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ABOUT COVER

Editorial board member of *World Journal of Clinical Cases*, Dr. Kvolik is a Professor in the School of Medicine, Osijek University, Croatia. She obtained her MD degree, with specialization in the field of anesthesiology, resuscitation and intensive care from the Zagreb Medical School, Croatia. Afterwards, she undertook postgraduate training in Clinical Pharmacology at the same institution, defending both a Master's thesis and PhD thesis. In 2006, she joined the Osijek University Medical Faculty as a lecturer and was promoted to Professor in 2009. In 2012, she was elected Head of the Department of Anesthesiology, Resuscitation, Intensive Care and Pain Therapy, a position she occupies to this day. She is also the current Head of the Intensive Care Unit at the Osijek University Hospital, Croatia. (L-Editor: Filipodia)

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Retrospective Study

Significance of serum fibroblast growth factor-23 and miR-208b in pathogenesis of atrial fibrillation and their relationship with prognosis

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Abstract

BACKGROUND

The incidence and prevalence of atrial fibrillation are increasing each year, and this condition is one of the most common clinical arrhythmias.

AIM

To investigate the levels and significance of serum fibroblast growth factor 23 (FGF-23) and miR-208b in patients with atrial fibrillation and their relationship with prognosis.

METHODS

From May 2018 to October 2019, 240 patients with atrial fibrillation were selected as an observation group, including 134 with paroxysmal atrial fibrillation and 106 with persistent atrial fibrillation; 150 patients with healthy sinus rhythm were selected as a control group. The serum levels of FGF-23 and miR-208b in the two groups were measured. In the observation group, cardiac parameters were determined by echocardiography.

RESULTS

The serum levels of FGF-23 and miR-208b in the observation group were 210.20 ± 89.60 ng/mL and 5.30 ± 1.22 ng/mL, which were significantly higher than the corresponding values in the control group ($P < 0.05$). In the observation group, the serum levels of FGF-23 and miR-208b in patients with persistent atrial fibrillation were 234.22 ± 70.05 ng/mL and 5.83 ± 1.00 ng/mL, which were significantly higher than the corresponding values in patients with paroxysmal atrial fibrillation ($P < 0.05$). The left atrial dimension (LAD) of patients with persistent atrial fibrillation was 38.81 ± 5.11 mm, which was significantly higher than that of patients with paroxysmal atrial fibrillation ($P > 0.05$). The serum levels of FGF-23

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and miR-208b were positively correlated with the LAD ($r = 0.411$ and 0.382 , $P < 0.05$). In the observation group, the serum levels of FGF-23 and miR-208b in patients with a major cardiovascular event (MACE) were 243.30 ± 72.29 ng/mL and 6.12 ± 1.12 ng/mL, which were significantly higher than the corresponding values in patients without a MACE ($P < 0.05$).

CONCLUSION

The serum levels of FGF-23 and miR-208b are increased in patients with atrial fibrillation and are related to the type of disease, cardiac parameters, and prognosis.

Key words: Fibroblast growth factor-23; MiR-208b; Atrial fibrillation; Prognosis

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Core tip: In this study, the authors investigated the levels and significance of serum fibroblast growth factor 23 and miR-208b in patients with atrial fibrillation and their relationship with prognosis.

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INTRODUCTION

The incidence and prevalence of atrial fibrillation are increasing each year, and this condition is one of the most common clinical arrhythmias^[1,2]. Atrial fibrillation leads to stroke, thromboembolic events, heart failure, myocardial infarction, and cognitive decline, as well as an increased risk of chronic kidney disease and other diseases^[3]. The identification of useful serological markers can provide guidance for the primary prevention of atrial fibrillation and for monitoring and evaluating treatment efficacy and prognosis^[4]. Fibroblast growth factor 23 (FGF-23) is a member of the FGF superfamily and is closely related to the development of cardiovascular disease^[5]. Most microRNAs (miRNAs) exhibit tissue specificity and are stable in serum, making them potential biomarkers for disease diagnosis^[6]. MiR-208b was found in the plasma of patients with acute myocardial infarction, which indicates that heart-specific miR-208b can be released from damaged cardiomyocytes into the blood circulation^[7]. The present study aimed to investigate the levels and significance of serum FGF-23 and miR-208b in patients with atrial fibrillation and their relevance to patient prognosis.

MATERIALS AND METHODS

General information

In total, 240 patients with atrial fibrillation treated at our hospital from May 2018 to October 2019 were selected as an observation group, including 134 patients with paroxysmal atrial fibrillation and 106 with persistent atrial fibrillation. The inclusion criteria were as follows: (1) Diagnosis with atrial fibrillation according to the diagnostic criteria of the 2016 ESC atrial fibrillation management guidelines; (2) Cardiac function NYHA grade I-III; and (3) Complete clinical follow-up data. The exclusion criteria were as follows: (1) Heart diseases such as valvular heart disease; (2) Malignant tumors, immune system diseases, circulatory system diseases, and other diseases; and (3) History of infection within the past month. At the same time, 150 patients with normal sinus rhythm as determined by clinical examination were selected as a control group, and general data for the observation group and control group are compared in [Table 1](#).

Table 1 Comparison of general data between the two groups, *n* (%)

Group	<i>n</i>	Male	Age (yr)	BMI (kg/m ²)	Smoking	Drinking
Observation	240	168 (70.00)	60.50 ± 9.28	23.30 ± 5.44	103 (42.92)	63 (26.25)
Control	150	98 (65.33)	59.22 ± 10.11	22.83 ± 6.50	56 (37.33)	34 (22.67)
<i>t</i> / χ^2		0.927	1.280	0.769	1.192	0.634
<i>P</i> value		0.336	0.201	0.442	0.275	0.426

BMI: Body mass index.

Detection methods

Three milliliters of venous blood was taken at each time point, placed into an EDTA anticoagulant tube, and centrifuged at 2000 r/min at 4°C for 20 min; then, the supernatant was removed and stored at -80°C. Real-time quantitative PCR (qRT-PCR) was used to detect miR-208b in serum. For total RNA extraction, the serum was placed in an RNase-free centrifuge tube, the lysis buffer was added, and the solution was mixed thoroughly to lyse the cells according to the kit instructions. The RNA concentration and purity were detected according to spectrophotometry. RNA was then used for cDNA synthesis. Reverse transcription was performed in a reaction system containing 2 μ L of RNA template, 2 μ L of RT primer, 5 μ L of 5 × RT buffer, 2 μ L of dNTP (2.5 mmol/L), 0.5 μ L of RNase inhibitor (40 U/ μ L), 0.5 μ L of reverse transcriptase (20 U/ μ L), and 24 μ L of ddH₂O. cDNA was generated following the steps in the reverse transcription kit. qRT-PCR was then performed in a 20- μ L reaction system containing 2 μ L of cDNA, 10 μ L of 2 × SYBR Green qPCR mixture, 1 μ L of forward primer, 1 μ L of reverse primer, and 6 μ L of diethylpyrocarbonate-treated water. Each target gene was analyzed in triplicate with the following cycling conditions: 95°C for 10 min, followed by 40 cycles of 95°C for 15 min, 60°C for 1 min, and 72°C for 30 s. The qRT-PCR instrument was programmed for PCR amplification, and the 2^{- $\Delta\Delta$ CT} method was used to calculate the relative expression of miR-208b. β -actin was used as the internal reference gene.

Five milliliters of venous blood was collected from the middle of the elbow vein of fasted patients on the morning after admission. After 20 min of natural condensation, serum was isolated within 2 h after blood collection by centrifugation at 3000 r/min for 10 min. The isolated supernatant was stored in an Eppendorf tube at -80°C. The serum FGF-23 concentration was detected by enzyme-linked immunosorbent assay.

Cardiac function and structural indexes were determined using a DW-CE540 color Doppler ultrasound instrument (Dawei Medical Co., Ltd.) and an S5-1 probe with a frequency of 2-5 MHz. The patient was placed in the left lateral position, with the left ventricle near the long axis of the sternum. We measured the left ventricular systolic diameter, left ventricular end diastolic diameter (LVEDD), left ventricular dysfunction, and the distance from the left posterior wall of the atrium to the front wall of the left atrium and the back of the aorta. The apical four-chamber and two-chamber heart sections were adopted, and the left ventricular ejection fraction (LVEF) was calculated using Simpson's method. The values were continuously evaluated for 5 to 7 cardiac cycles, and the average was recorded.

Statistical analysis

The data were analyzed with SPSS 22.0 software and are reported as the mean ± SEM or *n* (%), as appropriate. The χ^2 test and analysis of differences were used to compare groups, and Pearson's correlation analysis was used to identify correlations. Differences were considered statistically significant at *P* < 0.05.

RESULTS

Comparison of serum FGF-23 and miR-208b levels in the two groups

The relative levels of serum FGF-23 and miR-208b in the observation group were significantly higher than those in the control group (*P* < 0.05, Table 2).

Table 2 Comparison of serum fibroblast growth factor-23 and miR-208b levels in the two groups

Group	n	FGF-23 (ng/mL)	Relative expression of miR-208b
Observation	240	210.20 ± 89.60	5.30 ± 1.22
Control	150	110.04 ± 32.29	2.90 ± 0.88
<i>t</i>		13.162	20.926
<i>P</i> value		0.000	0.000

FGF: Fibroblast growth factor.

Comparison of serum FGF-23 and miR-208b levels and cardiac parameters in different subgroups of the observation group

In the observation group, the relative levels of serum FGF-23 and miR-208b in patients with persistent atrial fibrillation were significantly higher than those in patients with paroxysmal atrial fibrillation ($P < 0.05$), and the LAD was significantly higher in patients with persistent atrial fibrillation than in patients with paroxysmal atrial fibrillation ($P > 0.05$, Table 3).

Correlation analysis

The relative levels of serum FGF-23 and miR-208b were analyzed for correlations with the LAD, and the results showed a positive correlation ($r = 0.411$ and $r = 0.382$, $P < 0.05$).

Comparison of serum FGF-23 and miR-208b among patients in the observation group with different prognoses

Patients were grouped based on the occurrence of a major cardiovascular event (MACE) ($n = 58$) or not ($n = 182$) during follow-up. MACEs included cardiogenic death, nonfatal myocardial infarction, heart failure, readmission due to recurrence, *etc.* The serum FGF-23 and miR-208b levels in the MACE group were 243.30 ± 72.29 ng/mL and 6.12 ± 1.12 ng/mL, respectively, and these values were significantly higher than those in patients without a MACE ($P < 0.05$, Table 4).

DISCUSSION

The mechanisms of atrial fibrillation occurrence and maintenance are complex. The electrical pulse from the pulmonary ectopic excitatory focus is considered an important mechanism of atrial fibrillation, and atrial fibrosis is considered an important factor in the difficulty of maintaining and sustaining atrial fibrillation^[8-10]. It is important to identify biomarkers with a high specificity and sensitivity. Cardiovascular diseases are usually accompanied by changes in miRNA levels in the peripheral circulation. Acute obstruction of the coronary artery disrupts blood flow, causing ischemia, death, heart failure, and cardiomyocyte death; therefore, early diagnosis and effective treatment are vitally important to improve patient prognosis^[11,12].

FGF-23 requires binding to FGF receptors to exert biological activity, and relies on the alpha-Klotho protein as a coreceptor^[13]. The alpha-Klotho protein shows tissue-specific expression with predominant distribution in the kidney and parathyroid glands. It is the main target organ of FGF-23^[14]. FGF-23 is linked to the onset of many cardiovascular diseases, and current research has confirmed^[15] that elevated levels of FGF-23 are significantly associated with left ventricular hypertrophy, hypertension, cardiogenic death, and all-cause death. Research shows^[16] that high levels of FGF-23 are an independent predictor of cardiovascular events. However, there are few reports on the relationship between FGF-23 levels and atrial fibrillation.

MiRNA levels typically change in the peripheral circulation of patients with heart failure, myocardial infarction, coronary heart disease, and other cardiovascular diseases. Both miR-208a and miR-208b are cardiac tissue-specific miRNAs^[17]. The results of this study showed that the relative levels of serum FGF-23 and miR-208b were significantly higher in the observation group than in the control group; besides, both levels were higher in patients with a MACE and changed significantly as the

Table 3 Comparison of serum fibroblast growth factor-23 and miR-208b in different subgroups of observation group

Group	<i>n</i>	Man, <i>n</i> (%)	Age (yr)	FGF-23 (ng/mL)	Relative expression of miR-208b	LAD (mm)	LVEDD (mm)	LVESD (mm)	LVEF (%)
Paroxysmal atrial fibrillation	134	98 (73.13)	61.02 ± 10.11	191.20 ± 65.59	4.88 ± 1.02	34.03 ± 4.02	50.03 ± 6.03	32.10 ± 5.58	53.30 ± 7.81
Persistent atrial fibrillation	106	70 (66.04)	59.89 ± 9.92	234.22 ± 70.05	5.83 ± 1.00	38.81 ± 5.11	49.81 ± 5.82	32.01 ± 6.02	52.83 ± 8.10
<i>t</i>		1.419	0.867	-4.896	-7.227	-8.112	0.285	0.120	0.455
<i>P</i> value		0.234	0.387	0.000	0.000	0.000	0.776	0.905	0.649

FGF: Fibroblast growth factor; LAD: Left atrial dimension; LVEDD: Left ventricular end diastolic diameter; LVEF: Left ventricular ejection fraction; LVESD: Left ventricular end-systolic diameter.

Table 4 Comparison of serum fibroblast growth factor-23 and miR-208b in patients with and without a major cardiovascular event

Group	<i>n</i>	Man, <i>n</i> (%)	Age (yr)	FGF-23 (ng/mL)	Relative expression of miR-208b
MACE	58	43 (74.14)	61.19 ± 10.54	243.30 ± 72.29	6.12 ± 1.12
Non-MACE	182	125 (68.68)	62.28 ± 9.89	199.65 ± 71.43	5.04 ± 1.07
<i>t</i>		0.624	-0.719	4.041	6.619
<i>P</i> value		0.43	0.473	0.000	0.000

FGF: Fibroblast growth factor; MACE: Major cardiovascular event.

patient's condition deteriorated, suggesting that high FGF-23 levels in patients with atrial fibrillation are closely related to the maintenance of this condition. The increases in FGF-23 and miR-208b may be associated with factors such as atrial overload, atrial systolic dysfunction, and decreased cardiac function. Factors such as FGF-23 and miR-208b may act as independent predictors of adverse events in patients with atrial fibrillation. The mechanism of FGF-23 involves increasing the sodium current in atrial myocytes by activating the protein kinase C signal transduction pathway. The L-type calcium current and calcium transients lead to calcium and sodium imbalance. Through the oxidative stress pathway, there is excessive formation of mitochondrial reactive oxygen species in cardiomyocytes, which increases the incidence of arrhythmia originating in the pulmonary vein.

LAD, LVEDD, left ventricular end-systolic diameter, and LVEF may reflect the severity of atrial fibrillation^[7,18]. The results of this study showed that patients with atrial fibrillation had an elevated LAD and a corresponding increase in the severity of atrial fibrillation, similar to the effects of previous studies, suggesting that the LAD may be a risk factor for the occurrence and severity of atrial fibrillation. Potential reasons for this association are that inflammatory-mediated atrial structural remodeling and electrical remodeling in patients with atrial fibrillation may be essential factors for the maintenance and development of atrial fibrillation. The extent of left atrial enlargement is closely related to the recurrence of atrial fibrillation and thrombosis. Studies show^[19,20] that the occurrence and development of atrial fibrillation are influenced by many mechanisms and factors, especially those related to left atrial structural remodeling and electrophysiological modifications. This study not only examined the changes in cardiac parameters of the LAD but also compared the changes in serum FGF-23 and miR-208b in different patients; these factors were found to be related to the severity of atrial fibrillation, and our findings are helpful for the diagnosis, evaluation, and prognosis of patients with atrial fibrillation. However, this test also has some defects, such as failure to reveal the relationship between these factors and the linearity of atrial fibrillation or some other aspects. For this, we need to further conduct a large number of experiments

In summary, serum FGF-23 and miR-208b levels are elevated in patients with atrial fibrillation and correlate with disease type, cardiac parameters, and prognosis.

ARTICLE HIGHLIGHTS

Research background

The incidence and prevalence of atrial fibrillation are increasing each year, and this condition is one of the most common clinical arrhythmias

Research motivation

The identification of useful serological markers can provide guidance for the primary prevention of atrial fibrillation and for monitoring and evaluating treatment efficacy and prognosis

Research objectives

To investigate the levels and significance of serum fibroblast growth factor (FGF)-23 and miR-208b in atrial fibrillation and their relevance to patient prognosis

Research methods

A total of 240 patients with atrial fibrillation treated at our hospital from May 2018 to October 2019 were included.

Research results

The serum levels of FGF-23 and miR-208b in the observation group were significantly higher than the corresponding values in the control group. In the observation group, the serum levels of FGF-23 and miR-208b in patients with persistent atrial fibrillation were significantly higher than those in patients with paroxysmal atrial fibrillation. The left atrial dimension (LAD) of patients with persistent atrial fibrillation was significantly higher than that of patients with paroxysmal atrial fibrillation. The serum levels of FGF-23 and miR-208b were positively correlated with the LAD. In the observation group, the serum levels of FGF-23 and miR-208b in patients with a major cardiovascular event (MACE) were significantly higher than the corresponding values in patients without a MACE.

Research conclusions

Serum FGF-23 and miR-208b levels are elevated in patients with atrial fibrillation and correlate with disease type, cardiac parameters, and prognosis.

Research perspectives

Our findings may suggest a new method for evaluating the condition of atrial fibrillation.

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