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Original Article

Effect of nicorandil combined with trimetazidine on miR-223-3p and NRF2 expression in patients with coronary heart disease

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Abstract: Objective: The aim of this study was to explore the effect of nicorandil (NCR) combined with trimetazidine (TMZ) on miR-223-3p and NRF2 expression in patients with coronary heart disease (CHD). Methods: This study included 71 CHD patients admitted to our hospital from February 2017 to March 2019, including 33 cases in the control group (CG) treated with NCR and 38 cases in the research group (RG) treated with TMZ combined with NCR. Improvement in clinical efficacy after treatment was observed in the two groups; serum miR-223-3p and NRF2 levels pre- and post-treatment were compared, and the predictive value of the two for curative effect was analyzed. In addition, ST segment depression frequency and total duration, pre- and post-treatment cardiac function levels and blood lipid levels were recorded and compared. Results: RG had statistically more markedly effective cases and notably lower serum miR-223-3p and NRF2 expression than CG after treatment. Through receiver operating characteristic (ROC) curve analysis, it was found that the area under curves (AUCs) of miR-223-3p and NRF2 were 0.716 and 0.712 respectively. The post-treatment ST segment depression frequency and duration were lower in RG than in CG ($P<0.05$). Cardiac function and blood lipid levels were significantly better in RG as compared to those in CG after treatment ($P<0.05$). Conclusions: NCR combined with TMZ is more effective in patients with CHD, and miR-223-3p and NRF2 can be predictors of clinical efficacy.

Keywords: Coronary heart disease, nicorandil combined with trimetazidine, miR-223-3p, NRF2, efficacy

Introduction

Coronary heart disease (CHD) is considered the leading cause of death worldwide [1]. CHD, a heart disease with myocardial ischemia, hypoxia or necrosis triggered by atherosclerotic lesion of coronary artery which causes stenosis or obstruction of vascular lumen, presents high incidence in middle-aged and elderly people [2, 3]. Relevant statistical results showed that in 2015, its prevalence and mortality in Chinese population was 2.23% and 1.75% respectively, ranking among the top three in the world [4]. Therefore, attention to CHD, early diagnosis and active treatment, as well as rapid control of the cardiac function and blood lipid levels in patients with CHD is of great significance.

It is shown that statins can improve patients' clinical symptoms and reduce the incidence of ST-segment elevation myocardial infarction in the long run [5, 6]. However, statins also have some adverse drug reactions, such as hepatotoxicity and muscular toxicity [7, 8], so many scholars are searching for potential better drugs that can be used for CHD treatment. Nicorandil (NCR) is an antianginal drug with dual action mechanism. As far as we know, it is currently the only potassium channel opener with antianginal effect, which has effective dilatation effect on arteries, veins and coronary arteries without seriously affecting heart rate, myocardial contractility and conduction system [9-11]. While trimetazidine (TMZ) is another antianginal medicine, which can control the symptoms of myocardial ischemia through metabolic changes. Ciapponi et al. [12] reported that TMZ can safely and effectively alleviate the condition when first-line drugs cannot control the disease. However, whether NCR combined with TMZ can play an effective therapeutic effect as a combination drug therapy remains

Table 1. Primer sequences

	Upstream sequence	Downstream sequence
U6	5'-CTCGCTTCGGCAGCACA-3'	5'-AACGCTTCACGAATTTGCGT-3'
miR-223-3p	5'-TGCCAT-AGTCTCTGCTGCTCAAG-3'	5'-CCAACACCAC-GAACACGAAGTC-3'

to be studied. MiRNAs, a type of small non-coding RNA, are involved in the regulation of various physiological and pathological functions, and modulation of target gene expression by functioning as endogenous RNA interference [13]. Bioinformatics data indicate that one single miRNA can bind to hundreds of target mRNAs, thus interfering with various biological processes [14].

Therefore, by exploring the efficacy of NCR combined with TMZ in the treatment of patients with CHD, and the predictive value of miR-223-3p and NRF2, this experiment aims to provide deeper insights into CHD treatment for future clinical practice.

Materials and methods

Clinical data

This study collected 71 patients with CHD admitted to Xingtai People's Hospital from February 2017 to March 2019. Among them, 33 patients, aged 45-73 years, with an average age of 58.69 ± 4.28 years were included in the control group (CG) for NCR treatment. The rest 38 cases, with the age ranging from 45-72 years and a mean age of 59.13 ± 4.38 years, were assigned to the research group (RG) for NCR combined with TMZ treatment. The Medical Ethics Committee of Xingtai People's Hospital approved the study protocol.

Inclusion and exclusion criteria

Inclusion criteria: Aged 40-75 years, all the patients met the diagnostic criteria of *China Medicine* [15], and were free of communication barrier and mental illness, with a course of disease of 5-11 years and complete case data. The patients or their next of kin had provided the informed consent to give the consent for the participation.

Exclusion criteria: Patients withdrew from the experiment, with malignant tumors or severe organ dysfunction, a history of heart surgery or coronary intervention, infectious diseases, poor treatment compliance, physical disability, language impairment, or referred patients.

Treatment regimen

After diagnosis, all the patients were given routine measures such as improving internal circulation, controlling blood pressure and life guidance. In addition, patients in CG were injected intravenously with 24 mg NCR (Guangzhou Baiyunshan Mingxing Pharmaceutical, National Drug Approval No.: H44024571) in 100 mL of 0.9% sodium chloride solution at a rate of 10 to 15 mL per hour, once daily. On the basis of the above treatment, patients in RG were treated with TMZ (Suzhou Pharmaceutical Factory, Jiangsu Wuzhong Pharmaceutical Group, National Drug Approval No.: H20073709) orally, 20 mg each time, 3 times a day. All the patients were treated consecutively for 3 months.

Efficacy evaluation

The clinical efficiency, the ST segment depression frequency and total duration within 24 h were observed. Markedly effective: clinical symptoms disappeared after treatment, and cardiac function improved by 2 levels; Effective: clinical symptoms improved after treatment and cardiac function improved by 1 level; Ineffective: clinical symptoms did not improve or even worsened after treatment. Total effective = (markedly effective + effective) cases/total cases × 100%.

Main reagents

NRF2 kit (Wuhan Yipu Biotechnology, Cat. No.: MM-2402H1); TRIzol reagents and miRNA reverse transcriptase kits (Invitrogen, USA); EasyPure miRNA Kit (Beijing TransGen Biotechnology, ER601-01); TransScript ® Green miRNA Two-Step qRT-PCR SuperMix (Beijing TransGen Biotechnology, AQ202-01); KH19A desktop high-speed high-performance centrifuge (KAIDA Company); -80°C low temperature refrigerator (Thermo Fisher Scientific, United States). See **Table 1** for miR-223-3p's primer

Table 2. Basic clinical data [n (%)]

	Research group (n=38)	CG (n=33)	χ^2 or t	P
Age (years)	59.13 ± 4.38	58.69 ± 4.28	0.427	0.671
Exercise history			0.444	0.505
Yes	11 (28.95)	12 (36.36)		
No	27 (71.05)	21 (63.64)		
BMI	21.05 ± 1.24	21.02 ± 1.17	0.104	0.917
Smoking history			0.095	0.758
Yes	14 (36.84)	11 (33.33)		
No	24 (63.16)	22 (66.67)		
Drinking history			0.122	0.727
Yes	18 (47.37)	17 (51.52)		
No	20 (52.63)	16 (48.48)		
Residence			0.207	0.649
Urban	21 (55.26)	20 (60.61)		
Rural	17 (44.74)	13 (39.39)		
Dietary preference			4.703	0.995
Light	15 (39.47)	13 (39.39)		
Spicy	23 (60.53)	20 (60.61)		
Frequency of ST segment depression (24/h)	7.13 ± 1.22	7.23 ± 1.23	0.343	0.733
Total duration of ST segment depression (min)	53.86 ± 10.94	53.69 ± 10.93	0.065	0.948

sequences designed and synthesized by Shanghai Sangon Biotechnology.

QRT-PCR and ELISA detection

Before and after treatment, fasting venous blood (4 mL) was collected from patients in the early morning, left to rest for 30 min at room temperature and centrifuged for 10 min (3000 rpm/min). The obtained upper serum was aliquoted into non-enzyme EP tubes, some of which were used in the experiment, and the rest were preserved at -80°C. EasyPure miRNA Kit was employed for total RNA extraction, and UV spectrophotometer and agarose gel electrophoresis were utilized for purity, concentration and integrity determination. Then the reverse transcription of obtained total RNA was performed using TransScript Green miRNA Two-Step qRT-PCR SuperMix. cDNA was collected for PCR amplification experiments. Primer sequences are detailed in **Table 1**. qPCR amplification system: cDNA: 1 μL, upstream and downstream primers: 0.4 μL each, 2 × TransTaq® Tip Green qPCR SuperMix: 10 μL, Passive Reference Dye (50 ×): 0.4 μL, and ddH_2O in a volume of 20 μL. qPCR amplification conditions (40 cycles): 94°C/30 s, 94°C/5 s, 60°C/30 s. There were 3 replicate holes for each tested sample and the experiment was run in triplicate. The internal reference was U6, and $2^{-\Delta ct}$ was used for data analysis.

Outcome measures

Main endpoints: Improvement of clinical efficacy after treatment, serum miR-223-3p and NRF2 levels pre- and post-treatment and their predictive value for curative effect were observed.

Secondary endpoints: ST segment depression frequency and total duration, and pre- and post-treatment cardiac function blood lipid levels were observed.

Statistical methods

SPSS20.0 and GraphPad 7 were employed for statistical analysis and image processing of data respectively. The distribution analysis of the dose data was conducted by K-S test. Those conformed to normal distribution were represented by mean ± standard deviation (mean ± SD) and compared by independent sample t-test and paired t-test within the group and between groups respectively. The counting data, described in the form of percentage (%), were analyzed by the chi-square test (denoted by χ^2). The significance of miR-223-3p and NRF2 in predicting curative effect of CHD

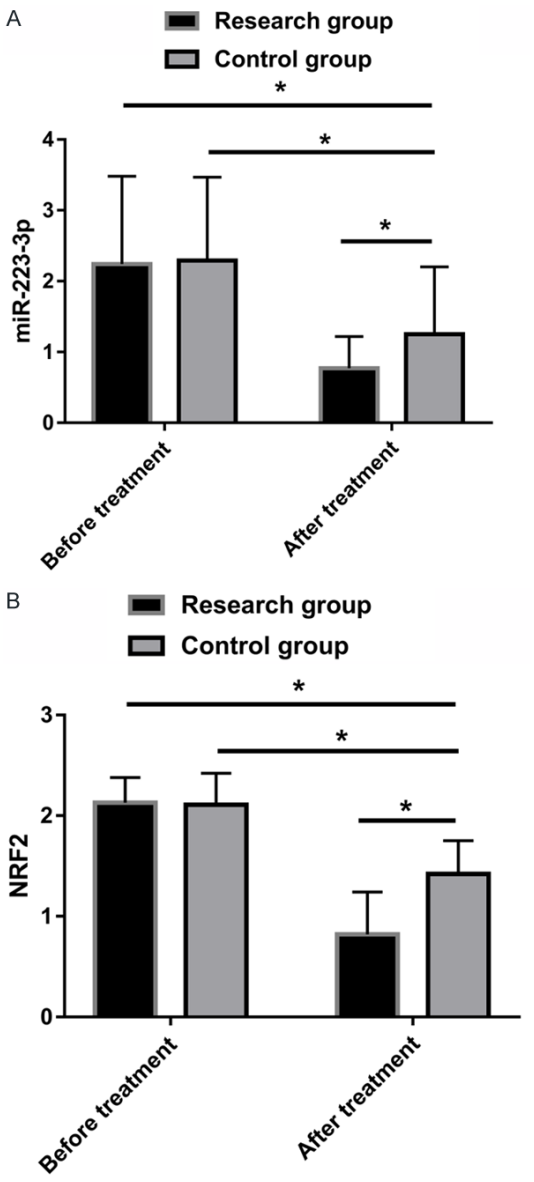

Figure 1. MiR-223-3p and NRF2 expression. A. The miR-223-3p level in the research group declined noticeably after treatment, and was lower than that in the control group. B. The NRF2 level in the research group reduced significantly after treatment, and was lower than that in the control group. Note: * indicates P<0.05 between the two groups.

patients was analyzed by receiver operating characteristic (ROC) curves. When P<0.05, there was a statistical difference.

Results

General information

Clinical data: The clinical data of CG, including age, hypertension history, BMI, smoking history, drinking history, place of residence, dietary preference, diastolic blood pressure, and systolic blood pressure, did not differ markedly from those of RG, which proved to be comparable (P>0.05) (**Table 2**).

Efficacy: In comparison with CG, the clinical efficacy of RG was remarkably higher in terms of markedly effectiveness, and notably lower in terms of ineffectiveness (P<0.05), while little difference was identified in effectiveness (P> 0.05). As to overall effectiveness, RG was also superior to CG (P<0.05).

Serum miR-223-3p and miR-210 expression pre- and post-treatment: No marked differences were identified in miR-223-3p and NRF2 between CG and RG before treatment (P> 0.05); however, miR-223-3p and NRF2 decreased dramatically after treatment in RG as compared to CG (P<0.05) (**Figure 1**; **Table 3**).

ROC curve: ROC curve analysis demonstrated that the AUCs of miR-223-3p and NRF2 were 0.716 and 0.712 respectively in predicting curative effect (**Table 4**; **Figure 2**).

ST segment depression frequency and total duration: No noteworthy differences were observed in ST segment depression frequency and total duration before treatment between RG and CG (P>0.05), but the post-treatment ST segment depression frequency and total duration were statistically lower in RG (P<0.05) (**Figure 3**).

Observation of cardiac function levels pre- and post-treatment: The cardiac function levels showed no evident differences between RG and CG before treatment (P<0.05), while the levels were statistically better in RG than in CG after treatment (P<0.05) (**Figure 4**).

Observation of serum lipid levels pre- and post-treatment: Statistical differences were absent in pre-treatment serum lipid levels between RG and CG (P<0.05), but the post-treatment lipid levels were statistically better in RG (P<0.05) (**Figure 5**).

Discussion

CHD is a major threat to human health and a prime cause of death and disability [16, 17]. Occlusion or stenosis of the coronary arteries causes myocardial dysfunction, or shortage of

Table 3. Comparison of efficacy between the two groups

	Cases (n)	Treatment efficacy [n (%)]			Effective treatment rate (%)
		Markedly effective	Effective	Ineffective	
Research group	38	24 (63.16)	13 (34.21)	1 (2.63)	97.37
CG	33	12 (36.36)	15 (45.46)	6 (18.18)	81.82
χ^2		5.073	0.935	4.806	4.806
P		0.024	0.334	0.028	0.028

Table 4. ROC curve

	miR-223-3p	NRF2
AUC	0.716	0.712
Std. Error	0.043	0.044
95% CI	0.631-0.801	0.626-0.797
P	0.001	0.001
Cut-off	11.040	1.710
Sensitivity (%)	78.87	64.79
Specificity (%)	61.97	73.24

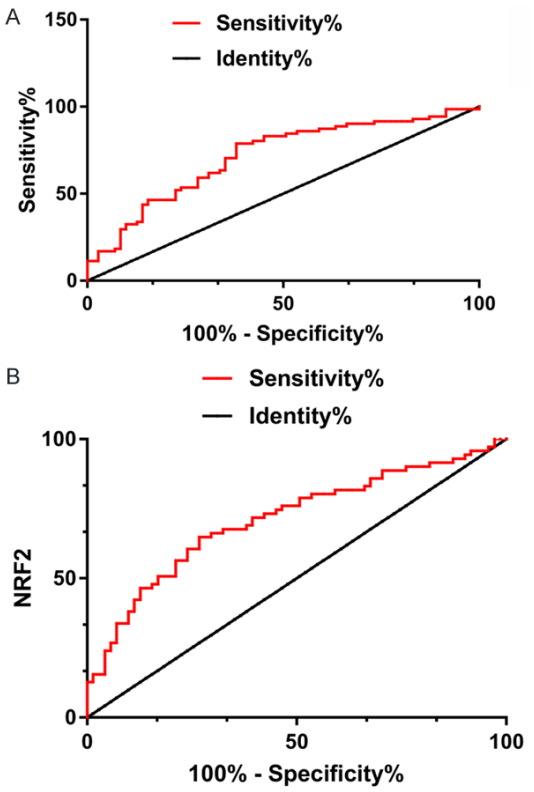

Figure 2. ROC curve analysis. A. The AUC of miR-223-3p was 0.716, the sensitivity was 78.87%, and the specificity was 61.97%. B. The AUC of NRF2 was 0.712, the sensitivity was 64.79%, and the specificity was 73.24%.

blood and oxygen supply, which leads to myocardial infarction, angina instability and heart failure [18, 19], and causes myocardial necrosis, ischemia and hypoxia [20]. Although great leap has been made in CHD diagnosis and treatment in the past decade, the deduction of morbidity and mortality remains disappointed.

NCR is a nitrate compound, which is capable of inhibiting intracellular calcium ion dissociation, increasing the permeability of cell membrane to potassium ions, dilating coronary vessels, continuously increasing coronary blood flow, and suppressing coronary artery spasm [21]. TMZ belongs to other categories of antiangina cardiovascular drugs. It prevents the decrease of intracellular ATP level by protecting cell energy metabolism under hypoxia or ischemia, thus ensuring the normal function of ion pump and the normal operation of membrane permeating sodium-potassium flow, and maintaining the stability of intracellular environment [22, 23].

In this study, we first compared post-treatment clinical efficacy between CG and RG. It demonstrated that the clinical efficacy and overall effective rate were statistically better in RG, with a lower ineffectiveness rate, which indicated that NCR combined with TMZ could improve the effective treatment rate of patients. There is numerous literature evaluating the incidence and risk factors of CHD, but little has been done to assess the correlation between clinical features and biological indicators pre- and post-treatment. MiRNAs, which influence and modulate approximately 30% of the coding proteins in the human body, are able to degrade target genes and inhibit target gene translation, thus achieving post-transcriptional silencing [24, 25]. In the present study, we observed no evident differences in serum miR-223-3p and NRF2 levels between CG and RG before treatment; however, their post-treatment expression levels declined statistically in RG compared to CG, indicating that NCR combined with TMZ can better inhibit miR-223-3p and NRF2 expression. Further, ROC curves

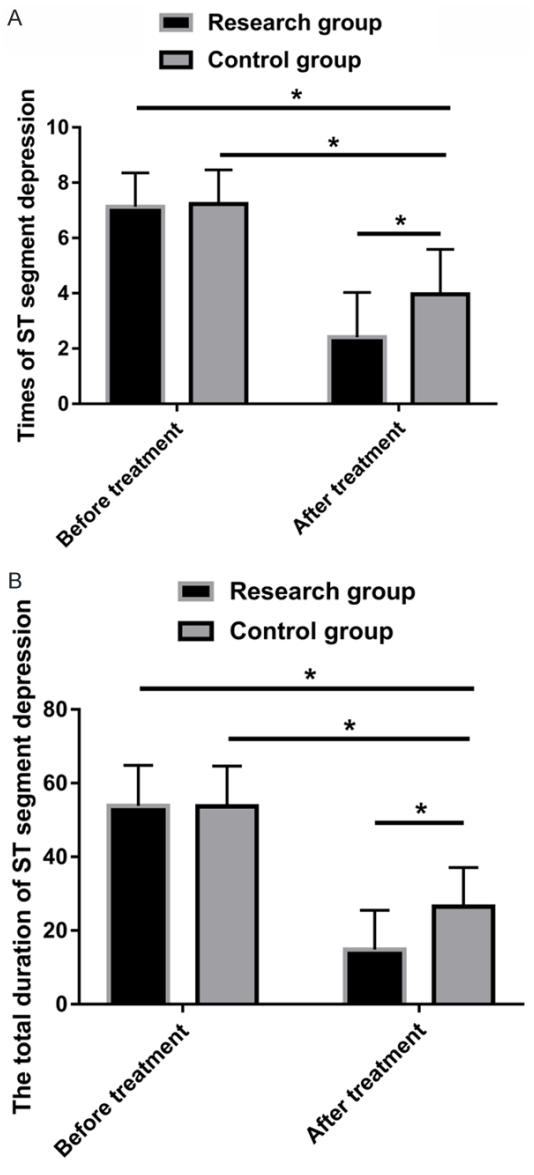

Figure 3. Frequency and total duration of ST segment depression. A. The post-treatment frequency of ST segment depression in the research group was significantly lower than that in the control group. B. The post-treatment total duration of ST segment depression in the research group was significantly lower than that in the control group. Note: * indicates P<0.05 between the two groups.

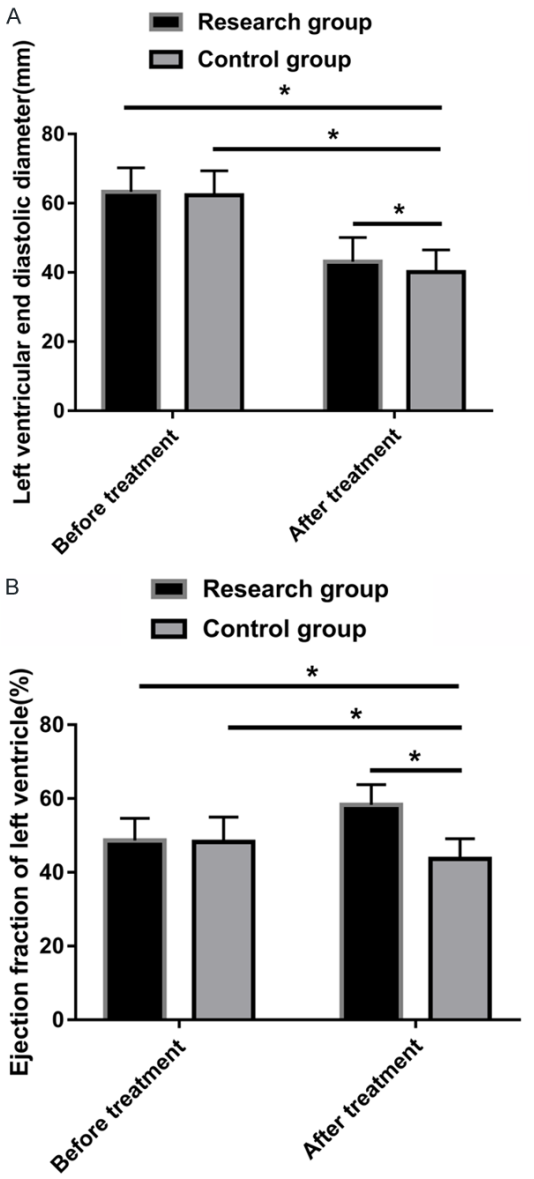

Figure 4. Cardiac function level. A. The left ventricular end-diastolic diameter in the research group decreased significantly after treatment, and was lower than that in the control group. B. The post-treatment left ventricular ejection fraction in the research group decreased significantly, and was lower than that in the control group. Note: * indicates P<0.05 between the two groups.

were drawn to find that the AUCs for miR-223-3p and NRF2 were 0.716 and 0.712 respectively, which indicated high clinical predictive value. We also detected cardiac function and blood lipid levels of patients pre- and post-treatment, and found that both left ventricular end-diastolic diameter and left ventricular ejection fraction were reduced significantly in RG after treatment, and were lower than those in CG; in addition, post-treatment triglycerides and total cholesterol were also statistically reduced in RG as compared to CG. It exhibited that the combination of NCR and TMZ could effectively improve the cardiac function and reduce blood lipid levels of patients. By observing ST segment depression frequency and total duration, it was found that there was no significant difference

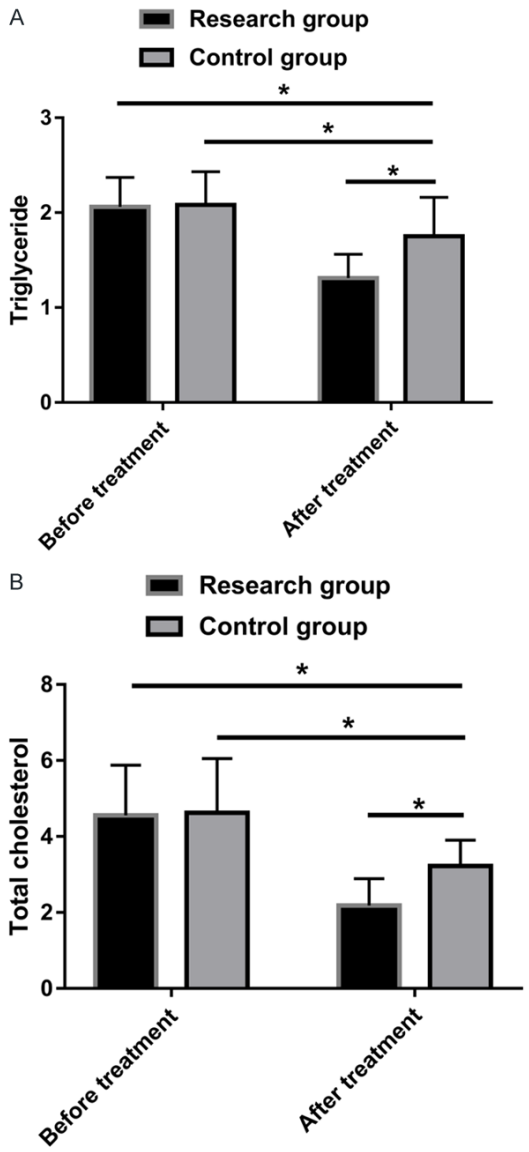

Figure 5. Serum lipid levels. A. The triglyceride in the research group reduced noticeably after treatment, and was lower than that in the control group. B. The total cholesterol in the research group dropped notably after treatment, and was lower than that in the control group. Note: * indicates P<0.05 between the two groups.

in these two parameters between RG and CG before treatment; however, post-treatment ST segment depression frequency and total duration were evidently better in RG, which further reflected the higher efficacy of NCR combined with TMZ regimen.

We have preliminarily demonstrated the predictive value of miR-223-3p and NRF2 and the clinical efficacy of NCR combined with TMZ in patients with CHD through the above studies. However, there are still some shortcomings. First, the drug dose used in this study is single, and we have not conducted an in-depth study on whether the treatment rate of patients can be improved by increasing the drug dose. Second, the prognostic follow up of patients was absent in the current study. In future studies, we hope to observe the clinical efficacy of patients by increasing the drug doses of different treatment regimens, and conduct random follow-up on the prognosis of patients to supplement our research results.

Taken together, the combination therapy of NCR and TMZ can improve the clinical efficacy and overall treatment rate of patients with CHD, and miR-223-3p and NRF2 can serve as predictors for clinical efficacy.

Disclosure of conflict of interest

None.

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