Comparison of apolipoprotein-A1 levels between paroxysmal atrial fibrillation patients and healthy subjects

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Introduction

Atrial fibrillation (AF) is a frequent supraventricular arrhythmia characterized by an irregular, disorganized, and rapid atrial activation.¹ Paroxysmal atrial fibrillation (PAF), which is a subtype of AF, is usually in an intermittent nature and terminates spontaneously or with intervention in less than 7 days. Recent studies have found that the inflammation might have a key role in the initiation and perpetuation of PAF.²⁻³ The elevation of some inflammatory markers, such as high-sensitivity C-reactive protein (hs-CRP) and interleukin-6, have been found in patients with PAF compared to healthy participants.⁴⁻⁵ Furthermore, higher hs-CRP levels have been found to be associated with longer AF duration in a previous study.⁶ In addition to the inflammation, oxidative stress is shown to be involved in the pathogenesis of PAF.⁷

Apolipoprotein-A1 (Apo-A1), which is the major apolipoprotein of high-density lipoprotein (HDL), seems to confer a major protection role against atherosclerosis development in the human body. Besides this important role, Apo-A1 has some anti-inflammatory and anti-oxidant properties, which can exert these affects through relevant enzymes.⁸⁻⁹ As the inflammatory status and oxidative stress are involved in the pathophysiology AF, we considered that Apo-A1 levels might be lower among PAF patients. Based on this data, the present study aimed to determine whether there is any difference of Apo-A1 levels in patients with PAF and healthy subjects.

Materials and Methods

Data collection

In this prospective, cross-sectional study, we enrolled PAF patients seen in a tertiary heart center emergency department between July 2012 and May 2013. The enrolled patients’ age was between 18 and 60 years. The patients with chronic diseases, such as hypertension, diabetes mellitus, coronary artery disease, congestive heart failure, etc., were excluded from the study. Finally, the study cohort was consisted of a total of 35 PAF patients. The control cohort was composed of 34 age-, sex-, and body mass index (BMI)-matched healthy subjects.

AF was diagnosed with a rapid and irregular heart rhythm without detecting P waves, which was present on the electrocardiography (ECG) taken during palpitation.
In patients in whom AF did not terminate spontaneously, normal sinus rhythm was achieved either with medical or electrical cardioversion. Following the restoration of normal sinus rhythm, either beta-blocker or calcium channel blocker or antiarrhythmic were prescribed to prevent AF attack. All patients were followed-up for AF attacks during 1 year study period. The follow-up of each participant was performed using either outpatient visits or telephone interviews.

**Echocardiographic assessment**
A trained investigator performed an echocardiographic examination within 30 days following AF attack. The left ventricular end-diastolic diameter (LV-dd), LV end-systolic diameter (LV-sd), LV posterior wall thickness (LV-pw) and inter-ventricular septal thickness (LV-sw) as well as the left atrium (LA) end-systolic dimension were measured using the M-mode method on the parasternal long-axis view. The Simpson's method was used to measure the LV ejection fraction (LV-ef).

**Laboratory analysis**
The blood samples of all patients were collected following 8-12 hours overnight fasting. The standard laboratory methods were used to analyze blood samples for glucose, hemoglobin, creatinine, triglyceride (TG), very low density lipoprotein (VLDL), total cholesterol (TC), HDL, and low-density lipoprotein (LDL) levels.

**Apolipoprotein analysis**
First, we collected a 5 ml of blood sample in a dried tube. Then, we centrifuged it at 1000 rpm for at least ten minutes in order to obtain serum sample. After that, we stored all serum samples at -80°C until the analysis. Immunophelometric method (Nephstar, Apo-A1 kit) was used to measure serum Apo-A1 level.

**Statistical analysis**
All statistical analyses were conducted using Number Cruncher Statistical System 2007 and Power Analysis and Sample Size 2008 statistical software (Utah, USA). The Shapiro-Wilk's test was used for testing data normality. The mean ± standard deviation was used to express the continuous variables. The student t test was used to compare the continuous variables with normal distribution, whereas the Mann-Whitney U was used to compare the continuous variables without normal distribution. In order to compare the qualitative data, either Fisher’s exact test or Yates continuity correction test were performed. Pearson correlation analysis was performed to assess correlation for variables showing normal distribution. On the other hand, Spearman’s correlation analysis was preferred to assess correlation for variables without normal distribution. It was considered statistically significant when a P value was less than 0.05.

**Results**
Table 1 is a presentation of baseline demographic features of all enrolled subjects included in the study. The study cohort mean age was 44.52 ± 7.32 years. A total of 48 (69.6%) of the patients were male and 21 of them (30.4%) were female. Both groups were indifferent in terms of demographic characteristics, including age, sex, BMI, etc. (P > 0.05 for each). In the study, the mean number of AF attacks was 3.60 ± 3.63 episodes and the mean AF duration was 13.17 ± 10.22 hours.

Echocardiographic findings of each subject were displayed in Table 2. LV-ef, LV-dd, LV-sw, and A wave velocity were not different between the groups (P > 0.05 for each). On the other hand, the LA dimension was higher and E wave velocity was lower in PAF patients (P= 0.02 and P = 0.07, respectively).

Comparison of laboratory findings of both groups are demonstrated in Table 3. In terms of lipid profile, only VLDL and TG levels were significantly different between the groups (P < 0.05 for each). The other lipid parameters were similar both in PAF patients and healthy subjects (P > 0.05 for each). Patients with PAF had significantly higher hs-CRP levels than healthy subjects (1.54 ± 1.99 vs. 1.06±2.01, respectively). Of note, patients with PAF had lower Apo-A1 levels (1.84 ± 0.74 vs. 2.55 ± 0.44, P = 0.001, respectively). There was no statistical difference between the groups in terms of apolipoprotein-B levels (1.08 ± 0.36 vs. 0.99 ± 0.38, P = 0.339, respectively).

**Table 1.** The comparison of demographic characteristics of patients with paroxysmal atrial fibrillation and control group

|                | Total (n=69) | Control (n=34) | Patients (n=35) | P value |
|----------------|-------------|---------------|----------------|---------|
| Age, years     | 44.52±7.32  | 45.76±5.18    | 43.31±8.84     | 0.164*  |
| BMI, kg/BSA    | 26.45±3.51  | 26.46±3.32    | 26.44±3.73     | 0.985*  |
| Female, n (%)  | 21 (30.4)   | 10 (29.4)     | 11 (31.4)      | 1.000*  |
| Male, n (%)    | 48 (69.6)   | 24 (70.6)     | 24 (68.6)      | 1.000*  |
| Smokers, n (%) | 9 (13)      | 4 (11.8)      | 5 (14.3)       | 1.000*  |
| Alcohol usage, n (%) | 2 (2.9) | 1 (2.9)      | 1 (2.9)       | 1.000*  |
| Obesity, n (%) | 13 (18.8)   | 6 (17.6)      | 7 (20)        | 1.000*  |

Abbreviations: BMI; body mass index, BSA; body surface area, AF; atrial fibrillation.

*Student t test, *Yates continuity correction, *Fisher’s exact test.
Table 2. Echocardiographic parameters of all participants

|                     | Total            | Control (n=34) | Patients (n=35) | P value |
|---------------------|------------------|----------------|----------------|---------|
| LVEF, %             | 61.75±2.47       | 61.88±2.36     | 61.63±2.6      | 0.673*  |
| LVSD, mm            | 27.64±4.02       | 26.44±2.58     | 28.80±4.79     | 0.014** |
| LVDD, mm            | 46.90±3.69       | 46.38±2.81     | 47.4±4.36      | 0.252a  |
| LAP, mm             | 34.3±0.38        | 32.68±3.75     | 35.89±4.42     | 0.002***|
| PW, cm              | 1.05±0.97        | 0.92±0.05      | 0.94±0.08      | 0.078a  |
| IVS, cm             | 0.92±0.07        | 0.91±0.06      | 0.94±0.08      | 0.002** |
| E wave velocity     | 0.85±0.13        | 0.89±0.1      | 0.81±0.15      | 0.007***|
| A wave velocity     | 0.50±0.10        | 0.48±0.11     | 0.52±0.09      | 0.102a  |

Abbreviations: LVEF; left ventricular ejection fraction, LVSD; left ventricular systolic dimension, LVDD; left ventricular diastolic dimension, LAP; left atrial size, IVS; interventricular septum.

* Student t test, Mann-Whitney U test, ** P < 0.05, *** P < 0.01.

Table 3. The comparison of biochemical and hematological measurements between paroxysmal AF and control group

|                     | Total            | Control (n=34) | Patients (n=35) | P value |
|---------------------|------------------|----------------|----------------|---------|
| HbA1c, mg/dL        | 5.69±0.33        | 5.62±0.36      | 5.75±0.30      | 0.123a  |
| LDL, mg/dL          | 122.07±28.41     | 122.21±28.25   | 121.94±28.98   | 0.970a  |
| VLDL, mg/dL         | 26.13±13.48      | 21.73±11.54    | 30.40±13.99    | 0.007***|
| TC, mg/dL           | 199.78±36.64     | 198.21±38.22   | 201.31±35.53   | 0.727a  |
| HDL, mg/dL          | 50.30±11.50      | 52.74±10.24    | 47.94±12.30    | 0.084a  |
| TG, mg/dL           | 129.3±64.22      | 111.91±59.48   | 146.2±64.95    | 0.025a  |
| BUN, mmol/L         | 14.39±3.07       | 15.12±2.96     | 13.69±3.06     | 0.052a  |
| Creatinine, mg/dL   | 0.84±0.15        | 0.82±0.13      | 0.85±0.16      | 0.347a  |
| AST, U/L            | 24.13±8.16       | 23.35±8.46     | 24.89±7.90     | 0.439a  |
| ALT, U/L            | 29.21±32.57      | 26.5±23.28     | 31.83±23.89    | 0.003***|
| Glucose, mg/dL      | 93.38±28.5       | 92.85±8.05     | 93.83±9.66     | 0.651a  |
| hs-CRP, mg/dL       | 2.08±0.65        | 1.06±0.01      | 1.54±1.99      | 0.016** |
| Apo-A1, mg/dL       | 2.19±0.70        | 2.55±0.44      | 1.84±0.74      | 0.001***|
| Apo-B, mg/dL        | 1.03±0.37        | 1.08±0.36      | 0.99±0.38      | 0.339a  |
| Apo-B/ Apo-A1       | 0.51±0.21        | 0.42±0.12      | 0.59±0.24      | 0.002***|
| Hemoglobin, g/dL    | 14.28±1.43       | 14.18±1.31     | 14.37±1.54     | 0.597a  |
| Hematocrit, %       | 42.85±4.08       | 42.45±3.78     | 43.23±4.39     | 0.433a  |
| WBC, x 10^3 µL      | 6.58±1.73        | 6.63±1.87      | 6.53±1.6       | 0.821a  |
| MPV, fl             | 8.93±0.85        | 8.79±0.82      | 9.08±0.87      | 0.160a  |

Abbreviations: LDL; low-density lipoprotein, VLDL; very-low density lipoprotein, HDL; high-density lipoprotein, TC; total cholesterol, TG; triglyceride, MPV; mean platelet volume, hs-CRP; high-sensitivity C-reactive protein, HCT; hematocrit, WBC; white blood cell, HbA1c; hemoglobin A1c, BUN; blood urea nitrogen, AST; aspartate aminotransferase, ALT; alanine aminotransferase.

* Student t test, Mann-Whitney U test, ** P < 0.05, *** P < 0.01.

In a correlation analysis, there was no correlation between Apo-A1 levels and AF attacks, AF duration, and the LA diameter (r = -0.166, P = 0.235, r = -0.070, P = 0.691, and r = -0.136, P = 0.436, respectively).

**Discussion**

In this observational and prospective study, we found the following: I-) PAF patients had lower Apo-A1 levels than healthy participants II-) there was no any relation between Apo-A1 levels and AF attacks, AF duration, and the LA diameter in patients with PAF III-) this study result might provide evidence that Apo-A1 levels might have an important role in the pathophysiology of AF.

Recent experimental and clinical studies have demonstrated the importance of inflammation in the pathogenesis of AF.10,11 The biopsy samples that were obtained from AF patients undergoing cardiac surgery revealed the presence of inflammatory infiltrates and fibrosis in the atrium.10 Additionally, studies have found that both hs-CRP and interleukin-6 levels were significantly elevated in patients with PAF.4,5 Similar to this published clinical studies, PAF patients had higher hs-CRP levels than healthy subjects in our study. Besides baseline elevated inflammatory status, oxidative stress...
and their markers have been linked to increased risk of AF occurrence. Experimental animal studies showed that oxidative modification of lipoprotein might cause a cardiac myocyte damage and electrophysiologic alterations via disrupting transmembrane ion currents.12,13 Hence, it was considered that decreased anti-oxidative functions as result of elevated lipid oxidation might be present in AF patients. Similar to these experimental studies, in a recent study, Samman et al reported that increased oxidative stress is related with more prevalent and incident AF.14

Lipid profiles in AF patients were investigated in a previous study.15 It was found that PAF patients had similar HDL and LDL levels. Similarly, a consistent finding was found in our study regarding these lipid parameters. These study findings may indicate that some other pathological mechanisms are involved in the AF development.

Apo-A1, which is the main apolipoprotein of HDL, has some important anti-inflammatory and anti-oxidant properties in a healthy physiological system. Prior studies have showed that decreased Apo-A1 levels are closely associated with increased risk of coronary artery disease, stroke, and acute coronary syndrome.16-18 Besides that, in a small study included only 11 female patients, Kim et al demonstrated that patients with PAF had an approximately 30% lower expression of Apo-A-1 compared to the healthy subjects.15 The present study, which was conducted in a larger sample size compared to the aforementioned study, also found that not only female PAF patients but also male PAF patients had lower Apo-A1 levels than healthy subjects. However, although Apo-A1 were decreased in such patients, we could not find any relation between Apo-A1 levels and AF attacks and duration. As the possible explanations of the study findings, we considered that patients with lower Apo-A1 levels may have a decreased anti-oxidant and anti-inflammatory capacity, which may result in a higher risk of AF. Therefore, further prospective studies with larger population are needed to assess the exact role of Apo-A1 levels in PAF patients.

Limitations of the study
Our study had following limitations. First, the study had a relatively small number of patients. Second, we only enrolled patients with PAF. Hence, the study results might not be generalized to all AF patients. Third, because the follow-up of each participant was performed using either outpatients visits or telephone interviews, we might miss some AF attacks in the study. Finally, we could not repeat Apo-A1 measurements during follow-up period.

Conclusion
The main finding of this study was that Apo-A1 levels were significantly lower in PAF patients compared to healthy participants. Based on our results, we considered that Apo-A1 may have a key role in the pathogenesis of PAF.

Competing interests
The authors declare that they have no conflict of interest.

Ethical approval
All procedures were in accordance with the ethical standards of the local committee and with the 1975 Declaration of Helsinki, as revised 2008. The design of the present was reviewed and approved by the Institutional Local Committee. An informed consent was obtained from each subject included in the study.

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