Diabetes, Metabolic Syndrome and Obesity

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ORIGINAL RESEARCH

Role of Decreased Expression of miR-155 and miR-146a in Peripheral Blood of Type 2 Diabetes Mellitus Patients with Diabetic Peripheral Neuropathy

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Objective: To Study the Correlations of microRNA-155 (miR-155) and microRNA-146a (miR-146a) Expression in Peripheral Blood of Type 2 Diabetes Mellitus (T2DM) Patients with Diabetic Peripheral Neuropathy (DPN), and Explore the Clinical Value of miR-155 and miR-146a in the Diagnosis and Treatment Outcomes of DPN.

Methods: The study included 51 T2DM patients without DPN (T2DM group), 49 T2DM patients with DPN (DPN group), and 50 normal controls (NC group). Quantitative real-time PCR was utilized to determine the expression levels of miR-155 and miR-146a. Clinical features and risk factors for DPN were assessed. Multivariate stepwise logistic regression analysis was conducted to confirm whether the expressions of miR-155 and miR-146a could independently predict the risk of DPN. ROC curve analysis evaluated their diagnostic value.

Results: The T2DM group exhibited significantly lower expression levels of miR-155 and miR-146a compared to the NC group ($P < 0.05$). Moreover, the DPN group exhibited a significantly decreased expression level of miR-155 and miR-146a compared to the T2DM group ($P < 0.01$). Multivariate logistic regression analysis indicated that higher levels of miR-155 and miR-146a might serve as protective factors against DPN development. ROC curve analysis revealed that miR-155 (sensitivity 91.8%, specificity 37.3%, AUC 0.641,) and miR-146a (sensitivity 57.1%, specificity 84.3%, AUC 0.722) possess a strong ability to discriminate between T2DM and DPN. Their combined use further enhanced the diagnostic potential of DPN (sensitivity 83.7%, specificity 60.8%, AUC 0.775). A multi-index combination can improve DPN diagnostic efficiency.

Conclusion: The decreased expression of miR-155 and miR-146a in the peripheral blood of T2DM patients is closely related to the occurrence of DPN, highlighting their potential as valuable biomarkers for diagnosing and prognosticating DPN.

Keywords: miR-155, miR-146a, diabetic peripheral neuropathy, type 2 diabetes mellitus, biomarkers

Introduction

In 2021, the global diabetic population reached 521 million, with type 2 diabetes mellitus (T2DM) accounting for 96.0% of cases. Remarkably, approximately one-quarter of these cases were from China, ranking it as the country with the highest number of patients with T2DM worldwide.[1,2] Diabetic Peripheral Neuropathy (DPN), a common microvascular complication of T2DM, affects approximately 50% of patients with T2DM, leading to chronic pain, foot ulcers, amputation, depression and sleep disorders, significantly reducing the quality of life.[3,4] Although the pathogenesis of DPN involves multiple risk factors and cascades of pathophysiological changes, including reduced expression of cell Na^+-K^+-ATPase, endoplasmic reticulum stress, mitochondrial dysfunction, DNA damage, enhanced inflammatory

Received: 28 March 2024
Accepted: 16 July 2024
Published: 23 July 2024

signalling and increased levels of inflammatory cytokines, it remains incompletely understood.[5] Therefore, exploring potential risk factors for DPN in T2DM is warranted and may aid in the development of preventative or ameliorative strategies.

MicroRNA (miRNA), endogenous non-coding small RNAs, play a crucial role in regulating gene expression by inhibiting the translation of target mRNAs or directly degrading them. Increasing evidence suggests their involvement in diabetes and its complications, particularly diabetic neuropathies.[6]

miR-155 regulates over 241 genes, playing a role in various physiological and pathological processes, including haematopoietic lineage differentiation, inflammatory responses, immunity, cancer, cardiovascular diseases and notably diabetes.[7–9]

In T2DM, reduced levels of miR-155 across various mediums, including plasma, peripheral blood cells, platelets and urine, have been consistently observed across different ethnicities.[8] This reduction may lead to the continuous over-activation of the Renin-Angiotensin-Aldosterone System (RAAS), resulting in harmful effects such as pro-oxidation, fibrosis, proliferation and inflammation.[10,11] However, miR-155 can inhibit key factors like Basic Leucine Zipper Transcription Factor 1 (BACH1) and Suppressor of Cytokine Signalling 1 (SOCS1), thereby synergistically enhancing cellular protective, antioxidative, anti-apoptotic and anti-inflammatory pathways. Additionally, it promotes the formation of a protective cellular environment, which is compromised when miR-155 is downregulated.[7,12] Concurrently, decreased levels of miR-155 have been observed in the leukocytes, sural nerves and the skin of the lower legs of patients with DPN, particularly those with painful neuropathy.[13] Furthermore, microRNA-155 mimics have been demonstrated to improve nerve conduction velocity in DPN mice and inhibit pro-inflammatory genes induced by high glucose levels.[14] These findings suggest the involvement of miR-155 in the development and progression of DPN.

miR-146a, pivotal in inhibiting inflammation escalation and maintaining immune homeostasis,[15] also shows reduced levels in patients with T2DM across different regions.[16–18] Notably, acute strength training has been shown to ameliorate this decrease, exhibiting a negative correlation with insulin resistance. This correlation could be attributed to its role in suppressing inflammatory responses.[19] Studies on DPN in mice have demonstrated that elevated blood glucose levels lead to decreased miR-146a expression, resulting in increased levels of TRAF6 and IRAK1 in dorsal root ganglion (DRG) neurons. miR-146a mimetics have been found to significantly inhibit neuronal death induced by high blood glucose levels[20] and exhibit protective effects against diabetic neuropathy and DPN. Thus, the therapeutic effects of miR-146a mimetics are speculated to stem from their ability to inhibit pro-inflammatory genes induced by high glucose levels.[21,22] Furthermore, in patients with DPN, particularly those with painful neuropathies, miR-146a levels are significantly diminished in leukocytes, the sciatic nerve and the skin of the lower leg, underscoring its critical role in DPN pathogenesis.[13]

However, while previous studies on the relationship between miR-155, miR-146a and DPN have primarily been conducted in animal models, clinical research on the correlation between peripheral blood miR-155 and miR-146a expression and DPN remains limited. Therefore, our study aims to investigate the changes in the expression levels of miR-155 and miR-146a in the peripheral blood of patients with DPN and their potential as useful biomarkers for the diagnosis and treatment of DPN.

Materials and Methods

Study Participants

Clinical data were collected from 100 hospitalised patients with T2DM at the Endocrinology Department of the First Affiliated Hospital of Anhui Medical University between September 2020 and May 2021. Patients were categorised into two groups based on the presence of DPN: 51 patients without DPN comprised the T2DM group (n = 51) and 49 patients with DPN formed the DPN group (n = 49). Within the DPN group, patients were further divided into subgroups: those experiencing symptoms such as numbness, burning or stabbing pain in the extremities were classified into the painful neuropathy subgroup (n = 16), while those without such symptoms were categorised into the non-painful neuropathy subgroup (n = 33). Additionally, 50 healthy individuals were selected as the Normal Control (NC) group (n = 50). The diagnosis of T2DM followed the standards set by the American Diabetes Association.[23] DPN diagnosis followed the

guidelines recommended by the American Diabetes Association and the Toronto Diabetic Neuropathy Consensus, considering the presence of clinical symptoms or signs and abnormalities in nerve conduction studies (NCS).[24,25] Exclusion criteria included patients with type 1 diabetes; gestational diabetes; other forms of diabetes; neuropathies from other conditions, such as cervical and lumbar spine diseases, Guillain-Barré syndrome, epilepsy and severe vascular diseases; severe comorbidities like progressive malignant tumours, acute infections, severe renal impairment, or heart failure; and medications affecting platelets (eg, aspirin) or cardiovascular or haematological diseases that could affect platelet-related indices. This study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Anhui Medical University (Ethics batch number: CDEC000004982), and informed consent was obtained from all participants before commencement.

Detection of Clinical Indicators

Trained staff collected clinical data, including age, sex, body mass index (BMI), systolic/diastolic blood pressure (SBP/DBP), diabetes duration, hypertension status, current smoking status, statin use and antidiabetic treatments, from all participants. Participants fasted for at least 10 hours before blood samples were collected from the elbow or forearm veins the next morning. Venous blood was drawn to measure Fasting plasma glucose (FPG), Fasting C-peptide (FCP), renal function, liver function, blood lipid composition, glycosylated haemoglobin A1c (HbA1c) and other indicators. Nerve conduction studies, including motor nerve conduction velocity (MCV) and sensory nerve conduction velocity (SCV) of the peroneal nerve, were performed using an electromyography-evoked potential system.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

qRT-PCR was used to detect miRNA expression in blood samples from all participants. Total RNA was extracted using TRIzol reagent (Thermo Fisher, Shanghai, China), and cDNA was synthesised using the PrimeScript RT kit (TaKaRa, Beijing, China). Micro RNA was analysed using SYBR™ Green PCR Master Mix (Servicebio, Wuhan, China). The relative levels were calculated using the $2^{-\Delta\Delta Ct}$ method, with β-actin as the internal reference. Primers were used as follows:

miR-155: forward primer, 5'-CAAGGTGGTTAATGCTAATCGTGATA-3' and reverse primer, 5'-GTGCAGGGTCCGAGGT-3';

miR-146a: forward primer, 5'-TAATCGTGTGAGAACTGAATTCCA-3' and reverse primer, 5'-TATGGTTTTGACGACTGTGTGAT-3';

U6: forward primer, 5'-GCT TCG GCA CAT ATA CTA AAA-3' and reverse primer, 5'-CGC TTC ACG AAT TTG CCT GTCAT-3'.

Statistical Analysis

Data were analysed using SPSS software version 26.0. For qRT-PCR results, the standard curve method was employed for quantification. Quantitative data conforming to normal distribution were expressed as mean ± standard deviation (±s), and comparisons among multiple groups were performed using analysis of variance (ANOVA). Non-normally distributed quantitative data were represented by the median [M (P25, P75)], with comparisons between two groups assessed using the Wilcoxon rank-sum test. Moreover, comparisons among three groups were performed using the Kruskal–Wallis *H*-test. Categorical data were presented as frequencies or percentages (%) and compared using the Chi-square test. Spearman correlation analysis was used to assess the relationship between miR-155 and miR-146a expression levels and clinical data. Logistic regression analysis identified risk factors for the DPN group. Furthermore, diagnostic sensitivity and specificity of plasma miR-155 and miR-146a for DPN were evaluated using the area under the ROC curve (AUC). A P value < 0.05 was considered statistically significant.

Results

Clinical Parameter Comparison Among the Three Groups

There were no statistically significant differences in gender, age, smoking history, alcohol history, family history of diabetes, BMI, TBIL, AST, ALT, TC, TG, LDL-C, HDL-C, VLDL and FCP between the NC, T2DM and DPN groups (P

> 0.05) (Table 1). However, patients in the T2DM group exhibited significantly higher levels of FPG and HbA1c compared to the NC group. Additionally, both miR-155 and miR-146a expression levels in peripheral blood were significantly decreased (P < 0.05). Patients in the DPN group exhibited higher levels of FPG, HbA1c, BUN and Scr, along with a significant reduction in the expression levels of miR-155 and miR-146a (P < 0.05) (Table 1). Compared to the T2DM group, patients in the DPN group had a significantly longer duration of diabetes (P=0.002), higher levels of FPG, HbA1c, BUN and Scr, and lower SCV and MCV (P < 0.05). Moreover, the expression levels of miR-155 and miR-146a in peripheral blood were significantly decreased in the DPN group compared to the T2DM group (P < 0.05) (Table 1).

Comparison of Clinical Parameters Among DPN Subgroups

The analysis revealed a significantly lower TBIL level in the painful neuropathy subgroup compared to the non-painful neuropathy subgroup (10.32±4.10 vs 13.74±4.55, P = 0.014). However, there were no significant differences between the two subgroups in terms of AST, ALT, TC, TG, LDL-C, HDL-C, VLDL, FCP, FPG, HbA1c, BUN, Scr, SCV, MCV, miR-155 and miR-146a (P > 0.05) (Table 2). Both the painful and non-painful neuropathy subgroups exhibited significantly reduced expression levels of miR-155 and miR-146a compared to the NC group (P<0.001). Compared to the T2DM group, the non-painful neuropathy subgroup exhibited a significant reduction in miR-146a expression levels (P < 0.001), whereas the painful

Table 1 Comparisons of Clinical Parameters Among the NC, T2DM and DPN Groups [n(%)/$\left(\bar{x}\pm s\right)$/M (P25, P75)]

Variables	DPN Group (n=49)	T2DM Group (n=51)	NC Group (n=50)	P value
Sex (male/female)	26/23	28/23	26/24	0.957
Age (year)	56.18±9.96	53.8±10.90	52.36±8.85	0.159
Smoking history (%)	12 (24.5)	11 (21.6)	13 (26.0)	0.869
Drinking history (%)	13 (26.5)	15 (29.4)	14 (28.0)	0.950
Family history of diabetes (%)	15 (30.6)	16 (31.4)	11 (22.0)	0.510
BMI (kg/m^2)	24.14±3.13	24.65±3.16	23.47±2.47	0.130
Duration of diabetes (year)	10 (7, 15.5)	7 (3, 10)	–	0.002*
TBIL (umol/L)	12.83±4.53	14.13±4.35	14.68±3.94	0.091
AST (U/L)	14.5 (12.0, 18.0)	15.0 (12.0, 21.0)	16.35 (14.47, 19.42)	0.091
ALT (U/L)	17.0 (12.0, 23.5)	20.0 (15.0, 31.0)	16.9 (10.13, 29.7)	0.091
BUN (mmol/L)	6.41 (4.96, 8.55) ■ ▲	5.12 (4.06, 6.30)	4.85 (4.30, 5.80)	<0.001*
Scr (umol/L)	66.0 (55.05, 83.0) ■ ▲	56.0 (47.3, 63.0)	57.65(47.28, 67.03)	0.001*
FPG (mmol/L)	10.5 (9.1, 12.64) ■ ▲	9.34 (6.8, 11.52) ▲	5.21 (4.89, 5.36)	<0.001*
HbA1c (%)	10.0 (8.73, 11.85) ■ ▲	9.1 (7.9, 10.0) ▲	4.9 (4.6, 5.2)	<0.001*
TC (mmol/L)	4.41±0.78	4.37±0.94	4.47±0.79	0.831
TG (mmol/L)	1.36 (1.13, 1.97)	1.38 (0.96)	1.22 (1.00, 1.69)	0.214
VLDL (mmol/L)	0.50 (0.35, 0.67)	0.47 (0.32, 0.63)	0.38 (0.26, 0.71)	0.428
LDL-C (mmol/L)	2.74 (2.09, 3.15)	2.74 (2.05, 3.25)	-	0.754
HDL-C (mmol/L)	1.12 (0.84, 1.305)	1.02 (0.89, 1.17)	-	0.336
FCP (ng/mL)	0.96 (0.525, 1.655)	1.01 (0.66, 1.33)	-	0.934
SCV (m/s)	35.1 (33.25, 44.15) ■	46.6 (42.4, 50.0)	-	<0.001*
MCV (m/s)	40.3 (36.65, 44.8) ■	45.5 (40.8, 49.0)	-	<0.001*
MiR-155	3.58 (3.41, 3.78) ■ ▲	3.77 (3.52, 3.89) ▲	3.86 (3.68, 4.04)	<0.001*
MiR-146a	3.01 (2.6, 3.73) ■ ▲	3.70 (3.26, 4.01) ▲	4.21 (3.82, 4.63)	<0.001*

Note: Data are presented as mean ± standard deviation or numbers (%) or median with IQR; differences among the three groups were analysed using one-way analysis of variance or x^2 test, and least-significant difference (LSD) analysis was used for comparison between the two groups. *P < 0.05; ■Compared to the T2DM group, P < 0.05; ▲Compared to the control group, P < 0.05.

Abbreviations: T2DM, Type 2 Diabetes Mellitus; DPN, Diabetic Peripheral Neuropathy; BMI, Body Mass Index; TBIL, Total Bilirubin; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; BUN, Blood Urea Nitrogen; Scr, Serum Creatinine; FPG, Fasting Plasma Glucose; HbA1c, Glycated Haemoglobin; TC, Total Cholesterol; TG, Triglycerides; VLDL, Very Low-Density Lipoprotein Cholesterol; LDL-C, Low-Density Lipoprotein Cholesterol; HDL-C, High-Density Lipoprotein Cholesterol; FCP, Fasting C-peptide; SCV, Sensory Nerve Conduction Velocity; MCV, Motor Nerve Conduction Velocity; miR-155, microRNA-155; miR-146a, microRNA-146a.

Table 2 Comparison of Clinical Examinations, Biochemical Indicators and miRNA Among the Subgroups of DPN $\left(\bar{x} \pm s\right)$)/M(P25, P75)]

Variables	PDPN Subgroup (n=16)	Non-PDPN Subgroup (n=33)	NC Group (n=50)	T2DM Group (n=51)	P value
Age (year)	55.13±10.08	56.70±10.02	26/24	53.8±10.90	0.610
Duration of diabetes (year)	9.0 (6.25, 20.0)	10.0 (7.5, 15.0)	-	7 (3, 10)	0.949
BMI (kg/m^2)	23.88±2.93	24.27±3.26	23.47±2.47	24.65±3.16	0.692
TBIL (umol/L)	10.32±4.10	13.74±4.55	14.68±3.94	14.13±4.35	0.014*
AST (U/L)	14.00 (12.00, 16.65)	15.00 (11.50, 18.50)	16.35 (14.47, 19.42)	15.0 (12.0, 21.0)	0.353
ALT (U/L)	13.00 (11.30, 20.25)	18.00 (13.50, 24.50)	16.9 (10.13, 29.7)	20.0 (15.0, 31.0)	0.156
BUN (mmol/L)	6.25 (5.10, 9.05)	6.57 (4.94, 8.20)	4.85 (4.30, 5.80)	5.12 (4.06, 6.30)	0.693
Scr (umol/L)	62.65 (48.50, 74.25)	68.60 (59.40, 91.85)	57.65(47.28, 67.03)	56.0 (47.3, 63.0)	0.141
FPG (mmol/L)	9.20 (8.74, 12.63)	11.20 (9.53, 12.75)	5.21 (4.89, 5.36)	9.34 (6.8, 11.52)	0.082
HbA1c (%)	9.75 (8.70, 11.93)	10.00 (8.90, 11.80)	4.9 (4.6, 5.2)	9.1 (7.9, 10.0)	0.685
TC (mmol/L)	4.42±0.75	4.40±0.81	4.47±0.79	4.37±0.94	0.921
TG (mmol/L)	1.26 (0.96, 1.90)	1.43 (1.20, 2.06)	1.22 (1.00, 1.69)	1.38 (0.96)	0.197
VLDL (mmol/L)	0.45±0.21	0.56±0.22	0.38 (0.26, 0.71)	0.47 (0.32, 0.63)	0.125
LDL-C (mmol/L)	2.47 (1.94, 3.19)	2.85 (2.25, 3.13)	-	2.74 (2.05, 3.25)	0.502
HDL-C (mmol/L)	1.25 (0.77, 1.48)	1.08 (0.88, 1.27)	-	1.02 (0.89, 1.17)	0.430
FCP (ng/mL)	1.16 (0.48, 1.68)	0.94 (0.61, 1.65)	-	1.01 (0.66, 1.33)	0.848
SCV (m/s)	34.40 (32.25, 41.23)	38.70 (34.00, 44.30)	-	46.6 (42.4, 50.0)	0.236
MCV (m/s)	39.79±4.52	40.85±4.63	-	45.5 (40.8, 49.0)	0.451
MiR-155	3.49 (3.15, 3.68) ▲	3.61 (3.50, 3.82) ▲	3.86 (3.68, 4.04)	3.77 (3.52, 3.89)	<0.001
MiR-146a	3.49 (2.77, 3.90) ▲	2.88 (2.50, 3.35) ■▲	4.21 (3.82, 4.63)	3.70 (3.26, 4.01)	<0.001

Notes: Within the DPN group, patients were further divided into subgroups: those experiencing symptoms such as numbness, burning or stabbing pain in the extremities were classified into the painful neuropathy subgroup (n = 16), while those without such symptoms were categorised into the non-painful neuropathy subgroup (n = 33). Data are presented as mean ± standard deviation or numbers (%) or median with IQR; differences among the three groups were analysed using one-way analysis of variance or x^2 test, and least-significant difference (LSD) analysis was used for comparison between the two groups. *P < 0.05; ■Compared to the T2DM group, P < 0.05; ▲Compared to the control group, P < 0.05.

Abbreviations: T2DM, Type 2 Diabetes Mellitus; PDPN subgroup, painful neuropathy subgroup; Non-PDPN subgroup, non-painful neuropathy subgroup; BMI, Body Mass Index; TBIL, Total Bilirubin; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; BUN, Blood Urea Nitrogen; Scr, Serum Creatinine; FPG, Fasting Plasma Glucose; HbA1c, Glycated Haemoglobin; TC, Total Cholesterol; TG, Triglycerides; VLDL, Very Low-Density Lipoprotein Cholesterol; LDL-C, Low-Density Lipoprotein Cholesterol; HDL-C, High-Density Lipoprotein Cholesterol; FCP, Fasting C-peptide; SCV, Sensory Nerve Conduction Velocity; MCV, Motor Nerve Conduction Velocity; miR-155, microRNA-155; miR-146a, microRNA-146a.

neuropathy subgroup did not demonstrate a significant difference. Furthermore, no significant differences were observed in miR-155 expression levels between the painful and non-painful neuropathy subgroups (Table 2).

Correlation Analysis Between Peripheral Blood miR-155 and miR-146a and Clinical Indicators in Different Groups

Spearman correlation analysis revealed significant associations. In the NC group, miR-155 showed a negative correlation with Scr (rs = −0.305, P = 0.031), and miR-146a was negatively correlated with alanine aminotransferase (ALT) (rs = −0.303, P = 0.033). In the T2DM group, miR-146a was negatively correlated with smoking history (rs = −0.288, P = 0.040), while in the DPN group, miR-155 was positively correlated with FPG (rs = 0.362, P = 0.011). Additionally, miR-146a displayed a negative correlation with ALT (rs = −0.402, P = 0.004) and a positive correlation with AST (rs = 0.362, P = 0.346) (Table 3).

Correlation Analysis Between Peripheral Blood miR-155, miR-146a and Clinical Indicators in Painful Neuropathy Subgroups and Non-Painful Neuropathy Subgroups

Spearman correlation analysis in DPN subgroups revealed significant correlations. In the painful neuropathy subgroup, miR-155 displayed a significant negative correlation with BMI (rs = −0.749, P = 0.001). In the non-painful neuropathy subgroup, miR-155 was positively correlated with FPG (rs = 0.444, P = 0.010), while miR-146a exhibited a positive correlation with FPG (rs = 0.376, P = 0.031) and negative correlations with BMI (rs = −0.368, P = 0.035), aspartate aminotransferase (AST) (rs = −0.470, P = 0.006) and ALT (rs = −0.437, P = 0.011) (Table 4).

Table 3 Correlation Analysis of Peripheral Blood miR-155, miR-146a and Clinical Indexes in the DPN, T2DM and NC Groups

Variables	DPN Group				T2DM Group				NC Group			
	miR-155		miR-146a		miR-155		miR-146a		miR-155		miR-146a	
	r_s	*P*	r_s	*P*	r_s	*P*	r_s	*P*	r_s	*P*	r_s	*P*
Sex(male/female)	−0.176	0.225	−0.036	0.805	−0.181	0.204	−0.037	0.794	0.051	0.723	0.050	0.731
Smoking history (%)	−0.185	0.204	0.084	0.567	0.136	0.341	−0.288	0.040*	0.267	0.061	−0.092	0.526
Drinking history (%)	−0.165	0.257	−0.034	0.815	−0.021	0.884	0.164	0.250	0.114	0.432	0.117	0.419
BMI (kg/m^2)	−0.271	0.059	−0.212	0.144	0.063	0.660	0.085	0.552	0.155	0.283	−0.096	0.509
Age (year)	−0.012	0.933	0.112	0.442	−0.176	0.217	0.055	0.701	−0.117	0.417	0.227	0.113
Duration of diabetes (year)	0.140	0.336	−0.023	0.875	−0.175	0.219	0.278	0.049*	-	-	-	-
TBIL (umol/L)	−0.050	0.733	0.048	0.744	0.028	0.848	−0.070	0.627	0.157	0.277	0.149	0.303
AST (U/L)	0.154	0.290	−0.346	0.015*	0.136	0.343	−0.156	0.273	0.167	0.246	−0.050	0.730
ALT (U/L)	0.068	0.643	−0.402	0.004*	0.203	0.152	−0.168	0.238	−0.222	0.122	−0.303	0.033*
BUN (mmol/L)	−0.224	0.121	0.068	0.643	0.053	0.712	−0.242	0.087	−0.123	0.395	0.137	0.344
Scr (umol/L)	−0.034	0.814	−0.123	0.398	0.176	0.217	0.037	0.796	−0.305	0.031*	0.044	0.763
FPG (mmol/L)	0.362	0.011*	0.109	0.455	−0.007	0.960	0.016	0.913	0.019	0.896	−0.168	0.243
HbA1c (%)	0.087	0.554	−0.114	0.435	−0.076	0.595	−0.213	0.134	0.115	0.426	−0.041	0.775
TC (mmol/L)	0.239	0.098	0.098	0.501	−0.003	0.983	0.116	0.417	−0.014	0.923	−0.142	0.324
TG (mmol/L)	0.221	0.127	0.066	0.653	0.127	0.375	−0.090	0.531	0.038	0.792	−0.187	0.193
VLDL (mmol/L)	0.145	0.319	−0.034	0.818	−0.054	0.709	−0.085	0.555	−0.274	0.055	−0.239	0.095
FCP (ng/mL)	−0.058	0.691	−0.066	0.654	0.169	0.236	−0.078	0.588	-	-	-	-
SCV (m/s)	0.025	0.864	0.017	0.906	−0.120	0.403	0.133	0.350	-	-	-	-
MCV (m/s)	0.234	0.106	−0.090	0.537	−0.009	0.950	0.151	0.289	-	-	-	-
miR-155	-	-			-	-	0.005	0.975	-	-	−0.019	0.896
miR-146a	−0.185	0.204	-	-	0.005	0.975	-	-	−0.019	0.896	-	-

Note: *$P < 0.05$.

Abbreviations:T2DM, Type 2 Diabetes Mellitus; DPN, Diabetic Peripheral Neuropathy; BMI, Body Mass Index, TBIL, Total Bilirubin; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; BUN, Blood Urea Nitrogen; Scr, Serum Creatinine; FPG, Fasting Plasma Glucose; HbA_{1c}, Glycated Haemoglobin; TC, Total Cholesterol; TG, Triglycerides; VLDL, Very Low-Density Lipoprotein Cholesterol; LDL-C, Low-Density Lipoprotein Cholesterol; HDL-C, High-Density Lipoprotein Cholesterol; FCP, Fasting C-peptide; SCV, Sensory Nerve Conduction Velocity; MCV, Motor Nerve Conduction Velocity; miR-155, microRNA-155; miR-146a, microRNA-146a.

Table 4 Correlation Analysis Between Peripheral Blood miR-155, miR-146a and Clinical Indexes in DPN Subgroups

	PDPN Subgroup				Non-PDPN Subgroup			
	miR-155		miR-146a		miR-155		miR-146a	
	r_s	*P*	r_s	*P*	r_s	*P*	r_s	*P*
Sex (male/female)	−0.161	0.552	−0.190	0.481	−0.066	0.714	−0.155	0.388
Smoking history (%)	−0.084	0.757	0.196	0.467	−0.111	0.537	−0.044	0.808
Drinking history (%)	−0.052	0.848	−0.052	0.848	−0.194	0.279	−0.062	0.730
BMI (kg/m^2)	−0.749	0.001*	0.254	0.343	−0.151	0.402	−0.368	0.035*
Age (year)	0.199	0.460	0.399	0.126	0.019	0.917	0.134	0.457
Duration of diabetes (year)	0.206	0.445	−0.155	0.566	0.147	0.413	−0.027	0.883
TBIL (umol/L)	−0.013	0.961	0.106	0.696	−0.227	0.204	0.214	0.232
AST (U/L)	0.239	0.373	0.103	0.704	0.061	0.737	−0.470	0.006*
ALT (U/L)	−0.173	0.521	0.004	0.987	0.020	0.913	−0.437	0.011*
BUN (mmol/L)	−0.174	0.520	0.341	0.196	−0.243	0.173	−0.099	0.585
Scr (umol/L)	−0.303	0.254	0.000	1.000	−0.068	0.705	−0.164	0.360
FPG (mmol/L)	−0.015	0.957	0.015	0.957	0.444	0.010*	0.376	0.031*
HbA1c (%)	0.027	0.922	−0.169	0.530	0.102	0.571	−0.038	0.832
TC (mmol/L)	0.241	0.368	0.059	0.829	0.293	0.098	0.079	0.661
TG (mmol/L)	−0.126	0.641	0.221	0.412	0.277	0.119	0.164	0.361
VLDL (mmol/L)	−0.179	0.506	0.197	0.464	0.147	0.415	0.048	0.790
FCP (ng/mL)	−0.182	0.499	0.269	0.313	−0.005	0.979	−0.189	0.292

(*Continued*)

Table 4 (Continued).

	PDPN Subgroup				Non-PDPN Subgroup			
	miR-155		miR-146a		miR-155		miR-146a	
	r_s	*P*	r_s	*P*	r_s	*P*	r_s	*P*
SCV (m/s)	−0.093	0.733	0.155	0.568	0.043	0.811	−0.022	0.903
MCV (m/s)	0.363	0.168	−0.208	0.440	0.154	0.393	−0.049	0.787
miR-155	-	-	−0.318	0.231	-	-	0.101	0.577
miR-146a	−0.318	0.231	-	-	0.101	0.577	-	-

Note: *$P < 0.05$.

Abbreviations: T2DM, Type 2 Diabetes Mellitus; PDPN subgroup, painful neuropathy subgroup; Non-PDPN subgroup, non-painful neuropathy subgroup; BMI, Body Mass Index; TBIL, Total Bilirubin; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; BUN, Blood Urea Nitrogen; Scr, Serum Creatinine; FPG, Fasting Plasma Glucose; HbA_{1c}, Glycated Haemoglobin; TC, Total Cholesterol; TG, Triglycerides; VLDL, Very Low-Density Lipoprotein Cholesterol; LDL-C, Low-Density Lipoprotein Cholesterol; HDL-C, High-Density Lipoprotein Cholesterol; FCP, Fasting C-peptide; SCV, Sensory Nerve Conduction Velocity; MCV, Motor Nerve Conduction Velocity; miR-155, microRNA-155; miR-146a, microRNA-146a.

Logistic Regression Analysis of Factors Affecting Different Clinical Outcomes

Logistic regression analysis for DPN risk factors: Using DPN as the dependent variable and disease duration, age, BMI, ALT, AST, FPG, HbA1c, BUN, Scr, miR-155, and miR-146a as independent variables, the analysis identified increased disease duration, elevated levels of FPG and Scr as independent risk factors for DPN, while higher levels of miR-155 and miR-146a appeared to be potential protective factors against the onset of DPN ($P < 0.05$) (Table 5).

Table 5 Logistic Regression Analysis of Risk Factors of Clinical Outcome

Clinical outcome	Independent variable	β	OR	95% CI	*P*
DPN	Duration of diabetes (year)	0.147	1.158	1.040~1.291	0.008*
	Age (year)	−0.021	0.979	0.923~1.040	0.495
	BMI	0.013	1.013	0.815~1.261	0.905
	ALT	−0.036	0.964	0.884~1.052	0.412
	AST	0.004	1.004	0.829~1.216	0.964
	FPG	0.317	1.374	1.059~1.782	0.017*
	HbA1c	0.240	1.271	0.878~1.840	0.204
	BUN	0.197	1.217	0.826~1.794	0.320
	Scr	0.047	1.048	1.006~1.091	0.023*
	miR-155	−1.976	0.139	0.032~0.592	0.008*
	miR-146a	−1.316	0.268	0.133~0.539	<0.001*
PDPN	Duration of diabetes (year)	0.101	1.106	0.967~1.265	0.140
	Age (year)	−0.056	0.946	0.865~1.035	0.225
	BMI	−0.083	0.921	0.716~1.184	0.519
	ALT	−0.005	0.995	0.899~1.102	0.928
	AST	−0.137	0.872	0.673~1.130	0.301
	FPG	−0.333	0.717	0.500~1.029	0.071
	HbA1c	0.279	1.332	0.857~2.038	0.206
	BUN	0.443	1.557	1.003~2.419	0.049*
	Scr	−0.025	0.975	0.948~1.003	0.077
	miR-155	−2.117	0.120	0.012~1.241	0.075
	miR-146a	0.530	1.699	0.677~4.264	0.259

Notes: *$P < 0.05$. Multivariate unconditional logistic regression analysis adjusted for the course of diabetes, age, BMI, ALT, AST, FPG, HbA1c, BUN, Scr, miR-155, and miR-146a showed that increased diabetes duration, higher FPG, and elevated Scr levels were independent risk factors for DPN, while higher levels of miR-155 and miR-146a were potential protective factors against DPN. Multivariate unconditional logistic regression analysis adjusted for the same factors revealed that higher BUN levels were an independent risk factor for the occurrence of PDPN, whereas miR-155 and miR-146a, among other variables, did not show significant statistical relevance.

Abbreviations: DPN, Diabetic Peripheral Neuropathy; PDPN, painful neuropathy; BMI, Body Mass Index; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; FPG, Fasting Plasma Glucose; HbA1c, Glycated Haemoglobin; BUN, Blood Urea Nitrogen; Scr, Serum Creatinine; miR-155, microRNA-155; miR-146a, microRNA-146a.

Table 6 Predictive Value of ROC Curve of miR-155, miR-146a, Course of the Disease and All the Indexes in the Peripheral Blood

Index	AUC	95% CI	Optimal Critical Value	Sensitivity (%)	Specificity (%)	*P* value
miR-155	0.641	0.532~0.750	3.835	0.918	0.373	0.015*
miR-146a	0.722	0.621~0.824	3.185	0.571	0.843	<0.001*
Duration of diabetes (year)	0.681	0.578~0.785	6.5	0.796	0.49	0.002*
miR-155 and miR-146a	0.775	0.685~0.866	-	0.837	0.608	<0.001*
miR-155 and disease duration	0.720	0.621~0.819	-	0.918	0.431	<0.001*
miR-146a and disease duration	0.788	0.695~0.881	-	0.694	0.863	<0.001*
miR-155, miR-146a and disease duration	0.818	0.733~0.903	-	0.796	0.784	<0.001*

Note: *$P < 0.05$.

However, in the subgroups of DPN, with the occurrence of painful neuropathy as the dependent variable and the same set of variables as independent factors, only an increase in BUN levels was identified as an independent risk factor for developing painful neuropathy. Other variables including miR-155 and miR-146a did not show a significant statistical relevance ($P > 0.05$) (Table 5).

The Diagnostic Value of miR155 and miR-146a in Peripheral Blood for DPN in T2DM

ROC curve analysis for DPN diagnosis using miR-155 and miR-146a: The ROC curve analysis was employed to evaluate the sensitivity and specificity of miR-155 and miR-146a in peripheral blood for diagnosing DPN. The analysis indicated that low expression levels of miR-155 had an AUC of 0.641 (95% Confidence Interval (CI): 0.532–0.750, sensitivity: 91.8%, specificity: 37.3%), while low levels of miR-146a displayed an AUC of 0.722 (sensitivity, 57.1%; specificity, 84.3%). Furthermore, the combined diagnostic value of both miR-155 and miR-146a yielded a higher AUC of 0.775, sensitivity of 83.7% and specificity of 60.8%. The AUC for the duration of the disease as a diagnostic factor for DPN was 0.681, with a sensitivity of 79.6% and specificity of 49%. The AUC for combinations of miR-155 with disease duration, miR-146a with disease duration, and all three combined were 0.720, 0.788, and 0.818, respectively. These findings suggest that a combination of multiple indicators, such as miR-155, miR-146a, and disease duration, provides a superior diagnostic value for DPN compared to individual factors ($P < 0.05$) (Table 6, Figures 1 and 2).

Discussion

The findings of our study corroborate previous research, demonstrating significantly lower expression levels of miR-155 and miR-146a in the peripheral blood of patients with T2DM compared to individuals with normal glucose tolerance. This observation is consistent across various regions and ethnic groups.[8,16–18] Furthermore, the decline in miR-155 and miR-146a expression is more pronounced in patients with DPN than those with T2DM alone. Advanced multivariate logistic regression analysis indicates that elevated levels of miR-155 and miR-146a may act as protective factors against the development of DPN. ROC curve analysis further highlights the discriminative ability of miR-155 and miR-146a in distinguishing between T2DM and DPN, with their combined diagnostic value for DPN being particularly enhanced when considering the duration of diabetes. Therefore, the decrease in miR-155 and miR-146a not only serves as a significant risk factor for the onset of DPN but also emerges as potential biomarkers for the assessment, treatment and prognosis of DPN. To the best of our knowledge, this is the first study to explore the relationship between changes in peripheral blood miR-155 and miR-146a expression and the incidence of DPN in patients with T2DM.

Our study also reveals a negative correlation between miR-146a levels and smoking history in patients with T2DM. Additionally, increased disease duration, FPG and Scr levels emerge as independent risk factors for DPN, while elevated BUN levels serve as independent risk factors for the development of painful neuropathy. These findings are consistent with Papanas N et al's study,[26] further reinforcing our research conclusions. Notably, our study highlights the independent association of Scr levels with an increased risk of DPN, whereas higher BUN levels were specifically associated with an elevated risk of developing painful neuropathic symptoms. This underscores the detrimental impact of prolonged hyperglycemia not only on DPN but also on diabetic nephropathy, another significant microvascular complication. Furthermore, our conclusions are supported by a multicenter cross-sectional study from Beijing,[27] wherein chronic

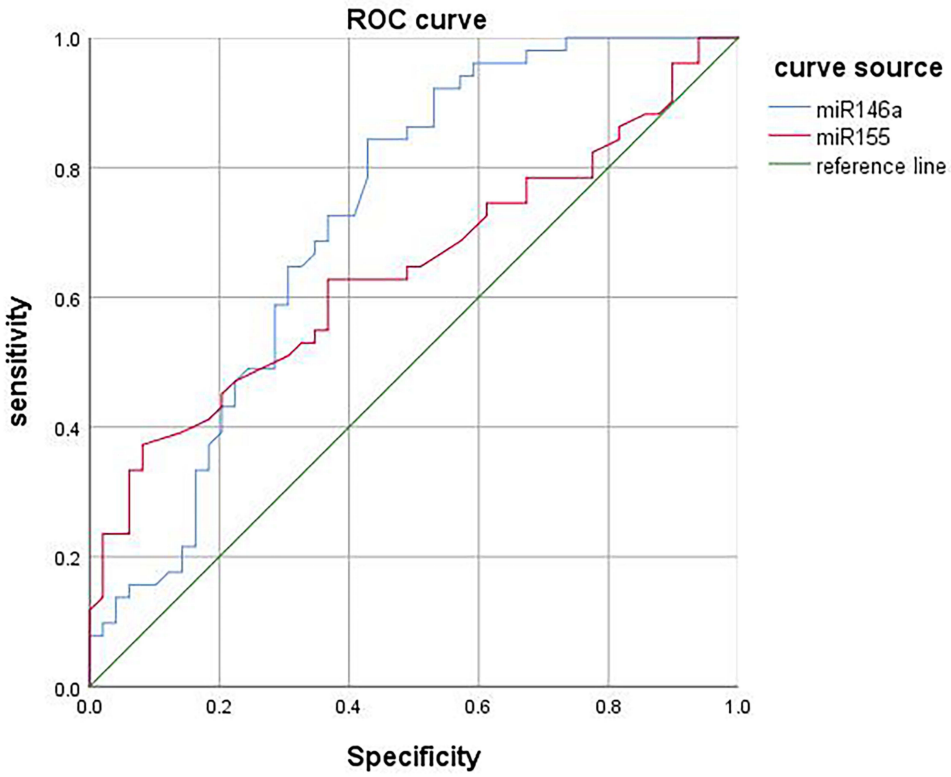

Figure 1 The biomarker potential of circulating miR-155 and miR-146a for distinguishing between DPN and T2DM was assessed through ROC curve analysis. Individually, circulating miR-155 showed a sensitivity of 91.8%, specificity of 37.3%, and AUC of 0.641(95% CI 0.532–0.750, P<0.05), while miR-146a had a sensitivity of 57.1%, specificity of 84.3%, and AUC of 0.722(95% CI 0.621–0.824, P<0.001).

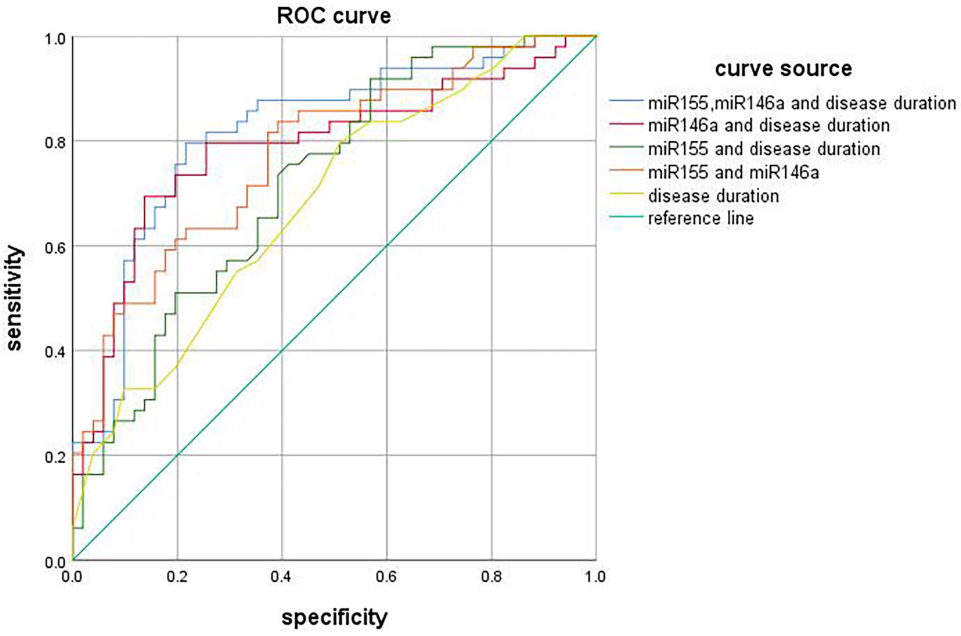

Figure 2 The Diagnostic Value of Combining Multiple Indicators for DPN;The AUC for the combined diagnostic use of miR-155 and miR-146a specific to DPN diagnosis reached 0.775 (95% CI 0.685–0.866, P < 0.001), with a sensitivity of 83.7% and a specificity of 60.8%. The AUC of disease duration specific to DPN diagnosis reached 0.681 (95% CI 0.578–0.785, P= 0.002), with a sensitivity of 79.6% and a specificity of 49%.The AUC for the combined use of miR-155 with disease duration, miR-146a with disease duration, and all three factors combined were 0.720(95% CI 0.621–0.819, P<0.001), 0.788(95% CI 0.695–0.881, P<0.001), and 0.818(95% CI 0.733–0.903, P<0.001), respectively.

kidney disease was identified as a risk factor for DPN, reflecting the shared pathological pathways between these conditions.

Further analysis within DPN subgroups revealed that patients with painful neuropathy exhibited lower TBIL levels compared to those with non-painful neuropathy. Notably, in the painful neuropathy subgroup, miR-155 was negatively correlated with BMI, whereas miR-146a was negatively correlated with BMI, AST and ALT in the non-painful neuropathy subgroup. Given the association between obesity and chronic inflammation,[28] the inverse relationships between miR-155, miR-146a and BMI, alongside the role of TBIL as an antioxidant, suggest that patients with painful neuropathy may experience a more enduring and severe inflammatory condition. Previous research has indicated miR-146a's beneficial role in liver conditions such as ischaemia/reperfusion injury, non-alcoholic fatty liver, fibrosis and hepatitis B, primarily through its regulatory effects on inflammation and immunity,[29–32] emphasising the protective functions of miR-155 and miR-146a in DPN.

In our subgroup analysis, a noteworthy observation was made: while the non-painful neuropathy subgroup exhibited a significant decrease in miR-146a expression compared to the T2DM group, no notable difference in miR-146a levels was observed in the painful neuropathy subgroup. Additionally, there was no significant difference in miR-155 expression levels between the painful and non-painful neuropathy subgroups. Other clinical studies have reported similar findings,[33] wherein peripheral blood miR-155 expression was downregulated in patients with T2DM but significantly increased in patients with Diabetic Foot Ulcer (DFU), possibly linked to the ongoing inflammatory state in DFU. Chen J et al[34] reported that silencing miR-155 could improve sciatic nerve damage, enhance angiogenesis and alleviate inflammation in DPN rats. Conversely, Ghada M[35] identified a detrimental role of spinal miR-155 expression in neuropathy. Studies also report that miR-155 mimics can improve nerve conduction velocity in DPN mice and inhibit pro-inflammatory genes induced by high glucose.[14] Furthermore, Fan et al[36] intervened in diabetic mice with exosomes rich in miR-146a (exo-146a), which significantly suppressed peripheral blood inflammation, monocyte and endothelial cell activation by inhibiting the Toll-like receptor (TLR)-4/NF-κB signalling pathway and enhancing nerve conduction speed, thereby improving neurological function recovery. However, these findings warrant further exploration to elucidate the underlying mechanisms of miR-155 expression changes in DPN. On the other hand, miR-146a, closely associated with inflammatory responses, has been consistently shown to be reduced in numerous animal experiments, highlighting its potential in improving neurological function.[20–22] However, our study indicates no significant difference in miR-146a expression levels between the painful neuropathy subgroup and the T2DM group. However, Ying et al[37] demonstrated that miR-146a-5p could alleviate neuropathic pain by inhibiting TRAF6 signalling in the spinal cord. Similarly, Wang et al[38] reported that an intrathecal injection of miR-146a-5p could alleviate mechanical and thermal hyperalgesia in chronic constriction injury (CCI) rats and reverse TRAF6 upregulation in the L4-L6 dorsal root ganglia and spinal dorsal horn induced by CCI. Similarly, Peter M et al[39] demonstrated that miR-146a could mitigate morphine-induced persistent neuropathic pain in male rats through Toll-like receptors. These discrepancies might stem from the limited sample size of the painful neuropathy subgroup and the possibility that painful and non-painful neuropathy represent different subtypes of DPN with distinct biological characteristics and heterogeneity, potentially affecting statistical differences. Nevertheless, further investigation is warranted to explore these aspects.

Studies have reported that miR-146a expression in peripheral blood[40] could serve as a biomarker for patients with non-small cell lung cancer, while miR-155[41] has been identified as a non-invasive biological marker for the presence, staging and prognosis of cancer. Collectively, our study's results indicate that elevated levels of miR-155 and miR-146a in peripheral blood could potentially act as protective factors against the development of DPN and are negatively correlated with various DPN risk factors. Furthermore, ROC curve analysis suggests that miR-155 and miR-146a have the capacity to distinguish between T2DM and DPN, with their combined assessment offering greater diagnostic value for DPN. Therefore, the expression levels of miR-155 and miR-146a in peripheral blood suggest their functionality in the diagnosis and prognosis of DPN. However, further research is needed to ascertain the reasons behind the decreased expression of miR-155 and miR-146a, particularly among patients with painful and non-painful neuropathy. Notably, as peripheral blood sampling poses minimal risk of trauma and miR-155 and miR-146a assessment is straightforward and convenient, we recommend utilising them to predict the onset and progression of DPN.

Conclusion

According to our study, the decreasing expression of miR-155 and miR-146a in the peripheral blood of patients with T2DM correlates significantly with the development of DPN. This association positions these microRNAs as promising biomarkers for both diagnosing and monitoring the progression of DPN (Figure 3). Despite these insights, the limitations of this study, including its single-center design and relatively modest sample size, may impact the reliability and applicability of the findings. To address these issues, future research should involve multicenter studies with larger sample sizes, which would help confirm our results and enable the classification of DPN into mild, moderate, and severe stages for enhanced clinical assessment. Moreover, our study could not determine the causal relationships between miR-155, miR-146a, and DPN, nor could it elucidate why their expression decreases in DPN patients. Therefore, further in-depth studies are essential to explore the molecular mechanisms of miR-155 and

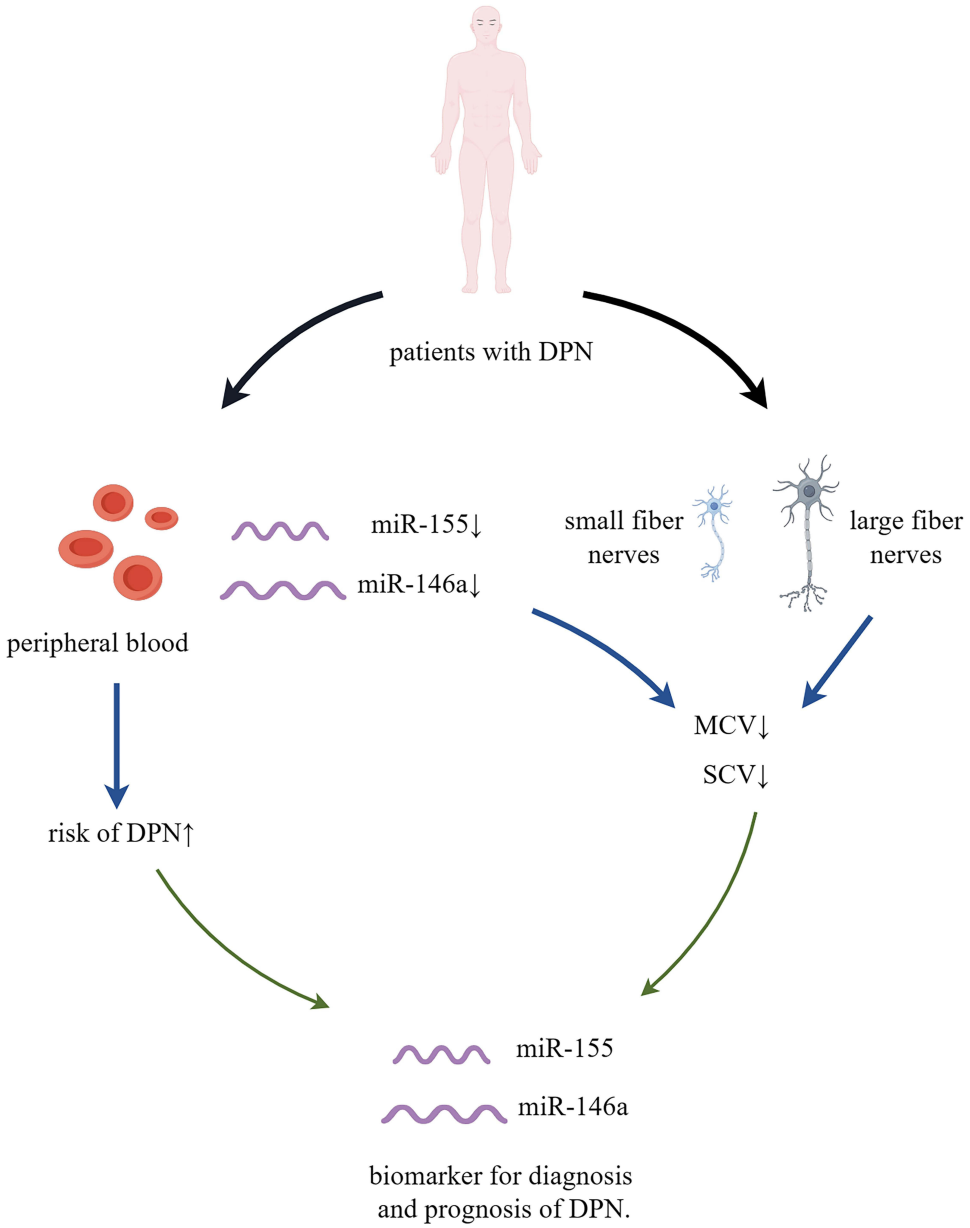

Figure 3 miR-155 and miR-146a effectively served as useful biomarkers for the diagnosis and prognosis of DPN.↑: increase; ↓: decrease.
Abbreviations: DPN, Diabetic Peripheral Neuropathy; SCV, Sensory Nerve Conduction Velocity; MCV, Motor Nerve Conduction Velocity; miR-155, microRNA-155; miR-146a, microRNA-146a.

miR-146a, and to evaluate their potential as new therapeutic targets for DPN, which could lead to novel treatment avenues.

Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

All procedures performed in this study involving human participants were in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This study was approved by the medical ethics committee of the First Affiliated Hospital of Anhui Medical University as CDEC000004982, and informed consent was obtained from the subjects.

Consent for Publication

All authors have declared their consent for this publication.

Acknowledgments

We are grateful to the all patients for participating in the study. We thank the participants of this study including the doctors, nurses, and researchers from the Department of Endocrinology in the First Affiliated Hospital of Anhui Medical University.

This paper has been uploaded to Research Square as a preprint: https://www.researchsquare.com/article/rs-3969442/v1

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This study was supported by the Natural Science Foundation of Anhui Province in China (2108085MH269), and the Natural Science Research Project of Colleges and Universities in Anhui Province (KJ2021A0274). The funding body had no role in the design of the study, or the collection, analysis, and interpretation of data, or in writing the manuscript.

Disclosure

The authors declare that they have no competing interests in this work.

References

1. GBD 2021 Diabetes Collaborators. Global, regional, and national burden of diabetes from 1990 to 2021, with projections of prevalence to 2050: a systematic analysis for the Global Burden of Disease Study 2021 [published correction appears in Lancet. 2023 Sep 30;402(10408):1132]. *Lancet*. 2023;402(10397):203–234. doi10.1016/S0140-6736(23)01301-6
2. Magliano DJ, Boyko EJ; IDF Diabetes Atlas 10th edition scientific committee. *IDF Diabetes Atlas*. 10th ed. International Diabetes Federation: Brussels; 2021.
3. Faselis C, Katsimardou A, Imprialos K, Deligkaris P, Kallistratos M, Dimitriadis K. Microvascular complications of type 2 diabetes mellitus. *Curr Vasc Pharmacol*. 2020;18(2):117–124. doi:10.2174/1570161117666190502103733
4. Zhang Y, Lazzarini PA, McPhail SM, van Netten JJ, Armstrong DG, Pacella RE. Global disability burdens of diabetes-related lower-extremity complications in 1990 and 2016. *Diabetes Care*. 2020;43(5):964–974. doi:10.2337/dc19-1614
5. Eid SA, Rumora AE, Beirowski B, et al. New perspectives in diabetic neuropathy. *Neuron*. 2023;111(17):2623–2641. doi:10.1016/j.neuron.2023.05.003
6. Feng J, Xing W, Xie L. Regulatory roles of MicroRNAs in diabetes. *Int J Mol Sci*. 2016;17(10):1729. doi:10.3390/ijms17101729

7. Papadopoulos KI, Papadopoulou A, Aw TC. Beauty and the beast: host microRNA-155 versus SARS-CoV-2. *Hum Cell*. 2023;36(3):908–922. doi:10.1007/s13577-023-00867-w
8. Palihaderu P, Mendis B, Premarathne J, et al. Therapeutic potential of miRNAs for type 2 diabetes mellitus: an overview. *Epigenet Insights*. 2022;15:25168657221130041.
9. Jankauskas SS, Gambardella J, Sardu C, Lombardi A, Santulli G. Functional role of miR-155 in the pathogenesis of diabetes mellitus and its complications. *Noncoding RNA*. 2021;7(3):39. doi:10.3390/ncrna7030039
10. Shou J, Chen PJ, Xiao WH. Mechanism of increased risk of insulin resistance in aging skeletal muscle. *Diabetol Metab Syndr*. 2020;12:14. doi:10.1186/s13098-020-0523-x
11. Frantz EDC, Prodel E, Braz ID, et al. Modulation of the renin-angiotensin system in white adipose tissue and skeletal muscle: focus on exercise training. *Clin Sci*. 2018;132(14):1487–1507. doi:10.1042/CS20180276
12. Faraoni I, Antonetti FR, Cardone J, Bonmassar E. miR-155 gene: a typical multifunctional microRNA. *Biochim Biophys Acta*. 2009;1792 (6):497–505. doi:10.1016/j.bbadis.2009.02.013
13. Leinders M, Üçeyler N, Thomann A, Sommer C. Aberrant microRNA expression in patients with painful peripheral neuropathies. *J Neurol Sci*. 2017;380:242–249. doi:10.1016/j.jns.2017.07.041
14. Chen J, Liu W, Yi H, Hu X, Peng L, Yang F. MicroRNA-155 mimics ameliorates nerve conduction velocities and suppresses hyperglycemia-induced pro-inflammatory genes in diabetic peripheral neuropathic mice. *Am J Transl Res*. 2019;11(6):3905–3918.
15. Olivieri F, Prattichizzo F, Giuliani A, et al. miR-21 and miR-146a: the microRNAs of inflammaging and age-related diseases. *Ageing Res Rev*. 2021;70:101374. doi:10.1016/j.arr.2021.101374
16. Balasubramanyam M, Aravind S, Gokulakrishnan K, et al. Impaired miR-146a expression links subclinical inflammation and insulin resistance in type 2 diabetes. *Mol Cell Biochem*. 2011;351(1–2):197–205. doi:10.1007/s11010-011-0727-3
17. Alipoor B, Ghaedi H, Meshkani R, et al. Association of miR-146a expression and type 2 diabetes mellitus: a meta-analysis. *Int J Mole Cell Med*. 2017;6(3):156–163. doi:10.22088/acadpub.BUMS.6.3.156
18. García-Jacobo RE, Uresti-Rivera EE, Portales-Pérez DP, et al. Circulating miR-146a, miR-34a and miR-375 in type 2 diabetes patients, pre-diabetic and normal-glycaemic individuals in relation to β-cell function, insulin resistance and metabolic parameters. *Clin Exp Pharmacol Physiol*. 2019;46 (12):1092–1100. doi:10.1111/1440-1681.13147
19. Morais Junior GS, Souza VC, Machado-Silva W, et al. Acute strength training promotes responses in whole blood circulating levels of miR-146a among older adults with type 2 diabetes mellitus. *Clin Interv Aging*. 2017;12:1443–1450. doi:10.2147/CIA.S141716
20. Wang L, Chopp M, Szalad A, et al. The role of miR-146a in dorsal root ganglia neurons of experimental diabetic peripheral neuropathy. *Neuroscience*. 2014;259:155–163. doi:10.1016/j.neuroscience
21. Liu XS, Fan B, Szalad A, et al. MicroRNA-146a mimics reduce the peripheral neuropathy in type 2 diabetic mice. *Diabetes*. 2017;66 (12):3111–3121. doi:10.2337/db16-1182
22. Luo Q, Feng Y, Xie Y, et al. Nanoparticle-microRNA-146a-5p polyplexes ameliorate diabetic peripheral neuropathy by modulating inflammation and apoptosis. *Nanomed Nanotechnol Biol Med*. 2019;17:188–197. doi:10.1016/j.nano.2019.01.007
23. ElSayed NA, Aleppo G, Aroda VR, et al. 2. Classification and diagnosis of diabetes: standards of care in diabetes-2023 [published correction appears in Diabetes Care. 2023 May 1;46(5):1106. doi: 10.2337/dc23-er05] [published correction appears in Diabetes Care. Diabetes Care. 2023 Sep 1;46(9):1715. doi: 10.2337/dc23-ad08]. *Diabetes Care*. 2023;46(Suppl 1):S19–S40. doi:10.2337/dc23-S002
24. Tesfaye S, Boulton AJ, Dyck PJ, et al. Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments [published correction appears in Diabetes Care. 2010 Dec; 33(12):2725]. *Diabetes Care*. 2010;33(10):2285–2293. doi:10.2337/dc10-1303
25. American Diabetes Association. 11. Microvascular complications and foot care: standards of medical care in diabetes-2019. *Diabetes Care*. 2019;42(Suppl 1):S124–S138. doi10.2337/dc19-S011
26. Papanas N, Ziegler D. Risk factors and comorbidities in diabetic neuropathy: an update 2015. *Rev Diabet Stud*. 2015;12(1–2):48–62. doi:10.1900/RDS.2015.12.48
27. Liu J, Yuan X, Liu J, et al. Risk factors for diabetic peripheral neuropathy, peripheral artery disease, and foot deformity among the population with diabetes in Beijing, China: a multicenter, cross-sectional study. *Front Endocrinol*. 2022;13:824215. doi:10.3389/fendo.2022.824215
28. Palella E, Cimino R, Pullano SA, et al. Laboratory parameters of hemostasis, adhesion molecules, and inflammation in type 2 diabetes mellitus: correlation with glycemic control. *Int J Environ Res Public Health*. 2020;17:300. doi:10.3390/ijerph17010300
29. Jiang W, Kong L, Ni Q, et al. miR-146a ameliorates liver ischemia/reperfusion injury by suppressing IRAK1 and TRAF6 [published correction appears in PLoS One. 2023 Jul 11;18(7):e0288672]. *PLoS One*. 2014;9(7):e101530. doi:10.1371/journal.pone.0101530
30. Li K, Zhao B, Wei D, et al. miR-146a improves hepatic lipid and glucose metabolism by targeting MED1. *Int J Mol Med*. 2020;45(2):543–555. doi:10.3892/ijmm.2019.4443
31. Ouyang Y, Fu X, Tan D, Peng S, Fu L. Expressions of miR-146a and miR-155 in different samples of chronic hepatitis B patients. *Zhong Nan Da Xue Xue Bao Yi Xue Ban*. 2019;44(8):845–849. doi:10.11817/j.issn.1672-7347.2019.190366
32. Zou Y, Cai Y, Lu D, Zhou Y, Yao Q, Zhang S. MicroRNA-146a-5p attenuates liver fibrosis by suppressing profibrogenic effects of TGFβ1 and lipopolysaccharide. *Cell Signal*. 2017;39:1–8. doi:10.1016/j.cellsig.2017.07.016
33. Xu M, Li Y, Tang Y, Zhao X, Xie D, Chen M. Increased expression of miR-155 in peripheral blood and wound margin tissue of type 2 diabetes mellitus patients associated with diabetic foot ulcer. *Diabetes Metab Syndr Obes*. 2022;15:3415–3428. doi:10.2147/DMSO.S376292
34. Chen J, Li C, Liu W, Yan B, Hu X, Yang F. miRNA-155 silencing reduces sciatic nerve injury in diabetic peripheral neuropathy. *J Mol Endocrinol*. 2019;63(3):227–238. doi:10.1530/JME-19-0067
35. El-Lithy GM, El-Bakly WM, Matboli M, Abd-Alkhalek HA, Masoud SI, Hamza M. Prophylactic L-arginine and ibuprofen delay the development of tactile allodynia and suppress spinal miR-155 in a rat model of diabetic neuropathy. *Transl Res*. 2016;177:85–97.e1. doi:10.1016/j.trsl.2016.06.005
36. Fan B, Chopp M, Zhang ZG, Liu XS. Treatment of diabetic peripheral neuropathy with engineered mesenchymal stromal cell-derived exosomes enriched with microRNA-146a provide amplified therapeutic efficacy. *Exp Neurol*. 2021;341:113694. doi:10.1016/j.expneurol.2021.113694
37. Lu Y, Cao DL, Jiang BC, Yang T, Gao YJ. MicroRNA-146a-5p attenuates neuropathic pain via suppressing TRAF6 signaling in the spinal cord. *Brain Behav Immun*. 2015;49:119–129. doi:10.1016/j.bbi.2015.04.018

38. Wang Z, Liu F, Wei M, et al. Chronic constriction injury-induced microRNA-146a-5p alleviates neuropathic pain through suppression of IRAK1/TRAF6 signaling pathway. *J Neuroinflammation*. 2018;15(1):179. doi:10.1186/s12974-018-1215-4
39. Grace PM, Strand KA, Galer EL, Maier SF, Watkins LR. MicroRNA-124 and microRNA-146a both attenuate persistent neuropathic pain induced by morphine in male rats. *Brain Res*. 2018;1692:9–11. doi:10.1016/j.brainres.2018.04.038
40. Dezfuli NK, Alipoor SD, Dalil Roofchayee N, et al. Evaluation expression of miR-146a and miR-155 in non-small-cell lung cancer patients. *Front Oncol*. 2021;11:715677. doi:10.3389/fonc.2021.715677
41. Jurkovicova D, Magyerkova M, Kulcsar L, et al. miR-155 as a diagnostic and prognostic marker in hematological and solid malignancies. *Neoplasma*. 2014;61(3):241–251. doi:10.4149/neo_2014_032