Am J Transl Res 2022;14(12):9001-9006
www.ajtr.org /ISSN:1943-8141/AJTR0145309

Original Article

Relationship of serum lncRNA XIST and miR-30d-5p levels with diabetic peripheral neuropathy in type 2 diabetes

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Received July 12, 2022; Accepted October 4, 2022; Epub December 15, 2022; Published December 30, 2022

Abstract: Objective: To investigate the relationship between serum long non-coding RNA (lncRNA) X inactive specific transcript (XIST) and microRNA-30d-5p (miR-30d-5p) expression levels in type 2 diabetic peripheral neuropathy (DPN). Methods: Clinical data of patients with only type 2 diabetes mellitus (pure T2DM group), DPN patients (DPN group) and healthy patients (control group) admitted to Inner Mongolia Forestry General Hospital from August 2019 to April 2022 were retrospectively analyzed, with 76 cases in each group. The serum lncRNA XIST and miR-30d-5p expression levels of each group were compared. The correlation between serum lncRNA XIST and miR-30d-5p in DPN patients was analyzed. The influencing factors of DPN occurrence were analyzed. Also, the diagnostic value of serum lncRNA XIST and miR-30d-5p for DPN was analyzed. Results: There were significant differences in the lncRNA XIST and miR-30d-5p expression levels among the pure T2DM group, DPN group, and control group. LncRNA XIST expression level was negatively correlated with miR-30d-5p in DPN patients ($P<0.05$). Triglycerides, hemoglobin A1c, miR-30d-5p were risk factors for the occurrence of DPN, and lncRNA XIST was a protective factor ($P<0.05$). The areas under the curve (AUC) of serum lncRNA XIST and miR-30d-5p for the diagnosis of DPN were 0.851 and 0.845, respectively, and the AUC of lncRNA XIST and miR-30d-5p combined was 0.932, with a sensitivity of 92.1%, and a specificity of 85.5%. Conclusion: Both lncRNA XIST and miR-30d-5p may be involved in the development of type 2 DPN. Therefore, detecting serum levels of both may be helpful for clinical diagnosis and treatment of type 2 DPN.

Keywords: MicroRNA-30d-5p, type 2 diabetic peripheral neuropathy, X inactive specific transcript

Introduction

Diabetic peripheral neuropathy (DPN) is a common complication of type 2 diabetes mellitus (T2DM) and can lead to disability and death if left untreated [1, 2]. Studies have found that DPN progression is related to oxidative stress, glucose and lipid metabolism disorders, genetic susceptibility, dysregulated expression of microRNA (miRNA)/long non-coding RNA (lncRNA), neuroinflammation, and autophagy [3, 4]. LncRNA and miRNA are stable in serum and can be used as targets for the diagnosis and treatment of DPN [3, 4]. The expression of lncRNA X inactive specific transcript (lncRNA XIST) is down-regulated in DPN mice, which can affect the DPN process by regulating the expression level of microRNA-30d-5p (miR-30d-5p) [5]. However, the lncRNA XIST and miR-30d-5p expression levels in DPN patients, and the value of both in the diagnosis of DPN are still unclear. Therefore, this paper aims to analyze the diagnostic value of lncRNA XIST and miR-30d-5p by measuring their expression levels in the serum of DPN patients.

Materials and methods

Baseline data

This was a retrospective study. Data of 76 patients with only T2DM (pure T2DM group) and 76 patients with DPN (DPN group) who were diagnosed and treated in Inner Mongolia Forestry General Hospital from August 2019 to April 2022 were analyzed. In addition, 76 healthy subjects were selected as a control group, without lung, heart, liver and kidney dis-

Table 1. Primer sequences of LncRNA XIST, miR-30d-5p and internal reference GAPDH and U6

Gene	Forward primer 5'-3'	Reverse primer 5'-3'
LncRNA XIST	AATGGAACGGGCTGAGTTTTAG	TCATCCGCTTGCGTTCATAG
GAPDH	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG
miR-30d-5p	CCTGTTGGTGCACTTCCTAC	TGCAGTAGTTCTCCAGCTGC
U6	ATGACGTCTGCCTTGGAGAAC	CACTTTGTCAGTTACCAACGTCA

XIST: X Inactive Specific Transcript; GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase.

eases, T2DM or rheumatic immune system disease. This study was approved by the ethics committee of Inner Mongolia Forestry General Hospital (ethics approval number: 20190046).

Inclusion criteria: (1) patients who met the diagnostic criteria of T2DM/DPN [6] and were diagnosed for the first time; (2) those with complete examination data; (3) patients who gave informed consent to this study; (4) those who did not receive neurotrophic or anti-inflammatory treatments 1 month before admission; (5) those with normal liver and kidney function.

Exclusion criteria: (1) those with limb movement/sensory disorders caused by bone and joint disease, cerebrovascular disease or lumbar/cervical vertebral disease, etc.; (2) those with neuropathy caused by ischemic neuropathy, peripheral neuropathy, uremia or other factors; (3) those with gestational diabetes mellitus; (4) those with tumor or anemia; (5) those with a history of acute or chronic infection in the past 2 weeks; (6) those with cardiovascular disease, Guillain-Barré syndrome or autoimmune diseases; (7) those with epilepsy or mental illness; (8) those with type 1 diabetes mellitus.

Methods

Subject data including age, sex, total cholesterol (TC), systolic blood pressure (SBP), high/low density lipoprotein-cholesterol (HDL-C/LDL-C), fasting blood glucose (FBG), diastolic blood pressure (DBP), triglycerides (TG) and glycosylated hemoglobin A1c (HbA1c) were collected for all three groups.

Sample collection

Peripheral blood (4-6 mL) was collected from DPN patients and T2DM patients on the day of diagnosis, and 4-6 mL of peripheral blood from healthy people on the day of physical examination. After standing for about 30 minutes, the serum was separated and stored in -70°C refrigerator.

QRT-PCR

Serum was thawed. Trizol LS Reagent (10296010, Invitrogen) was used to extract serum total RNA, and RevertAid First Strand cDNA Synthesis Kit (K1621, Fermentas) was used to prepare cDNA template. Then, SYBR Green Realtime PCR Master Mix (QPK-201, TOYOBO) and qRT-PCR instrument (MyGo Pro, IT-IS) were used to amplify cDNA and determine the cycle threshold (CT) of each gene. GAPDH and U6 were used as internal reference for lncRNA XIST and miR-30d-5p, respectively, and $2^{-\Delta\Delta CT}$ method was used for calculation. See **Table 1** for the primer sequences.

Statistical methods

SPSS 25.0 was used to analyze the data. Measurement data were expressed as (mean ± SD) and analyzed by one-way ANOVA, SNK-*q* test or independent sample *t* test. The counted data were represented by [*n* (%)], and compared by Chi-square. The correlation was analyzed by Pearson's correlation coefficient. The influencing factors were analyzed by multivariate regression analysis. The diagnostic value was evaluated by the receiver operating characteristic (ROC) curve. $P<0.05$ indicated a significant difference.

Results

Comparison of baseline data

The levels of FBG in the control group, pure T2DM group, and DPN group increased sequentially ($P<0.05$). The levels of TG, FBG and HbA1c in the DPN group were higher than those in the pure T2DM group ($P<0.05$) (**Table 2**).

Comparison of serum lncRNA XIST and miR-30d-5p expression levels

The levels of lncRNA XIST decreased sequentially ($P<0.05$), while the levels of miR-30d-5p increased sequentially in the control group, pure T2DM group, and DPN group ($P<0.05$) (**Figure 1**).

Table 2. Comparison of general data in each group [(mean ± SD)/*n*]

Clinical indicator	Control group (*n* = 76)	Pure T2DM group (*n* = 76)	DPN group (*n* = 76)	$t/\chi^2/F$	*P*
Age (years)	54.34±9.62	56.65±9.96	55.75±9.73	1.079	0.342
Male/Female (*n*)	40/36	37/39	35/41	0.667	0.716
DBP (mmHg)	73.33±9.41	75.09±9.82	74.67±9.68	0.691	0.502
SBP (mmHg)	112.27±13.65	113.48±13.50	115.94±13.89	1.420	0.244
LDL-C (mmol/L)	2.30±0.77	2.56±0.85	2.60±0.87	2.919	0.056
TG (mmol/L)	1.75±0.58	1.79±0.60	3.02±1.01*,#	69.235	<0.001
HDL-C (mmol/L)	1.19±0.40	1.13±0.38	1.07±0.36	1.891	0.153
TC (mmol/L)	4.68±1.56	4.72±1.57	4.95±1.65	0.635	0.531
FBG (mmol/L)	5.03±1.01	8.48±1.70*	9.17±1.83*,#	154.524	<0.001
HbA1c (%)	-	7.59±0.95	8.85±1.11#	7.518	<0.001

*Denotes comparison with the control group, *P*<0.05; #Denotes comparison with the pure T2DM group, *P*<0.05. TC: Total Cholesterol; DBP: Diastolic Blood Pressure; LDL-C/HDL-C: Low/High Density Lipoprotein-Cholesterol; SBP: Systolic Blood Pressure; FBG: Fasting Blood Glucose; TG: Triglycerides; HbA1c: Glycosylated Hemoglobin A1c; DPN: Diabetic Peripheral Neuropathy; T2DM: Type 2 Diabetes Mellitus.

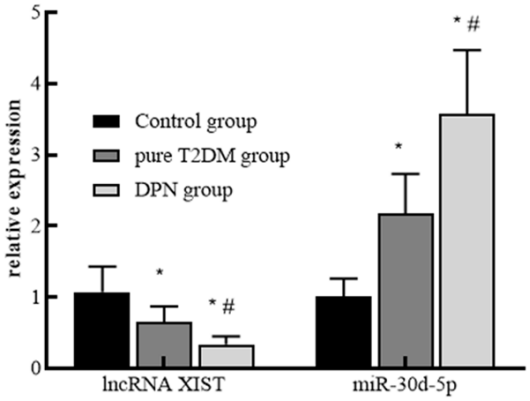

Figure 1. Comparison of serum lncRNA XIST and miR-30d-5p expression levels among the groups. *Denotes comparison with the control group, *P*<0.05; #Denotes comparison with the pure T2DM group, *P*<0.05. XIST: X Inactive Specific Transcript; DPN: Diabetic Peripheral Neuropathy; T2DM: Type 2 Diabetes Mellitus.

Correlation of serum lncRNA XIST and miR-30d-5p expression levels with TG, FBG, and HbA1c in DPN patients

The TG, FBG, and HbA1c in DPN patients were negatively correlated with the serum expression of lncRNA XIST (*P*<0.05), but positively correlated with the serum expression of miR-30d-5p (*P*<0.05) (**Table 3**).

Relationship between lncRNA XIST and miR-30d-5p expression level in DPN patients

The expression of serum lncRNA XIST was negatively correlated with the level of miR-30d-5p in DPN patients (*r* = -0.598, *P*<0.05) (**Figure 2**).

Multivariate logistic regression analysis

Taking TG, FBG, HbA1c, lncRNA XIST and miR-30d-5p as independent variables, and taking the occurrence of DPN as the dependent variable (no occurrence = 0, occurrence = 1), the results showed that TG, HbA1c and miR-30d-5p were risk factors affecting the occurrence of DPN, and lncRNA XIST was the protective factor (*P*<0.05) (**Table 4**).

Value of serum lncRNA XIST and miR-30d-5p in the diagnosis of DPN

The area under curve (AUC) of serum lncRNA XIST and miR-30d-5p in the diagnosis of DPN were 0.851 and 0.845, respectively, and the AUC of lncRNA XIST and miR-30d-5p combined was 0.932, which was higher than the diagnostic value of the two alone (*Z* = 2.212, 2.224, *P* = 0.027, 0.026). The combined diagnostic sensitivity of lncRNA XIST and miR-30d-5p was 92.1%, and the specificity was 85.5% (**Table 5**; **Figure 3**).

Discussion

Diabetic peripheral neuropathy (DPN) is a microvascular complication characterized by hypoesthesia and spontaneous pain, that affects patients' quality of life [7-9]. Therefore, finding markers related to the development of DPN has significance for timely intervention and improving quality of life.

LncRNAs can regulate neuroinflammatory responses, affect autophagy and regulate oxida-

Table 3. Correlation of serum lncRNA XIST and miR-30d-5p expression levels with TG, FBG, and HbA1c in DPN patients

Index	lncRNA XIST		miR-30d-5p	
	r	*P*	*r*	*P*
TG	-0.461	<0.001	0.442	<0.001
FBG	-0.587	<0.001	0.562	<0.001
HbA1c	-0.553	<0.001	0.495	<0.001

XIST: X Inactive Specific Transcript; FBG: Fasting Blood Glucose; TG: Triglycerides; HbA1c: Glycosylated Hemoglobin A1c.

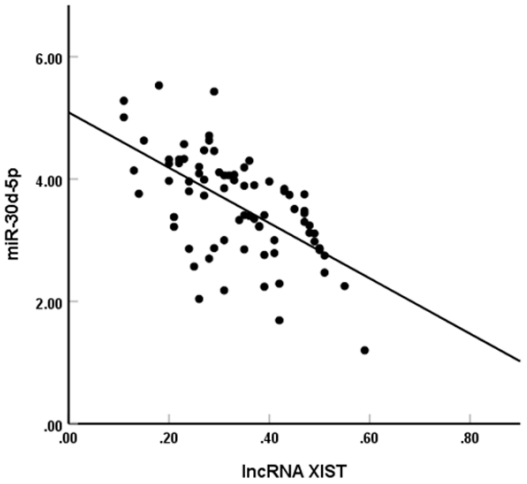

Figure 2. Correlation between serum lncRNA XIST and miR-30d-5p in DPN patients. XIST: X Inactive Specific Transcript; DPN: Diabetic Peripheral Neuropathy.

tive stress and other processes, and can be used for the diagnosis and treatment of T2DM and DPN [10-14]. Studies have shown that lncRNA XIST has low expression in diabetic nephropathy podocytes, and lncRNA XIST may affect the process of diabetic nephropathy by regulating the expression level of microRNA-30 [15]. In addition, Dong et al. [16] found that lncRNA XIST may provide protection against hyperglycemia-related damage in diabetic retinopathy through the competitive binding of hsa-miR-21-5p. Our results showed that the expression level of serum lncRNA XIST in the control group, pure T2DM group and DPN group decreased sequentially, similar to the results of Liu et al. [5], suggesting that a decreased level may affect progression of DPN lesions. It is speculated that lncRNA XIST may regulate the expression of related genes by targeting, thereby affecting autophagy and regulating the process of oxidative stress [5], and finally influencing the occurrence and development of DPN. ROC curve analysis found that the AUC of serum lncRNA XIST for the diagnosis of DPN was 0.851, suggesting that serum lncRNA XIST has a certain diagnostic value for DPN, and lncRNA XIST may become a target for the diagnosis and treatment of DPN.

Disorders of glucose and lipid metabolism are present in most patients with DPN. In this study, compared to the pure T2DM group, the DPN group had higher levels of TG, FBG, and HbA1c, which is similar to the findings by Li et al. [17]. It was also found that the serum lncRNA XIST expression level was negatively correlated with TG, FBG, and HbA1c in DPN patients, suggesting that lncRNA XIST and glycolipid metabolism may jointly affect the pathologic process of DPN.

MiRNAs can affect neuroinflammatory responses, regulate oxidative stress, mediate autophagy and other processes, and participate in the development of T2DM, DPN, and other pathologic processes [18-22]. MiR-30d-5p is overexpressed in type 1 diabetes and may participate in the pathogenesis of type 1 diabetes by affecting signal transduction and the inflammatory response [23]. This study showed that the serum miR-30d-5p expression level in the DPN group was higher, which is similar to the research of Liu et al. [5], suggesting that a high level may be related to the occurrence and development of DPN. It is speculated that miR-30d-5p plays a promoting role in the process of DPN pathology by binding to target genes, regulating related signal transduction and affecting autophagy [5, 23]. Our results also showed that the AUC of serum miR-30d-5p for the diagnosis of DPN was 0.845, indicating that miR-30d-5p may be a serodiagnostic indicator. Further analysis showed that the AUC of the combination of lncRNA XIST and miR-30d-5p was 0.932, with over 90% of sensitivity, suggesting that the combination of the two may be more helpful for the clinical diagnosis of DPN. Besides, the serum miR-30d-5p expression level in DPN patients was positively correlated with TG, FBG and HbA1c, suggesting that miR-30d-5p may jointly affect the pathologic changes of DPN with glycolipid metabolism.

Pearson's correlation coefficient showed that lncRNA XIST expression level of DPN patients was negatively correlated with miR-30d-5p,

Table 4. Multivariate logistic regression analysis

Index	*B*	*SE*	*Wald*	*P*	*OR*	95% CI
TG	1.674	0.489	11.700	0.001	5.333	2.044-13.916
HbA1c	1.452	0.411	12.485	0.000	4.272	1.909-9.560
lncRNA XIST	-5.276	2.035	6.721	0.010	0.005	0.000-0.276
miR-30d-5p	2.581	0.609	17.959	0.000	13.206	1.003-43.564

XIST: X Inactive Specific Transcript; TG: Triglycerides; HbA1c: Glycosylated Hemoglobin A1c.

Table 5. Diagnostic efficacy of various indicators for DPN

Index	AUC	95% CI	cut-off value	sensitivity (%)	specificity (%)	Youden index
lncRNA XIST	0.851	0.793-0.910	0.45	71.1	86.8	0.579
miR-30d-5p	0.845	0.780-0.910	2.67	77.6	86.8	0.644
lncRNA XIST, miR-30d-5p combination	0.932*,#	0.891-0.973	0.21	92.1*,#	85.5	0.776

Note: *Denotes compared with lncRNA XIST, $P<0.05$; #Denotes compared with miR-30d-5p, $P<0.05$. XIST: X Inactive Specific Transcript; DPN: Diabetic Peripheral Neuropathy; AUC: Area Under The Curve.

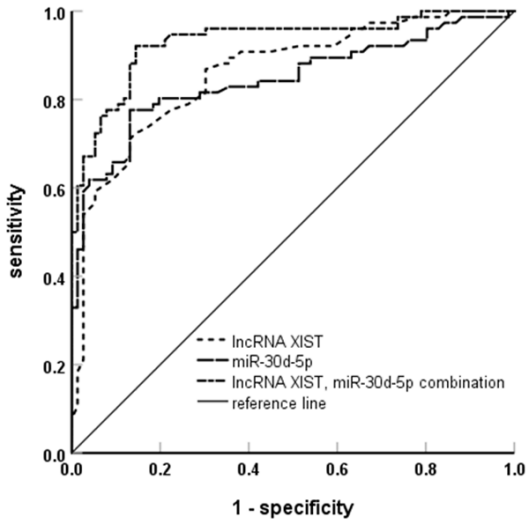

Figure 3. ROC curve of serum lncRNA XIST and miR-30d-5p in the diagnosis of DPN. ROC: Receiver Operating Characteristic; XIST: X Inactive Specific Transcript; DPN: Diabetic Peripheral Neuropathy.

suggesting that lncRNA XIST may synergize with miR-30d-5p to affect the progression of DPN. Multivariate logistic regression analysis found that serum levels of miR-30d-5p, TG and HbA1c increased, while decreased lncRNA XIST levels may increase the risk of DPN. Timely monitoring of serum index levels is beneficial for clinical prevention and treatment of DPN. However, the sample size in this study is small, and the mechanism of action of lncRNA XIST and miR-30d-5p in type 2 DPN is not sufficiently studied. In the later stage, the sample size will be expanded and further research will be carried out in combination with basic experiments.

In conclusion, the serum lncRNA XIST level was lower, and the miR-30d-5p level was higher in type 2 DPN patients, and the two were negatively correlated. Clinical detection of the two serum levels may have a guiding role for the diagnosis and treatment of type 2 DPN.

Acknowledgements

Serum lncRNA XIST and miR-30d-5p levels in peripheral neuropathy.

Disclosure of conflict of interest

None.

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