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Original Article

Dynamic changes and clinical value of Sirt6 in acute coronary syndrome (ACS) patients

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Abstract

This study aimed to investigate the role of Sirt6 and inflammatory cytokines in blood samples of patients with ACS. This is a retrospective randomized controlled clinical trial, a total of 30 patients from our hospital are included and divided into following two groups: control group and experimental group, and experimental group consists of 15 patients with ACS and control group consists of 15 patients with non-acute coronary syndrome. Sirt6 protein is detected by western blotting and Sirt6 mRNA is detected by real-time PCR, then inflammatory cytokines such as IL-1β, IL-18, TnI, and CK-MB are measured by ELISA and cytokines NT-proBNP are monitored by immunofluorescence. Our outcomes show that Sirt6 protein and Sirt6 mRNA in experimental group are remarkably lower than those in control group, and IL-1β, IL-18, TnI, CK-MB, and NT-proBNP in the experimental group are remarkably higher than those in control group. We can conclude that Sirt6 can prevent or inhibit the development of ACS and IL-1β, IL-18, TnI, CK-MB, and NT-proBNP can accelerate the development of ACS.

Keywords: Acute coronary syndrome; Sirt6; inflammatory cytokines

1. Introduction

Acute coronary syndrome (ACS), the most common disease in cardiovascular, seriously threats human health and lives, including unstable angina (UA), acute myocardial infarction (AMI) and sudden cardiac death (SCD), and it is characterized by an imbalance in lipid metabolism, rupture of arterial plaque and inflammatory response of arterial wall [1-3]. Currently, the detailed molecular mechanisms regarding the ACS is still unclear. Hence, further study of the molecular mechanisms underlying ACS is required to obtain an effective therapeutic target. To date, numerous drugs such as atorvastatin, ticagrelor, and aspirin enteric-coated tablets have been used to treat patients with ACS, and these drugs have certain protective effects on slowing or preventing the development progression of ACS. However, ACS is a disease that is caused by complicated factors, and the efficacy of these drugs on ACS still does not meet the patients' requirements [4-6]. Therefore, clear target regarding drugs in ACS is required to improve the efficacy of treatment. Those findings in previous studies suggest that Sirt6 is a key target participating in the development process of ACS, but the detailed mechanism of Sirt6 in ACS is still unclear [7].

Sirtuin6 (Sirt6), derived from the mammalian Sirtuins family, it is known as a highly conserved ADP-ribosylase and NAD+-dependent deacylase which participates in a wide range of cellular processes such as regulation of senescence, DNA repair, glucose metabolism, transcription regulation, etc. [8-10]. As a cellular pathway checkpoint controller, Sirt6 mainly participates in the regulation of cell proliferation and survival, so its abnormal expression is associated with a variety of diseases [11]. Sirt6 is expressed in the heart and has a key regulatory effect on cardiac function [12]. During sepsis myocardial injury, the expression of Sirt6 in myocardial is down-regulated and its activity is decreased, which can accelerate the progression of myocardial damage after sepsis myocardial injury, damage cardiac function, and affect the cardiac pARs signaling system. Up-regulated of Sirt6 by gene transfection can effectively improve cardiac function in animal models [12,13]. Therefore, Sirt6 has been recognized as a potential therapeutic target for the treatment of myocardial infarction. In recent years, studies have suggested that Sirt6 has a protective effect in animal models with ACS. In addition, this process leads to high expression of IL-1β, IL-18, TnI, CK-MB and NT-proBNP factors through Sirt6 during ACS [14].

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This study aims to investigate the regulation correlation of Sirt6 in patients with ACS, and IL-1β, IL-18, TnI, CK-MB and NT-proBNP cytokines are detected. We elucidate the mechanism of Sirt6 in ACS by detecting the expression of Sirt6, IL-1β, IL-18, TnI, CK-MB and NT-proBNP in the blood of patients with ACS.

2. Materials and methods

2.1. Patients

All patients included in this retrospective clinical study who have been diagnosed with ACS or non-acute coronary syndrome. All protocols in our study were prospectively reviewed and permitted by the ethics committee of our hospital, and written informed consent forms from all patients were obtained. A total of 30 patients from our hospital from January 2019 to December 2021 are included in this retrospective clinical study.

Inclusion criteria for ACS patients were as follows: (i) Patients with typical symptoms of exertional angina pectoris have a history of more than 1 year and are confirmed as coronary heart disease by coronary angiography and no obvious evidence of ischemic attack in the past 48 hours; (ii) Patients had coronary angiography evidence of coronary heart disease and a silent angina pectoris attack and the duration time is often more than 20 min; (iii) There was an initial angina pectoris attack within the past 1 to 2 months, and the slight activity will lead to angina pectoris and cause severe pain; (iv) based on exertional angina pectoris, angina pectoris symptoms last longer and more frequent, and pain is more severe. Electrocardiograms (ECG) or dynamic ECG shows ST-T ischemia changes; (v) Acute Myocardial Infarction (AMI) is marked with myocardial markers CK-MB, and significant myocardial ischemia symptoms persist for 30 min and unrelieved, in addition, the patients were accompanied by ST elevation on ECG or ST shift of more than 2 mm in adjacent two leads and T wave inversion (non-ST elevation AMI).

Exclusion criteria for all patients are as follows: (1) Patients who have an age of >85-year-old; (2) Patients who have infection, allergic reaction, endocrine dysfunction, malignant tumor, autoimmune disease, rheumatic heart disease, other heart disease such as atrial fibrillation, myocarditis, valvular heart disease etc.; (3) Patients who have severe liver disease; (4) Patients who have renal failure and have a history of anti-inflammatory and anti-immune drugs during 3 weeks.

2.2. Trial design

This study is a retrospective randomized control clinical trial, and all patients were divided into the following two groups: the experiment group and the control group. A total of 30 patients who have non-coronary heart disease outpatients or inpatients (NCA) were included in this retrospective clinical study based on the exclusion criteria and inclusion criteria. A total of 15 patients who had ACS are rolled in this clinical study as experiment group and 15 patients who had non-ACS are set as control group. 10 ml of peripheral blood in antecubital vein of experiment group and control group was taken, 2 ml of peripheral blood is utilized to analyze the gene levels in two groups by the real-time fluorescence quantitative PCR (qRT-PCR), and 2 ml of peripheral blood was used to detect the protein levels in two groups by Western blot (WB), and then 4 ml of peripheral blood was used to calculate the inflammatory cytokines levels by enzyme-linked immunosorbent assay (ELISA), finally, 2 ml of peripheral blood is also utilized to monitor cytokines levels by immunofluorescence.

2.3. Real time PCR analysis

The total RNA of the 2 ml of peripheral blood in experiment group and control group were extracted by TRIzol reagent (Bioworld Techmology, Inc, Shanghai, China), and the purity and concentration of the total RNA were calculated by a microspectrophotometer; the cDNA were obtained by using Transcriptor First Strand cDNA Synthesis Kit (KR106-02, TIANGEN, Inc., Beijing, China). Sirt6 gene (Forward: 5'-TGTGCCAAGTGTAAGACG-CAG-3' and Reverse: 5'-TTGCCTTAGCCACGGTG-CAG-3'), GAPDH gene (Forward: 5'-ATGGTGAAG-GTCGGTGTCGGTGTGAAC-3' and Reverse: 5'-GCCG-TGAGTGGAGTGGAGTCATACTG-3'), and the primer sequences were designed by Beijing Qingke Biotechnology Co., Ltd. (Beijing, China). Real-time quantitative PCR was conducted by CFX Real-time System (Bio-Rad, Hercules, CA, USA). All reactions for real-time quantitative PCR were conducted in triplicate by the following protocol: reverse transcription at 95°C for 3 min and at 95°C for 15 s followed by 40 cycles at 60°C for 60 s and 65°C for and 95°C for 5 min.

2.4. Western blot analysis

Protein extracts were obtained from the 2 ml of peripheral blood in the experiment group and control group. Firstly, the samples were fully ground in liquid nitrogen and then 1 mL mixture consisting of 100:1:10 Lysate-Protease Inhibitor-Phosphatase Inhibitor was added to the sample, and the proteins were lysed on ice for 1 h, then centrifuged at 12 000 r/min for 10 min at 4°C, then the supernatant was collected for protein concentration determination using BCA protein quantification kit (ASPEN Institute of Biotechnology). The protein samples were subjected to 10% SDS-PAGE and load, transferred to PVDF membrane, washed with TBST for 5 min, and then blocked with 5% skim milk powder for 1 h at room temperature. Anti-SIRT6 (1: 2000; Bioworld Techmology, Inc, Shanghai, China) and GAPDH (1: 1000; Bioworld Techmology, Inc, Shanghai, China) primary antibodies were added and incubated overnight at 4°C. After washing with TBST for 10 min and 3 times, a secondary antibody (1:10 000; Bioworld Techmology, Inc, Shanghai, China) was added and blocked for 2 h at room temperature. After washing with TBST for 10 min and 3 times, ECL luminescent reagent was added for development, and the gray value of the bands was analyzed by Image J software.

2.5. Immunofluorescence analysis

2 ml of peripheral blood in experiment group and control group was used for paraffin-embedded and sectioned at 5μm for immunofluorescence. Paraffin sections of samples were removed, dewaxed, rehydrated with gradient ethanol, and heated with citrate buffer for antigen repair. Primary antibody F4/80 (1:100) was added and incubated in a wet box at 4°C for 15 h overnight. A fluorescent secondary antibody was added for staining (red). After blocking with serum, primary antibodies NT-proBNP (1:100) were added and incubated in a wet box at 4°C for 15 h overnight. Fluorescent secondary antibody was added for staining (green), and the nuclei are counterstained.

2.6. Enzyme-linked immunosorbent assay analysis

Enzyme-linked immunosorbent assay (ELISA) was used to detect the IL-1 β, IL-18, TnI, and CK-MB levels in Serum of experiment group and control group. The 2 ml of peripheral blood samples in experiment group and control group were obtained and immediately separated using centrifugation at 12 000 r/min for 10 min at 4°C. A blank well, a standard well and a sample well were prospectively set up. 100 μL of sample diluent is added to blank wells, and 0, 31.25, 62.5, 125, 250, 500, 1000, 2000 pg·mL-1 of standard, 100 μL of sample were added to remaining wells. All wells were incubated at 37°C for 90 min and subsequently removed the liquid in the well, was dried and wash the wells. 100 μL of biotinylated antibody working solution was added to each well and incubated at 37°C for 60 min, and remove the liquid and wash the wells 3 times, and 100 μL of enzyme conjugate working solution was added to each well after that add film to the plate and the plate are incubated at 37°C for 30 min and then remove the liquid in the well, subsequently dry and wash the plate 5×times. 90 μL of chromogenic reagent was added to each well and the wells are incubated at 37°C for 15 min in the dark, and 50 μL of stop solution was added to each well to stop the reaction. The cytokines in the blood samples of two groups of patients were detected using commercially available ELISA kits (human IL-1 β, IL-18, TnI, CK-MB are provided by R and D System, Co., Minneapolis, MN, USA).

2.7. Statistical analysis

Statistic Package for Social Science (SPSS) 22.0 (IBM, Armonk, NY, USA) was used for statistical analysis. Measurement data were described by mean ± standard deviation, and One-way ANOVA was used for significance analysis. Non-parametric test (Kruskal-Wallis test) is used for significance analysis.

3. Results

3.1. The analysis of expression level of Sirt6 in experimental group and control group

The Sirt6 expression level in blood samples between experimental group and control group was monitored using WB and the outcomes are presented in Figure 1.

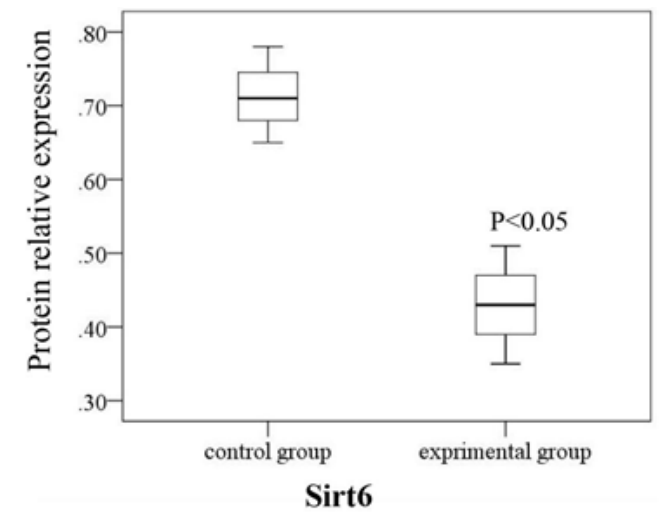

Fig. 1. The expression of Sirt6 protein in blood samples by western blot. Compared to the Sirt6 protein level in control group, the Sirt6 level in exprimental group is significantly down-regulated.

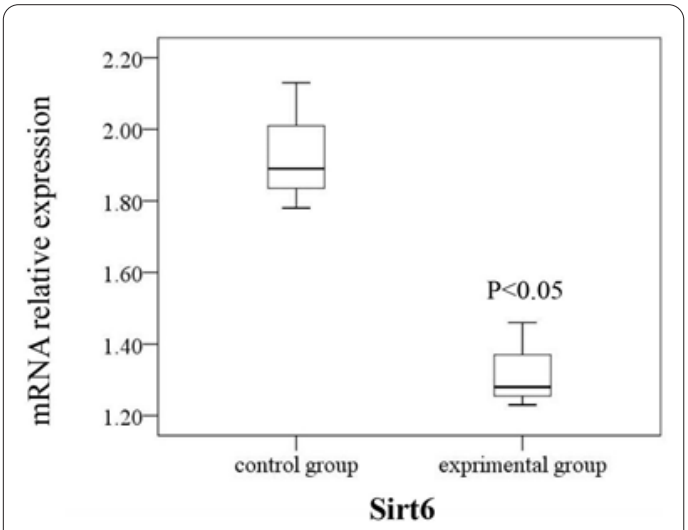

Fig. 2. The expression of Sirt6 mRNA in blood samples by real-time PCR. Compared to the Sirt6 mRNA level in control group, the Sirt6 mRNA level in exprimental group is significantly down-regulated.

Compared to Sirt6 protein level in control group, the Sirt6 protein level in experimental group was significantly down-regulated. Our study results revealed that Sirt6 may have association with the development progress of ACS.

3.2. The comparison of the Sirt6 mRNA expression between two groups is detected by real-time PCR

The Sirt6 mRNA levels in blood samples between experimental group and control group were detected using real-time PCR. As depicted in Figure 2. Compared to the Sirt6 mRNA level in the blood sample in control group, the level of Sirt6 mRNA in experimental group was clearly down-regulated. This study outcomes show the mRNA level of Sirt6 in the experimental group was obviously less than that in control group. These results indicate that Sirt6 mRNA may had association with the development progress of ACS.

3.3. The comparison of cytokines IL-1β, IL-18, TnI, CK-MB expression level between two groups are detected by ELISA

Patients with ACS can lead to inflammatory damage by generating numerous cytokines. Of which, the change of IL-1β, IL-18, TnI, CK-MB levels between the two groups were remarkable. As depicted in Figure 3. Present study outcomes suggest that IL-1β, IL-18, TnI, and CK-MB levels in blood samples in the experimental group were significantly higher compared to those in control group. These outcomes suggest that the cytokines IL-1β, IL-18, TnI, CK-MB in blood sample of patients with ACS were dramatically increased.

3.4. The comparison of the cytokines NT-proBNP expression between two groups are detected by immunofluorescence

As depicted in Figure 4. To investigate the efficacy of the cytokines NT-proBNP in patients with ACS, the cytokines NT-proBNP between two groups were detected by immunofluorescence. Compared to the control group, NT-proBNP level in control group is obviously up-regulated. These findings suggest that up-regulated of cytokines NT-proBNP can promote the development of ACS.

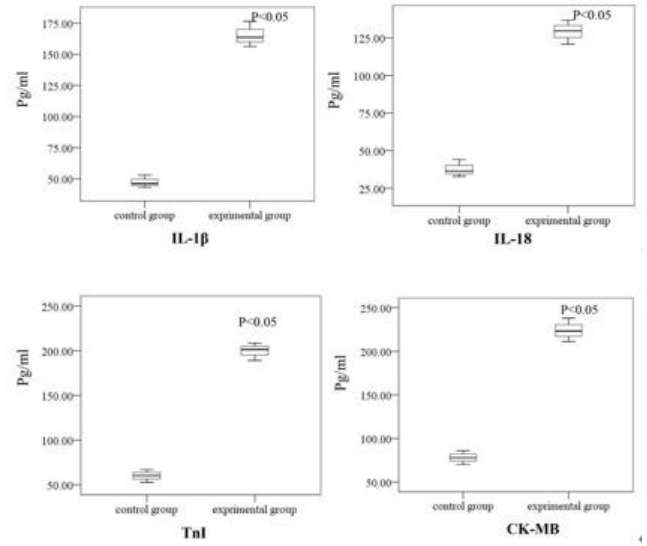

Fig. 3. The expression level of cytokines IL-1β, IL-18, TnI, CK-MB in blood samples between two groups by ELISA. The levels of IL-1β, IL-18, TnI, CK-MB in experimental group are remarkably clearly upregulated.

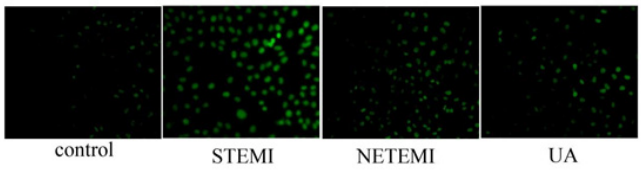

Fig. 4. The expression level of the cytokines NT-proBNP in blood samples from control group patients, STEMI patients, NETEMI patients and UA patients by immunofluorescence. The expression level of the cytokines NT-proBNP depicted in Figure 4. The expression level of NT-proBNP in STEMI patients, NETEMI patients and UA patients are remarkably up-regulated.

4. Discussion

Acute coronary syndromes easily lead to bleeding and subsequent myocardial infarction. Therefore, acute coronary syndromes increase the risk of long-term death. It is urgent to find an effective therapy method for ACS [15]. The regarding results have reported that Sirt6 signaling has been associated with the development of ACS, and this study suggests that Sirt6 can be a promising target to treat the ACS [7]. Sirt6, as a key cellular pathway checkpoint controller, participates in the development of ACS, and have a protective effect on numerous organs such as the heart, blood vessels and the brain, and Sirt6 may have an association with ACS. However, the detailed mechanism of Sirt6 in patients with ACS remains unclear. In present clinical study, we recruited the following two groups of patients: experimental group consists of patients with ACS and control group consists of patients with non-coronary heart disease outpatients or inpatients, and present study results suggest that Sirt6 protein and mRNA in ACS patients are significantly downregulated. Our study outcomes show that the up-regulated of Sirt6 may be involved in the development of ACS, and Sirt6 is a promising target to treat the ACS. In previous studies, Sirt6 protein expression in ACS patients by western blotting is also clearly up-regulated versus ordinary people, it can clearly be seen that present results are consistent with the corresponding studies [7,16], which indicates that Sirt6 is a promising target to treat the ACS.

Currently, it is reported that inflammatory processes are also involved in the occurrence and development of ACS, and they are considered to be the pathogenic factors of ACS [12,14]. Abnormal inflammatory cytokines such as IL-1 β, IL-18, TnI, CK-MB, NT-proBNP are involved in development of plaque instability and ACS, and these inflammatory cytokines are associated with atherosclerosis and are considered to be causative factors of ACS [17,18]. Therefore, IL-1 β, IL-18, TnI, CK-MB, NT-proBNP expression in our study are detected to investigate the relationship of these abnormal inflammatory cytokines in ACS. Present results show that the levels of IL-1 β, IL-18, TnI, CK-MB, NT-proBNP in ACS patients are obviously upregulated. These findings demonstrate that the up-regulated of these inflammatory cytokines can accelerate the development of ACS. These results are consistent with those previous corresponding clinical study report [19].

We can conclude from our study that Sirt6 levels in ACS patients may be involved in the development of ACS and abnormal up-regulated for inflammatory cytokines such as IL-1 β, IL-18, TnI, CK-MB, NT-proBNP can promote the development.

Competing interests

All authors declare no conflict of interest.

Consent to publish

All of the authors have Consented to publish this research.

Ethics approval and consent to participate

The ethic approval was obtained from the Ethic Committee of ShengLi Oil Field Central Hospital.

Availability of data and materials

The data are free access to available upon request.

Authors' contributions

Each author has made an important scientific contribution to the study and has assisted with the drafting or revising of the manuscript.

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