Abstract. – OBJECTIVE: Changes of microRNAs (miRNAs) may contribute to the pathogenesis and progression of postoperative atrial fibrillation (POAF) in patients undergoing cardiac valve surgery. This study aimed to measure the expression levels of miRNAs in peripheral blood, as well as their target mRNAs, in POAF patients and normal controls (non-POAF), and to evaluate the potential of miRNAs as promising biomarkers to predict POAF.

PATIENTS AND METHODS: The expression of miRNAs in peripheral blood, including miR-27b, miR-133a, miR-328, miR-499 and their target mRNAs, was analyzed in 109 POAF patients and 96 non-POAF patients via quantitative real-time polymerase chain reaction (RT-PCR). We compared differences between the two groups and also analyzed the treatment reaction to amiodarone.

RESULTS: All miRNAs in POAF patients were significantly highly expressed. Compared to non-POAF, the expression of miR-27b, miR-133a, miR-328, miR-499 increased in both groups of POAF patients, and miR-499 was only up-regulated miRNAs in the amiodarone - group versus amiodarone + group and non-POAF. Among the upregulated miRNAs, miR-499 expression significantly changed amiodarone + and amiodarone - patients (P < 0.005). The ROC curve analysis revealed that miR-499 might be a potential therapeutic response biomarker. The miRNA–mRNA interactions revealed 10 mRNAs regulated by miR-27b, miR-133a, and miR-499.

CONCLUSION: We found an expression pattern of miR-27b, miR-328, and miR-499 was significantly different between these groups. High expression being observed in patients compared to non-POAF patients. Further, the present results showed that miR-499 was significantly upregulated in amiodarone - patients, compared to non-POAF, and amiodarone + patients. This finding indicated that miR-499 may be a potential biomarker for predicting the occurrence of POAF after cardiac valve surgery and treat the reaction to amiodarone.

Key Words: MicroRNAs, Postoperative atrial fibrillation, Cardiac surgery.

Introduction

Atrial fibrillation (AF) is one of the main causes of stroke, heart failure, sudden death, and cardiovascular disease and is considered to be an independent factor that increases all-cause mortality. New-onset postoperative atrial fibrillation (POAF) is encountered after cardiac surgery with a high frequency, especially the valve surgery. The incidence of POAF was reported from 20% to 50%. Many risk factors are related with POAF, including body mass index, age, gender, chronic obstructive disease, valvular heart disease, epicardial adipose tissue, and even obstructive sleep apnea. But the exact mechanism remains unclear.

MicroRNAs (miRNAs) are short non-coding RNA and play an important role in many biological processes such as cellular proliferation, differentiation, inflammation, and apoptosis. The human genome encodes more than 1000 miRNAs, of which 30% were detected in the cardiovascular system. Many miRNAs are involved in the regulation of atrial contractility, ion channel performance, and automaticity. MicroRNAs are also related to AF; however, the full range of the miRNAs functionality remains unknown.

Irregular expression of miRNAs is associated with POAF and may play a key role in this disease. In addition, the presence of miRNAs in biological fluids, such as semen, saliva and blood, makes them more attractive as diagnostic and prognostic biomarkers of diseases. The
The purpose of this study was to measure and compare the expression levels of peripheral blood miRNAs, including miR-27b, miR-133a, miR-328 and miR-499, as well as their target mRNAs in POAF patients and normal controls (non-POAF) after valve surgery. The efficacy of miRNAs as biomarkers to monitor the response of POAF patients to amiodarone was also evaluated.

Patients and Methods

Patients

This study was approved by institutional Review Board at Huai’an First People’s Hospital, Jiangsu, China, and informed consent was obtained from all patients recruited in this study.

From January 2019 to July 2019, one hundred and nine POAF patients (40 males and 69 females) undergoing valve surgeries, with the mean age of 62.6 ± 7.7 years, were recruited in our study. A total of 96 non-POAF patients were selected for the study by matching the most relevant clinic variables such as age, sex, ethnicity, as well as possible clinical characteristics such as types of surgery, hypertension, body mass index, myocardial infarction, smoking, left ventricular ejection fraction, and cardiopulmonary bypass. POAF was defined as multiple AF lasting >30 seconds, recorded by electrocardiogram monitor from immediately after heart surgery to discharge, requiring anti-AF treatment (usually intravenous amiodarone). All patients were screened to ensure that they had never experienced AF preoperative by direct questioning and 12-lead electrocardiogram examination before surgery. Other exclusion criteria included a history of severe heart disease, nervous system disease, consumption disorders, or hepatic insufficiency. Anti-arrhythmic drugs, but not β-blockers, left ventricular ejection fraction <35%, or emergency procedure surgery.

In POAF group, amiodarone was used to treat new-onset POAF with normal protocol. It was defined as amiodarone positive (amiodarone +) when sinus rhythm came back before discharge. Otherwise, it was amiodarone negative (amiodarone -). The recommended dose (300 mg intravenous loading dose followed by 600 mg orally twice daily for 5 days) shown to be safe and effective.

Samples Collections

Total RNA, including miRNA, was extracted using the miRNeasy Serum/Plasma kit and spike-in control (Qiagen, Redwood City, CA, USA), according to the manufacturer’s protocols. For quality control of the RNA isolation, three synthetic RNA spike-ins (UniSp2, UniSp4, UniSp5) from the RNA Spike-in Kit (Qiagen, Redwood City, CA, USA) were added to the samples prior to purification and concentrations recommended by the manufacturer. cDNA was synthesized from purified miRNA according to the protocol supplied with the miRCURY LNA RT Kit (Qiagen, Redwood City, CA, USA) with the addition of spike-ins (UniSp6 and cel-miR-39-3p) intended as a cDNA synthesis control.

The target genes were predicted by miRWalk2.0, an online complementary prediction tool ($p < 0.01; \text{SUM score} > 5$). The miR-Tar Base was used as an experimentally validated tool, and protein-protein interactions of target genes were evaluated based on STRING11.0 database. Moreover, the bimolecular network model was plotted to discover the interactions of miRNAs with target genes, visualized on Cytoscape3.6.1. Overall, the constructed miRNA-gene network provides information about genes involved in POAF.

Real-Time Polymerase Chain Reaction (RT-PCR)

Expression levels of miRNAs were further determined by RT-PCR. RT-PCR experiments were performed as usual. Briefly, RT-PCR was performed according to the manufacturer’s protocol (Bioteke, Beijing, China). A twenty-microliter reaction contained 2.5 μl reverse transcription products diluted five-fold, 600 nM of each primer, 10 μl 2× SYBR Green PCR master mix, 0.4 μl ROX, and 4.1 μl water. The reactions were prepared in a 96-well plate, and the thermal cycler program was as follows: 95°C for 2 min, followed by 40 cycles at 95°C for 15 s, and then 62°C for 32 s, on an ABI 7500 Real-Time PCR System. The thermal denaturation protocol was run at the end of the PCR to determine the number of products that were present in the reaction. All reactions were run in triplicate.

Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Science v. 20.0 (IBM, Armonk, NY, USA). Parametric variables were analyzed using Student’s t-test and ANOVA followed by the Tukey’s test. Nonpara-
Table 1. Comparison of baseline characteristics between the groups of patients with POAF or non-POAF.

<table>
<thead>
<tr>
<th>Variables</th>
<th>POAF [n = 109]</th>
<th>non-POAF [n = 96]</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>40 (36.7%)</td>
<td>39 (40.6%)</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>62.6 ± 7.7</td>
<td>60.3 ± 5.1</td>
<td>0.07</td>
</tr>
<tr>
<td>BMI</td>
<td>25.9 ± 3.1</td>
<td>26.2 ± 2.3</td>
<td>0.329</td>
</tr>
<tr>
<td>Smoking</td>
<td>32 (29.4%)</td>
<td>28 (29.2%)</td>
<td>0.655</td>
</tr>
<tr>
<td>Hypertension</td>
<td>77 (70.6%)</td>
<td>71 (74.0%)</td>
<td>0.202</td>
</tr>
<tr>
<td>PCI</td>
<td>13 (11.9%)</td>
<td>11 (11.5%)</td>
<td></td>
</tr>
<tr>
<td>GLU (mmol/L)</td>
<td>6.4 ± 2.7</td>
<td>6.1 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>54.6 ± 8.5</td>
<td>57.2 ± 7.9</td>
<td>0.05</td>
</tr>
<tr>
<td>LVDd (mm)</td>
<td>50.5 ± 6.3</td>
<td>48.2 ± 5.9</td>
<td>0.106</td>
</tr>
<tr>
<td>LADs (mm)</td>
<td>39.3 ± 4.4</td>
<td>37.5 ± 3.7</td>
<td>0.096</td>
</tr>
<tr>
<td>NYHA heart function before surgery</td>
<td>2.5 ± 0.7</td>
<td>2.2 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Operation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MVR (%)</td>
<td>67 (61.5%)</td>
<td>(60.4%)</td>
<td>0.72</td>
</tr>
<tr>
<td>AVR (%)</td>
<td>26 (23.8%)</td>
<td>26 (26.0%)</td>
<td>0.259</td>
</tr>
<tr>
<td>DVR (%)</td>
<td>16 (14.7%)</td>
<td>16 (16.0%)</td>
<td>0.316</td>
</tr>
<tr>
<td>CPB (min)</td>
<td>97.7 ± 16.2</td>
<td>93.4 ± 16.7</td>
<td>0.074</td>
</tr>
<tr>
<td>Ventilation time (h)</td>
<td>17.5 ± 2.1</td>
<td>15.9 ± 2.2</td>
<td>0.117</td>
</tr>
<tr>
<td>β-blocker</td>
<td>82 (81.7%)</td>
<td>73 (76.0%)</td>
<td>0.081</td>
</tr>
</tbody>
</table>

Values are presented as n (%), mean ± standard deviation, or median (range). POAF, Postoperative atrial fibrillation; BMI, body mass index; PCI, percutaneous coronary intervention; GLU, fasting blood-glucose; LVEF, left ventricular ejection fraction; LVDd, left ventricular diastolic diameter; LADs, left atrial end systolic diameter; CPB, cardiopulmonary bypass; NYHA, New York Heart Association; MVR, mitral valve replacement; AVR, aortic valve replacement; D VR, double valves replacement.

Results

Clinical Data of the Study Population

One hundred and nine POAF patients (40 males and 69 females), with a mean age of 62.6 ± 7.7 years, as well as 96 sex and age-matched non-POAF (39 males and 57 females), with a mean age of 60.3 ± 5.1 years, were recruited and compared. Clinical and paraclinical parameters indicated no significant differences in terms of age, sex, smoking, hypertension, myocardial infarction, left ventricular ejection fraction, operation and cardiopulmonary bypass (Table I). The patients in POAF group were subdivided into two groups, based on the electrocardiogram at discharge: amiodarone + (n=76) and amiodarone - (n=33). And no adverse effect caused by amiodarone was observed at the recommended dose.

Target Genes and Network Establishment

The miRNA–mRNA interactions showed 14 mRNAs regulated by miR-27b, miR-133, and miR-499 (Figure 1). Ingenuity Pathway Analysis (IPA) detected no target or role for miR-328 in regulating POAF mechanisms.

Expression Levels of MiRNAs in POAF Patients Reacted to Amiodarone

To evaluate the expression levels of miRNAs in the peripheral blood of POAF patients...
with regard to the treatment of amiodarone, four miRNAs (miR-27b, miR-133a, miR-328, and miR-499) were analyzed. As indicated in Figure 2, all selected miRNAs were significantly upregulated in POAF patients, compared to non-POAF.

Among miRNAs with increased levels, miR-499 was significantly upregulated in amiodarone + patients versus non-POAF ($p < 0.05$), and its expression level was different between the amiodarone + and amiodarone - groups ($p = 0.005$). Moreover, the ROC curve analysis revealed that miR-499 might be a potential therapeutic response biomarker (Figure 3). The ROC curve analysis showed an optimal cut-off point of 0.3 for the relative expression of miR-499 to discriminate between amiodarone + and amiodarone - groups, with a specificity of 62% and sensitivity of 79% ($AUC = 0.72; p = 0.006$). Analysis of the expression level of other miRNAs showed no significant differences between amiodarone + and amiodarone - groups.

**Discussion**

miRNAs have been shown to be highly stable in biological fluids, such as plasma, which provides a non-invasive and accessible method to obtain samples. In addition, studies on the expression of miRNA in human plasma provide unique opportunities in monitoring and diagnosing various diseases, such as AF. Besides, patients with paroxysmal and persistent AF exhibit different miRNA expression profiles.

In the present study, we evaluated miRNA expression patterns in the peripheral blood of POAF patients in terms of amiodarone + or amiodarone - and compared the results with non-POAF. The selected miRNAs showed significantly high expression in both groups of POAF patients, compared to non-POAF.
pared to non-POAF. Compared to amiodarone + patients, miR-499 was the only upregulated miRNAs in the amiodarone - group with significantly high expression.

Cardiac electrophysiology deterioration can promote atrial remodeling by partial losing of the function of ion channels, which were basis for maintaining atrial fibrillation. MiR-499 functionally control the ion regulating the activity of Ca$^{2+}$ and K$^+$ channels$^{14,15}$. In this study, we found that the expression level of miR-499 elevated in both amiodarone + and amiodarone - groups of patients versus non-POAF, and the amiodarone + group showed a lower expression of miR-499, compared to the amiodarone - group ($p = 0.005$). Moreover, the ROC curve analysis showed the potential capacity of miR-499 in predicting the treat reaction to amiodarone with specificity of 62% and sensitivity of 79% (AUC = 0.72).

The bioinformatic analysis conducted in this study showed that the relationship between miR-27b, miR-133, and miR-499, and mechanisms closely related to POAF, such as fibrosis, hypertrophy and cardiogenesis. Additionally, the 10 mRNAs (NR3C1, RTN4, TIMP3, SMAD7, NTF3, UBE2V2, ABCC1, FASLG, MATN2, and UB2VI) that were the targets of the candidate four miRNAs in this study are known to be involved in heart disease. In previous literature, the mRNAs SMAD7 and FASLG are associated with atrial fibrillation$^{16}$. SMAD7 expression was shown to be related to the progression of atrial fibrosis. FAS/FASLG expression plays a role in the regulation of apoptosis and atrial fibrillation. However, experimental results showed that the role of miR-499 in the AF process, but the relationship was not detected in our study. In prescriptive, we first verified that miR-499 can predict the treat reaction of POAF to amiodarone.

**Conclusions**

Briefly, the expression of miR-133a, miR-27b, miR-133, and miR-499 was significantly different between these groups, with a highly specific difference being observed in POAF patients compared to non-POAF patients. Also, the prescriptive results showed that miR-499 was remarkably upregulated in amiodarone - patients, compared to non-POAF, and amiodarone + patients. This finding first indicates that miR-499 may be a potential biomarker for predicting the treat reaction to amiodarone in POAF patients.

**Conflict of Interest**

The Authors declare that they have no conflict of interests.

**Declaration**

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