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GPIIb/IIIa expression changes in atrial fibrillation post radiofrequency ablation

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Abstract. – OBJECTIVE: Atrial fibrillation (AF) is one of the most common arrhythmias affecting the patient's quality of life, and its complications of thromboembolism can lead to serious consequences. AF patients are often in hypercoagulation status that can affect the prognosis. GPIIb/IIIa is a fibrinogen receptor that can bind to the ligands of platelet and cause aggregation. Therefore, GPIIb/IIIa can be treated as a marker of hemagglutination. This work aims to analyze the changes of GPIIb/IIIa after radiofrequency ablation of atrial fibrillation, and to investigate its relationship with recurrence.

PATIENTS AND METHODS: A total of AF 80 patients in our hospital received radiofrequency ablation from January 2017 to August 2017. Peripheral blood was collected 1 week after surgery. A total of 40 healthy volunteers were enrolled as control group. GPIIb/IIIa was analyzed by enzyme-linked immunosorbent assay (ELISA). High-sensitivity troponin (hs-cTnT), fasting plasma glucose (FPG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), total cholesterol (TC), and triglyceride levels (TG) were analyzed by using electrochemical luminescence assay. Body mass index (BMI), smoking index, and age were recorded.

RESULTS: Compared with the non-recurrence group, GPIIb/IIIa, hs-cTnT, FPG, LDL, TC, and TG levels increased, whereas HDL level declined in the recurrence group ($p < 0.05$). There was a positive correlation between GPIIb/IIIa and hs-cTnT, FPG, LDL, TC, TG, BMI, and smoking index, and a negative correlation with HDL ($p < 0.05$). GPIIb/IIIa was positively correlated with postoperative recurrence ($p < 0.05$).

CONCLUSIONS: Increased GPIIb/IIIa expression after radiofrequency ablation of AF is associated with myocardial injury, suggesting a risk of postoperative recurrence.

Key Words:
Atrial fibrillation, Radiofrequency ablation, GPIIb/IIIa, Coagulation.

Introduction

Atrial fibrillation (AF) is a typical arrhythmia with high incidence and persistent characteristics[1]. Its incidence increased significantly for subjects older than 60 years[2,3]. In China, it was found that patients with atrial fibrillation occupied a large proportion of arrhythmias at all ages[4]. There are many inducing factor of AF, such as hypertension, valvular disease, heart failure, congenital heart disease, coronary heart disease, severe infection, and environmental and mental stress[5,6]. AF can aggravate myocardial ischemia and make the blood in the atria prone to stagnation and form left atrial thrombus, leading to deterioration of cardiac function. For example, thrombosis fall off can reach different organs along with circulation, resulting in embolism, such as cerebral embolism, pulmonary embolism, etc.[7,8]. Drug treatment for AF mainly focused on converting normal sinus rhythm. However, drug treatment for AF tends to induce arrhythmia[9]. Other commonly used treatment methods include electrical conversion, radiofrequency ablation, and surgical maze surgery, among which radiofrequency ablation is more common in the clinic[10,11]. Most AF patients can be treated with catheter radiofrequency ablation, which has the characteristics of less trauma, faster cure, and better acceptance[12].

However, AF patients are often in hypercoagulation status that can affect the prognosis[13]. GPIIb/IIIa is a fibrinogen receptor that can bind to the ligands of platelet and cause aggregation. Therefore, GPIIb/IIIa can be treated as a marker of hemagglutination[14,15]. We aim to analyze the changes of GPIIb/IIIa after radiofrequency ablation of atrial fibrillation, and to investigate its relationship with recurrence.

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Patients and Methods

General Information

There were 43 males and 37 females with average age at 59.3 ± 8.5 years old. Inclusion criteria: (1) age < 75 years old. (2) in line with AF catheter ablation indications. (3) left atrial diameter < 50 mm. (4) no thrombosis detected by transatrial transesophageal echocardiography in left atrial appendage[16]. Exclusion criteria: (1) valvular atrial fibrillation. (2) atrial fibrillation caused by hyperthyroidism. (3) associated with other severe organ damage. (4)severe cardiovascular disease such as rheumatic heart disease, hypertension, and coronary heart disease. (5) acute and chronic infectious diseases and other systemic diseases. (6) benign and malignant tumors. (7) unfinished or unsuccessful catheter ablation procedures. (8) failure to tolerate catheter ablation procedures or catheter ablation contraindications. (9) previous history of catheter ablation therapy[16]. Another 40 patients undergoing physical examination during the same period were enrolled as control, including 21 males and 19 females with mean age of 53.5 ± 7.2 years old.

This research was approved by the Ethics Committee of Beijing Ditan Hospital, Capital Medical University (Beijing, China). All subjects had signed informed consent and approved this study.

Main Reagents and Instruments

GPIIb/IIIa ELISA kit was purchased from Asaaypro (St. Charles, MO, USA). Other commonly used reagents were purchased from Sangon Biotech. Co. Ltd. (Shanghai, China). Labsystem Version 1.3.1 microplate reader was purchased from Bio-Rad Laboratories (Hercules, CA, USA). ACL TOP-700 automatic analyzer was purchased from Beckmann (Beckman, Brea, CA, USA).

Radiofrequency Ablation

Radiofrequency ablation was performed using cold saline perfusion method and three-dimensional mapping CARTO system. According to the 2012 ACC/AHA/ESC atrial fibrillation guidelines[17], the conducting wire went under the CARTO system guidance and ablation was performed through a pulmonary vein isolation catheter. The atrial vein side 0.5-2 cm was selected for ablation. The power of the anterior wall and other walls were adjusted to 35 W and 30 W, and the temperature was set to 43°C. Heparin saline flow was adjusted to 2 ml/min (non-ablation) and 17 ml/min (ablation) according to the ablation status. Continuous linear ablation was required in the presence of right tricuspid valve isthmus dependent atrial flutter typical symptoms. Tricuspid valve isthmus bidirectional block served as the end point of ablation.

Sample Collection

Blood samples were collected at 1 week after surgery. Fasting peripheral venous blood was drawn in the early morning. The blood was centrifuged at 3000 r/min for 15 min, and the serum was placed in an Eppendorf (EP) tube and stored at -20°C.

Index Detection

High-sensitivity troponin (hs-cTnT) was tested by enhanced chemiluminescence immunoassay. Fasting plasma glucose (FPG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), total cholesterol (TC), and triglyceride levels (TG) were analyzed by enhanced chemical luminescence assay.

Clinical Data Collection

Body mass index (BMI), smoking index, and age were recorded.

Enzyme-Linked Immunosorbent Assay (ELISA)

The serum level of GPIIb/IIIa in each group was detected by ELISA. The collected peripheral blood was centrifuged and the supernatant was taken. The experimental procedure was performed according to the ELISA kit instructions. The 50 μl diluted standard substance and samples were added to 96-well plate at 37°C for 30 min. After the plate was washed for 5 times, it was added with 50 μl reagent A and 50 μl reagent B at 37°C for 10 min. At last, the plate was added with 50 μl stop solution and tested on micro-plate reader. The standard curve was prepared based on the optical density (OD) value to calculate sample concentration.

Statistical Analysis

All data analyses were performed on SPSS 22.0 software (IBM, Armonk, NY, USA). Measurement data were described as mean ± standard deviation (± SD). Student's *t*-test was used to compare the differences between two groups. Tukey's post-hoc test was used to validate the ANOVA for comparing measurement data among the groups. Univariate analysis was performed

Table I. General conditions comparison.

	Non-recurrent group	Recurrent group
Age (year)	57.2 ± 9.1	60.7 ± 7.9
Gender (male/female)	25/17	18/20
BMI (kg/m^2)	21.1 ± 1.3	24.7 ± 2.6*
Smoking index	300.0 ± 10.0	450.0 ± 15.0*

*$p < 0.05$, compared with non-recurrent group.

based on Pearson linear correlation analysis. Multivariate analysis was performed based on multiple stepwise regression analysis. $p < 0.05$ was considered as statistically significance.

Results

Analysis of Postoperative Recurrence and General Conditions

Postoperative recurrence was defined as the presence of electrocardiographic atrial fibrillation, atrial tachycardia, atrial flutter or clinical symptoms of tachycardia; echocardiography with atrial systolic velocity, atrial flutter, or atrial fibrillation for more than 30 s[5]. Based on postoperative recurrence, the subjects were divided into recurrent and non-recurrent groups. A total of 42 patients exhibited postoperative recurrence with the rate of 50.25%. The age, sex, BMI, and smoking index were recorded in two groups. The results showed that BMI and smoking index were significantly higher in the recurrent group than in the non-recurrent group ($p < 0.05$). There was no statistical difference in age and gender between the two groups (Table I).

GPIIb/IIIa Expression Analysis After Radiofrequency Ablation

ELISA was used to detect the expression of GPIIb/IIIa before and after AF radiofrequency ablation. The results showed that compared with the normal control group, GPIIb/IIIa expression was significantly increased in AF patients after radiofrequency ablation ($p < 0.05$). Compared with the non-recurrent group, the expression of GPIIb/IIIa in the recurrent group was further elevated ($p < 0.05$) (Figure 1).

Hs-cTnT Expression Analysis After Radiofrequency Ablation

It was found that compared with the normal control group, hs-cTnT expression was markedly enhanced in AF patients after radiofrequency ablation ($p < 0.05$). Compared with the non-recurrent group, the expression of hs-cTnT in the recurrent group was further increased ($p < 0.05$) (Figure 2).

Plasma Glucose and Lipid Detection After Radiofrequency Ablation

FPG and blood lipids at 1 week after radiofrequency ablation were tested. It was observed that FPG, LDL, TC, and TG levels in the recurrent group were elevated, while HDL level was declined compared with the non-recurrent group ($p < 0.05$) (Table II).

Correlation Analysis of GPIIb/IIIa With hs-cTnT, FPG, Blood Lipids, and General Indicators In Postoperative Recurrence

The relationship between GPIIb/IIIa expression with hs-cTnT, FPG, blood lipids, and general information was analyzed in postoperative patients. It was confirmed that there was a positive correlation between GPIIb/IIIa and hs-cTnT,

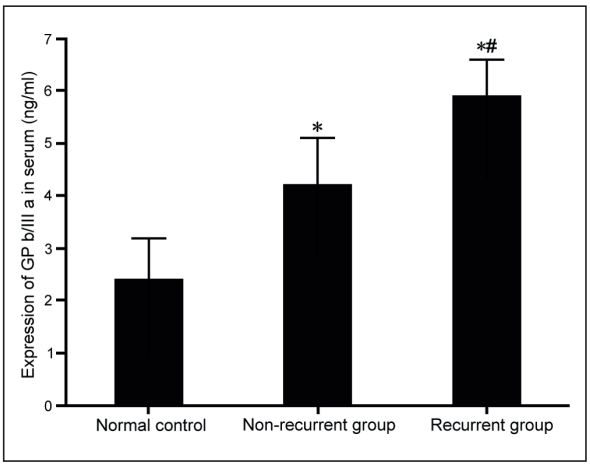

Figure 1. GPIIb/IIIa expression analysis after radiofrequency ablation. *$p < 0.05$, compared with normal control, #$p < 0.05$, compared with non-recurrent group.

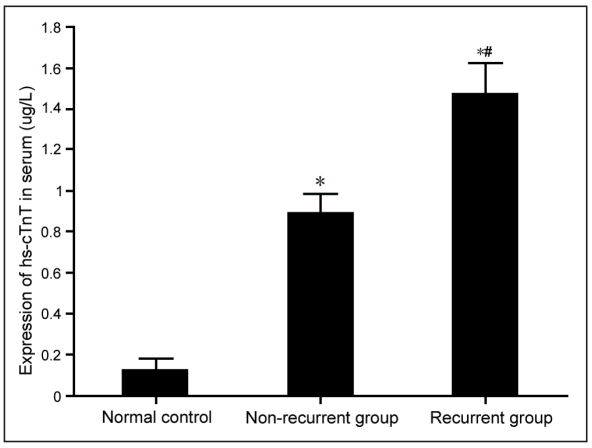

Figure 2. hs-cTnT expression analysis after radiofrequency ablation. *$p < 0.05$, compared with normal control $^{\#}p < 0.05$, compared with non-recurrent group.

FPG, LDL, TC, TG, smoking index, and BMI, and negative correlation with HDL ($p < 0.05$). There was a correlation between GPIIb/IIIa with postoperative recurrence ($r = 0.627$, $p < 0.05$) (Table III).

Discussion

Radiofrequency ablation is currently the first-line therapy for the treatment of AF, particularly for patients with poor efficacy of drug therapy and recurrent episodes[17]. However, even the application of radiofrequency ablation for the treatment of AF still has the risk of recurrence which can reach 60%[18]. AF patients are in hypercoagulation status after radiofrequency ablation. Therefore, coagulation status is a key factor affecting the recurrence of AF[19]. The platelet glycoprotein GPIIb/IIIa is expressed on the platelet membrane and is a marker of platelet activation. GPIIb/IIIa ligands are a series of adhesion proteins, including glassectin, fibrinogen, fibronectin, and von Willebrand factor, etc.[20,21]. Platelet activation exposes the ligand-binding site of GPIIb/IIIa, which forms a bridge between the sub-endothelium and platelet adhesion proteins. GPIIb/IIIa promotes platelet aggregation when combined with ligands. Under normal circumstances, since fibrinogen in plasma cannot bind to platelets, aggregation does not occur. Only when the endothelium damages and the sub-endothelial matrix are exposed, GPIIb/IIIa changes to receptor ligand binding state from the non-binding state, in turn, promoting platelet adhesion aggregation and thrombosis[22,23]. Platelet adhesion and aggregation can induce the occurrence of AF[24]. Therefore, we investigated the expression and significance of GPIIb/IIIa in AF after radiofrequency ablation. The results confirmed that the expression of GPIIb/IIIa, hs-cTnT, FPG, LDL, TC, and TG were increased, whereas the HDL was declined in the recurrence group compared with the non-recurrent group. GPIIb/IIIa was positively correlated with hs-cTnT, FPG, LDL, TC, TG, BMI, and smoking index, but negatively correlated with HDL. GPIIb/IIIa was positively associated with postoperative recurrence. Serum hs-cTnT can predict myocardial injury with higher sensitivity, thus had an important value in the acute myocardial infarction, coronary heart disease, and other cardiovascular disease[25]. In this study, hs-cTnT elevated in the recurrent group indicated the presence of myocardial injury. The expression trends of GPIIb/IIIa and hs-cTnT were similar and have a positive correlation, suggesting that GPIIb/IIIa was a potential factor for predicting myocardial problems. FPG, LDL, TC, TG, and HDL are important for the prediction of cardiovascular disease. Therefore, we analyzed the changes of blood glucose and lipids in recurrent and non-recurrent groups, and confirmed their association with GPIIb/IIIa. The patient states, including age, BMI, and smoking index also affected the efficacy of radiofrequency ablation on AF. Thus, we confirmed that age and BMI exhibited difference between

Table II. Plasma glucose and lipid detection after radiofrequency ablation.

	Non-recurrent group	Recurrent group
FPG (mmol/l)	6.2 ± 1.1	9.5 ± 3.5*
LDL (mmol/l)	3.7 ± 2.6	5.8 ± 3.1*
TC (mmol/l)	4.2 ± 0.8	8.7 ± 1.2*
TG (mmol/l)	1.7 ± 0.6	2.3 ± 1.2*
HDL (mmol/l)	2.1 ± 0.7	0.9 ± 0.7*

*$p < 0.05$, compared with non-recurrent group.

Table III. Correlation analysis of GPIIb/IIIa with hs-cTnT, FPG, blood lipids, and general indicators in postoperative recurrence.

	n	p
Age	80	0.112
Gender	80	0.120
BMI	80	0.199*
Smoking history	80	0.228*
FPG (mmol/l)	80	0.246*
LDL (mmol/l)	80	0.671*
TC (mmol/l)	80	0.423*
TG (mmol/l)	80	0.312*
HDL (mmol/l)	80	-0.572*
Postoperative recurrence	42	0.627*

*$p < 0.05$.

the recurrent and non-recurrent groups and had a correlation with GPIIb/IIIa, suggesting that are both factors that affect AF recurrence. This study confirmed for the first time that GPIIb/IIIa may be one of the important factors in evaluating the prognosis of AF after radiofrequency ablation, and analyzed the influencing factors associated with GPIIb/IIIa. The related role of GPIIb/IIIa in AF recurrence after radiofrequency ablation remains to be further analyzed.

Conclusions

We found that increased GPIIb/IIIa expression after radiofrequency ablation of AF is associated with myocardial injury, suggesting a risk of postoperative recurrence.

Acknowledgements

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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