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Platelet Toll-like receptor and its ligand HMGB-1 expression is increased in the left atrium of atrial fibrillation patients

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ABSTRACT

Background: Atrial fibrillation(AF) is the most common sustained arrhythmia. Its most feared sequelae are stroke and peripheral thromboembolism due to atrial thrombi formation. Mechanisms underlying the relationship between platelet activation and left atrial thrombi have not been clearly elucidated yet. We aimed to investigate whether immune-mediated platelet activation occurred in AF patients in this cross-sectional study.

Methods: Persistent and paroxysmal AF patients who underwent cryoballoon-based AF ablation between March 2015 and July 2016 were included as the patient group. Patients without AF in whom transseptal puncture was performed at the same period for purposes other than AF ablation were included as the control group. Peripheral and left atrial blood samples were obtained for determination of platelet Toll-like receptor(TLR)-2, TLR-4 and high mobility group box-1(HMGB-1) expression levels.

Results: A total of 75 subjects (53 patients with AF and 22 control subjects) [mean: 60.33 (SD: 6.14) years, 57.33% male] were included. Left atrial and peripheral TLR-2, 4 and HMGB-1 expression levels were significantly higher in the patient group when compared to the controls. Left atrial platelet TLR-2 and TLR-4 expression and serum HMGB-1 levels were higher in persistent AF patients compared to paroxysmal AF patients. In the patient group, left atrial expression of TLR-2, 4 and HMGB-1 were significantly higher than the peripheral expression levels.

Conclusion: Findings of our study suggest evidence for immune-mediated platelet activation in the left atria of AF patients.

1. Introduction

Atrial fibrillation (AF) is the most common sustained arrhythmia. The most feared sequelae due to AF are stroke and/or peripheral thromboembolism due to the formation of atrial thrombi, usually in the left atrial appendage (LAA) [1]. Several scores have been reported to determine the risk of cardioembolic stroke in AF [1]. Pathologic thrombosis is caused by a triad of factors, “Virchow’s triad”, that includes disturbance in blood flow, endothelial dysfunction and hypercoagulability. Most common site for thrombi formation in AF is the left atrium, and mainly the LAA. Disturbances in the blood flow in LAA are common, and are reflected with lower LAA flow velocities and observation of spontaneous echogenic contrast in echocardiographic examination [2,3]. Atrial structural remodeling in AF patients have been reported to result in alterations in the atrial endocardium, such as thickening of left atrial endocardial tissue, loss of endothelium, and thrombotic changes of the endothelium [4,5]. High levels of von Willebrand factor (vWF) have also been reported in non-valvular chronic AF patients, and elevated levels have been suggested to represent widespread and/ or local (endocardial) endothelial damage [6,7]. Regarding hypercoagulability, several studies have reported enhanced platelet activation existed in patients with LA thrombi [8–10].

Mechanisms underlying the relationship between platelet activation and left atrial thrombi have not been clearly elucidated yet. Toll-like receptors (TLRs) are of pattern recognition receptors that recognize pathogen-associated molecular patterns (PAMPs). One of the mechanisms of interactions of platelets in the process of thrombosis has been suggested to be the TLR expression of platelets on their surface and the

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Table 1
Baseline characteristics of the study population (n = 75).

	Study population (n = 75)	Patient group (n = 53)	Control group (n = 22)	p value
Age (years)	60.33, SD: 6.14	59.87, SD: 7.07	61.45, SD: 2.69	0.163
Gender: male (n, %)	43 (57.33)	30 (56.60)	13 (59.09)	1.000
Duration of AF (months)	37.50 (9.75–105.00)	37.50 (9.75–105.00)	–	NA
Hypertension (n, %)	41 (54.67)	28 (52.83)	13 (59.09)	0.799
Diabetes mellitus (n, %)	12 (16.00)	9 (16.98)	3 (13.64)	1.000
Hyperlipidemia (n, %)	27 (36.00)	21 (39.62)	6 (27.27)	0.429
Left ventricular end-diastolic diameter (cm)	4.85, SD: 0.44	4.86, SD: 0.44	4.83, SD: 0.46	0.780
Left ventricular ejection fraction (%)	65.09, SD: 2.95	65.02, SD: 3.12	65.23, SD: 2.64	0.792
Left atrial volume index (mL/m^2)	27.46, SD: 3.75	28.88, SD: 3.30	24.05, SD: 2.34	< 0.001[a]
$CHADS_2$ score	1.00 (0–1.75)	1.00 (0–1.75)	–	NA
CHA_2DS_2-VASc score	1.50 (1.00–3.00)	1.50 (1.00–3.00)	–	NA
C-reactive protein (mg/dL)	0.28 (0.16–2.49)	1.99 (0.33–4.05)	0.16 (0.15–0.18)	0.001[a]
White blood cell count ($\times 10^3/\mu L$)	6.50 (5.90–7.10)	6.40 (5.70–7.55)	6.50 (6.50–7.03)	0.371
Platelet count ($\times 10^3/\mu L$)	225.00 (197.00–264.00)	215.00 (195.25–264.50)	245.00 (205.00–264.00)	0.166
Mean platelet volume (fL)	9.07, SD: 1.11	9.03, SD: 1.13	9.21, SD: 1.05	0.552
Peripheral platelet TLR-2 expression (%)	15.00 (3.00–19.00)	18.00 (15.00–22.00)	3.00 (3.00–4.00)	< 0.001[a]
Peripheral platelet TLR-4 expression (%)	14.00 (4.00–19.00)	17.00 (15.00–24.00)	3.00 (3.00–4.00)	< 0.001[a]
Peripheral HMGB-1 expression (%)	3.00 (1.75–4.00)	3.80 (3.07–4.05)	1.70 (1.53–2.05)	< 0.001[a]
Left atrial platelet TLR-2 expression (%)	17.00 (5.00–32.00)	30.00 (18.00–38.00)	4.00 (3.00–5.00)	< 0.001[a]
Left atrial platelet TLR-4 expression (%)	20.00 (5.00–35.00)	31.00 (21.50–40.50)	4.00 (2.00–5.00)	< 0.001[a]
Left atrial HMGB-1 expression (%)	4.00 (2.20–5.00)	4.80 (4.13–5.18)	1.90 (1.50–2.20)	< 0.001[a]

AF atrial fibrillation, HMGB-1 high mobility group box-1, SD standard deviation, TLR Toll-like receptor.
[a] Denotes statistical significance.

wide spectrum of pro-inflammatory molecules that they secrete in response to the TLR stimulation, particularly at the interface between bacterial infections and thrombosis [11]. In this study, we aimed to investigate whether immune-mediated platelet activation via TLRs occurred in AF patients.

2. Methods

2.1. Study population

Persistent and paroxysmal AF patients who underwent cryoballoon-based AF ablation between March 2015 and July 2016 were included in this study as the patient group. Patients without AF in whom transseptal puncture was performed in the same time period for purposes other than AF ablation, such as percutaneous patent foramen ovale (PFO) closure and left-sided accessory pathway ablation, were included as the control group.

Patients with heart failure with valvular AF, moderate-severe valvular heart disease, coronary artery disease, reduced ejection fraction, left ventricular hypertrophy, chronic kidney disease, chronic inflammatory diseases, or recent infections (in the last three months) were not included in the study. None of the patients received antiaggregants.

Detailed medical history, laboratory and transthoracic echocardiographic (TTE) findings were recorded for all patients. $CHADS_2$ and CHA_2DS_2-VASc risk stratification scores were calculated in accordance with the recent guidelines [1]. Study protocol was approved by the institutional review board (Hacettepe University Non-Interventional Clinical Research Ethics Board, Number: GO 15/244-13 and 16969557-619) and informed consent was obtained from all patients.

2.2. Pre-procedural medications

None of the patients had chronic kidney disease. Anticoagulation was discontinued at least 48–72 h before the procedure for warfarin; 24–36 h before the procedure for dabigatran; and 24–28 h before the procedure for rivaroxaban. The pre-procedural interval was bridged with enoxaparin 1 mg/kg when sub-therapeutic INR levels were detected following suspension of warfarin, 12 h after suspension of dabigatran, and 24 h after suspension of rivaroxaban. Last dose of enoxaparin was given 12 h prior to the scheduled procedure. None of the patients were on antiaggregants prior to the procedure. Heparin was not administered before blood samples were drawn during the procedure, until a successful transseptal puncture was performed. Other drug use prior to the procedure included antiarrhythmic drugs. Treatment with antiarrhythmic drugs was discontinued for at least 3 days prior to the procedure.

2.3. Blood sample obtainment

Transseptal puncture during AF ablation or percutaneous PFO closure (in patient and control groups, respectively) was performed under conscious sedation using boluses of midazolam. Invasive arterial blood pressure, oxygen saturation, and electrocardiogram were monitored throughout the procedure. Right femoral vein puncture was performed with Seldinger technique. Peripheral blood samples for determination of peripheral platelet TLR-2, TLR-4 and HMGB-1 were obtained. Single transseptal puncture by modified Brockenbrough technique (BRK-1™, St Jude Medical) was performed under fluoroscopy and 8Fr transseptal sheath (Biosense Webster) was placed into the LA. Once LA access was obtained, blood samples for determination of LA platelet TLR-2, TLR-4 and HMGB-1 were obtained. The procedure was completed accordingly.

2.4. Determination of TLR-2, TLR-4 and HMGB-1 levels

Detection of TLR-2 and 4 levels was performed using flow cytometry in peripheral venous and left atrial blood samples collected in EDTA-anticoagulated tubes withdrawn from patients. Whole blood (5 ml) collected in citrate was centrifuged for 15 mins at 210*g*, and the Platelet Rich Plasma Fraction was collected. Platelets were washed twice with buffer (17.5 mM Na2HPO4, 8.9 mM Na2EDTA, 154 mM NaCl, pH 6.9, containing 0.1% bovine serum albumin by centrifuging them 5 min at 2310*g*. Platelets were re-suspended in HEPES medium (132 mM NaCl, 6 mM KCl, 1 mM MgSO4, 1.2 mM KH2PO4, 20 mM HEPES, pH 7.4, containing 5 mM glucose). TLR expression on platelets were measured on the flow cytometer (Beckman Coulter XL-MCL, CA, USA). Briefly, 10^6 platelets were incubated with 10 µl of TLR-2 FITC (CD282, cloneT2.5, Biolegend) and 10 µl of TLR-4 PE (CD284, HTA 125, Biolegend) antibodies and isotopic controls (MsIgG1 FITC, Biolegend and MsIgG1PE, Biolegend) for 30 mins at 4 °C in the dark and were washed once with PBS; then 10.000 events were acquired through a live gate drawn on forward light scatter (FSC) and side light scatter (SSC).