Original Article

MicroRNA-148a-3p reduces inflammatory response by inhibiting IRS-1 and LDLR in diabetic foot ulceration

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Abstract: Objective: This study aimed to investigate the serum expression levels of MicroRNA-148a-3p, IRS-1 and LDLR in patients with diabetic foot (DF) and their relationship with inflammatory response. Methods: Forty-two patients with DF admitted to our hospital were enrolled as the study group. 43 diabetic patients without DF were enrolled as the disease control group, and 58 healthy individuals were selected as the healthy control group. The levels of MicroRNA-148a-3p, IRS-1 and LDLR expression in the serum of all participants were detected, and correlation analysis was performed between the serum levels and the clinical parameters, grades of severity for DF and inflammatory response. Results: The expression levels of MicroRNA-148a-3p, IRS-1 and LDLR in the study group, disease control group and healthy control group were statistically significant (P<0.05). The serum levels of MicroRNA-148a-3p were lower, and IRS-1 and LDLR were higher in patients with DF who developed inflammatory response than those in patients without DF (P<0.05). As the DF worsened, the serum level of MicroRNA-148a-3p gradually decreased, and the levels of IRS-1 and LDLR gradually increased (P<0.05). Conclusion: MicroRNA-148a-3p showed low expression in the serum of patients with DF who developed inflammatory reactions, and it was clinically possible to inhibit IRS-1 and LDLR by increasing the expression level of MicroRNA-148a-3p, so as to reduce inflammatory reactions in patients with DF.

Keywords: Diabetic foot, MicroRNA-148a-3p, IRS-1, LDLR

Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia, which is caused by defective insulin secretion or other impaired biological function [1]. With the improvement of people’s living standard and the change of dietary structure, the incidence rate of diabetes is rising year by year, reaching 6.4% worldwide. By 2035, the number of people with diabetes worldwide is expected to exceed 380 million [2, 3]. DM, commonly known as diabetes, is a metabolic disease that causes high blood glucose [4]. The hormone insulin promotes transfer of sugar from the blood into cells for storage or use as energy. With diabetes, your body either doesn’t produce enough insulin or can’t effectively use the insulin. Untreated high blood glucose caused by diabetes can damage your nerves, eyes, kidneys, and other organs.

Diabetic foot (DF) is any pathology directly caused by peripheral arterial disease (PAD) and/or sensory neuropathy affecting the feet in DM. It is a long-term (or “chronic”) complication of DM. Long-term high blood glucose level will cause damage to vascular endothelial cells, increase the degree of atherosclerosis, reduce vascular blood flow, and induce ischemic hypoxia in the lower limbs, leading to the formation of DF. According to the statistics by the International Diabetes Federation in 2015, 9.1-26.1 million diabetic patients worldwide develop foot ulcers each year [5]. Patients with DF ulcers have a 2.5 times higher risk of death within 5 years than diabetic patients without DF [6]. Diabetic patients exhibit low autoimmu-
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nity. Gram-positive bacteria, negative bacteria, fungi, and even anaerobic bacteria that appear deep in the wound infection can trigger the inflammatory response in patients with DF. When patients with DF have inflammatory reactions in wounds, high blood glucose not only provides a good environment for bacteria growth, but also reduces the phagocytic capacity of white blood cells, making the wound difficult to heal. Antibiotics have been used to treat inflammatory reactions in patients with DF. However, the infection is mostly caused by mixed bacteria. Single antibiotic cannot achieve a good therapeutic effect, and over time, the strain tends to develop drug resistance or even mutation, reducing its efficacy. As the condition worsens, patients may experience discomfort, decreased quality of life, increased likelihood of hospitalization and amputation, and even death [7]. At present, domestic and foreign scholars aim to reduce the amputation rate of DF patients through early diagnosis and multidisciplinary team management of complications, timely removal of callus and control of infection [8, 9].

The aim of this study was to investigate the relationship between MicroRNA-148a-3p, IRS-1 and LDLR and the wound inflammatory response, so as to provide a theoretical basis for reducing the wound inflammatory response in patients with DF.

Materials and methods

General information

Forty-two patients with DF diagnosed by the endocrinology department in our hospital from January 2018 to December 2019 were enrolled as the study group, including 8 cases of grade 2, 14 cases of grade 3, 12 cases of grade 4, and 8 cases of grade 5. Forty-three diabetic patients without DF during the same period were selected as the disease control group. In addition, 58 cases of healthy individuals were recruited as the healthy control group. The baseline data of the three groups of patients in terms of gender, age, BMI index, etc. showed no statistically significant difference (P>0.05).

Inclusion criteria: patients who met the diagnostic criteria for DF were classified to grades 2, 3, 4 and 5 according to classification standards of Wagner for diabetes [10].

Exclusion criteria included patients presenting with autoimmune system diseases, malignant tumors, severe cardiovascular diseases, severe psychiatric diseases, and other systemic infectious diseases [11].

A personal profile was established for the three groups of subjects, and information such as name, gender, age, contact telephone number, and residential address were registered, and an informed consent form was signed by each subject. This study was approved by the ethics committee of our institution.

Methods

Determination of LDLR levels: Peripheral venous blood was collected from the three groups of subjects for laboratory testing. EDTA was added as an anticoagulant and was mixed with the blood samples at room temperature for 15-20 min, followed by centrifugation at 1800 r/min at 2-8°C for 20 min. The supernatant was placed in the refrigerator at -80°C. If precipitates appeared in the storage process, centrifugation was performed again.

Determination of LDLR levels using ELISA: LDLR levels were determined according to the instructions of the LDLR ELISA kit (96-well plate, Shanghai Shuang Sheng Biotechnology Co.).

Determination of IRS-1 levels: Peripheral venous blood was collected from the three groups of subjects. Heparin as anticoagulant was added and centrifuged at 1000 r/min for 20 min at room temperature. The supernatant was stored in the refrigerator at -80°C.

The plasma levels of IRS-1 were determined using ELISA. The IRS-1 level was determined according to the instructions of Human Insulin Receptor Substrate 1 (IRS1) ELISA kit (96-well plate, Shanghai Jinma Experimental Equipment Co.).

Determination of MicroRNA-148a-3p level: Peripheral venous blood samples were collected, and real-time PCR was used to determine the level of MicroRNA-148a-3p [12].

(i) The content of total RNA in plasma was measured according to the Trizol LS kit (Invitrogen, USA) instructions, and the purity of RNA was determined by measuring absorbance with a

(ii) The RNA was reverse transcribed into cDNA by reverse transcription kit (WD3126, Beijing Hua Yue Yang Biotechnology Co., Ltd.).

(iii) The expression level of MicroRNA-148a-3p was determined with cDNA as the template and U6 as the internal reference according to the instructions of the fluorescent quantitative PCR kit (BL705A, Biosharp).

Observation indicators

Comparison of MicroRNA-148a-3p expression levels in the three groups: MicroRNA-148a-3p was associated with the regulation of diabetes, lipid metabolism and inflammation. According to the expression level of MicroRNA-148a-3p in cells of the three groups of subjects, the level of lipid metabolism and the inflammation can be determined. The high expression of MicroRNA-148a-3p indicates good regulation of lipid metabolism and the inflammatory response in patients with DF [13-16].

Comparison of expression levels of IRS-1 in the three groups: IRS-1 is a major substrate for the insulin receptor and other tyrosine kinases. It plays a key role in eliciting many actions of insulin, including the binding and activation of phosphatidylinositol (PI) 3-kinase and the subsequent increase in glucose transport. When IRS-1 expression is reduced, the insulin signaling is attenuated [17, 18]. IRS-1 is also a docking protein involved in angiogenesis, and when IRS-1 is inhibited, the inflammatory response is also suppressed, thereby alleviating the inflammatory response in wounds of patients with DF.

Comparison of LDLR levels in the three groups: LDLR is a transmembrane glycoprotein synthesized in the liver to regulate cholesterol metabolism in the body. The organism’s inflammatory response can cause damage to feet of patients with DF through negative feedback regulation of lipid metabolism. When LDLR expression is downregulated, the inflammatory response of wounds in patients with DF is alleviated [19-21].

Statistical analysis

Statistical analysis was performed using SPSS 23.0. The measurement data were expressed in the form of mean ± standard deviation (x ± sd), and the t-test was used to compare the differences between groups. P<0.05 indicates significant difference.

Results

Comparison of baseline data in the three groups

The three groups of subjects were comparable in terms of baseline data such as sex, age, BMI, and course of disease (P>0.05) (Table 1).

Comparison of serum MicroRNA-148a-3p, IRS-1, and LDLR levels in the three groups

The study group showed significantly lower levels of MicroRNA-148a-3p, and higher levels of IRS-1 and LDLR than the disease control group (P<0.05) (Figure 1).

The disease control group showed lower serum levels of MicroRNA-148a-3p and higher levels of IRS-1 and LDLR than the healthy control group (P<0.05) (Figure 2).

Comparison of serum MicroRNA-148a-3p, IRS-1 and LDLR levels with regard to inflammatory response

The serum levels of MicroRNA-148a-3p in patients with DF and inflammatory response were lower while the levels of IRS-1 and LDLR...
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The expression levels of MicroRNA-148a-3p, IRS-1 and LDLR in the serum of subjects. The expression level of MicroRNA-148a-3p in serum of subjects in study group was significantly lower than that of subjects in disease control group (P<0.05), and the expression levels of IRS-1 and LDLR of subjects in study group were significantly higher than those in disease control group (P<0.05). * Representing significant difference between the two groups.

Comparison of serum MicroRNA-148a-3p, IRS-1 and LDLR levels with regard to DF grading

The serum levels of MicroRNA-148a-3p, IRS-1 and LDLR among the patients with varying severity of DF were significantly different (P<0.05). With the increase of DF grading, MicroRNA-148a-3p levels gradually decreased while IRS-1 and LDLR levels gradually increased, and the differences in these indicators among the patients with different grading were statistically significant (P<0.05) (Figures 4-6).

Discussion

With the change in dietary habits, the incidence of diabetes continues to rise. It is incurable regardless of whether it belongs to type I or type II. The treatment options currently focus on controlling the patient’s condition and preventing serious complications. DF is the most common complication, affecting about 30-40% of patients with diabetes [22, 23]. When patients develop DF, the risk of amputations and mortality rate as well as medical costs increase, which not only brings psychological
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MicroRNAs are highly conserved endogenous, single-stranded, non-coding RNAs with a length of 18-25 nucleotides, which are involved in physiological processes such as cell proliferation, differentiation, and apoptosis, and play a regulatory role in diabetes, tumors, cardiovascular and inflammatory responses [25]. Negative regulation of target gene expression is achieved by identifying the 3'UTR of the target gene through complementary base pairing [26]. The body usually promotes the release of anti-inflammatory factors when a wound appears, leading to automatic healing within 1-4 days. In patients with DF, the expression of inflammatory factors was increased, the duration of inflammatory was prolonged, angiogenesis was decreased, and an increased inflammatory response to wounds was observed in patients with DF [27, 28].

This study was conducted by measuring the expression levels of MicroRNA-148a-3p, IRS-1 and LDLR, and the results showed that the healthy control group had higher expression levels of MicroRNA-148a-3p and lower levels of IRS-1 and LDLR (P<0.05) than the disease control and study groups. Patients with wound inflammation showed lower MicroRNA-148a-3p expression levels and higher IRS-1 and LDLR expression levels than patients without wound inflammation in the study group. As the grade of DF patients increased, MicroRNA-148a-3p expression levels gradually decreased, and IRS-1 and LDLR expression levels gradually increased (P<0.05). It was demonstrated that MicroRNA-148a-3p can significantly reduce the expression levels of IRS-1 and LDLR, thus inhibiting the wound inflammatory response for patients with DF.
In the last two decades, microRNA targeted therapies have been developed rapidly, which can modulate multiple gene targets simultaneously and have become therapeutic options for cancer, diabetes, renal diseases, etc. The selection of targets was the key step for successful MicroRNA-targeted therapy. Several studies have confirmed that MicroRNAs have a regulatory role in wound inflammatory response, angiogenesis, and re-epithelialization [29-31]. MicroRNA-targeted drugs, e.g. Miricrasen, RG-101, RG-125/AZD4076, MRG-106, and MRX34, have entered the process of clinical trials for chronic diseases, but there is no known MicroRNA-targeted drug for DF at present.

In summary, MicroRNA-148a-3p regulates expression levels of IRS-1 and LDLR through negative feedback to reduce inflammatory responses in wounds of patients with DF. The novelty of this study is that it gave up the traditional anti-inflammatory regimen for DF and chose to study the serum expression levels of MicroRNA-148a-3p to explore the relationship between MicroRNA-148a-3p, IRS-1 and LDLR and the inflammatory response, so as to provide a theoretical basis for the development of MicroRNA-targeted drugs to inhibit the inflammatory response in patients with DF. The shortcomings of this study are as follows: (1) The sample size is small and geographically diverse, making the results less generalizable. (2) Only three groups were enrolled, and the results obtained may be biased. We will carry out in-depth studies with larger sample size to obtain more representative and scientific conclusions and provide more detailed theoretical basis for the treatment of DF.

Disclosure of conflict of interest

None.

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