



Serum miR-155 as a biomarker for risk assessment and prediction of chronic complications in type 2 diabetes mellitus

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Abstract

Introduction and Objective. T2DM is a chronic disease with high prevalence. It can lead to a series of complications, causing severe damage to the body. The aim of the study is to examine the expression of miR-155 in patients with prediabetes and T2DM, and assessment of its diagnostic value.

Materials and Method. The biochemical indices of 96 prediabetic patients, 96 patients with T2DM, and 60 healthy subjects were measured using related instruments. The serum expression level of miR-155 in these three groups was detected through RT-qPCR. The early diagnostic value of miR-155 for T2DM was evaluated using a Receiver Operating Characteristic (ROC) curve. The predictive value of miR-155 for chronic complications in T2DM patients was assessed using a Kaplan-Meier curve. A logistic regression analysis was used to further assess the impact of miR-155 and other factors on T2DM complications.

Results. The expression level of miR-155 was found to be down-regulated in the serum of patients with pre-diabetes and T2DM. MiR-155 demonstrated a significant ability to differentiate between prediabetic, T2DM, and healthy individuals. T2DM patients with low miR-155 expression were found to be more susceptible to complications. Therefore, miR-155 can be considered a risk factor for T2DM complications.

Conclusions. The study highlighted the potential use of miR-155 in the early diagnosis and prognosis of T2DM. Additionally, miR-155 is somewhat associated with the onset and progression of T2DM complications.

Key words

T2DM, miR-155, complication, diagnostic value

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic disease characterized by a low-grade inflammatory response [1]. Complications of T2DM are classified into acute and chronic complications, which can be further divided into macroangiopathy and microangiopathy [2, 3]. The primary cause of disability and death in T2DM is microvascular disease, which includes diabetic retinopathy, diabetic nephropathy, and diabetic peripheral neuropathy [4]. Diabetic retinopathy is the most common microvascular complication of diabetes, occurring in up to 78% of patients with a history of more than 15 years. It can lead to blindness in severe cases [5]. Late-stage diabetic kidney disease can result in uraemia and lead to renal failure. Peripheral neuropathy may manifest as autonomic nerve dysfunction, abnormal sweating, or fluctuating gastrointestinal issues like diarrhea and constipation [5]. Overall, if T2DM is not detected and treated promptly, it can lead to a variety of complications, significantly reducing the patient's quality of life and posing serious risks to their health [6].

MicroRNAs (miRNAs) are crucial in cell differentiation, biological development, and disease, drawing the attention of many researchers [7, 8]. Over the past few decades, the role of miRNA in diseases, including tumours and cancer, has been extensively studied. Research indicates that miRNAs regulate autophagy in autoimmune diseases [9]. Previous studies suggest miRNAs may also have roles in renal disease, cardiovascular disease, and chronic obstructive pulmonary disease [10–12]. These findings point to miRNAs as potential biomarkers for disease diagnosis and prognosis.

MiR-155 has been identified as a factor closely tied to physiological and pathological processes and may serve as a potential therapeutic target for inflammatory diseases [13, 14]. Research indicates that miR-155 could have a significant role in the onset of T2DM retinopathy by regulating Treg cells via TGF- β . Furthermore, plasma miR-155 levels have been linked with diabetic retinopathy [15]. These findings imply that abnormal miR-155 expression could lead to diabetes-related complications. However, the clinical relevance of miR-155 in T2DM remains uncertain. The current study, therefore, investigates the potential of serum miR-155 as a diagnostic marker for T2DM patients. This research provides a new approach for the early detection of T2DM, aiming to prevent the onset and progression of T2DM complications.

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MATERIALS AND METHOD

Patients and sample collection. This study involved 96 prediabetic individuals and 96 patients with T2DM. Additionally, 60 healthy individuals undergoing physical examinations during the same period were chosen as the control group. Venous blood samples were collected from the 3 groups on admission, centrifuged at 1,500 r/min for 5 minutes, and the serum retained for use.

The study received approval from the Ethics Committee of Qinhuangdao Hospital of Integrated Traditional Chinese and Western Medicine, Hebei Port Group Co., LTD. All participants and their families provided informed consent before the commencement of the study.

Patient follow-up and prognosis. All subjects were monitored for 5 years post-enrollment. The occurrence and progression of chronic complications, such as retinopathy, diabetic nephropathy, and peripheral neuropathy in T2DM patients, were recorded during this period.

Detection of biochemical indicators. Fasting venous blood Haemoglobin A1c (%) (HbA1c), Fasting Blood Glucose (FBG), High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), Triglycerides (TG), and Total Cholesterol (TC) were measured in all subjects. Subsequently, these measurements were statistically analyzed.

RNA extraction and polymerase chain reaction (PCR). Total serum RNA was extracted following the instructions of the TRIzol LS kit (Invitrogen; 10296010). Afterward, 2 μ l of the RNA sample was dissolved in 98 μ l 1 \times TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), and the concentration measured using NanoDrop (with 1 \times TE buffer serving as a blank control, A260/280=2.0). The concentration should reach 1.0 μ g/ μ l – 1.4 μ g/ μ l. Residual genomic DNA was removed using a 5 \times gDNA digester Mix included in the reverse transcription kit (Yeaston, China; RR036B). The cDNA was synthesized by reverse transcription according to the instructions of the reverse transcription kit (Yeaston, China). The PCR reaction was implemented following the illustrations of TB Green[®] Premix Ex Taq[™] II (Takara, Japan; RR820A). All PCR reactions were performed in triplicate for each sample. The expression of miR-155 in each group was calculated using the 2-DeltaDeltaCt method with U6 as the endogenous control. The primer sequence of miR-155 was: (forward) 5'-AAAGTTCCAGATCAAGGTCCTG-3'; (reverse) 5'-AGAGACACCAGAATTCCTTTC-3'.

Statistical analysis. Statistical analyses were performed using GraphPad Prism 9.0 and SPSS 26.0 software. The data with normal distribution were expressed as (mean \pm SD). Comparisons between 2 groups were conducted using the independent samples Student's *t*-test. One-way analysis of variance (ANOVA) followed by the LSD *post hoc* test was used for multiple group comparisons, while the Kruskal-Wallis H test was applied for non-normally distributed data. ROC curve analysis was performed to evaluate the clinical significance of miR-155. The predictive value of miR-155 for the occurrence and development of chronic complications in patients with T2DM using the Kaplan-Meier method. Binary logistic regression analysis was performed to assess the effects of miR-155 and other variables on chronic complications, with

collinearity diagnostics used to exclude multicollinearity. Statistical significance was defined as $p < 0.05$.

RESULTS

Subject baseline characteristics. The healthy control group comprised 31 men and 29 women, with an average age of 62.08 \pm 10.23 years. The prediabetes group, with an average age of 63.97 \pm 9.30 years, included 52 men and 44 women. The group with T2DM had an average age of 65.09 \pm 7.79 years and consisted of 46 men and 50 women. There was no significant difference between the 3 groups regarding gender, age, body mass index (BMI), smoking history, drinking history, and hypertension. ($p > 0.05$) (Tab. 1).

Table 1. Demographic characteristics of control and case groups

Variable	Healthy (n=60)	Pre-diabetes (n=96)	T2DM (n=96)	F	P value
Age (years)	62.08 \pm 10.23	63.97 \pm 9.30	65.09 \pm 7.79	2.066	0.129
Gender (male / female)	31 / 29	52 / 44	46 / 50	0.375	0.687
BMI (kg/m ²)	23.97 \pm 2.98	24.42 \pm 3.18	24.79 \pm 3.20	1.263	0.285
Smoking yes / no	27 / 33	39 / 57	51 / 45	1.507	0.224
Drinking yes / no	25 / 35	45 / 51	51 / 45	1.008	0.367
Hypertension yes / no	34 / 26	44 / 52	52 / 44	1.070	0.344

BMI - Body Mass Index.

Physiological and biochemical indicators of the subjects. In the group with pre-diabetes and T2DM, both FBG, HbA1c (%) and TG were significantly higher compared to the healthy control group ($p < 0.001$). However, there was no significant difference in HDL, LDL, and TC between all 3 groups ($p > 0.05$) (Tab. 2).

Table 2. Laboratory data analysis of control and case groups

Variable	Healthy (n=60)	Pre-diabetes (n=96)	T2DM (n=96)	F	P value
FBG (mmol/L)	5.94 \pm 1.05	8.89 \pm 0.40	13.81 \pm 2.30	533.1	<0.001***
HbA1c (%)	5.23 \pm 0.71	5.43 \pm 0.84	7.43 \pm 1.47	107.0	<0.001***
HDL (mmol/L)	1.35 \pm 0.09	1.31 \pm 0.20	1.29 \pm 0.21	2.673	0.071
LDL (mmol/L)	2.39 \pm 0.17	2.40 \pm 0.20	2.45 \pm 0.22	2.313	0.101
TG (mmol/L)	1.41 \pm 0.27	1.88 \pm 0.52	1.92 \pm 0.51	25.60	<0.001***
TC (mmol/L)	4.60 \pm 0.74	4.70 \pm 0.67	4.77 \pm 0.58	1.287	0.278

FBG, fasting blood glucose; HbA1c, glycosylated hemoglobin; HDL, high density lipoprotein; LDL, Low-density lipoprotein; TG, triglycerides; TC, serum total cholesterol. *** $P < 0.001$

Expression and clinical significance of miR-155. The expression of miR-155 was significantly lower in patients with pre-diabetes and T2DM, compared to healthy controls ($p < 0.001$). Among all groups, T2DM patients had the lowest level of miR-155 (Fig. 1 A).

The diagnostic value of miR-155 in pre-diabetes and T2DM was assessed using an ROC curve. The results demonstrated that miR-155 could differentiate between healthy controls and pre-diabetes with an area under the curve (AUC) of 0.767 (Fig. 1 B). Additionally, miR-155 was able to effectively

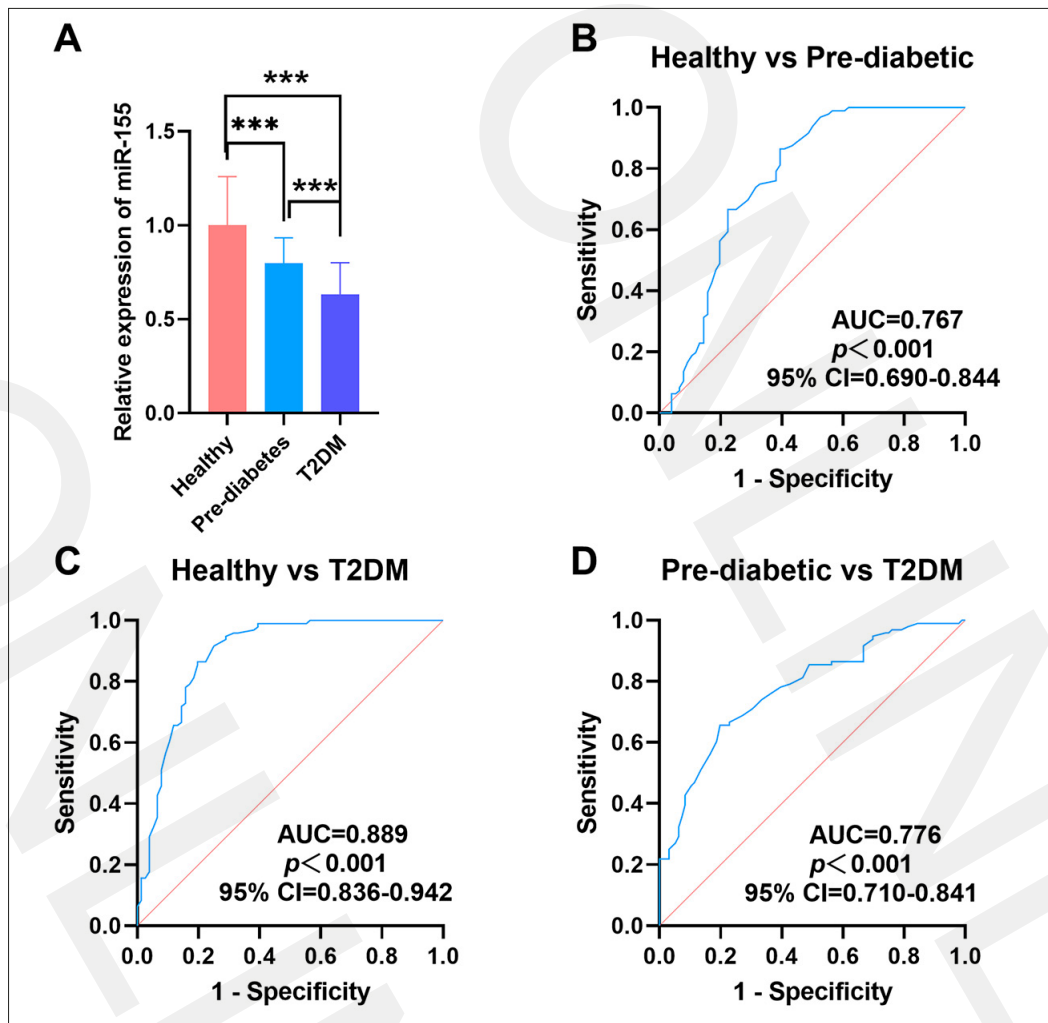


Figure 1. Expression and Clinical Significance of miR-155. (A) The expression level of miR-155 was significantly decreased in pre-diabetes and T2DM patients ($***P < 0.001$). (B) MiR-155 can effectively distinguish prediabetic patients from healthy individuals (AUC=0.767, 95% CI=0.690–0.844; $P < 0.001$). (C) MiR-155 can also better distinguish T2DM from healthy individuals (AUC=0.889, 95% CI=0.836–0.942; $P < 0.001$). (D) MiR-155 can also differentiate pre-diabetes patients from those with T2DM (AUC=0.776, 95% CI=0.710–0.841; $P < 0.001$)

differentiate between healthy controls and T2DM patients, and an AUC of 0.889 (Fig. 1 C). Remarkably, miR-155 could also distinguish pre-diabetes from T2DM with an AUC of 0.776 (Fig. 1 D).

Relationship between miR-155 and complications in patients with T2DM. The follow-up results indicated that none of the 96 patients with T2DM experienced acute complications, or died. However, complications were observed in 35 patients, including 19 cases of microvascular complications, 12 cases of microalbuminuria, and 4 cases of retinopathy. Patients were divided into low- and high-miR-155 level groups, based on the mean miR-155 expression level (0.63) in T2DM patients. Kaplan-Meier survival results demonstrated that patients with different miR-155 expression had a significant difference in the cumulative risk of chronic complications over time (log-rank $p < 0.001$). Specifically, patients in the low miR-155 expression group had an event-free survival probability of approximately 15% at 5 years, compared with approximately 55% in the high miR-155 expression group (Fig. 2), suggesting that high miR-155 levels may be associated with a lower cumulative risk of chronic complications.

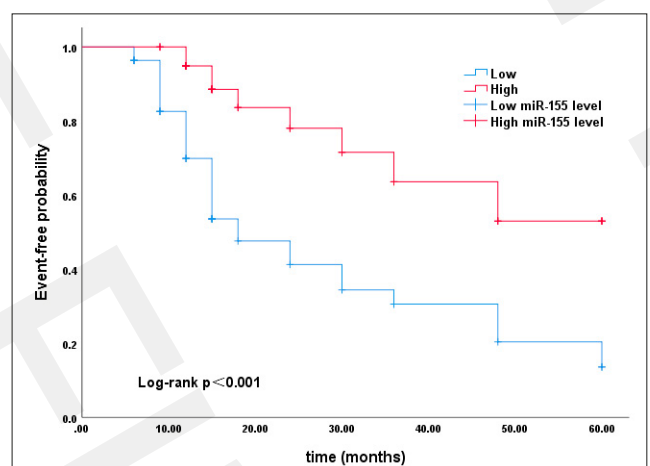


Figure 2. Kaplan-Meier analysis displayed that patients with low miR-155 expression had more chronic complications in T2DM patients (log-rank $p < 0.001$)

With the presence or absence of microvascular lesions as the dependent variable and miR-155 expression and clinical data as independent variables, a binary logistic regression analysis was performed. The correlation coefficient test

showed that there was a linear relationship between the dependent variables and the independent variables, and there was no high correlation between the independent variables. Logistic regression analysis indicated that miR-155 is the most significant risk factor for T2DM complications ($p < 0.05$). Furthermore, FBG and TG were also identified as risk factors for T2DM complications ($p < 0.05$). However, HbA1c (%) and hypertension showed no statistical significance ($p > 0.05$) (Tab. 3).

Table 3. Logistic regression was used to analyze the risk factors of T2DM complications

Variable	SE	Wald χ^2	HR	95 % CI for HR		P value
				Lower	Upper	
miR-155 expression	0.621	5.804	0.224	0.066	0.757	0.016*
age	0.528	0.168	1.242	0.441	3.496	0.682
gender	0.549	0.095	0.845	0.288	2.477	0.758
BMI	0.586	1.232	1.915	0.608	6.036	0.267
smoking	0.596	1.027	1.830	0.569	5.891	0.311
drinking	0.548	0.086	1.174	0.401	3.436	0.770
hypertension	0.576	0.650	1.591	0.514	4.924	0.420
FBG	0.675	4.872	0.226	0.060	0.846	0.027*
HbA1c (%)	0.571	1.828	2.163	0.707	6.620	0.176
HDL	0.551	0.011	0.944	0.321	2.778	0.917
LDL	0.536	0.007	1.047	0.366	2.994	0.932
TG	0.620	4.149	0.283	0.084	0.953	0.042*
TC	0.544	0.384	0.714	0.246	2.074	0.535

SE, standard error; BMI, body mass index; FBG, fasting blood glucose; HbA1c, glycosylated hemoglobin; HDL, high density lipoprotein; LDL, Low-density lipoprotein; TG, Triglycerides; TC, Serum total cholesterol. * $P < 0.05$

DISCUSSION

T2DM is characterized by insulin resistance and an eventual decline in insulin secretion, leading to high levels of glucose in the blood. It is a prevalent, chronic disease and long-term hyperglycaemia can result in complications causing severe and irreversible organ damage [16]. Through the analysis of clinical information, the presented study portrays the occurrence and developmental trend of chronic complications in T2DM patients. Without early intervention and appropriate management, more T2DM patients will develop a variety of chronic complications [17]. However, the most serious problem is that the current clinical screening methods for T2DM chronic complications are relatively complex, expensive, and have poor patient compliance [18]. Thus, finding a simple, effective, and reliable method for early diagnosis or prediction is of utmost importance.

As the mechanism of miRNA is further investigated using the latest technical means, it may provide a new tool for the treatment of human diseases by studying the relationship between miRNA and disease. MiR-155, a well-characterized member of the miRNA family, has been shown to potentially trigger insulin resistance and β -cell loss when down-regulated. Studies like those conducted by El et al. have shown that insulin resistance and T2DM in chronic hepatitis C virus (HCV) are closely related to miR-155 [19]. In the current study, a significant reduction found in serum miR-155 levels in patients with T2DM, which is consistent with

previous reports. Interestingly, a significant decrease was also observed in serum miR-155 levels in pre-diabetic patients, suggesting its potential as an early biomarker for T2DM diagnosis and prevention. Furthermore, the results obtained in the current study showed that miR-155 could differentiate between T2DM patients and healthy individuals, and more importantly, pre-diabetic patients from healthy individuals and T2DM patients. Thus, the presented study provides stronger evidence for the early diagnostic or predictive value of miR-155 in T2DM.

Research indicates that both reduction of miR-155 and its inhibition can prevent pathologic retinal neovascularization and mitigate visual loss [20, 21]. Silencing miRNA-155 has been shown to alleviate sciatic nerve damage caused by diabetic peripheral neuropathy in rats [22]. Studies suggest that miR-155-mediated autophagy can reduce cell damage in diabetic peripheral neuropathy [23, 24]. These findings reflect context-dependent roles of miR-155 in controlled systems. Prior research has implied a correlation between miR-155 and the onset and progression of T2DM complications [25].

The current study showed that patients with lower miR-155 expression experienced more chronic complications. Moreover, further analysis determined that miR-155 is a significant risk factor for T2DM complications. This apparent discrepancy highlights the complexity of miR-155's role in human diabetes, where its expression may reflect disease activity, or tissue-specific responses, rather than a straightforward pathogenic driver.

Limitations of the study. This study has several limitations. Firstly, the sample size was relatively small and may not be representative of broader T2DM populations, limiting generalizability. Secondly, the study focused on correlational analysis without exploring the molecular mechanisms of miR-155 in diabetic complications. Thirdly, the progression of prediabetic patients to T2DM or reversion to euglycemia over the 5-year period was not documented, restricting the analysis of miR-155 dynamics across disease stages. Finally, the failure to systematically collect detailed data on T2DM patients' treatment regimens, medication usage, and dynamic changes in blood glucose, blood pressure, and blood lipid levels may introduce confounding factors when employing miR-155 as a predictor of complications.

CONCLUSION

In conclusion, miR-155 levels were significantly higher in the serum of both pre-diabetes and T2DM patients. It has the potential to distinguish between prediabetes patients, T2DM patients, and healthy individuals. In the future, miR-155 is anticipated that it will be a predictive biomarker for chronic complications risk in T2DM patients.

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