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The clinical role of miR-10b-5p in vascular calcification of diabetic nephropathy undergoing maintenance haemodialysis

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Abstract

Introduction and Objective. The aim of the study is to investigate the clinical role of miR-10b-5p in vascular calcification (VC) of diabetic nephropathy (DN) patients undergoing maintenance haemodialysis (MHD).

Materials and Method. In this study, 156 patients with DN undergoing MHD were enrolled into the study. Based on coronary angiography results, they were divided into the VC group (n = 93) and the non-VC group (n = 63). miR-10b-5p levels were obtained by RT-qPCR. The relationship between miR-10b-5p and PTH, CRP, and Ca-P product levels was analyzed by Pearson correlation analysis. Multivariate logistic regression was utilized to analyze the independent risk factors of VC in DN patients undergoing MHD. ROC curves were utilized to analyze the efficacy of serum miR-10b-5p combined with PTH, CRP, and Ca-P product levels in predicting VC in DN patients undergoing MHD.

Results. miR-10b-5p was decreased in DN patients undergoing MHD with VC, while PTH, CRP, and Ca-P product were significantly elevated. In addition, in VC patients, miR-10b-5p was negatively correlated with PTH, CRP, and Ca-P product. Multivariate regression analysis revealed that miR-10b-5p, PTH, CRP, and Ca-P product are independent risk factors of VC occurrence in DN patients undergoing MHD. The ROC curves showed that serum miR-10b-5p combined with PTH, CRP, and Ca-P product levels had high diagnostic value for VC occurrence in DN patients undergoing MHD.

Conclusions. A low level of miR-10b-5p may be a potential risk factor for VC occurrence in DN patients undergoing MHD. The combination of miR-10b-5p with PTH, CRP, and Ca-P product shows potential as clinical diagnostic biomarkers for the occurrence of VC in DN patients undergoing MHD.

Key words

diabetic nephropathy, maintenance haemodialysis, vascular calcification, miR-10b-5p

INTRODUCTION

Diabetic nephropathy (DN), a major diabetes complication, manifests as gradual kidney function deterioration and sustained proteinuria in affected individuals [1]. Currently, maintenance haemodialysis (MHD) serves as a key therapeutic approach for end-stage DN patients [2]. This method is effective in removing toxic substances accumulated in the body to improve prognosis, but the incidence of vascular calcification (VC) remains high in such patients [3]. The risk of death from adverse cardiovascular events caused by VC remains high [4]. The pathophysiological mechanisms of VC are complex, and traditional views focus on the synergistic effects of calcium and phosphorus metabolism disorders and chronic inflammation, such as elevated parathyroid hormone (PTH), excessive calcium-phosphate product (Ca-P product), and raised C-reactive protein (CRP) levels [5]. However, in recent years, microRNAs (miRNAs) have been confirmed as a possible key factor in regulating VC.

Recently, with the in-depth study of emerging molecular markers related to clinical medicine, it has been demonstrated that miRNAs regulate both physiological and pathological processes [6]. In disease states, the imbalance of miRNA expression can lead to changes of disease-related molecular mechanisms, and the change of its level is often a hallmark of disease. For example, miR-34a has been shown to be a key molecular target in age-related vascular diseases, such as atherosclerosis and vascular calcification, by regulating vascular cell senescence, inflammatory responses, and metabolic disorders [7]. Current research indicates that under hypoxic conditions, miR-10b-5p alleviates myocardial cell apoptosis by targeting and inhibiting PTEN expression [8]. In addition, diabetes-related research has identified that the absence of miR-10b-5p leads to diabetes and intestinal motility disorders by disrupting intestinal barrier function [9]. miR-10b-5p is dramatically down-regulated in interstitial cells of Cajal (ICCs) of diabetic mice, and its absence leads to degeneration of ICCs and β cells, thereby triggering diabetes [10]. A bioinformatics study identified miR-10b-5p down-regulated in a uraemic VC model [11]. However, the clinical function of miR-10b-5p and its interactions with PTH, CRP, and Ca-P product in VC in DN patients undergoing MHD has not yet been elucidated.

This study is the first to investigate the clinical role of miR-10b-5p in VC in DN patients undergoing MHD, analyze the correlation of miR-10b-5p with PTH, CRP, and Ca-P product, and verify the diagnostic efficacy of combined detection. The research results provide theoretical guidance for the diagnosis and treatment of VC in DN patients undergoing MHD.

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MATERIALS AND METHOD

Research subjects. The study collected clinical data from 156 patients with DN undergoing MHD who were treated at Beijing Yangfangdian Hospital starting in 2023. The inclusion criteria for the patients were as follows: the primary disease was diagnosed as DN; regular haemodialysis duration of ≥ 3 months; age was > 18 years; all patients underwent coronary angiography to assess the degree of coronary artery calcification, and complete data. The following were excluded: patients undergoing haemodialysis combined with peritoneal dialysis, those transitioning from haemodialysis to peritoneal dialysis; transitioning from peritoneal dialysis to haemodialysis; congenital valve malformations or who have undergone artificial valve replacement; who have received a kidney transplant; those with rheumatic heart valve disease, infective endocarditis, severe liver disease, severe heart failure, autoimmune diseases, or acute infections; those with thyroid dysfunction or malignant tumours.

VC was assessed using a clinically validated visual semiquantitative scoring method. Two cardiovascular radiologists who were unaware of the clinical information on the patients reviewed the images independently. The severity of VC was divided into four grades according to angiographic imaging: grade 0 (no calcification); Grade 1 (mild) – calcification < 10 mm in length and/or radian < 90°; Grade 2 (moderate) – calcification 10–20 mm in length and/or 90°-180° in radian; and Grade 3 (severe) – calcification > 20 mm in length and/or radian > 180°. The two reviewers demonstrated good agreement, with cases of disagreement were resolved by arbitration from a third senior expert to determine the final grade. The study defined the presence of VC as the observation of any detectable calcification (≥ Grade 1) on angiographic imaging. Based on the results of coronary angiography, patients were divided into VC (n=93) and non-VC group (n=63).

Clinical data and serum PTH, CRP, and Ca-P product detection. All clinical data of patients were collected through case retrieval, including gender, age, body mass index (BMI), history of smoking, drinking, and hypertension, blood lipids (low-density lipoprotein cholesterol, LDL-C; high-density lipoprotein cholesterol, HDL-C; triglyceride, TG; total cholesterol, TC), albumin, haemoglobin, and dialysis duration.

Fasting venous blood (5 ml) was collected from all patients. The blood was centrifuged at 3,000 g for 10 min at 4 °C, and the supernatant was stored at –80 °C. Serum PTH levels were detected by an automatic microparticle chemiluminescence immunoanalyzer (Beckman Coulter, DxI 800 Access, USA). Serum CRP, blood calcium, and blood phosphorus levels were detected by an automatic biochemical analyzer (Beckman Coulter, AU5800, USA), and the Ca-P product was calculated.

RNA extraction. The 1mL of TRIzol reagent (Thermo Fisher, USA) was added to tested serum and mixed thoroughly. Chloroform was then added, vortexed for 15 s, and placed for 3 min. Subsequently, it was placed at 4 °C and centrifuged at 12,000 g for 15 min to obtain upper aqueous phase. Isopropanol was added, left at -20 °C for 30 min, and precipitate was obtained after centrifugation. They were washed twice with 75% alcohol, dried and dissolved in DEPC water. RNA concentration and purity were determined using a NanoDrop 2000 (Thermo Fisher, USA). The RNA purity test results for A260/A280 were 1.8–2.0. RNA was stored at -80 °C for subsequent experiments.

RT-qPCR detection. One μg of total RNA was used for reverse transcription using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher, USA). The reaction system was prepared on ice, and the reaction was performed according to the instructions. The reaction system was configured according to the instructions of SuperReal PreMix Plus (TIANGEN, China), and the qPCR amplification reaction was performed. Reactions were performed on a QuantStudio 5 real-time PCR system (Thermo Fisher, USA). The primer sequences used to detect miR-10b-5p and the reference gene U6 were as follows: miR-10b-5p forward, 5'-GGGUACCCUGUAGAACCGAA-3' and reverse, 5'-AGTGCAGGGTCCGAGGTATT-3'; U6 forward, 5'-CTCGCTTCGGCAGCACA-3' and reverse, 5'-AACGCTTCACGAATTTGCGT-3'. The reaction procedure was 95 ° for 15s, 60 ° for 30s, 72 ° for 20s, totalling 40 cycles. The relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis. Statistical analysis and graphing were conducted using SPSS 21.0 and GraphPad 9.0. Count data were expressed as n (%) and compared by chi-square test. Measurement data were expressed as mean ± SD and compared by t-test. Logistic regression was used to analyze the independent risk factors of VC in DN patients undergoing MHD. Pearson correlation was utilized to analyze association between miR-10b-5p and PTH, CRP, and Ca-P product in the serum of VC patients. A prediction model containing miR-10b-5p, PTH, CRP, and Ca-P product was constructed by logistic regression, and ROC curves were drawn using the predicted probabilities generated by this model. These curves were utilized to analyze efficacy of serum miR-10b-5p combined with PTH, CRP, and Ca-P product levels in predicting VC in DN patients undergoing MHD. $P < 0.05$ indicated a statistically significant difference.

RESULTS

Comparison of clinical data between the two groups. Clinical data for VC and non-VC groups are shown in Table 1. The VC group consisted of 93 patients – 57.0% male and 43.0% female. The non-VC group consisted of 63 patients – 60.3% male and 39.7% female. The average age patients in the VC group – 58.27 ± 11.42 years, while that of patients in non-VC group – 53.81 ± 8.72 years. Statistics showed that there was a difference in age ($P = 0.010$) between two groups, but no significant difference in gender ($P = 0.681$). In addition, there were significant differences in smoking history ($P = 0.034$), hypertension history ($P = 0.003$), ALB ($P < 0.001$), HGB ($P = 0.011$), and dialysis duration ($P < 0.001$), while the differences in BMI ($P = 0.774$), drinking history ($P = 0.348$), LDL-C ($P = 0.223$), HDL-C ($P = 0.130$), TG ($P = 0.114$), and TC ($P = 0.195$) were not apparent.

Comparison of serum PTH, CRP, Ca-P product, and miR-10b-5p levels between the two groups. To compare the levels of biomarkers associated with VC,, we measured PTH, CRP, and Ca-P product in VC and non-VC groups. The results indicated that serum PTH, CRP, and Ca-P product were

Table 1. Comparison of clinical data between the two groups

	Non-VC n=63	VC n=93	P value
Gender			
Male	38 (60.3%)	53 (57.0%)	0.681
Female	25 (39.7%)	40 (43.0%)	
Age (years)	53.81±8.72	58.27±11.42	**0.010**
BMI (kg/m^2)	21.85±3.76	22.03±3.97	0.774
Smoking	16 (25.4%)	39 (42.0%)	**0.034**
Drinking	23 (36.5%)	41 (44.1%)	0.348
Hypertension	37 (58.7%)	75 (80.1%)	**0.003**
Blood lipids			
LDL-C (mmol/L)	2.39±0.46	2.49±0.50	0.223
HDL-C (mmol/L)	1.08±0.24	1.02±0.23	0.130
TG (mmol/L)	1.72±0.25	1.79±0.27	0.114
TC (mmol/L)	3.72±0.93	3.96±1.20	0.195
ALB (g/L)	35.01±5.04	32.04±5.11	**<0.001**
HGB (g/L)	105.79±11.83	100.48±13.23	**0.011**
Dialysis duration (years)	5.43±2.75	7.33±1.91	**<0.001**

BMI – Body mass index; LDL-C – Low-density lipoprotein cholesterol; HDL-C – High-density lipoprotein cholesterol; TG – Triglyceride; TC – Total cholesterol; ALB – Albumin; HGB – Haemoglobin

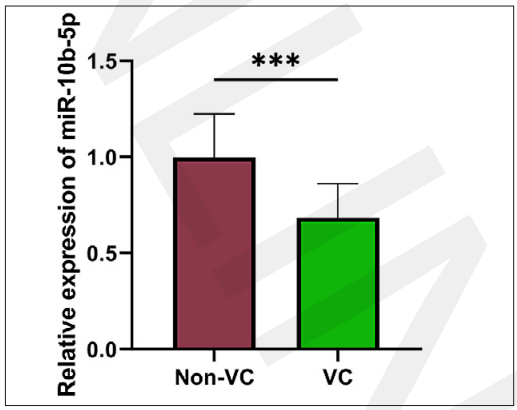

Figure 1. miR-10b-5p levels were significantly reduced in the VC group.
***$P < 0.001$

remarkably higher in the VC group than in the non-VC group ($P < 0.001$) (Tab. 2). Furthermore, miR-10b-5p expression in serum was detected, and it showed that miR-10b-5p levels were dramatically reduced in the VC group ($P < 0.001$) (Fig. 1).

Table 2. Comparison of serum PTH, CRP, and Ca-P product levels between the two groups

	Non-VC n=63	VC n=93	P value
PTH (pg/mL)	214.35±44.28	274.34±63.45	<0.001
CRP (mg/L)	25.23±4.37	31.07±7.78	<0.001
Ca-P product (mg^2/dL^2)	57.48±14.77	75.20±17.34	<0.001

PTH – Parathyroid hormone; CRP – C-reactive protein; Ca-P product – Calcium-phosphorus product.

Correlation analysis between serum miR-10b-5p and PTH, CRP, and Ca-P product in VC of DN patients undergoing MHD. To verify the relationship between serum miR-10b-5p and PTH, CRP, and Ca-P product in the VC process, we conducted a Pearson correlation analysis of the indicators in VC patients. The results are shown in Figure 2 A-C. In VC patients, miR-10b-5p was negatively correlated with PTH (r=-0.638; $P < 0.001$), CRP (r=-0.672; $P < 0.001$), and Ca-P product (r=-0.763; $P < 0.001$).

Multivariate logistic regression analysis of VC occurrence in DN patients undergoing MHD. To analyze the risk factors for VC in DN patients undergoing MHD, a multivariate logistic analysis was carried out. The outcomes results revealed that dialysis duration (OR = 4.481, 95%CI = 1.387–14.475; $P = 0.012$), PTH (OR = 4.783, 95%CI = 1.495–15.298; $P = 0.008$), CRP (OR = 5.193, 95%CI = 1.597–16.884; $P = 0.006$), Ca-P product (OR = 5.745, 95%CI = 1.849–17.847; $P = 0.003$), and miR-10b-5p (OR = 0.097, 95%CI = 0.032–0.300; $P < 0.001$) are independent risk factors for the occurrence of VC (Tab. 3).

Table 3. Multivariate logistic regression analysis of the occurrence of VC in DN patients undergoing MHD.

	OR	95% CI	*P* value
Age	1.967	0.644–6.003	0.235
Smoking	1.979	0.611–6.406	0.255
Hypertension	2.650	0.691–10.159	0.155
ALB	0.380	0.125–1.155	0.088
HGB	0.396	0.133–1.177	0.095
Dialysis duration	4.481	1.387–14.475	**0.012**
PTH	4.783	1.495–15.298	**0.008**
CRP	5.193	1.597–16.884	**0.006**
Ca-P product	5.745	1.849–17.847	**0.003**
miR-10b-5p	0.097	0.032–0.300	**<0.001**

OR – Odds ratio; 95% CI – 95% Confidence interval; ALB – Albumin; HGB – Haemoglobin; PTH – Parathyroid hormone; CRP – C-reactive protein; Ca-P product – Calcium-phosphorus product

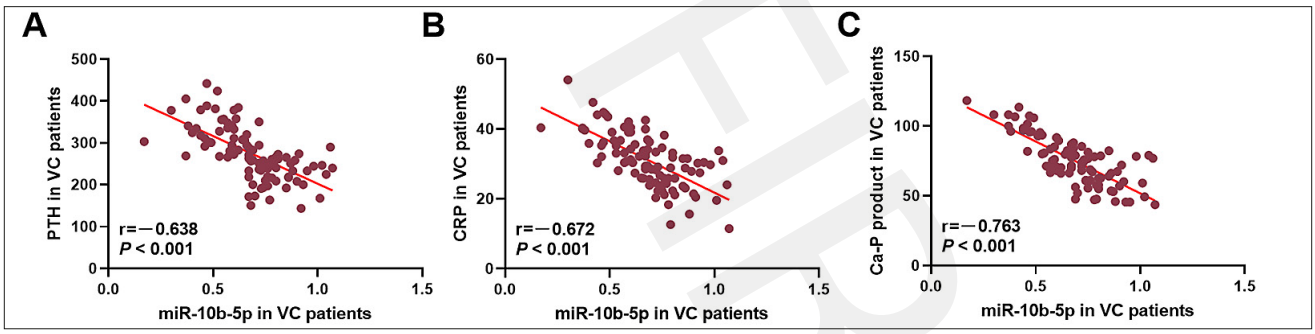

Figure 2. Correlation between miR-10b-5p and PTH, CRP, and Ca-P product in VC patients. miR-10b-5p was negatively correlated with (A) PTH, (B) CRP, and (C) Ca-P product in VC patients

Table 4. ROC curve analysis of miR-10b-5p, PTH, CRP, Ca-P product and their combination for the diagnostic ability in VC in DN patients undergoing MHD

	AUC	95% CI	Sensitivity	Specificity	*P* value
miR-10b-5p	0.859	0.799–0.919	80.65%	77.78%	<0.001
PTH	0.777	0.705–0.850	63.44%	84.13%	<0.001
CRP	0.742	0.666–0.819	64.52%	79.37%	<0.001
Ca-P product	0.783	0.710–0.856	72.04%	77.78%	<0.001
Combination	0.961	0.933–0.989	93.55%	88.89%	<0.001

PTH – Parathyroid hormone; CRP – C-reactive protein; Ca-P product – Calcium-phosphorus product

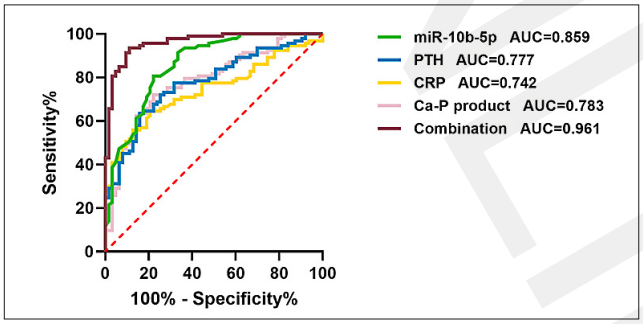

Figure 3. miR-10b-5p combined with PTH, CRP, and Ca-P product showed high diagnostic value in predicting VC in DN patients undergoing MHD

Efficacy analysis of serum miR-10b-5p combined with PTH, CRP, and Ca-P product in predicting VC in DN patients undergoing MHD. To demonstrate the clinical role of miR-10b-5p in the development of VC in DN patients undergoing MHD, ROC curves analysis was conducted. The results are presented in Figure 3 and Table 4. In DN patients undergoing MHD, the AUC of miR-10b-5p for diagnosing VC was 0.859 (95%CI = 0.799–0.919), with a sensitivity of 80.65% and specificity of 77.78% (P <0.001). Moreover, the AUC of PTH, CRP and Ca-P product in the diagnosis of VC was 0.777, 0.742 and 0.783, respectively (P <0.001). However, miR-10b-5p combined with PTH, CRP, and Ca-P product showed the most significant diagnostic ability, with an AUC of 0.961 (95%CI = 0.933–0.989), sensitivity of 93.55%, and specificity of 88.89% (P <0.001).

DISCUSSION

Patients with DN undergoing MHD have a general risk of VC. At present, VC is mainly detected through imaging techniques, including CT, X-ray, and coronary angiography [12]. However, these detection methods are not only costly but may also cause further damage to kidney function in patients [13]. To a certain extent, the detection of biomarkers can reflect the changes of a patient's condition, and is convenient for review examinations as appropriate [14]. Therefore, it has been a primary aim of clinical research to explore find more specific and sensitive biomarkers for assessing the severity of VC in DN patients undergoing MHD.

The miRNA-targeted therapy is a research hotspot and one of the most promising directions of research. miRNAs have become key biomarkers for various diseases, including diabetes. Serum miR-23a-3p expression in patients with type 2 diabetic nephropathy is significantly decreased and related to the severity, which may be used as a potential marker for early diagnosis and progression of the disease [15].

As a key regulator of vascular diseases, miR-125b participates in the process of various vascular diseases by affecting the function of endothelial and vascular smooth muscle cells, and has both a pathogenic role and diagnostic biomarker potential [16]. Studies have shown that miR-10b-5p plays a role in cardiovascular disease. Down-regulation of miR-10b-5p combined with miR-193a-5p and miR-1-3p can specifically identify patients with chronic heart failure with multiple hormone deficiency syndrome [17]. Moreover, Chao et al. revealed that down-regulation of miR-10b-5p may be associated with VC in uraemia [11]. The current study confirms that miR-10b-5p was markedly down-regulated in VC in DN patients undergoing MHD, which is similar to previous studies on its expression in cardiovascular diseases. These results suggest that miR-10b-5p may be involved in VC occurrence in DN patients undergoing MHD.

Although the specific mechanisms are not explored in the presented study, they may reasonably speculated based on existing knowledge in the field. The core mechanism of VC involves the phenotypic transformation of vascular smooth muscle cells into osteoblasts. It is hypothesized that down-regulation of miR-10b-5p in DN microenvironment may participate in this process by releasing suppression of pro-calcification target genes. For example, it may affect genes or signalling pathways related to osteogenic differentiation and calcium and phosphorus metabolism, thereby regulating the process of VC. In addition, its significant negative correlation with CRP, PTH and Ca-P product also suggests that miR-10b-5p may be at the common regulatory node connecting metabolic disorders, inflammation and VC. However, subsequent studies are needed to test this hypothesis.

PTH testing is used to assess the functional status of the parathyroid glands in patients. Elevated PTH levels indicate secondary hyperparathyroidism, which leads to increased bone resorption, enhanced calcium and phosphorus release, and exacerbated VC [18]. By monitoring PTH levels, the severity of calcium and phosphorus metabolism disorders can be identified, thereby detecting renal osteodystrophy and cardiovascular complications [19]. CRP can be used to quantify the levels of systemic inflammation. As a sensitive inflammatory marker, CRP is helpful in assessing the inflammation-related risk of VC [20]. In patients with MHD, CRP is positively correlated with the severity of VC [21]. In addition, evaluation of Ca-P metabolic balance and measurement of Ca-P product levels are independent predictors of VC. A high Ca-P product is a direct indicator of reduced vascular compliance and arterial stiffness, increasing the risk of cardiovascular death. In MHD and VC clinical studies, PTH, CRP, and Ca-P product testing are commonly involved. In the current study, PTH, CRP, and Ca-P product levels were dramatically elevated in VC patients, and showed a significant negative correlation with miR-10b-5p. These results indicate that miR-10b-5p, similar to PTH, CRP, and Ca-P product, has predictive ability for VC occurrence in DN patients undergoing MHD.

CONCLUSIONS

Combined diagnosis greatly improves the diagnostic efficiency of diseases by integrating multi-dimensional information of biomarkers. For example, epigenetic research

has shown that miR-126 combined with existing clinical indicators improves the early prediction and precision intervention of cardiovascular risk in diabetic patients [22]. In deep venous thrombosis research, miR-125a-5p and miR-223-3p combined with D-dimer, significantly improve the diagnostic efficacy of this disease [23]. Research has revealed that a combination of miR-4505, miR-4743-5p, and miR-4750-3p exhibits high diagnostic efficacy for type 2 diabetes mellitus complicated by coronary artery disease, and may serve as novel biomarkers [24]. The current study shows that the combination of miR-10b-5p, PTH, CRP, and Ca-P product had high diagnostic value for VC occurrence in DN patients undergoing MHD. These findings suggest that miR-10b-5p, PTH, CRP, and Ca-P product may serve as clinical biomarkers for diagnosing whether DN patients undergoing MHD have developed VC.

Limitations of the study. The study has several limitations. Although this single-centre cross-sectional study revealed an association between biomarkers and VC, but it cannot infer causality. The sample source was single and the size was limited, which may affect the generalization of the conclusions. The assessment of VC primarily relied on coronary angiography, which failed to comprehensively reflect the systemic calcification burden. In addition, the constructed diagnostic model has not been externally validated. In the future, multi-centre and prospective cohort studies are needed to verify the predictive value and causality of these markers. It is necessary to test the generalizability of the conclusions in a broader population, and to use more systematic imaging evaluation methods. Furthermore, in the future, external validation of diagnostic models in independent cohorts will be required to advance their clinical translation.

In conclusion, decreased serum miR-10b-5p level and increased PTH, CRP, and Ca-P product levels are independent risk factors for VC in DN patients undergoing MHD. Their combined detection is highly effective in predicting VC in DN patients undergoing MHD. This study provides a theoretical basis for the early screening of VC in DN patients undergoing MHD, and offers multidimensional intervention targets for targeted therapy.

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