Int J Clin Exp Med 2018;11(7):7165-7173
www.ijcem.com /ISSN:1940-5901/IJCEM0067527

Original Article

Expression and clinical significance of microRNA 146a in peripheral blood mononuclear cells from type 2 diabetic neuropathy patients

Guo-Feng Wang[1,2], Ning Xu[1,2]

[1]*Department of Endocrinology Medicine, Lianyungang First People's Hospital, Affiliated Hospital of Nanjing Medical University, Affiliated Hospital of Xuzhou Medical University, Lianyungang 222000, Jiangsu Province, China;* [2]*The Affiliated Hospital of Kangda College of Nanjing Medical University, Jiangsu Province, China*

Received October 18, 2017; Accepted February 27, 2018; Epub July 15, 2018; Published July 30, 2018

Abstract: MicroRNAs (miRNAs) have emerged as important regulatory factors in the development of diabetes and its chronic complications. We investigated the expression levels and clinical significance of miRNA 146a (miR-146a) in peripheral blood mononuclear cells (PBMCs) of type 2 diabetes (T2D) patients with or without diabetic peripheral neuropathy (DPN) and healthy controls. SYBR quantitative real-time PCR (qRT-PCR) was used to examine expression levels of miR-146a in PBMCs of 37 T2D patients with non-diabetic peripheral neuropathy (NDPN), 44 T2D patients with DPN, and 34 healthy controls. Receiver operating characteristic (ROC) curves were drawn to evaluate the diagnostic value of miR-146a expression in PBMCs for DPN patients. The correlation between miR-146a expression and clinical parameters was analyzed. Expression levels of miR-146a in the NDPN group decreased slightly compared with those in the healthy control group ($p < 0.05$), and the levels further decreased in the DPN group ($p < 0.05$). The area under the ROC curve was 0.807 (95% CI = 0.720-0.893), and the sensitivity for DPN diagnosis and specific degrees were 85.3% and 75.1%, respectively. Expression levels of miR-146a negatively correlated with tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) but were not related to other clinical parameters. This study revealed that decreased miR-146a expression from PBMCs is associated with DPN, suggesting a mechanism wherein decreased miR-146a expression activates the NF-κB target genes, leading to increased TNF-α and IL-6 production. This may serve as a novel molecular biomarker for early diagnosis and disease severity evaluation of DPN.

Keywords: microRNA146a, diabetic peripheral neuropathy, type 2 diabetes, biomarker

Introduction

Diabetic peripheral neuropathy (DPN) has been defined as a symmetric, length-dependent sensorimotor polyneuropathy attributable to metabolic and microvascular alterations resulting from chronic hyperglycaemia exposure and cardiovascular risk covariates [1]. They are among the most common complications of diabetes and are a significant source of morbidity and mortality. Almost 50% of diabetic patients develop neuropathy with symptoms including spontaneous pain, hypoesthesia, allodynia, hyperalgesia, recurrent foot infections, ulcers leading to amputations, and Charcot joints. It accounts for more hospitalization than all the other diabetic complications combined, resulting in a huge economic burden for diabetes care.

A timely and accurate diagnosis of DPN is essential for early intervention in order to decrease the rate of associated disability and death. For the past couple of years, tremendous efforts have been made to identify DPN, and currently, several diagnostic tools are available. However, a biomarkers specific to neural damage remain unknown. Previous studies have suggested that microRNAs (miRNAs) can serve as potential markers and therapeutic targets for diabetes and its chronic complications [2, 3]. Although many studies have shown abnormal miRNA expression in human diabetic retinopathy and nephropathy [4, 5], few studies to date have investigated the association between miRNA expression and DPN.

Emerging data indicate that miRNAs are enriched within axons and locally regulate metab-

olism and axonal [22]. Thus, miRNAs regulate the biological functions of dorsal root ganglia (DRG) neurons and play a crucial role in the development of DPN. miRNA 146a (miR-146a) is an important modulator in autoimmunity and innate immune response, where it regulates the innate immune system through downregulation of IL-1 receptor-associated kinase 1 (IRAK1) and tumor necrosis factor receptor-associated factor 6 (TRAF6) genes. It has been extensively studied in the fields of inflammation, immunity, and cancer development. As one of the regulators and biomarkers of inflammation, miR-146a also plays an important role in the pathophysiological complications of diabetes and has been one of the hotspots in the study of diabetic complications. miR-146a contributes to transcriptional regulation of the extracellular matrix protein fibronectin, which is involved in the occurrence and development of diabetic retinopathy [19]. It plays an anti-inflammatory role in the pathogenesis of diabetic nephropathy [20]. It also exacerbates diabetic wound healing by increasing the expression of its pro-inflammatory target genes [21]. Wang et al. [17] demonstrated that hyperglycaemia downregulates miR-146a in DRG neurons and upregulates neuronal apoptosis caused by suppressed miR-146a expression by reducing IRAK1 and TRAF6 expression. In contrast to the above mentioned molecular mechanism, Yousefzadeh et al. [18] found that a defect in regulation of IRAK1 and TRAF6 can weaken the miR-146a regulatory negative feedback loop and cause sustained activation of nuclear factor-kappa B (NF-κB) and its targets to promote cell apoptosis during hyperglycaemia. Although there is no consensus on the underlying molecular mechanisms, these *in vitro* studies provide new insights into the role of miR-146a in the pathogenesis of DPN.

Given that miR-146a plays a key role in the progression of DPN, abnormal expression of peripheral blood mononuclear cells (PBMCs) may serve as an early biomarker for the diagnosis and prediction of disease severity. To evaluate this, the present study was designed to determine the clinical significance of miR-146a in PBMCs from type 2 diabetic neuropathy (T2D) patients. Here, we analyzed miR-146a expression levels in PBMCs obtained from healthy controls and T2D patients with and without DPN as well as in tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and C-reactive protein (CRP) and studied the correlation between miR-146a expression levels and clinical indicators.

Materials and methods

Subject selection

Subjects with T2D registered consecutively as inpatients at our hospital and healthy controls registered at our hospital's physical examination centre from Doctor 2013 to May 2014 were randomly enrolled into the study. T2D was biochemically confirmed according to the World Health Organization diagnostic criteria for the classification of diabetes [6]. The presence of both clinically evident DPN and abnormal nerve conduction studies was required to confirm DPN [7]. Informed consent form was signed by all the subjects. The study was approved by the scientific and ethics committees of the hospital and university.

Patients with alcohol abuse, uraemia, hypothyroidism, vitamin B12 deficiency, peripheral arterial diseases, trauma, cancer, compression due to vertebral disk herniation, inflammatory and infectious diseases, substance abuse, HIV infections, unexplained weight loss, neurotoxic drug use, cardiovascular diseases, severe liver diseases (e.g. aspartate aminotransferase (AST), or alanine aminotransferase (ALT) greater than three times the normal level), central nervous system disorders, and other chronic diabetic complications (such as diabetic retinopathy, diabetic nephropathy and diabetic foot syndrome) were excluded.

Alcoholics, smokers, overweight/obese individuals, and those presenting a family history of diabetes, infections, hypertension, or long-term medication use were excluded from the control group.

Neuropathy assessment and physical examination

A complete history of neurological symptoms was first obtained from all the subjects, followed by a physical examination. DPN was assessed by Dr. Hui using a standardized evaluation method to identify symptoms, signs, and nerve conduction abnormalities. The Toronto Clinical Scoring System (TCSS) [8] and Michigan Neuropathy Screening Instrument (MNSI) were used for analysis.

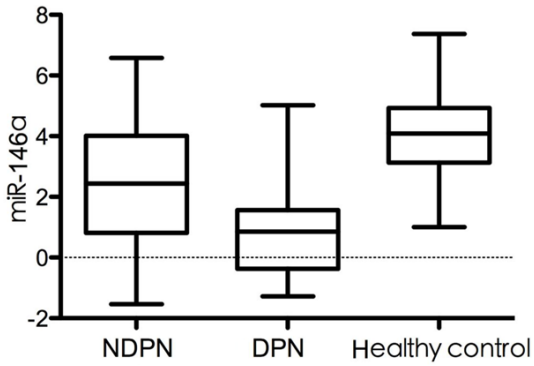

Figure 1. Relative expression of miR-146a in PBMCs of healthy controls and T2D patients without and with DPN.

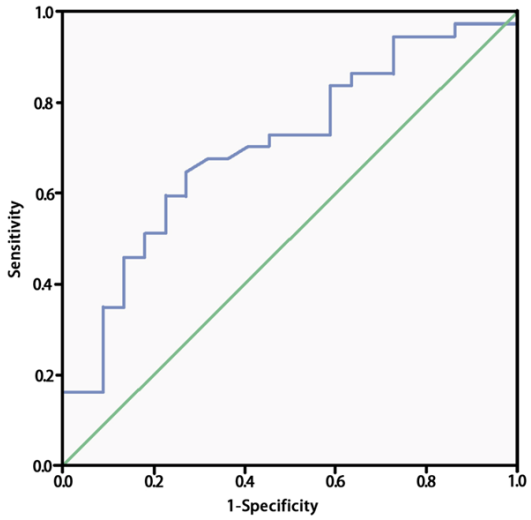

Figure 2. Receiver operating characteristic (ROC) curve analysis using PBMC miR-146a for discriminating patients with DPN from those with NDPN. The area under the ROC curve (AUC) was 0.807 (95% CI, 0.720-0.893) with a specificity and sensitivity of 85.3% and 75.1%, respectively.

Tibial, median, sural, and peroneal nerve conduction velocities (NCVs) were evaluated on the right side of each subject using EMG (key point, medronet 4, Minneapolis, MN) and a standardized technique [9]. Electrophysiological examination was considered abnormal if one abnormal attribute (among amplitude, conduction velocity and distal or F-wave latency) was observed in at least two nerves.

Clinical feature measurement

Demographic and clinical data were recorded, and retinal conditions were assessed by ophthalmologists using a combination of clinical examination, stereoscopic retinal photographs and fluorescein angiography.

Blood samples were analyzed for metabolic parameters such as glucose, insulin, cholesterol, and triglycerides (TGs). Plasma glucose, total cholesterol (TC), TG, high-(HDL-C) and low-density lipoprotein cholesterol (LDL-C), creatinine (Cr), AST, ALT, and r-glutamine aminotransferase (r-GTT) levels were measured using an automatic analyzer (UniCel DxH 800), and serum insulin levels were determined with the Phadebas insulin test (Pharmacia, Uppsala, Sweden) using a radioimmunosorbent technique. Insulin resistance was estimated using the homeostasis model assessment-insulin resistance (HOMA-IR), calculated as fasting plasma insulin (uU/ml) × fasting plasma glucose (mmol/l)/22.5 [19]. Glycosylated hemoglobin (HbA1c) was determined using the BIO-RAD D-10TM Kit (USA). ELISA was performed to measure serum levels of TNF-α, CRP, and IL-6.

Quantitative miR-146a

Peripheral blood samples (10 ml) from all the subjects were collected in EDTA-K2 tubes and processed within 2 h of collection. Isolation of PBMCs was performed using Ficoll-Hypaque density gradient centrifugation (Sigma, St. Louis, MO) and miRNA extraction was performed using the miRNeasy Mini Kit (QIAGEN, GmbH, Hilden, Germany). After the extraction, quantitation was performed using a NanoDrop ND-1000 spectrophotometer (NanoDrop Products, Wilmington, DE), and integrity was evaluated by microfluidic electrophoresis using the RNA 6000 Nano Kit and 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). RNA samples that were protein- and phenol-free and presented an RNA integrity number (RIN) of ≥ 7.0 were considered for microarray analysis.

Quantitative real-time PCR (qRT-PCR) was performed using the miScript II RT Kit (Qiagen, Germany). All the procedures were performed according to the instructions provided by the manufacturer. Specific primer assays for each miRNA were obtained from Qiagen: Hs_miR_146a (Catalogue number: MS00003535). The RT reaction was performed in an ABI PRISM 7300 thermal cycler, and RT-PCR was performed in the ABI PRISM 7500 Real-time PCR

Table 1. Comparison of baseline characteristics and miR-146a levels in diabetic patients with and without DPN (x±s)

	Health control	[1]P	NDPN	[2]P	DPN	[3]P
Number (n)	34		37		44	
Age (year)	49.08±11.09	N	49.44±8.69	N	54.10±9.22	N
Sex (Male/Female)	16/17	N	18/19	N	21/23	N
FPG (mmol/l)	4.86±0.47	0.00	8.89±2.01	0.00	9.03±2.45	N
HbA1c (%)	5.12±0.35	0.00	8.12±3.11	0.00	10.41±3.10	0.01
Duration (years)	-	-	5.22±0.86	-	8.42±1.17	0.02
BMI (Kg/m^2)	22.88±1.56	N	23.42±1.82	N	22.49±1.87	N
SBP (mmHg)	117.2±12.13	0.00	128.2±10.34	0.03	131.4±15.67	0.02
DBP (mmHg)	75.21±9.68	N	78.67±7.61	N	80.10±8.39	0.04
TC (mmol/L)	4.38±0.74	N	4.54±1.05	N	4.62±1.04	N
TG (mmol/l)	1.49±0.61	N	1.69±1.23	N	1.97±1.15	N
HDL-c (mmol/l)	1.20±0.19	N	1.18±0.21	N	1.10±0.31	N
LDL-c (mmol/l)	2.65±0.60	N	2.71±0.78	N	2.80±0.73	N
Scr (umol/l)	72.87±14.11	N	72.98±21.71	N	78.65±20.71	N
UA1b/Ucr (mg/g)	12.13±2.81	0.01	13.10±6.87	N	20.62±5.13	N
BUN (mmol/l)	4.72±1.53	N	5.54±1.61	N	6.14±1.87	N
HOMA-IR	1.78±0.45	0.00	3.31±1.39	0.01	3.69±1.96	N
AST (U/L)	18.93±10.12	N	19.23±14.67	N	22.78±12.25	N
ALT (U/L)	22.74±7.61	N	21.92±8.67	N	24.24±17.79	N
ALP (U/L)	86.73±38.54	N	89.45±40.32	N	100.81±75.02	N
r-GGT (U/l)	36.67±11.78	N	37.12±26.65	N	41.73±26.16	N
UA (mmol/l)	267.13±22.34	N	277.21±26.23	N	281.13±20.32	N
miR-146a	4.15±0.27	0.00	2.51±0.32	0.00	1.21±0.33	0.01
TNF-α	9.22±2.66	0.00	12.93±3.51	0.00	15.73±3.81	0.03
IL-6	3.82±1.88	0.00	5.94±2.27	0.00	8.34±2.24	0.00
CRP	0.66±0.39	0.00	1.54±1.08	0.00	2.79±1.61	0.01
FINS (mU/L)	8.25±2.14	0.00	8.63±3.65	0.00	9.12±3.63	N

NDPN: non-diabetic peripheral neuropathy; DPN: diabetic peripheral neuropathy; BMI: body mass index; DBP: diastolic blood pressure; SBP: systolic blood pressure; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglycerides; LDL-C: low-density lipoprotein cholesterol; FPG: fasting plasma glucose; HbA1c: glycosylated hemoglobin; Scr: serum creatinine; BUN: blood urea nitrogen; AST: aspartate aminotransferase; ALT: alanine aminotransferase; r-GGT: r-glutamine aminotransferase; UA: uric acid; HOMA-IR: homeostasis model assessment-insulin resistance; FIN: fasting insulin; N, insignificant ($p > 0.05$). [1]P: patients with DPN vs. healthy controls. [2]P: patients without DPN vs. healthy controls. [3]P: patients with DPN vs. patients without DPN.

System. Each reaction was run in duplicate, and the average raw quantification cycle (Cq) values of > 30 were removed from all data sets and performed again.

Statistics

The relative miRNA expression (Ct miRNA of interest) relative to the internal control gene (Ct internal control miRNA), was termed Δct. Continuous data are expressed as mean ± standard deviation (SD). Sensitivity, specificity, and area under the curve (AUC) for miR-146a distinguished patients with DPN and non-diabetic peripheral neuropathy (NDPN) and was determined using receiver operating characteristic (ROC) curve analysis. Multiple comparisons among groups were assessed using ANOVA for variables and percentages were compared using the χ^2 test. Pearson analysis was performed to determine the relationship between gene expression level and clinical parameters. A binary logistic regression analysis was performed using 'presence of DPN' as the dichotomous dependent variable and other independent covariants. Stepwise multiple linear regression was performed to determine the correlation and stages of DPN. Data were analyzed

Table 2. Pearson's correlation analysis between miR-146a and other clinical indicators (r)

MiR-146a	Duration	TCSS	FPG	HbA1c	Age	SBP	DBP	UA1b/Ucr	LDL-c	HDL-c	TG
r	-0.53	-0.69	-0.62	-0.55	-0.04	-0.22	-0.20	-0.42	-0.21	0.16	-0.14
P	0.04	0.02	0.02	0.03	0.77	0.13	0.95	0.04	0.13	0.24	0.30
MiR-146a	HOMA-IR	TNF-α	IL-6	MCV	SCV	Scr	BUN	MNSI	ALT	AST	ALP	TC
r	-0.62	-0.60	-0.61	0.79	0.62	-0.22	-0.07	-0.66	-0.06	0.10	-0.13	-0.20
P	0.02	0.02	0.02	0.04	0.04	0.14	0.61	0.04	0.65	0.48	0.36	0.14

using SPSS version 22.0. A *p*-value of < 0.05 was considered statistically significant.

Results

Relative miR-146a expression in PBMCs from healthy controls and T2D patients with and without DPN

In total, 37 T2D patients with NDPN, 44 T2D patients with DPN and 34 healthy controls were included in this study. Five subjects withdrew from the study due to either withdrawal of consent or medical care affordability.

The expression pattern of miR-146a in PBMCs from healthy control, DPN and NDPN groups was analyzed using qRT-PCR to determine whether miR-146a was abnormally expressed in the DPN group. **Figure 1** shows that patients with DPN exhibited decreased expression levels of miR-146a compared with healthy controls and patients with NDPN.

To investigate the power of miR-146a to distinguish patients with DPN from those with NDPN, ROC curves were generated and AUCs were calculated. These revealed that miR-146a may serve as a valuable biomarker for differentiating DPN from NDPN with AUC of 0.807 (95% CI = 0.720-0.893). Furthermore, the sensitivity for DPN diagnosis and specific degrees were 85.3%, and 75.1%, respectively (**Figure 2**).

Statistical analysis of patient characteristics

Anthropometric and clinical characteristics of the subjects are shown in **Table 1**. There were no obvious differences among the three groups in the following variables: age, sex ratio, BMI, lipid profile, BUN, Scr, and liver function. Neuropathy was identified in diabetic patients with increased levels of blood glucose and HbA1c, longer durations of diabetes, and higher levels of IL-6, TNF-α, and CRP. MiR-146a levels in PBMCs were significantly decreased in the DPN group compared with the NDPN group. Details are shown in **Table 1**.

Associations between miR-146a and other clinical indicators

We performed a linear regression analysis by considering miR-146a as the dependent variable and sex, age, BMI, disease duration, HbA1c, Homa-IR, and other clinical indicators as the independent variables. The results showed that expression levels of miR-146a in T2D patients were negatively associated with disease duration, HbAlc, TCSS, TNF-α, IL-6, UA1b/Ucr, MNSI, and Homa-IR (**Table 2**) and positively associated with NCV. Multiple linear regression analysis showed HOMA-IR, HbAlc, disease duration and TNF-α as miR-146a-independent factors (β = -23.45, -21.01, -23.50 and -35.25, respectively, $p < 0.05$).

Final model of binary logistic regression analysis (stepwise) used DPN as dependent variable: We performed a binary analysis using logistic regression analysis (stepwise method) considering the presence or absence of DPN as the dependent variable to evaluate the overall contribution of miR-146a and clinical variables to the risk of DPN development. This analysis confirmed involvement of disease duration, HbA1c, IL-6, TNF-α, UA1b/Ucr, Scr, UA and miR-146a in the susceptibility to DPN, showing that the miR-146a indeed contributed to DPN (R2 = 25.4%, **Table 3**).

Relative expression levels of miR-146a and clinical indicators between mild, moderate and severe DPN groups: As shown in **Table 1**, there was a significant association between miR-146a and DPN. To explore whether a link existed between the qualified miR-146a and DPN severity, further analysis of the relationship between relative miR-146a expression and DPN severity was performed. According to TCSS, patients with DPN were divided into mild (6-8 points), moderate (9-11 points) and severe

Table 3. Final model of binary logistic regression analysis with DPN as dependent variables

	B	SE	Wald	p	OR (95% CI)
UA1b/Ucr	0.94	0.38	6.11	0.013	2.55 (1.21, 5.36)
IL-6	1.05	0.31	11.25	0.001	2.85 (1.55, 5.2)
TNF-α	0.47	0.19	5.97	0.015	1.62 (1.03, 1.91)
Duration	0.17	0.07	5.14	0.02	1.19 (1.02, 1.38)
miR-146a	-0.60	0.24	6.55	0.01	0.55 (0.35, 0.87)
HbA1c	0.19	0.85	0.84	0.001	1.2 (1.89, 2.62)
Scr	0.39	0.76	0.65	0.045	1.08 (1.02, 3.24)
UA	0.21	0.07	0.58	0.049	1.01 (1.00, 4.02)
Constant	-2.37	1.57	2.27	0.132	

(1219 points) groups. As shown in **Table 4**, expression levels of miR-146a decreased and those of UA1b/Ucr, Scr, UA and SBP increased with the stages of neuropathy.

Correlation between miR-146a and DPN severity

The relationship between miR-146a levels and stages of DPN was also assessed using a multivariate model (**Table 3**). Co-variables that may potentially influence the miR-146a level or neuropathy severity, including HbA1c, duration of diabetes, TNF-α, UA1b/Ucr, and IL-6, were controlled. After adjustment, we observed that the miR-146a level was still independently associated with DPN (r = 0.894, R2 = 72.3%).

Multivariate linear regression analysis of factors influencing miR-146a

To further explore the factors influencing miR-146a, multivariate linear regression was conducted. The results showed that disease duration, HbA1c, FPG, TNF-α, MNSI, TCSS, MCV, and SCV influenced miR-146a (**Table 5**).

Discussion

Our study demonstrated that miR-146a is a potential biomarker of DPN. We used qRT-PCR to examine expression levels of miR-146a in PBMCs from T2D patients with or without DPN and healthy controls. miR-146a was decreased in DPN patients compared with NDPN patients and healthy controls. Relative expression levels of miR-146a can distinguish patients with DPN from those with NDPN via ROC analysis. Decreased expression levels of miR-146a were independently and negatively associated with TNF-α, IL-6, TCSS, duration of diabetes, UA1b/Ucr, HOMA-IR, FPG, and HbA1c but were not related to other biochemical clinical parameters. More importantly, decreased miR-146a in PBMCs was closely associated with DPN severity. Thus, miR-146a in PBMCs may be indicative of DPN. Our findings provide new insights into the molecular mechanisms involved in DPN.

DPN is a common complication of diabetes with complex, multifactorial pathogenetic mechanisms that are far from being completely understood. Chronic hyperglycemia seems to be the major culprit in the initiation of DPN, given that it enhances glucose oxidation and advanced glycation end products and activates protein kinase C, hexosamine and polyol pathway fluxes and activates NF-κB, which increases expression of pro-inflammatory cytokines such as TNF-α and IL-6 [10]. Several studies have shown that NF-κB plays a key role in the development and progression of DPN [11, 12], while elevated levels of TNF-α have been reported in the serum of patients with DPN [13].

NF-κB has been proposed to regulate its own activation partly through separate negative feedback loops by the transactivation of several miRNAs such as miR-146a, miR-155, miR-21, and miR-34. In this context, NF-κB activation is thought to promote miR-146a transactivation during the processing and maturation, enter the cytoplasm, and prevent the translation of IRAK1 and TRAF6 mRNAs into proteins. Indeed, emerging studies have shown that miR-146a expression is altered in diabetic patients and animals. miR-146a, as a regulator of NF-κB, can impair NF-κB activity and inhibit expression of IRAK1, TRAF6 [14], and pro-inflammatory cytokines such as IL-6, IL-8 and TNF-α [15]. Thus, it may play an important role in DPN.

Currently, several studies suggest that miR-146a is involved in the pathogenesis of diabetes and its chronic complications. Balasubramanyam et al. [15] found that expression levels of miR-146a are significantly decreased in patients with T2D compared with healthy controls, showing that impaired miR-146a expression links subclinical inflammation and insulin resistance in diabetic patients. The study by Ciccacci et al. [16] found that the C allele of rs2910164 SNP in miR-146a is associated with a lower risk of DPN development. Wang et

Table 4. Comparison of baseline characteristics and miR-146a levels in DPN patients with different disease severity

Group	Male/Female	Age (year)	Duration (year)	BMI (Kg/m^2)	SDP (mmHg)	DBP (mmHg)
Mild	40 (22/18)	60.91±9.9	12.8±4.7	24.4±3.7	134.12±16.07	76.67±12.03
Moderate	36 (19/17)	63.41±8.14	13.9±4.5	24.5±3.5	140.13±21.12[a]	79.89±11.13
Severe	33 (12/21)	66.22±11.8	13.9±5.1	22.8±2.3	143.23±19.71[a]	82.54±8.92
Group	HbA1c (%)	U-mALb/Ucr (mg/g)	BUN (mmol/l)	Scr (umol/l)	TC (mmol/l)	TG (mmol/l)
Mild	9.2±2.3	44.1±33.2[a]	7.7±1.6*	92.9±11.7	5.21±1.65	2.02±1.69
Moderate	9.3±2.4	105.7±180.9[a]	8.1±2.1	93.7±16.2	5.19±1.86	2.12±1.89
Severe	9.5±3.4	460.6±313.1[a]	8.8±2.8	104.6±20.7[a]	5.32±1.35	2.21±2.31
Group	LDL-C (mmol/l)	HDL-C (mmol/l)	FPG (mmol/l)	ALT (U/l)	AST (U/l)	ALP (U/l)
Mild	3.31±1.21	1.0±0.32	10.46±4.79	19.84±7.25	19.54±7.67	98.22±28.42
Moderate	3.20±1.27	0.97±0.32	10.26±4.39	21.94±7.95	18.54±7.59	99.22±27.38
Severe	3.22±1.26	1.2±0.32	10.01±4.21	21.94±6.91	18.64±7.72	101.22±26.51
Group	r-GGT (U/l)	TNF-α	IL-6	CRP	FINS (mU/L)	HOMA-IR
Mild	39.91±2.12	13.21±4.15	6.98±2.63	2.31±1.01	8.46±2.32	3.01±1.26
Moderate	41.91±3.01	14.19±5.82[a]	8.12±1.73	2.81±1.45	8.97±2.46	3.61±2.11
Severe	41.78±3.56	15.92±1.16[a]	8.02±2.14	2.72±1.52	9.21±2.31	3.58±1.98
Group	MiR-146a	UA (umol/l)	Tibial MCV	Peroneal MCV	Median SCV	Sural SCV
Mild	2.09±1.13	279.66±56.53	41.66±5.62	44.23±4.98	42.56±4.21	36.23±4.23
Moderate	1.41±0.98[a]	308.93±52.96[a]	39.27±5.96[a]	42.98±5.12[a]	40.12±5.08[a]	43.12±5.07[a]
Severe	0.98±0.25[a]	330.24±48.79[a]	36.24±8.79[a]	39.69±4.24[a]	39.24±3.79[a]	39.69±4.21[a]

The abbreviation are the same as **Table 1**; vs Mild DPN group, [a]$P < 0.05$.

Table 5. Multivariate linear regression analysis of influencing factors for miR-146a

	B	SE	Wald	p	OR (95% CI)
Constant	-1.95	6.22			
MNSI	0.03	0.02	4.00	0.04	0.97 (0.93, 0.99)
TCSS	0.36	0.79	0.54	0.04	0.55 (0.35, 0.87)
Tibial MCV	0.19	0.85	0.84	0.01	1.19 (1.02, 1.38)
Peroneal MCV	0.21	0.79	0.73	0.02	1.21 (1.02, 1.87)
Median SCV	0.39	0.76	0.65	0.04	2.85 (1.55, 5.20)
Sural SCV	0.38	0.80	0.79	0.04	2.79 (1.52, 4.02)
UA1b/Ucr	-0.34	0.82	0.82	0.03	0.65 (0.01, 0.88)
Duration	-0.35	0.59	0.42	0.03	0.61 (0.12, 0.98)
TNF-α	-0.47	0.69	0.59	0.02	0.58 (0.04, 0.99)
HbA1c	-0.42	0.70	0.04	0.04	0.82 (0.61, 0.91)
FPG	-0.26	0.74	0.42	0.04	0.63 (0.21, 0.94)
HOMA-IR	0.27	0.89	0.63	0.04	0.52 (0.21, 0.79)

al. [17] showed that hyperglycaemia downregulates miR-146a expression and leads to cultured DRG apoptosis by increasing IRAK1 and TRAF6 protein levels in db/db mice at 20 weeks, whereas sildenafil, a phosphodiesterase type 5 (PDE-5) inhibitor, reversed the effects of hyperglycaemia on miR-146a and its target gene expression in DRG neurons. It suggested that decreased miR-146a levels cannot inhibit NF-κB activity and are involved in the pathogenesis of DPN. However, Yousefzadeh et al. [18] found increased expression levels of miR-146a, IRAK, TRAF6, and NF-κB in the sciatic nerve of diabetic Sprague Dawley rats at 2 months, suggesting that defects in the NF-κB-miR-146a negative feedback loop are associated with DPN. They further confirmed the involvement of miR-146a and its adapter proteins (TRAF6 and IRAK) in the pathogenesis of DPN. The difference in the molecular mechanism in the aforementioned studies can be explained by the spatiality of miR-146a, type of specimen and animal models.

Our results are in agreement with those of Wang et al., who showed that hyperglycemia, longer disease duration and HOMA-IR lead to a decreased level of miR-146a. This consequently activates NF-κB activity and increases the inflammatory cytokines, further promoting the occurrence and development of DPN.

Some limitations of this study should be acknowledged. First, we assessed the clinical

relationship between miR-146a levels and DPN, and the results may be influenced by the number of individuals enrolled. Second, expression levels of miR-146a may be affected by multiple factors. Third, a low sensitivity of the cutoff value of miR-146a for the indication of DPN may limit the clinical and research efficacy of the test. Larger sample sizes would be needed to confirm and verify whether the results can be used in the early detection and evaluation of DPN severity. Notwithstanding, these findings add new insights into this intriguing and novel research field, showing that miR-146a could play a role in the risk of DPN development. The identification of potential genetic biomarkers could be useful in identifying patients more susceptible to DPN development, thus providing an additional approach to guide the management of DPN.

In conclusion, we observed that miR-146a in PBMCs is elevated in diabetes and is related to DPN. Our results further demonstrate that miR-146a plays an important role in DPN development and progression by regulating TNF-α and IL-6 gene expression. This may serve as a potential blood biomarker for DPN. Future studies should further confirm whether miR-146a rs2910164 is a susceptibility gene for DPN as well as reveal the effects of intervention of miR-146a expression on the behaviour of DPN.

Acknowledgements

This study was supported by the Lianyungang Health Bureau Program, Jiangsu Provincial in China (Bureau 1402, Bureau 2510).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Guo-Feng Wang, Department of Endocrinology Medicine, Lianyungang First People's Hospital, Tongguan North Road 182#, Lianyungang City, Jiangsu Provincial, China. Tel: +86+18961327083; E-mail: wanggfvip@163.com

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