Relationship between serum miR-297a level and vascular calcification in maintenance hemodialysis patients

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Abstract: The study aimed to explore the relationship between serum miR-297a levels and vascular calcification in Maintenance Hemodialysis (MHD) patients. One hundred and forty stable MHD patients, treated for at least six months, were categorized into three groups based on vascular calcification severity: no calcification (37 cases), mild calcification (55 cases), and severe calcification (48 cases). The research involved analyzing various blood parameters like calcium, phosphorus, urea, creatinine, albumin, alkaline phosphatase, hemoglobin, and parathyroid hormone levels using biochemical analyzers and ELISA. The Agatston score was employed to assess calcification. MiR-297a levels were measured through miRNA quantitative PCR. The findings revealed a significant decrease in miR-297a levels in the mild and severe calcification groups compared to the non-calcification group. Additionally, logistic regression indicated that serum alkaline phosphatase and miR-297a are risk factors for vascular calcification in MHD patients. These results suggest a link between miR-297a level changes and vascular calcification, proposing miR-297a as a potential regulator of cardiovascular risk in MHD patients. This discovery could lead to new targeted strategies for cardiovascular healthcare in these patients.

1. Introduction

Maintenance Hemodialysis(MHD) is a key treatment, which is used to maintain the life of patients with advanced chronic kidney disease. However, after long-term MHD treatment, patients are often accompanied by the continuous progress of vascular calcification, which poses a serious threat to their quality of life and survival rate. Vascular calcification represents a prevalent complication in individuals with chronic kidney disease and those undergoing regular MHD treatment. This condition gives rise to an array of cardiovascular issues, such as hypertension, coronary heart disease, and stroke [1]. As a result, investigating strategies to mitigate or prevent vascular calcification in MHD patients holds paramount clinical importance.

In recent years, more and more studies show that MicroRNA (MiRNAs) plays an important role in regulating vascular calcification. MiRNAs are short non-coding RNA molecules, which can

affect cell functions and signal pathways by inhibiting the expression of specific genes. MiR-297a is a newly discovered miRNA, which shows potential biological significance in many diseases [2-3]. Especially in cardiovascular diseases, miR-297a has been found to be related to key processes such as inflammation, apoptosis and cell proliferation [4-5]. However, so far, the relationship between serum miR-297a level and vascular calcification in patients with maintenance MHD is still not well understood. There is evidence that [6-7], MHD treatment itself may affect the expression of miRNA, but the specific mechanism of miR-297a related to vascular calcification is still unclear.

The purpose of this study is to explore the relationship between the changes of serum miR-297a level and vascular calcification in maintenance MHD patients, and to try to reveal the mechanism of miR-297a in this process. Through in-depth study on the expression characteristics of miR-297a in MHD patients and its potential relationship with vascular calcification, we are expected to uncover the new mechanism of cardiovascular complications in maintenance MHD patients and provide scientific basis for developing more effective intervention measures in the future, so as to reduce the cardiovascular risk of maintenance MHD patients and improve their quality of life. This will have a positive impact on clinical practice and provide better treatment and care for patients with chronic kidney disease.

2. Materials and methods

2.1. Research objects

140 patients, 79 males and 61 females, aged (57.17 ± 12.68) years, who received MHD treatment for more than 6 months and were in stable condition in the nephrology department of the hospital were selected. The patients were tested by X-ray and color Doppler ultrasound. According to the degree of vascular calcification, the patients were divided into three groups: 37 patients with no calcification, 55 patients with mild calcification and 48 patients with severe calcification. The contents of this study all follow the requirements of the Medical Ethics Committee, and obtain the informed consent of the subjects, all of whom signed the informed consent form.

The subjects were all between 18 and 80 years old. All of them were outpatients or hospitalized patients with CRFMHD (dialysis time ≥3 months). Exclusion criteria: there were a large number of patients with blood loss in the past year; Have a history of serious infection, fracture and heart failure within 1 year; Patients with malignant tumor; In this study, corticosteroids were used within 3 months; There are primary parathyroid glands, bone metabolism and other metabolic diseases that may affect calcium and phosphorus; Severe hepatobiliary parenchymal lesions. This study was approved by the Ethics Committee of our hospital.

2.2. Research method

(1) Dialysis scheme

German Senyus F80 dialyzer was used, and standard bicarbonate hemodialysis was used. The concentration of Ca^{2+} was 1.25 mmol/L, the blood flow was 200 $\sim$ 350 mL/min, and the dialysate flow was 500 mL/min, 4 h/ time, 2 ~ 3 times a week. Each dialysis ultrafiltration can meet the clinical evaluation target dry mass standard.

(2) Biochemical index detection

Serum calcium (Ca^{2+}), phosphorus (P^{3-}), urea, creatinine (CRE), albumin (Alb) and alkaline phosphatase (ALP) were detected by automatic biochemical analyzer. The level of serum hemoglobin (HGB) was detected by blood analyzer. Serum parathyroid hormone (PTH) level was detected by ELISA.

(3) Vascular calcification score

A CT scanner was used to scan the patient's chest to obtain an image. Calcification score adopts Agatston score, which considers the area and density of calcification [8]. According to the calculated vascular calcification score and image analysis results, the vascular health of patients was evaluated. The results are reported as Agatston score or other standard score. According to the evaluation results, the patients were followed up regularly to monitor the change of calcification degree and the progress of the disease.

(4) Detection of serum miR-297a level

Collect serum samples from patients or subjects, and pack them into centrifuge tubes. Serum was separated by standard centrifugation, and then stored in a freezer at-80 C to avoid repeated freezing and thawing. Total RNA was extracted from serum, and after passing the test, the extracted miRNA was transcribed into cDNA by using the specific reverse transcription kit of miRNA. MiRNA-297a expression level was measured by mirna-specific primers and probes. Prepare PCR reaction solution, including miRNA cDNA, primer, probe and PCR Master Mix. PCR amplification reaction is carried out, including initial denaturation, cyclic amplification and fluorescence detection. The expression level of miR-297a was calculated by standard curve method or relative quantitative method. QPCR data were analyzed and the expression level of miR-297a was calculated.

2.3. Statistical method

We utilized SPSS 26.0 statistical software for our analysis. For normally distributed measurement data denoted as $\bar{x} \pm s$, group comparisons were conducted using one-way analysis of variance, while independent sample t-tests were employed for pairwise comparisons. Spearman correlation analysis was applied to assess the relationship between vascular calcification and clinical indicators, and Logistic regression was employed to identify risk factors associated with vascular calcification. Statistical significance was considered at $P < 0.05$.

3. Result

3.1. Comparison of serum miR-297a levels in MHD patients

The serum miR-297a levels were notably lower in both the severe and mild calcification groups compared to the non-calcification group ($P < 0.05$). Additionally, the serum miR-297a levels were significantly lower in the mild calcification group compared to the non-calcification group ($P < 0.05$). Please refer to Table 1 for more details.

Table 1: Comparison of serum miR-297a levels in MHD patients

group	n	miR-297a/U6
Non-calcified group	37	1.01±0.27
Mild calcification group	55	0.64±0.24*
Severe calcification group	48	0.39±0.11*△
t		89.47
P		0.000

Note: Compared with the group without calcification, * $P < 0.05$; Compared with mild calcification group, △$P < 0.05$

3.2. Logistic regression analysis of vascular calcification in MHD patients

The age, serum levels of Ca^{2+}, P^{3-},Alb, ALP and miR-297a were included in the Logistic regression analysis. The results showed that serum ALP and miR-297a were the risk factors for

vascular calcification in MHD patients (P < 0.05). See Table 2.

Table 2: Logistic regression analysis of vascular calcification in MHD patients

impact factor	β	SE	Wald	P	OR	CI 95%
age	0.507	0.344	1.625	0.143	1.557	0.634~3.087
Ca2 +	0.485	0.534	1.673	0.063	1.738	0.648~5.838
P3－	0.569	0.309	1.412	0.263	1.609	0.636~3.606
Alb	0.218	0.153	1.127	0.063	1.358	0.532~4.637
ALP	0.915	0.356	8.629	<0.001	2.058	1.634~7.357
miR-297a	1.489	0.561	4.731	0.023	3.041	1.631~5.89

4. Discussion

MHD patients refer to those patients with advanced chronic kidney disease who need regular hemodialysis treatment to maintain their lives. Chronic kidney disease is a progressive disease, which eventually leads to the loss of renal function and can not effectively eliminate metabolites and maintain fluid balance in the body. Therefore, MHD has become a necessary treatment to replace renal function, remove waste in the body and maintain electrolyte balance. MHD patients are usually at high risk of cardiovascular complications such as vascular calcification, which is due to metabolic disorder caused by uremia, chronic kidney disease and other factors. MiRNAs are a kind of small non-coding RNA, which has been widely studied and plays a key role in many physiological and pathological processes. MiR-297a is a newly identified miRNA, which is involved in some inflammatory and metabolic pathways, but its role in MHD patients is still unclear.

MHD usually needs to be carried out on a regular basis, usually three times a week or more frequently, and each dialysis process lasts for several hours to ensure effective removal of waste and water in the body. This is a complicated medical process, which requires professional medical teams, including nephrologists, dialysis nurses and clinical nutritionists, to monitor patients' health, manage the dialysis process, and adjust drugs and diet to ensure patients' quality of life and longevity.

MHD patients usually face a series of health problems, including cardiovascular complications, bone problems, anemia, electrolyte disorders, etc. Therefore, these patients need comprehensive medical management and monitoring. In addition, due to the lifestyle limitation of dialysis itself and the complexity of the treatment process, the quality of life of MHD patients may be affected to some extent, so psychological and social support is also important. Studying the relationship between the biomarkers of MHD patients, such as the level of miR-297a, and the health status of patients can help to better understand and manage the condition of these patients, and provide more accurate treatment and prevention strategies.

Serum miR-297a level refers to the concentration or content of miR-297a microRNA measured in the patient's serum (plasma). MiR-297a is a specific microRNA, which is a non-coding RNA, and its length is usually between 20 and 25 nucleotides. These micrornas play an important regulatory role in cells, which can affect gene expression by inhibiting or promoting gene transcription or translation. The change of serum miR-297a level may be related to different physiological and pathological processes, including the development and progress of diseases. In research, scientists usually use molecular biology techniques, such as quantitative PCR, to determine the concentration of miR-297a in serum. By comparing the levels of miR-297a in different patients or the same patient at different time points, researchers can explore the relationship between miR-297a and specific diseases or physiological processes. Relevant researchers are paying attention to the changes of serum miR-297a level in MHD patients and

trying to determine whether there is a correlation between these changes and vascular calcification [9-10]. This study is helpful to understand the potential role of miR-297a in cardiovascular health of MHD patients and whether it can be used as a biomarker to predict or monitor the risk of vascular calcification.

This study revealed a significant reduction in serum miR-297a levels in both the severe and mild calcification groups compared to the non-calcification group ($P < 0.05$). Furthermore, the serum miR-297a levels were significantly lower in the mild calcification group than in the non-calcification group ($P < 0.05$). Logistic regression analysis identified serum ALP and miR-297a as risk factors for vascular calcification in MHD patients ($P < 0.05$). These findings suggest a connection between decreased serum miR-297a levels in MHD patients and an increased risk of vascular calcification. This underscores the potential significance of miR-297a in modulating patients' cardiovascular risk. However, the biological mechanisms of miRNA are intricate, necessitating further research to elucidate the precise role of miR-297a in vascular calcification. Moreover, given the relatively small sample size in this study, larger-scale research is essential to validate these findings.

5. Conclusions

There is a correlation between the change of serum miR-297a level and vascular calcification in MHD patients, suggesting that miR-297a may play a role in regulating cardiovascular risk in these patients. This study provides a preliminary clue for further exploring the role of miR-297a and its potential application in patient management, but more research is needed to confirm and deepen understanding. This discovery is expected to provide a new targeted strategy for cardiovascular health care of MHD patients.

Acknowledgements

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