Study on peripheral blood miR-155 expression in patients with type 2 diabetes mellitus and its relationship with vascular complications

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ABSTRACT

Objective:

To evaluate the expression of miR-155, nuclear factor kappa B (NF-κB) and soluble intercellular adhesion molecule-1 (sICAM-1) in the peripheral blood of patients with type 2 diabetes mellitus (T2DM) and assess its relationship with diabetic vascular lesions.

Methods:
A total of 165 T2DM patients and 60 healthy patients were included. Patients were divided into two groups: a simple T2DM group and a T2DM with vascular lesion group. The latter was further divided into a microvascular lesion group, a macrovascular lesion group and a microvascular combined with macrovascular lesion group. Albuminuria levels were divided into three groups as follows: <30 mg / 24 h, normal albuminuria group (NAU group); 30-300 mg / 24 h, microalbuminuria group (MAU group); and ≥300 mg / 24 h, large albuminuria group (LAU group). Levels of peripheral blood miR-155, NF-κB, and sICAM-1 were detected by
RT-PCR
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Results:
①Compared with that in the control group, the expression of miR-155, NF-κB, and sICAM-1 in the T2DM group was significantly increased [(1.00±0.12) vs. (1.82±0.71), (1.04±0.33) vs. (2.28±0.66), (1.03±0.30) vs. (1.88±0.80), respectively; P<0.05].
②The levels of miR-155, NF-κB, and sICAM-1 were significantly higher in the T2DM vascular lesion group than in the simple T2DM group [(1.95±0.73) vs. (1.34±0.29), (2.40±0.65) vs. (1.65±0.16), (2.01±0.77) vs. (1.07±0.41), respectively; P<0.05].
③The expression of miR-155 in the microvascular combined with macrovascular lesion group was higher than that in the macrovascular lesion group [(2.36±0.61) vs. (1.77±0.59); P<0.05], and the level of NF-κB in the former group was significantly higher than those in the microvascular and macrovascular lesion groups (P<0.05).
④Compared with that in the NAU group, the level of miR-155 was significantly increased in the MAU and LAU groups [(1.04±0.20) vs. (1.41±0.49) vs. (2.64±0.52), respectively; p<0.05], and the miR-155 level in the LAU group was higher than that in the MAU group (P<0.05).
⑤Pearson correlation analysis showed that miR-155 was positively correlated with NF-κB and sICAM-1 (R=0.259, 0.67; P<0.01).
Conclusions:

miR-155 expression was increased in T2DM patients and vascular lesions, consistent with the increasing trends observed for NF-κB and sICAM-1. These findings suggest that miR-155 may be involved in the occurrence and development of diabetic chronic vascular disease.

PROTOCOL STATUS

Working
We use this protocol in our group and it is working

MATERIALS TEXT

Subjects and Methods

One hundred sixty-five T2DM patients were randomly selected at the endocrinology department of the General Hospital of Tianjin Medical University and Teda International Vascular Hospital from June 2016 to June 2018. The subjects included 90 males and 75 females, and their average age was 61.79±10.55 years. At the same time, 60 nondiabetic subjects matched for age, sex and body mass index (BMI) were selected. All subjects provided written consent to participate in the study. -size:14.0pt;line-height:200%'>onsent to participate in the study. The study protocol was approved by the Independent Ethics Committee (IEC) of Teda International Cardiovascular Hospital. The registration institution is the Chinese Clinical Trial Registry, and the registration

number is ChiCTR-ROC-17010468.

Diabetes diagnostic criteria:
All subjects met the WHO diagnostic criteria for T2DM (1999).
Complications diagnostic criteria:
T2DM vascular lesions were diagnosed according to type 2 diabetes prevention and treatment strategies of China (2013) as follows: macrovascular lesions confirmed by electrocardiogram / coronary angiography, head CT/MRI or peripheral vascular color Doppler ultrasonography or a history of cardio cerebral vascular disease; fundus examination (e.g., microangioma, bleeding, exudation) or 24-h urine albumin (24 h UAlb) quantitative examination to assure microvascular lesions.
Exclusion criteria:

Subjects with acute complications of diabetes, infection, tumor, primary liver disease or nondiabetic nephropathy were excluded from the study.
Grouping:
Participants were allocated into a simple T2DM group and a T2DM with vascular lesion group, and the latter group was further divided into a microvascular lesion group, a macrovascular lesion group, and a microvascular combined with macrovascular lesion group. Participants were also classified into a normal albuminuria group (NAU group), a microalbuminuria group (MAU group) and a large albuminuria

SAFETY WARNINGS

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Methods

5 General data: All subjects were asked for details about their height, weight, blood pressure and BMI. Lipid, fasting plasma glucose, and glycosylated hemoglobin levels as well as liver and kidney functions were detected. The 24-h urine albumin level was measured, and fundus photography was examined. Information that identified individual participants during or after data collection was obtained when needed.

6 RT-PCR methods:(1) Extraction of peripheral blood total RNA: The TRIzol method was used to extract total RNA according to the manufacturer's instructions. The RNA concentration was measured using an ultraviolet spectrophotometer, and samples with a 260/280 ratio between 1.8 and 2.1 were preserved at -80ºC.(2) cDNA product: miR-155 and internal reference U6 primers were purchased from Guangzhou Ribo Biotechnology Company. The reverse transcriptase system (in which miR-155 uses the stem ring

method) comprised the following: 1 μl of dNTP (10 mM), 0.5 μl of the target gene reverse transcriptase primer, 0.5 μl of the internal parameter reverse transcriptase primer, 0.5 μl of RNase inhibitor, 1 μl of M-MLV, 4 μl of 5× buffer and 2 μg of total RNA in DEPC water at a total volume of 12.5 μl; the mixture was incubated at 42ºC for 60 min and at 70ºC for 15 min. The cDNA product was maintained at -20ºC.(3) The fluorescence quantitative RT-PCR system (cDNA diluted 10 times) comprised the following: 9 μl of 2.5× SYBR Green Master Mix, 1 μl of the upstream primer, 1 μl of the downstream primer, 3 μl of cDNA, and 6 μl of DEPC water. The RT-PCT conditions for amplifying miR-155, U6 and sICAM-1 were as follows: predenaturation at 95°C for 20 s; 40 cycles of denaturation at 95°C for 10 s, annealing at 60°C for 20 s, and extension at 72°C for 15 s; and extension at 72°C for 5 min. The NF-κB and GAPDH annealing conditions were 57°C for 20 s; all other conditions were as described above.