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The Importance of Nitric Oxide and Oxidative Stress in Atrial High-Rate Episodes in Patients with Cardiac Devices

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ABSTRACT

Objective: Atrial High Rate Episodes (AHRE) are subclinical atrial tachyarrhythmias detectable by cardiac implantable electronic devices (CIEDs). AHREs have been associated with an increased risk of developing atrial fibrillation (AF), thromboembolism, cardiovascular and cerebrovascular events, and mortality. Although recent studies have assessed the value of oxidative stress markers in patients with AF, the relationships between AHRE and oxidative stress markers, including nitric oxide, has not yet been elucidated. This study aims to investigate the relationship between these markers and AHRE.

Method: This prospective, cross-sectional study comprised 180 patients with CIEDs. The study population was divided into two groups based on the presence (n = 78) and absense (n = 102) of AHRE to analyze its association with biomarkers.

Results: The AHRE (+) group was significantly older, had a higher prevalence of hypertension, higher NT-proBNP (508.8 ± 249 pg/mL vs. 415.3 ± 292.1; *P* = 0.037), malondialdehyde (MDA) levels (20.9 ± 4.1 μmol/L vs. 19.1 ± 3.1 μmol/L; *P* = 0.006), and inducible nitric oxide synthase (iNOS) activity (1,935.9 ± 326.1 pg/mL vs. 1,677.4 ± 363.2 pg/mL; *P* < 0.001). Logistic regression analysis identified age, hypertension, MDA (odds ratio [OR]: 1.131, 95%CI: 1.009 - 1.268, *P* = 0.035), iNOS activity (OR = 1.002, 95% CI = 1.001 - 1.003, *P* < 0.001), and endothelial nitric oxide synthase (eNOS) activity (OR = 0.990, 95% CI = 0.986 - 0.984, *P* < 0.001) as independent predictors of AHRE.

Conclusion: The study findings indicated that plasma levels of NT-proBNP, MDA, nitric oxide, and the expression of iNOS and eNOS were significantly associated with AHRE. Moreover, elevated plasma MDA concentrations, increased iNOS activity, and decreased eNOS activity were identified as independent predictors of AHRE.

Keywords: Atrial high rate episodes, nitric oxide, malondialdehyde, inducible nitric oxide synthase, endothelial nitric oxide synthase

ÖZET

Amaç: Atriyal Yüksek Hızlı Epizotlar (AYHE), kardiyak implante edilebilir elektronik cihazlar (KİEC) ile tespit edilebilen subklinik atriyal taşiaritmilerdir. AYHE; atriyal fibrilasyon (AF) tromboembolizm, kardiyovasküler ve serebrovasküler olaylar ve mortalite riskinde artma ile ilişkilendirilmiştir. Oksidatif stres belirteçlerinin AF'li hastalardaki önemi son zamanlarda değerlendirilmiş ve aralarındaki ilişki gösterilmiştir. Ancak, AYHE ile oksidatif stres belirteçleri ve nitrik oksit arasındaki ilişki henüz aydınlatılmamıştır. Bu bağlamda, bu çalışmanın amacı, KİEC'li hastalarda bu belirteçler ile AYHE arasındaki ilişkiyi araştırmaktır.

Yöntem: Bu prospektif, kesitsel çalışmaya KIEC'li 180 hasta alındı. Çalışma popülasyonu, AHRE'nin varlığı (n = 78) ve yokluğuna (n = 102) göre iki gruba ayrılarak AYHE'nin biyobelirteçlerle ilişkisi incelendi.

Bulgular: AYHE (+) grubu anlamlı derecede daha yaşlıydı, hipertansiyon prevalansı, NT-proBNP (508,8 ± 249 pg/mL karşı 415,3 ± 292,1; *P* = 0,037), malondialdehit (MDA: 20,9 ± 4,1 μmol/L karşı 19,1 ± 3,1 μmol/L; *P* = 0,006) ve nitrik oksit sentaz (iNOS) aktivitesi (1.935,9 ± 326,1 pg/mL karşı 1.677,4 ± 363,2 pg/mL; *P* < 0,001) daha yüksekti. Lojistik regresyon analizinde yaş, hipertansiyon, MDA (odds oranı [OR]: 1,131, %95GA: 1,009 - 1,268, *P* = 0,035), iNOS aktivitesi (OR = 1,002, %95 GA = 1,001 -1,003, *P* < 0,001) ve endotel nitrik oksit sentaz (eNOS) aktivitesi (OR = 0,990, %95 GA = 0,986 - 0,984, *P* < 0,001) AYHE'nin bağımsız belirleyicileri olarak belirlendi.

Sonuç: Çalışma bulguları, NT-proBNP, MDA, nitrik oksit plazma düzeylerinin ve iNOS ile eNOS ekspresyonunun AYHE ile anlamlı düzeyde ilişkili olduğunu gösterdi. Ayrıca, yüksek plazma MDA konsantrasyonları, iNOS aktivitesi artışı ve eNOS aktivitesi azalması, AYHE'nin bağımsız belirleyicileri olarak tanımlandı.

Anahtar Kelimeler: Atriyal yüksek hız epizodları, nitrik oksit, malondialdehit, indüklenebilir nitrik oksit sentaz, endotelyal nitrik oksit sentaz

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Subclinical atrial tachyarrhythmias, referred to as Atrial High Rate Episodes (AHRE), can be identified by cardiac implantable electronic devices (CIEDs) such as pacemakers (PM), internal cardiac defibrillators (ICD), or cardiac resynchronization therapy (CRT) devices with biventricular pacing.[1] These episodes of atrial tachyarrhythmia are usually asymptomatic and are often diagnosed incidentally during regular follow-up of patients with CIEDs. AHREs are widely considered precursors to atrial fibrillation (AF). Indeed, numerous studies have demonstrated that the presence of AHRE is associated with an increased risk of developing clinical AF, thromboembolism, cardiovascular and cerebrovascular events, and mortality.[1-3] Given these adverse effects of AHREs, current clinical practice guidelines recommend the detection and management of AHREs in patients with CIEDs.[1]

Many studies conducted in recent years have demonstrated the importance of oxidative stress in the pathophysiological mechanisms potentially leading to left atrial fibrosis and AF.[4] In one such study, Mihm et al. [5] demonstrated an elevated oxidative modification of myofibrillar proteins in atrial myocytes of AF patients. This alteration resulted in impaired function of fibrillar proteins, highlighting the significant role of oxidative stress in this context. In another study, Samman Tahhan et al.[6] showed that increased oxidative stress (OS), as measured by the redox potentials of glutathione, was associated with AF and AF-related adverse events. Cai et al.[7] demonstrated in an animal study that AF was associated with a noticeable reduction in endothelial nitric oxide synthase (eNOS) expression and nitric oxide (NO) bioavailability in the left atrium. Wu et al.[8] found that increased oxidative stress was associated with AF, and malondialdehyde (MDA) was a significant independent predictor for AF. In summary, sufficient experimental and clinical data indicate that AF involves very complex pathophysiological processes, including changes in the autonomic nervous system, atrial dilatation and enlargement, Ca^{2+} overload, inflammation, apoptosis, and fibrosis.[9-11] The value of oxidative stress markers in patients with AF has been assessed and demonstrated in several of the studies mentioned above. However, the relationships between AHRE, considered a precursor of AF, and MDA, NO, inducible NOS (iNOS), and eNOS have not yet been elucidated. In this context, the objective of this study is to examine the association between these markers and AHRE in patients with CIEDs.

ABBREVIATIONS

AF	Atrial fibrillation
AHRE	Atrial high rate episodes
ANOVA	Analysis of variance
AT	Atrial tachyarrhythmias
AUC	Area under the curve
CIEDs	Cardiac implantable electronic devices
CRT	Cardiac resynchronization therapy
ECHO	Echocardiographic
ELISA	Enzyme-linked immunoassay
eNOS	Endothelial nitric oxide synthase
HT	Hypertension
ICD	Internal cardiac defibrillators
iNOS	Inducible nitric oxide synthase
MDA	Malondialdehyde
NO	Nitric oxide
NT-proBNP	N-terminal pro-natriuretic peptide
OS	Oxidative stress
PM	Pacemakers
ROC	Receiver operating characteristic

Materials and Methods

Population and Sample

This cross-sectional, prospective study assesses patients with cardiac implanted devices (PMs, ICDs, CRT). Patients who visited the Kafkas University Hospital Cardiology Clinic for cardiac device monitoring between January 2021 and September 2022 were included. On the admission date, blood samples were collected, electrocardiographic records were obtained and preserved, and echocardiographic measurements were taken and stored. Patients were then retrospectively assessed for the presence of AHRE, regardless of their initial status. Patients with single-chamber pacemakers or ICDs, a documented history of atrial fibrillation/flutter (AF/AFL), moderate or severe valve disease, or heart valve prostheses were excluded from the study. The study was conducted in accordance with the ethical principles outlined in the Helsinki Declaration, and informed consent was obtained from the patients. The study protocol was approved by the Ethics Committee of Kafkas University Faculty of Medicine (Approval Number: 80576354-050-099/256, Date: 24.02.2023).

Definition of Atrial High Rate Episodes

AHRE refers to asymptomatic atrial arrhythmias characterized by an atrial rate exceeding 175 bpm for a duration of at least 30 seconds, detected by CIEDs (Figure 1). The devices' diagnostic information was interrogated to assess whether patients had developed AHREs since the last device check-up. Device diagnostic data on AHREs were reviewed by an experienced electrophysiologist who was blinded to clinical outcomes. Atrial electrograms corresponding to detected AHREs were categorized as either adequate or inadequate detections. Inadequate detections, such as artifacts/double counts, ventricular far-field R-wave detection, or repetitive non-reentrant ventricular-atrial synchrony, were excluded.[12]

Data Collection

Baseline demographic variables of the patients were obtained from their medical records. Patients' initial demographic information was retrieved from their medical records. Hemogram tests and measurements of biochemical markers were performed on blood samples collected from all patients during their outpatient clinic visits. The automated hematology and biochemical analyzer (Roche Diagnostics Cobas 8000 c502, Indianapolis, USA) was used for conducting hemogram tests and measuring biochemical markers.

Blood samples collected for MDA and NO measurements were transferred into tubes and then centrifuged at 4000 g and 4°C for 10 minutes. The resulting sera were separated and subsequently stored at -25°C until analysis. For MDA concentration, an indicator of lipid peroxidation, measurements were taken at 535 nm following the method outlined by Yoshioka et al.[13] This method relies on the reaction between thiobarbituric acid and MDA. NO levels were assessed using the method described by Miranda et al.[14] In this procedure, nitrate is converted to nitrite by VCl3, followed by the reaction of nitrite with sulfonyl amide in an acidic environment, producing a colored diazonium compound measured at 540 nm.

Inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS) were quantified using commercially available

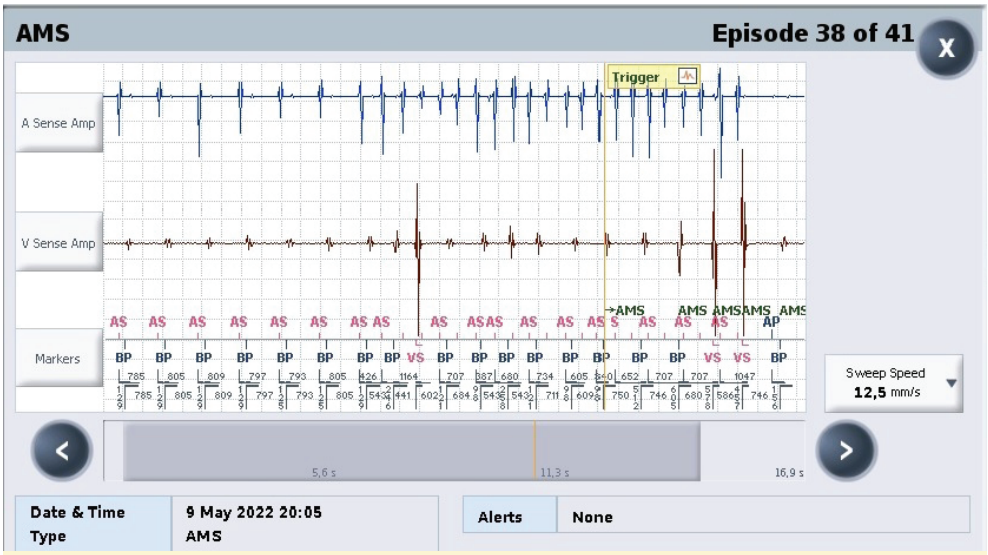

Figure 1. Example of an electrogram of an Atrial High Rate Episode.

enzyme-linked immunoassay (ELISA) kits provided by Elabscience Biotechnology, Beijing, China. N-terminal pro-natriuretic peptide (NT-proBNP) levels were assessed using ELISA kits from ELK Biotechnology, Wuhan, China. All measurements were performed using a spectrophotometer (Epoch, Biotek, USA).

Echocardiographic (ECHO) assessments were conducted using the General Electric Vivid-7 ultrasound machine (General Electric, Milwaukee, WI, USA). Subsequently, all ECHO images were reviewed offline and independently analyzed by two cardiologists in a blinded manner, utilizing proprietary software (EchoPAC, version 202, GE, Norway). The ECHO measurements were conducted in accordance with the guidelines established by the American Society of Echocardiography.[15]

Statistical Analysis

Statistical analysis was performed using the SPSS 24.0 software package (Statistical Product and Service Solutions for Windows, Version 24.0, IBM Corp., Armonk, NY, U.S., 2016). Statistical significance was indicated by probability (p) values of ≤ 0.05. For continuous variables exhibiting a normal distribution, analysis included histograms along with Kolmogorov-Smirnov and Shapiro-Wilk tests. These normally distributed continuous variables were presented as mean ± standard deviation (SD) and analyzed via Student's t-test or analysis of variance (ANOVA). Continuous variables not following a normal distribution were presented as median values (25^{th}-75^{th} percentile) and assessed using Mann-Whitney U or Wilcoxon's rank-sum tests. Categorical variables were expressed in absolute values and percentages, and analyzed using Pearson's chi-square or Fisher's exact tests. Variables showing statistically significant p-values in univariate analysis were further examined via multivariate logistic regression analysis to identify independent predictors of AHRE. Variables with statistically significant p-values in multivariate analysis were subjected to receiver operating characteristic (ROC) curve analysis. NT-proBNP levels underwent logarithmic transformation for Cox regression analysis to normalize the distribution of the variable. The logarithmic transformation was executed using logarithm10. Optimal cut-off values for these variables, in terms of estimating AHRE, were determined using Youden's index. Additionally, the comparison of Area Under the Curve (AUC) values between MDA, iNOS, and eNOS variables was conducted using MedCalc statistics software (MedCalc Software demo, version 11.4, Ostend, Belgium).

Results

The current study consisted of 180 patients with CIEDs. The mean age of the study group was 69 ± 12 years. Patients with AHRE (n = 78) were included in the AHRE (+) group, and patients without AHRE (n = 102) were included in the AHRE (-) group. The AHRE (+) group was significantly older than the AHRE (-) group (73 ± 10 years vs. 69 ± 12 years; $P < 0.001$). In terms of comorbidities, patients with AHRE had a higher prevalence of hypertension (HT) [n = 62, (79.5%) vs. n = 62, (60.8%); $P = 0.007$]. Patients' baseline demographic characteristics according to the AHRE groups are shown in Table 1. Patients with AHRE had significantly higher NT-proBNP (mean [SD], 508.8 ± 249 pg/mL vs. 415.3 ± 292.1; $P = 0.037$) and MDA (mean [SD], 20.9 ± 4.1 µmol/L vs. 19.1 ± 3.1 µmol/L; $P = 0.006$) levels, iNOS activity (mean [SD], 1,935.9 ± 326.1 pg/mL vs. 1,677.4 ± 363.2 pg/mL; $P < 0.001$), and left atrium-anteroposterior (LA-AP) diameter (mean [SD]; 45 ± 6 mm vs. 43 ± 7 mm; $P = 0.044$). In contrast, they had significantly lower eNOS activity (mean [SD], 207.9 ± 107.1 pg/mL vs. 310.2 ± 100.3 pg/mL; $P < 0.001$), and NO levels (mean [SD], 29.2 ± 10.3 µmol/L vs. 37.6 ± 9.5 µmol/L; $P < 0.001$). The distribution of the laboratory and echocardiographic characteristics of the study sample by the AHRE groups is shown in Table 2.

Univariate analyses revealed significant correlations between AHRE and age, HT, high MDA and NT-proBNP levels, and iNOS activity, as well as low NO levels and eNOS activity. These variables were further analyzed with multivariate regression analysis, and the results were adjusted to account for the significant differences between the groups. Consequently, age (odds ratio [OR]: 1.041, 95% Confidence interval [CI]: 1.004 - 1.079, $P = 0.031$), HT (OR = 3.059, 95% CI = 1.250 - 7.484, $P = 0.014$), MDA (OR = 1.131, 95% CI = 1.009 - 1.268, $P = 0.035$), iNOS activity (OR = 1.002, 95% CI = 1.001 - 1.003, $P < 0.001$), and

Table 1. Demographic Variables of Patients with and without AHRE

	Based on the Presence of AHRE			*P*
	Patients with AHRE, n = 78	**Patients without AHRE, n = 102**	**Total, n = 180**	
Age, years	73 ± 10	66 ± 12	69 ± 12	**<0.001**
Male, n (%)	41 (52.6)	66 (64.7)	107 (59.4)	0.101
Smoking, n (%)	12 (15.4)	16 (15.7)	28 (15.6)	0.956
DM, n (%)	29 (37.2)	26 (25.5)	55 (30.6)	0.092
CAD, n (%)	62 (79.5)	72 (70.6)	134 (74.4)	0.176
COPD, n (%)	23 (29.5)	20 (19.6)	43 (23.9)	0.125
HT, n (%)	62 (79.5)	62 (60.8)	124 (68.9)	**0.007**
HL, n (%)	44 (56.4)	46 (45.1)	90 (50)	0.134
Stroke/TIA, n (%)	11 (14.1)	6 (5.9)	17 (9.4)	0.062
Beta-blocker, n (%)	54 (69.2)	82 (80.4)	136 (75.6)	0.085
ACEI/ARB, n (%)	57 (73.1)	84 (82.4)	141 (78.3)	0.135
Statin, n (%)	36 (46.2)	59 (57.8)	95 (52.8)	0.121
CIED, type				
PM, dual chamber	38 (48.7)	49 (48)	87 (48.3)	0.462
ICD, dual chamber	15 (19.2)	33 (32.4)	48 (26.7)	
CRT-D	25 (32.1)	20 (19.6)	45 (25)	
CIED, indication				
AV-block	29 (37.2)	42 (41.2)	71 (39.4)	0.271
HF	38 (48.7)	54 (52.9)	92 (51.1)	
Other	11 (14.1)	6 (5.9)	17 (9.4)	
Cum % AP	23 ± 20	18 ± 16	20 ± 18	0.192
Cum % VP	73 ± 39	64 ± 42	68 ± 41	0.315
LA-AP, (cm)	45 ± 6	43 ± 7	44 ± 7	**0.044**
LVEDD, (cm)	52 ± 9	50 ± 7	51 ± 8	0.129
EF, (%)	49 ± 11	46 ± 9	47 ± 10	0.108

ACEI, Angiotensin Converting Enzyme Inhibitors; ARB, Angiotensin Receptor Blockers; CAD, Coronary Artery Disease; CIED, Cardiac Implantable Electronic Devices; COPD, Chronic Obstructive Pulmonary Diseases; CRT-D, Cardiac Resynchronization Therapy-Defibrillator/Pacemaker; Cum % AP, Cumulative percentage of atrial pacing; Cum % VP, Cumulative percentage of ventricular pacing; DM, Diabetes Mellitus; HF, Heart Failure; HL, Hyperlipidemia; HT, Hypertension; ICD, Intracardiac Defibrillator; LA-AP, Left Atrium Antero-posterior diameter; PM, Pacemaker; TIA, Transient Ischemic Attack.

eNOS activity (OR = 0.990, 95% CI = 0.986 - 0.984, *P* < 0.001) were found to be independent predictors of AHRE (Table 3).

ROC curve analysis revealed the optimal cut-off values for variables that were found to be independent predictors of AHRE in the multivariate analysis. Accordingly, MDA values > 22.9 µmol/L predicted AHRE with a sensitivity of 39.7% and specificity of 86.3% (AUC = 0.619, 95% CI = 0.544 - 0.691, *P* < 0.001), iNOS values > 1,526.8 pg/mL predicted AHRE with a sensitivity of 89.7% and specificity of 45.1% (AUC = 0.697, 95% CI = 0.625 - 0.764, *P* <0.001), and eNOS values ≤ 177.8 pg/mL predicted AHRE with a sensitivity of 61.5% and specificity of 81.4% (AUC = 0.762, 95% CI = 0.693 - 0.822, *P* < 0.001) (Figure 2).

Discussion

The study findings revealed that plasma levels of MDA and iNOS activity were significantly higher, while eNOS activity was notably lower in patients with AHREs compared to those without AHREs. In addition, serum MDA level, and iNOS and eNOS concentrations were found to be independent predictors of AHRE in patients with CIEDs.

The increasing use of cardiac implanted electronic devices (CIEDs) has enhanced our ability to assess the type and burden of atrial arrhythmias. These devices can detect episodes of subclinical atrial tachyarrhythmias (AT), referred to as atrial high-rate episodes (AHREs).[1] AHREs are reportedly precursors of AF and are associated with poor cardiovascular and cerebrovascular outcomes and mortality.[1,3] Therefore, the detection and management of AHRE is crucial for survival.

The relationship between AHRE and age has been demonstrated in recent studies. Miyazawa et al.[16] reported that aging and a history of AF/AFL were risk factors for the existence of AHRE. Li et al.[17] found that age and HT were the most significant risk factors for AHRE and also that age > 75 was an independent predictor for AHRE. Age-related atrial remodeling, characterized by decreased contractile functions and impaired relaxation, can lead to AHRE as well as AF, with pathological compensatory mechanisms such as hypertrophy, interstitial fibrosis, and electrical remodeling.[18] Similarly, in our study, the prevalence of hypertension (HT) was significantly higher among patients with detected AHRE compared to those without

Table 2. Laboratory and Echocardiographic Characteristics of Patients According to AHRE

	Based on the Presence of AHRE			
	Patients without AHRE, n = 102	**Patients with AHRE, n = 78**	**Total, n = 180**	***P***
WBC count, (/1000)	8.1 ± 2.5	7.7 ± 1.9	7.9 ± 2.3	0.343
Neutrophil, (/1000)	4.7 (3.9-6.1)	4.66 (3.8-5.6)	4.7 (3.85-5.85)	0.438
Hemoglobin, g/dL	13.6 ± 1.8	13.9 ± 1.7	13.7 ± 1.7	0.320
Platelet, 100/L	222 ± 60	234 ± 66	227 ± 63	0.161
Sodium, mmol/L	136 ± 3	139 ± 3	139 ± 3	0.231
Potassium, mmol/L	4.26 ± 0.49	4.37 ± 0.46	4.31 ± 0.48	0.226
Magnesium, mg/dL	1.89 ± 0.3	2.16 ± 1.09	2.01 ± 1.4	0.295
Glucose, mg/dL	111.5 (97-128)	109.5 (93-129)	111 (95.5-128)	0.362
Creatinine, mg/dL	1.1 ± 0.41	1.05 ± 0.35	1.08	0.214
Albumin, g/L	40 ± 5	39 ± 5	40	0.893
LDL-C, mg/dL	100.6 ± 38.2	109.9 ± 33.9	104.7	0.068
TG, mg/dL	113.5 (86-153)	128 (97-190)	119.5 (88-167.5)	0.098
HDL-C, mg/dL	46.2 ± 9.9	45.3 ± 10.9	45.8 ± 10.3	0.465
Total Cholesterol, mg/dL	173.3 ± 47.5	183.2 ± 39.5	177.6 ± 44.4	0.125
hs-CRP, mg/dL	3.48 (1.34-6.45)	4.37 (2.27-7.72)	3.88 (1.58-6.9)	0.078
Uric acid, mg/dL	5.74 ± 1.48	6.33 ± 1.98	6.01 ± 1.73	0.105
TSH, mIU/L	1.46 (1.01-2.21)	1.25 (0.72-1.93)	1.34 (0.92-2.04)	0.092
NT-proBNP, pg/mL	415.3 ± 292.1	508.8 ± 249	468.3 ± 271.8	**0.037**
MDA, µmol/L	19.1 ± 3.1	20.9 ± 4.1	19.9 ± 3.7	**0.006**
iNOS, pg/mL	1,677.4 ± 363.2	1,935.9 ± 326.1	1,789.4 ± 369.8	**<0.001**
eNOS, pg/mL	310.2 ± 100.3	207.9 ± 107.1	265.7 ± 114.8	**<0.001**
NO, µmol/L	37.6 ± 9.5	29.2 ± 10.3	34.1 ± 10.6	**<0.001**
LA-AP, (mm)	43 ± 7	45 ± 6	44 ± 7	**0.044**
LVEDD, (mm)	50 ± 7	52 ± 9	51 ± 8	0.129
EF, (%)	46 ± 9	49 ± 11	47 ± 10	0.108

EF, Ejection Fraction; eNOS, Endothelial Nitric Oxide Synthase; HDL-C, High Density Lipoprotein Cholesterol; hs-CRP, High-Sensitivity C-Reactive Protein; iNOS, Inducible Nitric Oxide Synthase; LA-AP, Left Atrium Antero-Posterior; LDL-C, Low Density Lipoprotein-Cholesterol; LVEDD, Left Ventricular End-Diastolic Diameter; MDA, Malondialdehyde; NO, Nitric Oxide; NT-proBNP, N-terminal prohormone of Brain Natriuretic Peptide; TG, Triglyceride; TSH, Thyroid Stimulating Hormone; WBC, White Blood Cell.

Table 3. Logistic Regression Analysis for AHRE Predictors

	Univariate			Multivariate		
	OR	**95% CI**	***P***	**OR**	**95% CI**	***P***
Age	1.054	(1.024-1.085)	<0.001	1.041	(1.004-1.079)	**0.031**
HT	2.501	(1.269-4.926)	0.008	3.059	(1.250-7.484)	**0.014**
LA-AP	1.032	(0.988-1.077)	0.155		-	
NT-proBNP	0.272	(0.108-0.682)	0.006		-	
MDA	1.142	(1.050-1.243)	0.002	1.131	(1.009-1.268)	**0.035**
iNOS	1.002	(1.000-1.003)	<0.001	1.002	(1.001-1.003)	**<0.001**
eNOS	0.991	(0.988-0.994)	<0.001	0.990	(0.986-0.994)	**<0.001**
NO	0.920	(0.890-0.951)	<0.001		-	

eNOS, Endothelial Nitric Oxide Synthase; HT, Hypertension; iNOS, Inducible Nitric Oxide Synthase; LA-AP, Left Atrium Antero-Posterior; MDA, Malondialdehyde; NO, Nitric Oxide; NT-proBNP, N-terminal prohormone of Brain Natriuretic Peptide.

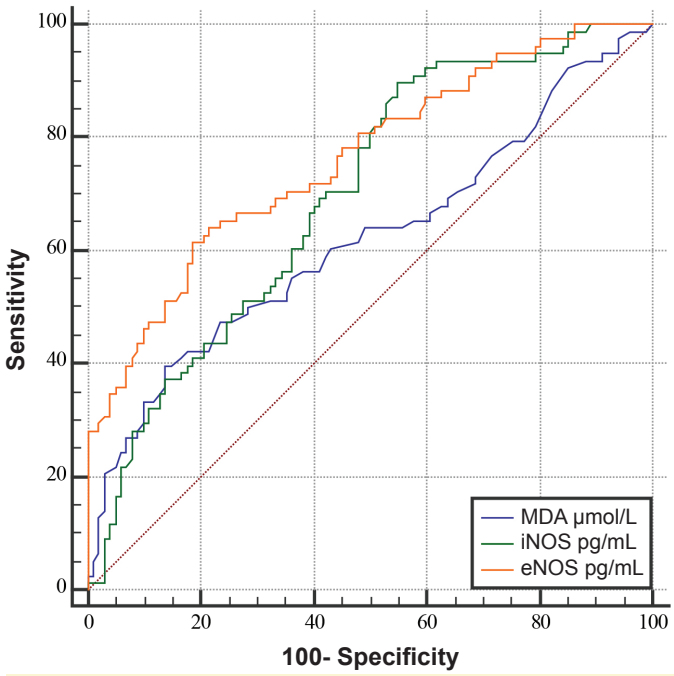

Figure 2. Receiver Operating Characteristic (ROC) curve analysis for MDA, iNOS and eNOS.
AHRE, Atrial High Rate Episodes; eNOS, Endothelial Nitric Oxide Synthase; iNOS, Inducible Nitric Oxide Synthase, MDA, Malondialdehyde.

AHRE. Suvro Banerjee revealed that HT was significantly and independently associated with AHRE development.[19] Additionally, other research demonstrated that HT was associated with AHRE and had a strong impact on the development of AHRE lasting more than 24 hours.[20] HT is associated with changes in left atrial structure, including left atrial enlargement, functional changes, altered left atrial electrophysiology, and enhanced ectopic atrial activity. Atrial remodeling due to these changes may also lead to AHRE development.[21] Furthermore, recent studies revealed that left atrial (LA) diameter was associated with AHRE.[16,22] In line with the literature, patients with AHRE in our study had significantly longer LA diameters. Increased LA volumes are accompanied by progressive interstitial fibrosis and changes in LA function, which may initiate and sustain AHRE.[23]

Patients with AHRE in this study also had significantly higher NT-proBNP levels compared to those without AHRE. NT-proBNP is a well-established laboratory marker for heart failure. Several studies have demonstrated that elevated brain natriuretic peptide (BNP) levels were predictors of AF prevalence and incidence.[24] Elevated levels of natriuretic peptides reflect the myocyte response to atrial wall stretch, increased wall tension, and atrial pressure. Enhanced atrial pressures lead to atrial myopathy, dysfunction, and remodeling. The role of atrial remodeling in the development of AHRE is well established. Therefore, it is not surprising that NT-proBNP was found to be significantly increased in patients with AHRE.[25]

On the other hand, this is the first study to date to report MDA levels, and iNOS and eNOS activities as independent predictors for AHRE development. Patients with AHRE had significantly increased serum MDA levels and iNOS activities, and considerably decreased eNOS activities compared to patients without AHRE. Previous studies have shown that oxidative stress may induce structural and electrical remodeling of the atrium, thus playing a vital role in the pathogenesis of AF.[26,27] MDA is one of the most common markers of lipid peroxidation. Elevated levels of MDA imply enhanced oxidative damage to lipid biomolecules and serve as an indirect indicator of increased levels of reactive oxygen species (ROS). Oxidative stress occurs as a result of increased oxidative processes and reduced defense antioxidant mechanisms.[28] Increased oxidative stress leads to structural atrial remodeling, which may play a role in the development of AHRE.[29,30] In comparison, patients with AHRE exhibited increased plasma levels of NO and iNOS activity, along with decreased expression of eNOS. NO is an endothelium-derived vasodilator and a signaling molecule associated with cardiovascular conditions such as hypertension (HT), myocardial infarction (MI), and heart failure (HF). Both Minamino et al.[31] and Han et al.[32] have demonstrated significant reductions in plasma NO levels among patients with AF. Han et al.[32] also reported upregulation of iNOS expression in patients with permanent AF. Dohi et al. [33] showed that heightened iNOS expression could induce apoptosis. In line with this, Ramasamy et al.[34] reported that targeted iNOS inhibition resulted in reduced myocyte apoptosis and diminished cardiac damage. Cai et al.[7], in an animal study, showed that AF was associated with a notable decrease in eNOS expression and NO bioavailability. Expanding on this, Agnoletti et al.[35] indicated that reduced eNOS expression correlated with increased apoptosis in patients with severe congestive heart failure. Therefore, the imbalanced expression of iNOS and eNOS may contribute to structural remodeling via an apoptosis mechanism, leading to atrial remodeling and the development of AHRE.[32] Additionally, turbulent flow conditions in the atrium and altered endothelial cellular functions may result in reduced eNOS activity and decreased plasma NO levels, facilitating the progression and persistence of AHRE in patients with CIEDs.[31]

Limitations of the Study

This study had several limitations. Firstly, the number of patients who developed AHRE in this study was limited. Therefore, further prospective, multicenter, large-scale studies are required to confirm the findings of this study. Secondly, the study did not include a follow-up period. Hence, future studies with adequate follow-up periods should be conducted to observe the long-term effects of MDA, NO, iNOS, and eNOS on the prognosis of patients with AHRE, particularly in terms of the incidence of cardiovascular or thromboembolic events. Finally, this study examined the relationship between laboratory results and retrospective events, which constitutes a limitation of our research.

Conclusion

In conclusion, the study findings indicate that plasma levels of NT-proBNP, MDA, NO, and the expression of iNOS and eNOS were significantly associated with the existence of AHRE. Additionally, elevated plasma MDA concentrations, increased iNOS activity, and decreased eNOS activity were identified as independent predictors of AHRE.

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