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The Relationship between Oxidative Stress Parameters and Diastolic Function Indices Determined with Tissue Doppler Echocardiography

Gültekin Günhan Demir[1*]
Aycan Fahri Erkan[2]
Berkay Ekici[2]
Gamze Yıldırım[3]
Gürkan İş[4]
Utku Kütük[2]
Hasan Fehmi Töre[2]

[1]Department of Cardiology, Medipol University, Istanbul, Turkey
[2]Department of Cardiology, Ufuk University, Ankara, Turkey
[3]Mardin State Hospital, Mardin, Turkey
[4]Department of Cardiology, Başkent University, Ankara, Turkey

Abstract

Background and Aim: Oxidative stress, imbalance between ROS production and antioxidant capacity, has been shown to play a crucial role in the pathogenesis of numerous acute and chronic diseases. In this study, we aimed to investigate the relationship between diastolic dysfunction and oxidative stress parameters; advanced oxidation protein products (AOPP) and malondialdehyde (MDA).

Methods and Results: A total of 42 patients (male: 18, mean age 60 ± 13) were included. AOPP and MDA levels, oxidative stress parameters, were measured by spectrophotometric method from venous blood samples. Diastolic functions of the patients were evaluated with mitral inflow velocities and tissue Doppler imaging indices. For the statistical analysis, we included the significant variables determined by univariate regression analysis in two different multivariate linear regression analysis models to test the effects of echocardiographic parameters on AOPP and MDA levels. Logarithmic transformation was used for some variables such as ejection fraction, high-sensitive C reactive protein (HsCRP), glucose, AOPP because of skewed distribution Univariate linear regression showed that Log-HsCRP (t: 2.589, *p*: 0.016), Log-ejection fraction (t: -2.110, *p*: 0.041), E/A ratio (t: -2.430, *p*: 0.020), E' wave (t: -3.562, *p*: 0.001), A' wave (t: 2.082, *p*: 0.044) were significantly associated with Log-AOPP levels. Additionally, for MDA levels, E wave (t: -2.161, *p*: 0.037) and isovolumetric contraction time (t: 2.133, *p*: 0.039) were found to be significant by univariate linear regression analysis. Two different multivariate linear regression analysis models were presented for independent predictors of oxidative stress. First model showed that Log-AOPP was negatively associated with E' wave (t: -3.225, *p*: 0.003) and second multivariate regression model showed that E wave (t: -2.315, *p*: 0.026) was negatively correlated with MDA levels.

Conclusion: Diastolic dysfunction may be associated with increased levels of AOPP and MDA, two different markers of oxidative stress.

Keywords

Oxidative stress parameters; Diastolic function indices; Tissue doppler echocardiography

Introduction

Considerable data indicate that reactive oxygen species and oxidative stress are important features of cardiovascular diseases including atherosclerosis, hypertension, and congestive heart failure [1-3]. Oxidative stress may result from either an overproduction of reactive oxygen species (ROS) relative to antioxidant capacity, a decrease in antioxidant ability to limit ROS accumulation, or a combination of both mechanisms [4].

Since the measurement of ROS *in vivo* is not possible, there are several oxidative stress parameters for assessment of ROS. In this study, oxidative stress was assessed using two parameters; advanced oxidation protein products (AOPP) which is a marker of protein oxidation, and malondialdehyde (MDA) which is a marker of lipid peroxidation. Heart failure (HF) is a clinical syndrome symptoms and signs of which are due to increased extravascular water and decreased tissue/organ perfusion. The definition of the mechanisms inducing HF needs the measurement of both left ventricular (LV) systolic and diastolic function since HF may occur in patients with either normal or abnormal LV ejection fraction (EF) [5].

Left ventricular diastolic dysfunction (DD) and diastolic heart failure, that is symptomatic DD, are due to alterations of myocardial diastolic properties. These alterations involve relaxation and/or filling and/or distensibility. Arterial hypertension associated to LV concentric remodelling is the main determinant of DD but several other cardiac diseases, including myocardial ischemia, and systemic disorders involving the heart are other possible

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*Corresponding author:

Gültekin Günhan Demir, MD
Department of Cardiology, Medipol University, Bağcılar 34214 İstanbul, Turkey
Fax: +90 212 460 70 70
Tel: +905336320488
E-mail: ggdemir@gmail.com

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causes. In the majority of the studies, isolated diastolic HF has been made equal to HF with preserved systolic function (=normal ejection fraction) but the true definition of this condition needs a quantitative estimation of LV diastolic properties. Despite an apparently lower death rate in comparison with LV systolic HF, long-term follow-up (more than 5 years) show similar mortality between the two kinds of HF [6].

The aim of this study was to evaluate the relationship between diastolic function indices measured by transthoracic echocardiography and oxidative stress markers such as AOPP and MDA. The association of hypertension with oxidative stress and diastolic dysfunction separately is well known but the direct relationship between oxidative stress and diastolic dysfunction has not been evaluated before.

Methods

Study population

A total of 42 patients aged between 18 and 60 years were enrolled in our study after obtaining informed consent. Baseline demographic and clinical characteristics are presented in Table 1. 21 patients with documented diastolic dysfunction were classified as the case group and 21 patients without diastolic dysfunction were classified as control group. The case group was divided into two subgroups according to the stage of diastolic dysfunction. Patients with Grade I diastolic dysfunction were classified as subgroup I whereas patients with Grade II diastolic dysfunction were classified as subgroup II. The study was performed between March and May 2011. The protocol was approved by local ethical review board.

Eligible patients were between 18 and 60 years, having fine acoustic window for echocardiographic evaluation.

Exclusion criteria included: documented coronary artery disease, systolic heart failure (LVEF<55%), chronic renal failure, obesity (BMI>30), aortic stenosis, any severe valvular disease, myocardial disease, deposition disease, pericardial disease, chronic consumption of dietary supplements or drugs having antioxidative properties.

Oxidative stress parameters

Measurement of advanced oxidation protein products (AOPP): AOPP levels were studied in the AU 2700 autoanalyzer (Olympus Diagnostics Inc. Melville, NY) with spectrophotometry method as described by Witko-Sarsat et al [7]. Blood samples were drawn from cubital veins in tubes with ethylene diamine tetra acetate (EDTA), and centrifuged at 1660 × g at 4°C for 10 min. The blood samples were divided in aliquots and kept in Eppendorf tubes at −20°C. All samples were analyzed within approximately 30 days. After preparation of chloramine-T stock solution (10 mmol/l), they were diluted 100 times with PBS (20 mmol/l, pH:7.4) to obtain a main standard solution at 100 µmol/l concentration (standard 1). Chloramine-T main standard solution (standard 1:100 µmol/l) was diluted with PBS (20 mmol/l, pH 7.4) to get a 5-point calibration curve, and also to prepare Chloramine-T standards at 75, 50, 25, and 12.5 µmol/l concentrations. PBS (160 µl) was added to 40 µl standard or plasma, mixed, and incubated for 25 s. The absorbance of the mixture was read at 340 nm, and then 20 µl of acetic acid was added and incubated for 25 s. Finally, 10 µl KI solution was added and reincubated for 25 s, and its absorbance was read again. All steps were completed at 37°C in a single cuvette. Time intervals were arranged at every step as 25 s or longer based on the program characteristics of the analyzer. A calibration curve was formulated using absorbance (A) values corresponding to the concentrations of 5 standard solutions. AOPP concentration was reported as µmol Chloramine-T/l, corresponding to the absorbance measured.

Measurement of malonyl dialdehyde (MDA): MDA was determined in plasma by the 2-thiobarbituric acid reactive substances (TBARS) method [8]. Blood samples drawn from the cubital vein were placed in blood tubes containing EDTA as an anticoagulant. Plasma samples were separated rapidly, and cryopreserved at −70°C. A 140 µl standard, sample, and reagent blank, were placed individually into microcentrifuge tubes. "Reagent" (455 µl) was then added into each tube and vortexed. HCl (105 µl; 12 N [37%]) was added into each tube as well. The tubes were stirred thoroughly, tapped close, and incubated at 95°C for 60 min in a milieu of acidity provided by HCl. Then, 1 molecule of MDA and 2 molecules of reagent (N-methyl-2-phenylenilindol) reacted with each other to yield a stable chromophor product (colored product), which might provide a maximal absorbance spectrum at a 586 nm wavelength. Centrifugation at 13,000 × g for 15 min yielded a clear supernatant sample. This sample (150 µl) was placed in each well. Their fluorescent activities were measured on a microplate reader (Synergy™ 2 Multi-Mode; BioTek Instruments, Inc., Vinooski, VT) at 500 nm (± 30) excitation, and 586 nm (± 30) emission. Using a y=ax+b formula derived from the absorbance-concentration correlation of standards used for the construction of the MDA standard curve, and absorbance data obtained, the analyzer automatically calculated MDA concentrations, and the results were expressed as "µmol/l."

Echocardiography

Echocardiographic examination was performed with the patient in the left lateral decubitus position, using a commercially available system (Vivid 7, General Electric-Vingmed, Horton, Norway). Images were obtained, with a simultaneous ECG signal, using a 3.5 MHz transducer at a depth of 16 cm in the parasternal and apical views. Pulsed-wave Doppler of the mitral valve was obtained by placing Doppler sample volume between the tips of mitral leaflets. The early (E) and late (A) peak diastolic velocities and E-wave deceleration times were measured. The E' velocity was measured using color-coded tissue Doppler imaging at the septal and lateral side of the mitral annulus in the apical four-chamber view.

Diastolic function was graded according to the most recent recommendations of the American Society of Echocardiography. Diastolic function was graded as normal, when septal E' was ≥ 8 cm/s and lateral E' was ≥ 10 cm/s. Diastolic dysfunction was graded as Grade I (mild), when septal E' was <8, E/A ratio <8 and deceleration time >200 ms; Grade II (moderate), when septal E' was <8, E/A ratio 0.8-1.5 and deceleration time 160-200 ms. Grade III (severe) diastolic dysfunction was defined , when septal E' was <8, E/A ratio ≥ 2 and deceleration time < 160 ms.

Statistical analysis

All statistical tests were performed using the SPSS 18.0 (Statistical Package for the Social Sciences for Windows, Chicago, IL, USA) software. Continous variables were expressed as mean ± standard deviation and categorical variables were expressed as percentages. Correlation analysis was performed by using the Spearman's correlation test. p values <0.05 were considered statistically significant.

Results

General characteristics of the case and control group patients are shown in Table 1. Patients in the case group were significantly younger than the ones in the control group (39.71 ± 8.37 *vs.* 49 ± 8.84, p=0.003). The percentage of female patients was significantly lower in the case group than control group (42.9% *vs.* 61.5%, p<0.04) just as in the case of the number of hypertensive patients (9.5% *vs.* 38.1%, p<0.03).

In the case group 9 of 21 patients (42.8%) had Grade I diastolic dysfunction (subgroup I) where as 12 of 21 patients (57.2%) had Grade II diastolic dysfunction (subgroup II). None of the patients had Grade III diastolic dysfunction.

Laboratory findings of both groups are shown in Table 2. Fasting blood glucose (FBG) was significantly higher in the case group (121 ± 61 mg/dl *vs.* 90 ± 10 mg/dl, p<0.002). Uric acid levels were significantly higher in the case group than the control group (5 ± 1 *vs.* 3.8 ± 1, p<0.001). HDL levels did not differ significantly between groups, however, LDL, triglyceride and total cholesterol levels were significantly higher in the case group when compared with control group (145 ± 33 mg/dl *vs.* 108 ± 31 mg/dl, p<0.002 for LDL, 151 ± 83 mg/dl *vs.* 102 ± 77 mg/dl, p<0.002 for triglyceride, 223 ± 37 mg/dl

	Group				
	Case		Control		
	N	%	N	%	*p*
Age (years)	39. 71 ± 8.37		49 ± 8.84		0.003
Female gender	9	42.9 %	15	61.5 %	0.04
HT	2	9.5%	8	38.1%	0.03
DM	1	4.8%	3	14.3%	NS
HL	6	28.6%	4	19.0%	NS
Smoking	12	57.1%	10	47.6%	NS
ACEI	0	0%	0	0%	NS
ARB	0	0%	1	4.8%	NS
CCB	0	0%	2	9.5%	NS
BB	0	0%	1	408%	NS
Statin	2	9.5%	0	0%	NS

ACEI: Angiotensin converting enzyme inhibitor; ARB: Angiotensin receptor blocker; BB: Beta blocker; CCB: Calcium channel blocker; DM: Diabetes Mellitus; HL: Hyperlipidemia; HT: Hypertension; NS: Statistically not significant

Table 1: Demographic and clinical characteristics of the case and control groups

	Case		Control		
	Mean	Standard Deviation	Mean	Standard Deviation	*p*
Mean Platelet Volume	8.2286	0.9865	7.7190	0.9185	NS
Fasting blood glucose (mg/dl)	121	61	90	10	<0.002
Creatinine	0.6881	0.1465	0.6262	0.1562	NS
Uric acid	5.0062	0.9695	3.8695	1.0332	<0.001
LDL (mg/dl)	145	33	108	31	<0.002
HDL (mg/dl)	70	37	54	15	NS
Triglyceride (mg/dl)	151	83	102	77	<0.002
Total cholesterol (mg/dl)	223	37	178	33	<0.002
MDA (pmol/ml)	9.1733	2.1521	8.3617	1.7938	NS
AOPP (µmol/L)	171.8196	86.2251	88.2490	32.6775	<0.001
Hs CRP (mg/dl)	3.9525	3.3221	2.3322	2.5135	<0.04

AOPP: Advanced oxidation protein products; HsCRP: High sensitive C-reactive protein; HDL: High density cholesterol; LDL: Low density cholesterol; MDA: Malondialdehyde; NS: Statistically not significant

Table 2: Comparison of laboratory findings of the case and control groups

vs. 178 ± 33 mg/dl, $p<0.002$ for total cholesterol). High sensitive CRP levels were significantly higher in the case group when compared with control group (3.9 ± 3.3 mg/dl *vs.* 2.3 ± 2.5 mg/dl, $p<0.04$).

AOPP levels were significantly higher in the case group than those of the control group (171 ± 86 µmol/L *vs.* 88 ± 32 µmol/L, $p<0.001$) whereas MDA levels did not differ significantly between the case and control groups (9.1 ± 2.1 pmol/ml *vs.* 8.3 ± 1.7 pmol/ml, $p=0.204$). AOPP and MDA levels did not differ significantly between patients having Grade I and Grade II diastolic dysfunction (subgroups I and II) in the case group.

Number of hypertensive patients was significantly higher in the control group as stated above; however AOPP and MDA levels were not different between normotensive and hypertensive individuals across the whole study population.

E/A ratio was significantly lower in the control group. In the whole study group E/A ratio had a significant and inverse correlation with AOPP levels ($p=0.02$, r=-0.358). In the whole study group E' value did not have a significant relationship with AOPP but in the case group there was trend towards an increase in AOPP levels as E' value decreased ($p=0.44$, r=-305). There was a statistically significant relationship when E' values of subgroups were compared (0.09 ± 0.04 cm/s for subgroup I and 0.07 ± 0.03 cm/s for subgroup II, $p=0.04$).

Discussion

In this study we sought to assess the relationship between oxidative stress and diastolic dysfunction and their relevance with hypertension as well. AOPP was one of the two parameters we used for measuring oxidative stress intensity and we detected AOPP levels significantly higher in the patients who had diastolic dysfunction (case group).

Despite the fact that the case group was significantly (approximately 10 years) younger than the control group, the case group had diastolic dysfunction and significantly higher AOPP levels. This situation can be explained by the fact that oxidative stress is more strictly related to metabolic parameters having negative effects on vascular integrity and endothelial function (i.e. high fasting blood glucose, dyslipidemia, hyperuricemia) than aging which was previously shown to increase oxidative stress.

The other parameter used for assessment of oxidative stress was MDA and we did not find a significant relationship between MDA levels and diastolic dysfunction. MDA is a marker of lipid peroxidation and the low sensitivity and specificity of the method may influence the reliability of the results. Therefore, oxidative protein products are increasingly used instead of lipid species in the evaluation of oxidative stress [9].

Oxidative stress is a consequence of the imbalance between ROS

production and antioxidant capacity. This can occur as a result of either heightened ROS generation, impaired antioxidant system, or a combination of both. In the presence of oxidative stress, uncontained ROS attack, modify, and denature functional and structural molecules leading to tissue injury and dysfunction. Reduced nitric oxide bioavailability has an important role in the process of endothelial dysfunction and hypertension [10]. Oxidative stress and its constant companion, inflammation, play a critical part in the pathogenesis of many acute and chronic illnesses including hypertension and its long-term complications. There is compelling evidence that oxidative stress, inflammation, and hypertension are involved in a self-perpetuating vicious cycle which, if not interrupted, culminates in progressive target organ injury and dysfunction [11]. In this study, despite the number of hypertensive patients was significantly lower in the case group, oxidative stress may have triggered diastolic dysfunction which is believed to be precursor of diastolic heart failure at an earlier age.

Oxidative stress has been shown to play an important role in the pathophysiology of cardiac remodeling and heart failure (HF). It induces subtle changes in intracellular pathways, redox signaling, at lower levels, but causes cellular dysfunction and damage at higher levels. ROS are derived from several intracellular sources including mitochondria, NAD(P)H oxidase, xanthine oxidase, and uncoupled nitric oxide synthase. The production of ROS is increased within the mitochondria from failing hearts whereas normal antioxidant enzyme activities are preserved [12].

Inflammation triggered by oxidative stress is the cause of much, perhaps even most, chronic human disease including human aging. The oxidative stress originates mainly in mitochondria from reactive oxygen and reactive nitrogen species (ROS/RNS) and can be identified in most of the key steps in the pathophysiology of atherosclerosis and the consequential clinical manifestations of cardiovascular disease [13].

The main limitation of this study is the relatively small sample size. Lack of follow-up in order to detect changes in diastolic dysfunction and oxidative stress, and their relationship over time may also be considered a limitation. We conclude that oxidative stress, as evidenced by elevated serum AOPP levels, is related to diastolic dysfunction from relatively early ages, even in the absence of hypertension. Oxidative stress may be the factor that directly causes endothelial dysfunction and vascular damage in the presence of metabolic abnormalities such as hyperglycemia, hyperuricemia or dyslipidemia. Vascular dysfunction in turn may lead to cardiac diastolic dysfunction. On the other hand, it may be argued that diastolic dysfunction is the earliest stage of heart failure, and subcellular abnormalities of the myocardium, especially those involving the mitochondria, may trigger oxidative stress. Thus, diastolic dysfunction per se may cause increased oxidative stress. Futher studies are warranted to clarify this issue.

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