Relation of inflammatory and oxidative markers to the occurrence and recurrence of persistent atrial fibrillation

Israrcı atriyum fibrilasyonu oluşumu ve nüksünün serum oksidatif stres ve yangı belirteçleri ile ilişkisi

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ABSTRACT

Objectives: There is increasing evidence linking inflammation and oxidative stress to atrial fibrillation (AF). In this study, we tested the hypothesis that C-reactive protein (CRP) and oxidative stress markers can predict the recurrence of persistent AF after successful pharmacological cardioversion. A possible relationship with AF occurrence was also investigated.

Study design: Using a case-control study design, CRP, catalase, superoxide dismutase (SOD), and malondialdehyde (MDA) levels of 42 patients (23 female, 19 male; mean age 58.4±13.6 years) with documented persistent AF episodes were compared with 21 controls (9 female; 12 male; mean age 58.1±6.9 years).

Results: Overall AF patients were followed for 6 months, and 17 showed recurrence. Then, they were divided into two groups (recurrence and no recurrence) and compared with each other. CRP, SOD, and MDA levels were significantly higher in AF patients compared with controls. However, only CRP levels were significantly higher in patients with AF recurrence compared to those without recurrence.

Conclusion: Increased markers of inflammation and oxidative stress are found in patients with persistent AF, suggesting that inflammation and oxidative stress may be associated with the presence of arrhythmia.

ÖZET

Amaç: Atriyum fibrilasyonu (AF) ile yangı ve oksidatif stres arasında giderek artan sayıda kanıtlar ortaya çıkmaktadır. Bu çalışmada, C-reaktif protein (CRP) ve oksidatif stres belirteçlerinin, ısrarcı AF'nin başarılı farmakolojik kardiyoversiyonu sonrası nüksü öngörebileceği yönündeki hipotez test edildi. Ayrıca, bu belirteçlerin AF gelişimi ile olası ilişkisi de araştırıldı.

Çalışma planı: Olgu-kontrol yöntemi kullanılarak, kanıtlanmış ısrarcı AF atağı geçiren 42 hastanın (23 kadın, 19 erkek; ort. yaş 58.4±13.6 yı) CRP, katalaz, süperoksit dismutaz (SOD) ve malondialdehit (MDA) seviyeleri, 21 olguluk kontrol grubu (9 kadın, 12 erkek, ort. yaş 58.1±6.9 yıl) ile karşılaştırıldı.

Bulgular: AF'li tüm hastalar 6 ay boyunca izlendi ve bu süre boyunca 17'sinde AF nüks etti. Böylece, AF'li hastalar nüks gösteren ve göstermeyenler olmak üzere iki gruba ayrıldı ve aynı belirteçler kullanılarak birbiriyle karşılaştırıldı. CRP, SOD ve MDA seviyeleri kontrol grubuna göre AF'li hastalarda anlamlı olarak yüksekti. Bununla birlikte, sadece CRP seviyeleri nüks etmeyenlerle karşılaştırıldığında nüks görülen AF'li hastalarda anlamlı olarak yüksekti.

Sonuç: Yangı ve oksidatif stres belirteçlerinin ısrarcı AF'li hastalarda yüksek bulunması, aritminin varlığı ile yangı ve oksidatif stresin ilişkili olabileceğini düşündürmektedir.

Atrial fibrillation (AF) is the most common arrhythmia encountered in clinical practice. Its prevalence has continuously increased during the past few decades, mainly due to the aging of the population and the improved survival of patients with other cardiovascular diseases.[1,2] Although it appears to be the result of a complex interaction between electrophysiologic, metabolic, ischemic, and hemodynamic disorders,

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along with genetic factors, etiology and pathophysiology have not been fully clarified yet.[3-5] Despite the use of antiarrhythmic agents for sinus rhythm maintenance, a considerable proportion of patients experience a relapse of AF.[6,7] In the past, classic factors known to influence AF history included the presence of left ventricular dysfunction, left atrial (LA) enlargement, age, arrhythmia duration, and history of hypertension.[8] It has been shown that atrial remodeling is responsible for high recurrence rates after cardioversion. Inflammation might play an important role in this process,[9] though conflicting data exist in the literature. Several studies have demonstrated that C-reactive protein (CRP) might predict recurrence after successful cardioversion;[10-13] however, other studies have failed to reproduce these results.[14-16]

In this study, we aimed to investigate the relationship of inflammation and oxidative stress to AF.

PATIENTS AND METHODS

Forty-two patients (23 female/19 male, mean age 58.4±13.6 years) diagnosed with persistent AF according to the European Society of Cardiology (ESC) 2010 AF guidelines 17 and 21 age and gender matched healthy volunteers (12 male/9 female, mean age 58.1±6.9 years) were prospectively enrolled in the study. Exclusion criteria were as follows: left ventricular ejection fraction <50%, unsuccessful pharmacological cardioversion, chronic inflammatory disease, current inflammatory disease, acute or chronic infection, acute coronary syndrome, uncontrolled hypertension, any operation in the last 60 days, any degree of stenotic valve disease, severe valve regurgitation, LA diameter >50 mm, hyperthyroidism, chronic obstructive pulmonary disease, chronic kidney disease, chronic liver disease, cerebrovascular disease, and malignancy.

All patients provided a detailed history and underwent a physical examination, 12-lead ECG, and transthoracic echocardiography. Pharmacological cardioversion with oral propafenone (600 mg single bolus and 150 mg three times daily) and the appropriate parenteral heparin dose were then administered. Restoration of sinus rhythm in 24 hours was considered successful cardioversion. All study subjects provided signed informed consent, and the study protocol was approved by the ethics committee of our institution.

Abbreviations:

AF	*Atrial fibrillation*
CRP	*C-reactive protein*
ESC	*European Society of Cardiology*
LA	*Left atrial*
MDA	*Malondialdehyde*
SOD	*Superoxide dismutase*

All patients were followed for six months while being treated with beta blockers and propafenone. Per the recommendation of the ESC 2010 AF guidelines,[17] warfarin or acetylsalicylic acid was added. After one month, all of the patients were reevaluated with 12-lead ECG and provided a detailed history; they were asked about palpitation episodes, adjustment to medications, and possible side effects. All of the patients were reevaluated monthly by telephone. If they had suspicious symptoms, 12-lead ECG and, if necessary, ambulatory ECG was performed. It was considered a recurrence if an AF episode was detected on 12-lead ECG or ambulatory ECG.

After six months of follow-up, 24 patients (15 male/9 female, mean age 55.8±16.5 years) were still maintaining sinus rhythm, while 17 patients (13 female/4 male, mean age 62±7.2 years) showed AF recurrence. Thus, patients were divided into two groups: group 1, persistent AF patients with normal sinus rhythm, and group 2, persistent AF patients with AF recurrence.

Blood samples

Blood samples were collected from all patients before cardioversion. All blood samples were drawn from a vein in the forearm and collected into 5-ml Vacutainer tubes containing potassium ethylenediaminetetraacetate (EDTA). The samples were centrifuged at 1000 g at 4°C for 10 minutes to separate the pellets and the supernatant. The supernatant was removed cautiously. Erythrocytes were washed three times using 0.9% NaCl solution to remove residuals. The mixed solution containing erythrocytes and saline was centrifuged at 1000 g at 4°C for 10 minutes following each process. Hemolysates were prepared directly from washed red cells to measure biochemical parameters.

CRP levels were assessed by means of particle enhanced immunonephelometry using BN Systems (Dade Behring BN 100, Germany). Erythrocyte catalase (CAT) activity was measured using the Beutler test at 230 nm based on the rate of hydrogen peroxide decomposition by CAT; activity was expressed as units per gram hemoglobin (U/gHb).[18] Superoxide dismutase (SOD) activity was measured using Fridovich's method.[19] This method uses xanthine

and xanthine oxidase to produce superoxide radicals which react with p-iodonitrotetrazolium violet to generate a red formazan measured at 505 nm. The result was expressed as units per gram hemoglobin (U/gHb). Lipid peroxidation of plasma samples was measured by the method of Ohkawa et al.[20] based on thiobarbituric acid-reactive malondialdehyde (MDA) formation by absorption at 532 nm; lipid peroxidation was expressed as nmol/ml.

Statistical analysis

All data were processed using the SPSS statistical software (version 11.0), and a *p* value of less than 0.05 was considered significant. The Mann-Whitney U-test was used to analyze the statistical significance of measurable values, and the chi-square test was used for non-measurable values.

RESULTS

Basic characteristics

During the six months of follow-up, 24 of 42 patients maintained sinus rhythm, while 17 patients showed recurrence. One patient died during the study period. Basic patient characteristics are shown in Table 1. The control group and the overall AF group were statistically similar for the following parameters: age, gender, body mass index, smoking, hypertension, diabetes mellitus, left ventricular ejection fraction, angiotensin-converting enzyme, and angiotensin receptor blocker medications. Left atrium diameter (38.1±5.2 and 33.3±2.7; p<0.001) was significantly higher in the overall AF group compared with the controls.

Age, body mass index, smoking, hypertension, diabetes mellitus, LA diameter, angiotensin-converting enzyme, and use of angiotensin receptor blocker were similar for group 1 and group 2. There were more female patients in group 2 compared with group 1 (13 [76.5%] and 9 [37.5%], respectively; p=0.025).

Relationship of AF to oxidative stress parameters and CRP

Oxidative stress parameters and CRP levels are shown in Table 1 and Table 2. CRP levels were significantly higher in overall AF patients compared with the controls. MDA and SOD levels were also significantly higher in overall AF patients compared with the controls. However, in the AF subgroups, only CRP levels were significantly higher in the AF recurrence group compared with the AF group with no recurrences. Although oxidative stress parameter levels were different for groups 1 and 2, the difference was not statistically significant.

Table 1. Basic patient characteristics

	Controls (n=21)			Overall AF group (n=42)			*p*
	n	%	Mean±SD	n	%	Mean±SD	
Age (years)			58.1±6.9			58.4±13.6	NS
Female	9	42.9		23	54.8		NS
Body mass index (kg/m^2)			29.1±2.1			29.8±5.4	NS
Smoking	2	9.5		5	11.9		NS
Hypertension	9	42.9		18	42.9		NS
Diabetes mellitus	2	9.5		5	11.9		NS
Ejection fraction (%)			64.5±3.4			64.4±5.2	NS
Left atrium diameter (mm)			33.3±2.7			38.1±5.2	**<0.001**
ACE, ARB	9	42.9		22	52.4		NS
C-reactive protein			3.41±0.36			7.41±4.23	**<0.001**
Catalase			0.51±0.44			0.64±0.52	NS
Malondialdehyde			6.54±7.17			12.6±11.4	**0.006**
Superoxide dismutase			24347.7±33662.1			31538.1±26073.2	**0.001**

AF: Atrial fibrillation; ACE: Angiotensin converting enzyme; ARB: Angiotensin receptor blocker; NS: Non-significant.

Table 2. Comparison of demographic data, inflammation and oxidative stress parameters between groups

	Group 1 (n=24)			Group 2 (n=17)			*p*
	n	%	Mean±SD	n	%	Mean±SD	
Age (years)			55.3±17			57.1±4.5	NS
Female	9	37.5		13	76.5		0.025
Body mass index (kg/m^2)			29.2±6.3			30.9±5.2	NS
Smoking	4	16.7		1	5.9		NS
Hypertension	10	41.7		7	41.2		NS
Diabetes mellitus	4	16.7		1	5.9		NS
Ejection fraction (%)			63.4±5.4			66.1±5.8	0.061
Left atrium diameter (mm)			38.2±4.9			37.8±5.4	NS
ACE, ARB	10	41.7		12	70.6		NS
C-reactive protein			5.04±1.02			11.41±4.55	**<0.001**
Catalase			0.51±0.36			0.84±0.62	NS
Malondialdehyde			14.1±14.3			10.5±6.4	NS
Superoxide dismutase			32109.7±30473.4			30731±19054.3	NS

AF: Atrial fibrillation; ACE: Angiotensin converting enzyme; ARB: Angiotensin receptor blocker; NS: Non-significant.

DISCUSSION

In this study, we investigated the relationship of oxidative stress parameters and CRP levels to persistent AF occurrence and recurrence. In conclusion: (1) CRP, MDA and SOD levels were higher in persistent AF patients; (2) only CRP levels were higher in recurrent persistent AF patients; (3) recurrence was more common in female patients; and (4) LA diameters were higher in overall persistent AF patients compared with the control group.

The results of this study suggest that inflammation and oxidative stress are related to persistent AF occurrence.

Inflammation and AF

In the past few years, much attention has been devoted to assessing the role of inflammation in AF. Several studies using markers of inflammation, such as CRP and interleukin-6, have indicated higher levels of both parameters in AF patients compared with sinus rhythm controls.[21-23] It has been demonstrated that AF is associated with high sensitivity CRP levels and larger LA dimensions, and higher inflammation status during sinus rhythm potentially increases the risk of AF occurrence during the follow-up period.[24] Furthermore, previous studies found that high sensitivity CRP levels were significantly higher in patients with paroxysmal and chronic AF than in normal controls. High sensitivity CRP levels were also higher in chronic AF patients than in the paroxysmal AF group.[25] CRP has also been independently associated with early AF recurrence in the first month after successful cardioversion.[11]

Oxidative stress and AF

Existing data in the literature regarding oxidative stress and AF are not as clear as data for inflammation and AF. Fewer studies on oxidative stress and AF have been completed. It has been found that patients with post-operative AF after cardiac surgery had significantly higher serum total peroxide levels compared with patients in sinus rhythm six hours after surgery.[26] Furthermore, Leftheriotis et al.[27] postulated that MDA and nitrotyrosine were reliable predictors of sinus rhythm maintenance after the first cardioverted episode of persistent lone AF. Our study findings support a relationship between AF development and oxidative stress. Conversely, the levels of oxidative stress markers (SOD and MDA) seem to be unrelated to AF recurrence.

In conclusion, most studies of inflammation, oxidative stress, and AF have been performed with heterogeneous groups of patients, and many of those pa-

tients have had underlying heart diseases which might have confounded inflammation and oxidative stress marker results. Also, patients with long-lasting AF have been included. Usually, electrical cardioversion has been preferred.[13,27-30]

We tried to choose a homogenous patient population; only persistent AF patients with successful pharmacological cardioversion using only propafenone were enrolled. The aim was to investigate the predictive role of inflammation and oxidative stress markers in detecting recurrence rates after successful pharmacological cardioversion using only one type of antiarrhythmic agent in patients with only one type of AF.

Limitations

Due to the relatively small number of patients included in this study, the study may have lacked sufficient statistical power to detect certain associations. Measurements of marker levels before the onset of AF could not be obtained, since our patients were urgently admitted to the hospital and AF onset time could not be predicted. Insufficient data on AF duration is also an important limitation of this study. In addition, we cannot exclude the possibility that asymptomatic episodes of self-limited AF occurred in the sinus rhythm patient group after cardioversion.

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Key words: Anti-arrhythmia agents; arrhythmias, cardiac; atrial fibrillation/therapy; C-reactive protein; case-control studies; inflammation mediators; oxidative stress/physiology.

Anahtar sözcükler: Antiaritmi ajanları; aritmiler, kardiyak; artiyum fibrilasyonu/tedavi; C-reaktif protein; olgu-kontrol çalışması; yangı belirteçleri; oksidatif stres/fizyoloji.