data of subjects in the two groups were comparable.

Criteria for inclusion: Patients conforming to the diagnosis criteria for end-stage renal disease (6); patients aged below 80 years old; patients with vascular calcification confirmed by the X-ray plain film and vessel images by spiral computed tomography (CT) for the radial artery; patients or whose family signed the written informed consents after they were informed of the content of this study.

Criteria for exclusion: Patients with the complications of systemic disease, including systemic vasculitis, anaphylactoid purpura, autoimmune diseases, or those in the active phase of infection, or those with nerve or language dysfunction, or those who failed to cooperate with the staff well.

Evaluation of vascular calcification

Philips X-ray DuraDiagnost was used to obtain the lateral film of the abdomen. Development of vascular calcification was marked for the films with the incrassate local arterial wall towards the lumen, with the incrassation over 50% when compared to the adjacent part, with uneven echo or evident incrassation. Subsequently, X-ray plain film was divided into 8 parts, and the part with no mark was scored as 0 points, while the part with a mark as 1 point, with a total score of 8 points. The degree of vascular calcification was divided as follows: mild vascular calcification for 0 to 4 points, moderate vascular calcification for 5 to 6 points and severe vascular calcification for 7 to 8 points.

Measurement of the expression of miR-129 and miR-29a-5p

For all subjects, 5 mL of fasting venous blood was taken and then subjected to centrifugation at 300 r/min for 15 min to obtain the supernatant. The supernatant was then transferred into the Eppendorf tubes and stored at -20°C for detection. The expression of miR-129 and miR-29a-5p in serum was measured through the real-time polymerase chain reaction (RT-PCR) as follows. In brief, samples were treated with TRIzol reagent at 37°C for 10 min, and after the samples were dissolved sufficiently, they were stirred with the 600 μL trichloromethane until the color of the solution turned milk white. The milk-white solution, after being placed at 4°C for 10 min, was subjected to centrifugation to obtain the supernatant, which was further centrifuged with isopropanol in a volumetric ratio of 1:1 for 15 min. Total RNA was then isolated by adding 1 mL 75% ethanol. Following the measurement of purity and content of total RNA, it was subjected to reverse transcription to obtain the cDNA. Primers were designed by Primer 5.0 software. The expression of target genes was quantified by the method of $2^{-\Delta\Delta Ct}$, with U6 as endogenous control. Conditions for reverse transcription were set as follows: 25°C for 10 min, 40°C for 60 min and 85°C for 5min. Conditions for amplification were set as follows: 94°C for 20 s, 72°C for 30 s and 60°C for 30 s, for 35 cycles. Primer sequence of U6: forward 5'- CTTAGTTGCATGCAG-3', reverse 5'- AATCGTGTATAAGTC-3'. Primer sequence of miR-129: forward 5'-ACCCAGGGGAAGACCCAAA-3', reverse 5'-CCTCTTGGCCGTTTTTCTCCA-3'. Primer sequence of miR-29a-5p: forward 5'-GGAGCGAGATCCCTCCAAAAT-3', reverse 5'-AGCGAGCATCCCCCAAAGTT-3'.

Statistical analysis

SPSS 20.0 software was utilized to perform the data analysis. Measurement data were expressed in the form of mean ± standard deviation (SD). Statistical significance of difference was ascertained by the *t*-test. Count data were expressed in the form of ratio or frequency. Statistical significance of the difference between the two groups was validated by the chi-square test or F value test. Correlations of miR-129 and miR-29a-5p with vascular calcification in end-stage renal disease were validated by Pearson's correlation analysis. $P < 0.05$ suggested that the difference had statistical significance.

Results

Expression of miR-129 and miR-29a-5p in serum of patients with end-stage renal disease and healthy subjects

As shown in Table 1, we found that in comparison with the healthy subjects, abnormal upregulation of miR-129 and miR-29a-5p was noted in the patients with end-stage renal disease ($P < 0.05$).

Expression of miR-129 and miR-29a-5p in subjects with vascular calcification or no vascular calcification

As shown in Table 2, the expression of miR-129 and miR-29a-5p in the non-vascular calcification subjects was much lower than those in the vascular calcification subjects ($P < 0.05$).

Table 1. Expression of miR-129 and miR-29a-5p in serum of patients with end-stage renal disease and healthy subjects ($\overline{x} \pm s$).

Group	Cases (n)	MiR-129	MiR-29a-5p
Healthy subjects	97	0.34±0.10	0.29±0.08
Patients with end-stage renal disease	97	1.17±0.35	3.06±0.21
t		22.460	121.400
P		0.001	0.001

Table 2. Expression of miR-129 and miR-29a-5p in vascular calcification and non-vascular calcification subjects ($\bar{x} \pm s$).

Group	Cases (n)	MiR-129	MiR-29a-5p
Non-vascular calcification group	58	1.19±0.37	3.10±0.25
Vascular calcification group	39	2.61±0.52	5.01±0.34
t		18.210	37.170
P		0.001	0.001

Table 3. Expression of miR-129 and miR-29a-5p in patients with vascular calcification at different degrees ($\bar{x} \pm s$).

Severity	Cases (n)	MiR-129	MiR-29a-5p
Mild	10	2.49±0.51	4.84±0.29
Moderate	16	2.68±0.56	5.03±0.46
Severe	3	3.02±0.61	5.61±0.52
F		6.915	15.421
P		0.001	0.001

Table 4. Correlations of miR-129 and miR-29a-5p expression with the vascular calcification of end-stage renal disease.

Index	Vascular calcification of end-stage renal disease	
	r	*P*
MiR-129	0.649	0.001
MiR-29a-5p	0.529	0.001

Expression of miR-129 and miR-29a-5p in patients with vascular calcification at different degrees

From Table 3, we noted that in comparison with mild vascular calcification, patients with moderate or severe vascular calcification presented the upregulation of miR-129 and miR-29a-5p, while compared to the patients with the moderate vascular calcification, those with mild vascular calcification had lower expression of miR-129 and miR-29a-5p, but those with severe vascular calcification had the quite the opposite changes; besides, when comparing to the severe vascular calcification, those with mild or moderate vascular calcification had lower expression of miR-129 and miR-29a-5p (all $P < 0.05$).

Correlation between the expression of miR-129 and miR-29a-5p in the vascular calcification of end-stage renal disease

As indicated in Figure 1, we found a positive correlation between the expression of miR-129 and miR-29a-5p in the vascular calcification of end-stage renal disease (r = 5.426, P = 0.001).

Correlations of miR-129 and miR-29a-5p expression with the vascular calcification of end-stage renal disease

From Table 4, we found that miR-129 was in a positive correlation with the vascular calcification of end-stage renal disease (r = 0.649, P = 0.001), and so was the miR-29a-5p (r = 0.529, P = 0.001).

Diagnostic value of miR-129 and miR-29a-5p for vascular calcification of end-stage renal disease

In Figure 2, miR-129 or miR-29a-5p alone performed poorly in the diagnosis of vascular calcification of end-stage renal disease, while the combination of them performed well.

Discussion
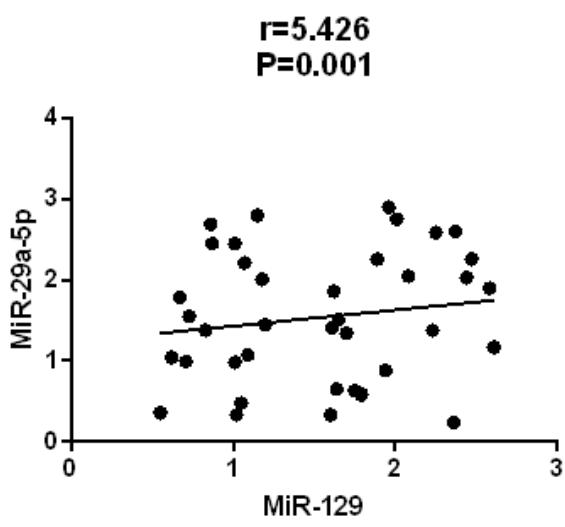
Vascular calcification, as one of the most frequent

Figure 1. Correlation between the expression of miR-129 and miR-29a-5p in the vascular calcification of end-stage renal disease.
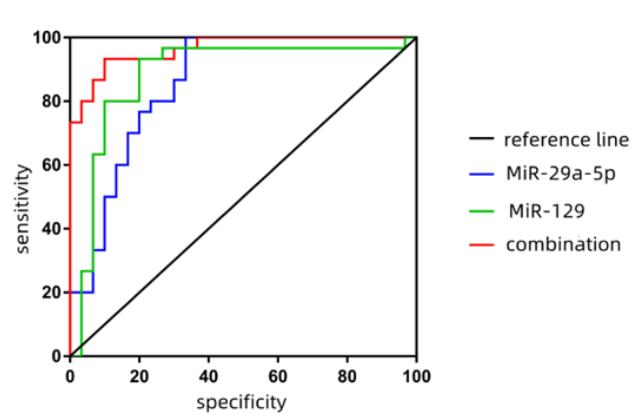

Figure 2. Diagnostic value of miR-129 and miR-29a-5p for vascular calcification of end-stage renal disease.

cardiovascular complications in renal diseases, has been regarded as the major cause responsible for the morbidity rate and mortality rate of these patients (7). The clinical survey has shown that vascular calcification is a quite complicated course that is modulated by multiple factors, similar to osteogenesis (8). The phenotypic transition from the contractile phenotype of vascular smooth muscle cells under the effect of pro-calcium factors, as the major pathological changes in vascular calcification, could trigger the abnormal deposition of minerals on the vascular wall by promoting the expression of downstream bone-related proteins (9, 10). Currently, CT, MRI or X-ray examination has been the major method for the detection of vascular calcification. However, these methods are still limited either by the high cost or the poor performance in the early diagnosis of vascular calcification (11, 12).

MiRNAs, as the clinically significant regulator for gene expression, are mainly found in biological fluids, like blood or urine (13). Existing data have shown that miRNAs could serve as biological markers for a variety of diseases, including renal diseases, for the advantages of stability and easiness in detection (14). A previous study on rats has shown that miR-302b could improve the metabolism of calcium and phosphorus in rats, thereby inhibiting the development and progression of vascular calcification. Recently, people have been focusing more on the role of miRNAs in the treatment of vascular calcification of renal diseases (15, 16). In addition, a great number of miRNAs could get involved in the modulation of vascular calcification by regulating the phenotypic transition of vascular smooth muscle cells (17, 18). *In vitro* experiments also showed that miR-129 and miR-29a-5p could also be involved in vascular calcification by targeting the genes (including Runx2) or multiple signal pathways (19). Nevertheless, there remains no study suggesting the expression patterns or the correlation of miR-129 and miR-29a-5p in vascular calcification of end-stage renal disease. In this study, we found that when compared to the healthy subjects, the serum of patients with end-stage renal disease presented the abnormal upregulation of miR-129 and miR-29a-5p, suggesting their abnormal expression of them in the end-stage renal stage and the underlying correlation.

Previous literatures have shown that miRNAs are capable of regulating the activity of vascular smooth muscle cells to modulate vascular calcification (20). In addition, analysis of the miR-129 and miR-29a-5p through the gene pool demonstrated the potential target correlation between them, which has also been proved by the *in vivo* and *in vitro* studies (21, 22). In this study, we found that miR-129 and miR-29a-5p may be the potential downstream target genes that are responsible for the regulation of vascular calcification. Besides, detections for the patients with vascular calcification in varying degrees also revealed that miR-129 and miR-29a-5p expression increased as the severity of vascular calcification deteriorated, suggesting the close association of miR-129 and miR-29a-5p with the vascular calcification. The part of correlation analysis on the miR-129 and miR-29a-5p also showed the positive correlation between them and the underlying positive correlation of them with the vascular calcification in end-stage renal disease, suggesting that they might be potentially correlated with the vascular calcification of end-stage renal diseases.

Although we have clarified the expression patterns of miR-129 and miR-29a-5p in the vascular calcification of end-stage renal disease and the underlying correlations, this study is also limited by the small size of the sample, which may result in the bias of the conclusion. As such, we will expand the sample size in future studies so as to solidate the conclusion of this study and provide the evidence for the clinical study.

In conclusion, miR-129 and miR-29a-5p are highly expressed in end-stage renal disease, and as the vascular calcification deteriorates, upregulation of miR-129 and miR-29a-5p is further enhanced, presenting the positive correlation in the vascular calcification of end-stage renal disease.

Acknowledgments

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

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