Plasma and tissue oxidative stress index in patients with rheumatic and degenerative heart valve disease

Romatizmal ve dejeneratif kalp kapak hastalarında doku ve plazma oksidatif stres indeksi

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Objectives: We investigated whether patients with rheumatic and degenerative heart valve disease (HVD) differed with regard to plasma and tissue oxidative stress index (OSI).

Study design: The study included 56 patients who underwent valve replacement due to rheumatic (n=32; 15 males; mean age 47±10 years) and degenerative (n=24; 13 males; mean age 55±12 years) HVD. Plasma and tissue total oxidative status (TOS) and total antioxidative capacity (TAC) levels were measured and OSI was calculated.

Results: Patients with degenerative HVD had significantly higher age, increased interventricular septum thickness, and higher frequency of aortic stenosis, whereas the incidence of mitral stenosis was higher in patients with rheumatic HVD (p<0.05). Plasma oxidative characteristics did not differ between the two HVD groups (p>0.05). Tissue TAC was significantly lower in patients with rheumatic HVD (p=0.027), whereas tissue TOS and OSI were similar between the two HVD groups (p>0.05). In bivariate analysis, plasma OSI did not show any correlation with clinical, laboratory, and echocardiographic variables (p>0.05).

Conclusion: Our data show that plasma and tissue OSI levels are similar in patients with rheumatic and degenerative HVD.

Key words: Antioxidants; biological markers; heart valve diseases; oxidative stress; rheumatic heart disease.

Amaç: Bu çalışmada, romatizmal ve dejeneratif kalp kapak hastalığında (KKH) plazma ve doku oksidatif stres indeksi (OSİ) açısından farklılık olup olmadığı araştırıldı.

Çalışma planı: Çalışmaya kapak replasmanı yapılan 56 hasta alındı. Bunların 32'sinde (15 erkek; ort. yaş 47±10) romatizmal, 24'ünde (13 erkek; ort. yaş 55±12) dejeneratif KKH vardı. Plazma ve dokuda total oksidatif durum (TOD) ve total antioksidan kapasite (TAK) seviyeleri ölçüldü ve OSİ hesaplandı.

Bulgular: Dejeneratif KKH grubunda yaş, interventriküler septum kalınlığı ve aort darlığının sıklığı anlamlı derecede fazlaydı; romatizmal KKH grubunda ise mitral darlığı sıklığı daha yüksek bulundu (p<0.05). Plazma oksidatif parametreleri iki KKH grubu arasında farklılık göstermedi (p>0.05). Doku örneklerinde ise, romatizmal KKH grubunda TAK anlamlı derecede daha düşük bulunurken (p=0.027), TOD ve OSİ değerleri iki grupta benzerdi (p>0.05). İkili korelasyon analizinde, incelenen hiçbir klinik, laboratuvar ve ekokardiyografik parametre OSİ ile anlamlı ilişki göstermedi (p>0.05).

Sonuç: Bulgularımız, plazma ve doku OSİ seviyelerinin romatizmal ve dejeneratif KKH'de benzer olduğunu göstermektedir.

Anahtar sözcükler: Antioksidan; biyolojik belirteç; kalp kapağı hastalığı; oksidatif stres; romatizmal kalp hastalığı.

Heart valve disease (HVD) is a multifactorial process and its pathophysiology has not been fully understood. Degenerative and rheumatic HVD are caused by the interaction of several risk factors such as genetic, inflammatory, autoimmune, infectious, and oxidative stress.[1-3]

Heart valves are composed mainly of extracellular matrix, smooth muscle cells, fibroblasts, and endothelial cells.[4] Fibrosis and calcification are characteristic features of degenerative aortic valve lesions and the extent of lesion calcification is correlated with both a more rapid disease progression and worse clini-

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cal outcomes.[5] Since coronary atherosclerosis and valvular sclerosis are similar processes, they may occur coincidentally.[6] Risk factors for atherosclerosis produce lesions in the cardiac valves.[7] Aortic valve disease and coronary disease have many common risk factors.[8] Oxidative modification of low-density lipoprotein by free oxygen radicals has been reported to influence initiation and progression of valve lesions.[9]

Rheumatic HVD has the characteristics of autoimmune diseases and the pathogenesis of the disease remains obscure. The role of oxidative stress and systemic inflammation in rheumatic HVD is well-known.[9] Previous studies have shown that the inflammatory response still persists in the chronic phase of the disease.[10,11]

Plasma concentrations of antioxidants can be measured separately in the laboratory, but these measurements are time-consuming, labor-intensive, and costly. The number of different antioxidants in plasma, serum, urine, or other biological samples makes it difficult to measure each antioxidant separately. Therefore, several methods have been developed to determine the antioxidative capacity of various biological samples.[12] Since antioxidative effects of antioxidant components of plasma are additive, the measurement of total antioxidant capacity (TAC) can reflect the antioxidative capacity of plasma.[13] Individual metabolites may not necessarily reflect the whole condition. Therefore, when seeking a specific relationship between oxidative metabolism and suggested diseases, an evaluation of total antioxidant-oxidant capacity is essential. A novel automated colorimetric measurement method for measuring total oxidant status (TOS) developed by Erel provides us with a whole evaluation.[14]

Although some molecules of free radicals have been investigated in HVD, to the best of our knowledge, no study examined the levels of TAC and TOS from a general antioxidant-oxidant aspect of view. In the present study, we aimed to assess the relationship between rheumatic and degenerative HVD and oxidative and antioxidative parameters.

PATIENTS AND METHODS

Patient groups. The study included a total of 56 consecutive patients with echocardiographically documented predominant mitral or aortic stenosis. Of these, 32 patients had rheumatic HVD (15 males; mean age 47±10 years) and 24 patients had degenerative HVD (13 males; mean age 55±12 years). Patients with predominant mitral or aortic regurgitation or those with tricuspid valvular disease were excluded. Valvular lesions were defined as rheumatic or degenerative on the basis of echocardiographic and surgical findings. Exclusion criteria were heart failure, use of antioxidant medications, hypertension, diabetes mellitus, hyperlipidemia, cerebrovascular disease, malignant tumor, smoking, chronic respiratory insufficiency, rheumatoid arthritis, cirrhosis, osteoporosis, and renal disease. Patients with coronary artery disease were also excluded.

All participants were assessed with a detailed medical history, complete physical examination, and electrocardiographic evaluation before valve replacement. Body mass index was calculated as weight divided by height squared (kg/m^2).

The study protocol complied with the principles outlined in the Declaration of Helsinki and was approved by ethic committee of our hospital. Informed consent for participation in the study was obtained from all patients.

Blood sample collection. Blood samples were obtained following an overnight fasting state before valve surgery. Samples were withdrawn from a cubital vein into blood tubes and stored at −80 °C. The serum was separated from the cells by centrifugation at 3,000 rpm for 10 min and then analyzed.

Tissue sampling and homogenization. Before biochemical assays, all mitral and aortic valve tissues were weighed and placed in empty glass tubes. Per 1 gram of tissue, 10 ml of 140 mM KCl solution was added to each tube, then all tissues were homogenized in a motor-driven homogenizer. The homogenate was centrifuged at 2,800 g for 10 min at 4 °C. The resultant supernatant was used for the levels of TAC and TOS. All homogenized tissues were placed in labelled vials and stored at –80 °C.

Measurement of total oxidant status (TOS) of plasma. Total oxidant status of plasma was measured using a novel automated colorimetric measurement method for TOS developed by Erel.[14] In this method oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are

expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 Eq/l).

Measurement of total antioxidant capacity (TAC) of plasma. Total antioxidant capacity of plasma was measured using a novel automated colorimetric measurement method for TAC developed by Erel.[13] In this method, the hydroxyl radical, the most potent biological radical, is produced by the Fenton reaction and it reacts with the colorless substrate O-dianisidine to produce the dianisyl radical, which is bright yellowish-brown in color. Upon the addition of a plasma sample, the oxidative reactions initiated by the hydroxyl radicals present in the reaction are suppressed by the antioxidant components of the plasma, preventing the color change and thereby providing an effective measurement of TAC. The assay results were expressed as mmol Trolox Eq/l, and the precision of this assay is excellent, being lower than 3%.[15]

Determination of oxidative stress index (OSI). The ratio of TOS to TAC was accepted as the oxidative stress index (OSI). For calculation, the resulting unit of TAC was converted to mmol/l, and the OSI value was calculated according to the following formula:[16] OSI (arbitrary unit) = TOS (μmol H_2O_2 Eq/l) / TAC (mmol Trolox Eq/l).

Statistical analysis. The results were presented as mean ± standard deviation (SD) or percentages, where appropriate. Comparisons were made using the chi-square test and independent samples t-test. Correlations between plasma OSI and clinical and laboratory parameters were sought by the Pearson correlation test. A *P* value of less than 0.05 was considered statistically significant. Data were analyzed using the SPSS (ver. 11.5 for Windows) software.

RESULTS

Clinical characteristics and laboratory findings of patients are presented in Table 1. There were no differences in sex, body mass index, diastolic blood pressure, and systolic blood pressure between the two groups (p>0.05). Baseline medications, ejection fraction, left atrial diameter, left ventricular end-diastolic and end-systolic diameters were similar in patients with degenerative and rheumatic HVD (p>0.05). Patients with degenerative HVD significantly differed from those with rheumatic HVD in terms of higher age, increased interventricular septum thickness, and higher frequency of aortic stenosis, whereas the incidence of mitral stenosis was higher in patients with rheumatic HVD (Table 1).

Plasma and tissue oxidative characteristics of the two groups are shown in Table 2. Plasma oxidative characteristics did not differ between the two HVD groups (p>0.05). Tissue TAC was significantly lower in patients with rheumatic HVD (p=0.027), whereas tis-

Table 1. Clinical, laboratory, and echocardiographic findings

	Rheumatic HVD (n=32)			Degenerative HVD (n=24)			
	n	%	Mean±SD	n	%	Mean±SD	*p**
Age (years)			47±10			55±12	0.008
Gender							0.394
Male	15	46.9		13	54.2		
Female	17	53.1		11	45.8		
Body mass index (kg/m²)			27±5			26±4	0.464
Blood pressure (mmHg)							
Systolic			107±16			111±14	0.514
Diastolic			63±9			66±8	0.338
Medications							
Beta-blocker	13	40.6		13	54.2		0.662
ACE/ARB inhibitor	22	68.8		21	87.5		0.596
Diuretic use	25	78.1		12	50.0		0.206
Left ventricle							
Ejection fraction (%)			59±6			55±10	0.194
End-diastolic diameter (cm)			5.4±0.9			5.5±0.7	0.582
End-systolic diameter (cm)			3.6±0.8			3.8±0.9	0.539
Left atrial diameter (cm)			5.3±0.9			5.0±0.9	0.251
Intraventricular septum (cm)			1.1±0.2			1.3±0.1	<0.001
Aortic stenosis	8	25.0		16	66.7		0.002
Mitral stenosis	24	75.0		8	33.3		<0.001

*For continuous variables: Student's t-test, for categorical variables: Pearson chi-square test; HVD: Heart valve disease; ACE: Angiotensin-converting enzyme; ARB: Angiotensin II receptor blocker.

Table 2. Comparison of plasma and tissue oxidative characteristics

	Rheumatic HVD	Degenerative HVD	*p**
Total antioxidant capacity (mmol Trolox Eq/l)			
Plasma	2.97±0.97	2.77±1.25	0.516
Tissue	0.070±0.02	0.085±0.02	0.027
Total oxidative status (μmol H_2O_2 Eq/l)			
Plasma	9.9±1.2	9.2±1.8	0.136
Tissue	0.223±0.04	0.249±0.05	0.070
Oxidative stress index (AU)			
Plasma	3.7±1.4	3.8±1.5	0.762
Tissue	3.36±0.83	3.05±0.78	0.170

*Student's t-test; HVD: Heart valve disease.

sue TOS and OSI were similar between the two HVD groups (p>0.05).

In bivariate analysis, plasma OSI did not show any correlation with clinical, laboratory, and echocardiographic variables (p>0.05; Table 3).

DISCUSSION

The present study investigated plasma and tissue oxidative stress markers, TAC, TOS, and OSI. Our study was the first to determine tissue oxidant-antioxidant levels in patients with rheumatic and degenerative HVD. Tissue TAC was higher in patients with degenerative HVD, but tissue TOS and OSI were similar in the two HVD groups.

Both arteries and heart valves consist of the endothelium, intima, and connective tissue. There is a well-known relationship between heart valve damage and the pathogenesis of valve fibrosis with immunoinflammatory response.[17] Degenerative valve lesions have many features characteristic to active pathobiological processes, including chronic inflammation,[10] lipoprotein deposition,[9] active calcification,[18] and renin-angiotensin system activation.[19] The course of rheumatic fever is characterized by the expression of immunological and biochemical disorders.[20] Although the role of oxidative stress in the pathophysiology of HVD has been suggested, little is known about the underlying mechanisms and a number of questions still remain to be clarified.

Table 3. Bivariate analysis of clinical and laboratory variables in relation to plasma oxidative stress index

	r	*p*
Age	-0.024	0.870
Body mass index	-0.004	0.982
Systolic blood pressure	-0.075	0.654
Diastolic blood pressure	-0.005	0.977
ACE/ARB use	0.145	0.730
Beta-blocker use	0.126	0.304
Diuretic use	-0.066	0.649
Left ventricular end-diastolic diameter	-0.197	0.249
Left ventricular end-systolic diameter	-0.114	0.509
Left atrial diameter	-0.136	0.345
Left ventricular ejection fraction	-0.052	0.785
Intraventricular septum	0.207	0.240
Tissue oxidative stress index	0.013	0.930

ACE: Angiotensin-converting enzyme; ARB: Angiotensin II receptor blocker.

Plasma TAC, TOS, and OSI, which reflect the redox balance between oxidation and antioxidation, showed no differences between rheumatic and degenerative HVD groups. On the basis of these findings, it seems that degenerative or rheumatic etiology have no additional influence on oxidative status in HVD patients.

Chiu-Braga et al.[21] examined oxidative status of patients with rheumatic HVD using advanced oxidation protein products. They found that levels of advanced oxidation protein products were significantly elevated in rheumatic HVD patients compared to controls and that these elevated levels were not correlated with the severity of mitral disease. It is well-known that various antioxidants in plasma have an additive effect, protecting the organism from free radicals.[22] In this respect, measurement of TAC provides information about the antioxidant capacity of the organism.[13] In addition, OSI, the ratio of the total plasma TOS level to TAC, is an indicator of oxidative stress, reflecting the redox balance between oxidation and antioxidation.[14,16]

In conclusion, this is the first study to evaluate oxidative and antioxidative status of patients with rheumatic and degenerative HVD using both plasma and tissue TAC, TOS, and OSI. Our findings suggest that the levels of plasma and tissue oxidative and antioxidative parameters are similar in patients with rheumatic and degenerative HVD.

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