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Serum prolidase activity in patients with degenerative and rheumatic heart valve diseases

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Aim: It is known that renin-angiotensin system, inflammation, signaling pathways, and genetic predisposition play a major role in the pathogenesis of heart valve disease. Scarring and collagen deposition in the valves and destruction of myocytes may result from the combined effects of rheumatic and degenerative process in heart valve diseases. Prolidase plays an important role in collagen metabolism, matrix remodeling and cell growth. The aim of the study was to comparatively assess the serum prolidase activity in rheumatic and degenerative heart valve diseases.

Materials and methods: The study included 26 patients who had undergone valve replacement due to rheumatic etiology (Group I), 24 patients who had undergone valve replacement due to degenerative etiology (Group II) and 20 healthy volunteers (Group III). Prolidase activity was determined in all subjects. High sensitive C-reactive protein and white blood cell count were studied in only valve patients.

Results: Prolidase activity was significantly greater in control group than in patients group (51.23.± 5.9 U/L vs. 39.40 ± 4.6 U/L, respectively; $P < 0.001$). There was no statistically significant difference between Group I and Group II ($P = 0.721$). No correlation was detected between serum prolidase activity and severity of valve disease ($P > 0.05$).

Conclusion: These results suggested that rheumatic and degenerative heart valve diseases seem to be associated with decreased serum prolidase activity.

Key words: Prolidase activity, rheumatic valve disease, degenerative valve disease

Dejeneratif ve romatizmal kalp kapak hastalarına serum pirolidaz aktivitesi

Amaç: Renin-anjiyotensin sistemi, inflamasyon, sinyal yolakları ve genetik yatkınlığın kalp kapak hastalığının patogenezinde önemli rol oynadığı bilinmektedir. Kapaklarda skar oluşumu ve kolajen birikimi miyositlerin hasarlanması, kalp kapak hastalığındaki romatizmal ve dejeneratif süreçlerin birleşik etkisinden sonuçlanabilir. Prolidaz, kolajen metabolizmasında, matriks yeniden-yapılanmasında ve hücre büyümesinde önemli rol oynar. Bu çalışmanın amacı, serum prolidaz aktivitesinin romatizmal ve dejeneratif kalp kapak hastalıklarında karşılaştırmalı değerlendirmesidir.

Yöntem ve gereç: Çalışmaya, romatizmal etiyoloji nedeniyle kapak replasmanı geçirmiş olan 26 hasta (Grup I), dejeneratif etiyoloji nedeniyle kapak replasmanı geçirmiş olan 24 hasta (Grup II) ve 20 sağlıklı gönüllü (Grup III) katıldı. Prolidaz aktivitesi tüm deneklerde ölçülürken, yüksek duyarlıklı C-reaktif protein ve beyaz küre sayımı sadece kapak hastalarında ölçüldü.

Bulgular: Prolidaz aktivitesi, kontrol grubunda hasta gruplarındakinden anlamlı şekilde daha yüksekti (sırasıyla 51,23.± 5,9 U/L ve 39,40 ± 4,6 U/L; $P < 0,001$). Serum prolidaz aktivitesi bakımından Grup I ve Grup II arasında istatistiksel olarak anlamlı bir fark yoktu ($P = 0,721$). Serum prolidaz aktivitesi ve kapak hastalığının ağırlığı arasında herhangi bir korelasyon saptanmadı ($P > 0,05$).

Sonuç: Bulgular ışığında, romatizmal ve dejeneratif kalp hastalığının azalmış serum prolidaz aktivitesi ile ilişkili olduğu görülmektedir.

Anahtar sözcükler: Prolidaz aktivitesi; romatizmal kalp hastalığı; dejeneratif kalp hastalığı.

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Introduction

Degenerative (DHVD) and rheumatic heart valve diseases (RHVD) are multifactorial processes that appear to be caused by the interaction of risk factors such as genetic, inflammatory, autoimmune, infectious and oxidative stress; however, their pathophysiology are not completely understood (1-3). Fibrosis and calcification are defining features of degenerative and rheumatic valve lesions, and the extent of lesion calcification correlates both with more rapid disease progression and worse clinical outcomes (4-6). Fibrosis is an excessive deposition of extracellular matrix (7). Matrix-metalloproteinase and the natural inhibitors of matrix-metalloproteinase molecules typically are expressed in inflammatory and fibrosing illnesses (1). Degenerative heart valve lesions contain a number of matrix-metalloproteinases (8, 9), which degrade various components of the extracellular matrix. Normal human heart valves contain collagen types I, III and V in extracellular matrix (10).

Prolidase (E.C.3.4.13.9) is a cytosolic exopeptidase, and cleaves imidodi- and imidotripeptides with C-terminal proline or hydroxyproline. (11). Prolidase plays an important role in collagen metabolism, matrix remodeling and cell growth (12). Its activity has been documented in plasma, erythrocytes, leukocytes, dermal fibroblasts and in various organs such as kidney, brain, heart, thymus and uterus (13). Prolidase activity has been investigated in various disorders such as chronic liver disease (14), osteoporosis (15) and osteoarthritis (16). Increased serum prolidase activity is associated with the presence of hypertension independent of the presence of left ventricular hypertrophy (17). Many studies have shown that the levels of inflammatory indices, mainly C-reactive protein, plasma endothelin-1 levels (18), plasma osteopontin concentrations (19) and collagen III expression in atrial tissue were increased in patients with mitral valve disease (20, 21).

However, there is no data available in the literature about the role of serum prolidase activity in the pathogenesis of valve diseases. We hypothesized that serum prolidase activity may be responsible for the increased valvular fibrosis and calcification in patients with RHVD and DHVD. Therefore, this study was designed to determine whether the prolidase activity can serve as a marker of susceptibility to rheumatic and degenerative valve diseases.

Materials and methods

Patients and controls: Patients with mitral and aortic stenosis admitted to cardiology and cardiovascular surgery clinics between September 2006 and March 2008 were included in the study. The sample included 26 patients who underwent mitral valve surgery due to rheumatic etiology (Group I; 10 males; mean age: 45 ± 11 years), 24 patients who underwent aortic valve surgery due to degenerative etiology (Group II; 14 males; mean age: 51 ± 12 years) and 20 gender- and age-matched healthy volunteers (Group III; 8 males; mean age: 46 ± 7 years).

Patients with echocardiographically documented predominantly mitral and aortic stenosis and sinus rhythm were enrolled in this study. Patients with combined valvular diseases, predominant mitral and aortic regurgitation or those with coronary artery diseases were excluded. Valvular lesions were defined on the basis of echocardiographic findings; supported by the evidence of past streptococcal throat infection and/or history of acute rheumatic fever. Patients without degenerative and rheumatic etiology were excluded from the study. Other exclusion criteria were presence of heart failure, cerebrovascular diseases or malignant tumor, antioxidant drug consumption, hypertension, hyperlipidemia, diabetes mellitus, smoking, chronic respiratory insufficiency, rheumatoid arthritis, cirrhosis, osteoporosis and renal diseases.

All participants were assessed by detailed medical history-taking, complete physical examination and electrocardiographic evaluation. Body mass index (BMI) was computed as weight divided by square of height (kg/m^2). The leukocytes and the levels of C-reactive protein were measured by a commercial kit (Abbott) using an automatic analyzer (Aeroset, USA).

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a *priori* approval by our institution's human research committee. Informed consent was obtained from all individuals.

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. The plasma was separated from the cells by centrifugation at 3000 rpm for 10 min and stored on at −80°C until analysis of prolidase.

Prolidase assay: Serum was diluted 40-fold with 2.5 mmol/L Mn^{2+}, 40 mmol/L trizma HCl buffer (pH: 8.0) and preincubated at 37°C for 2 h. The reaction mixture containing 30 mmol/L gly-pro, 40 mmol/L trizma HCl buffer (pH: 8.0) and 100 µL of preincubation serum in 1 mL was incubated at 37°C for 30 min. Incubation reaction was stopped after adding 0.5 mL of 20 % trichloroacetic acid solution. The supernatant was used for measurement of proline by the method proposed by Myara (22), which is a modification of Chinard's method (23). Intra-assay CV of the assay was 3.8 %.

Statistical analysis: Results are presented as mean ± SD or frequency values expressed as percentages. Continuous variables were compared using Student's t test and ANOVA with a least square difference post-hoc test, and categorical variables were compared using Pearson chi-square test. Associations among quantitative variables were assessed by Pearson's correlation coefficient. $P < 0.05$ was considered statistically significant. Data were analyzed by using SPSS 11.5 (SPSS for Windows 11.5, Chicago, IL).

Results

Patient clinical and some laboratory characteristics are shown in Table 1. There were no difference in age, sex, body mass index and systolic blood pressure. Only diastolic blood pressure was higher in the control group than the patients group ($P < 0.05$). Baseline medications in patients with degenerative and rheumatic groups are shown in Table 1. Ejection fraction, left ventricular end-diastolic and end-

Table 1. Comparison of demographic and clinical characteristics of the groups.

	RHVD n = 26	DHVD n = 24	Control n = 20	P value[a]	ANOVA P
Age (years)	45 ± 11	51 ± 12	54 ± 11	-	0.196
Male/Female (n)	10/16	14/10	8/12	-	0.308
BMI (kg/m^2)	27 ± 4	26 ± 4	25 ± 3	-	0.553
SBP (mmHg)	105 ± 14	110 ± 14	115 ± 15	-	0.135
DBP (mmHg)	64 ± 9[b]	70 ± 10	78 ± 9[c]	-	<0.001
Beta-blocker use (%)	16	12	-	0.199	-
ACEI/ARB use (%)	15	20	-	0.019	-
Diuretic use (%)	19	12	-	0.124	-
Leukocytes (10^{3}cells/µl)	6.9 ± 2.1	7.5 ± 1.2	-	0.255	-
C-reactive protein (mg/dl)	0.56 ± 0.43	0.86 ± 0.56	-	0.120	-
LVEF (%)	58 ± 7	55 ± 10	-	0.327	-
Left atrial diameter (cm)	5.1 ± 0.8	4.1 ± 0.3	-	0.002	-
LVDD (cm)	5.4 ± 1.0	5.5 ± 0.7	-	0.607	-
LVSD (cm)	3.7 ± 0.8	3.8 ± 0.9	-	0.682	-
IVS (cm)	1.09 ± 0.21	1.38 ± 0.13	-	<0.001	-

[a] Continuous variables were compared using Student's t test, and categorical variables were compared using Pearson chi-square test (RHVD vs. DHVD). Values are mean ± S.D. or %.
[b] P = 0·010 vs. DHVD
[c] P < 0.001 vs. RHVD and P= 0.004 vs DHVD
ACEI: angiotensin-converting enzyme inhibitor; ARB: angiotensin II receptor blocker; BMI: body mass index; DHVD: degenerative heart valve disease; DBP: diastolic blood pressure; IVS: intraventricular septum; LVEF: left ventricular ejection fraction; LVDD: left ventricular end-diastolic diameter; LVSD: left ventricular end-systolic diameter; RHVD: rheumatic heart valve disease; SBP: systolic blood pressure.

systolic diameter, C-reactive protein and leukocyte counts were similar in patient groups (all P > 0.05). Diastolic blood pressure and intraventricular septum thickness were significantly higher in patients with DHVD than RHVD patients. But left atrial diameter was significantly bigger in patients with RHVD than DHVD patients (Table 1).

A bar graph of prolidase activity in three study groups is shown in Fig. 1. Serum prolidase activity was significantly greater in control than in patients group (51.23.± 5.9 U/L vs. 39.40 ± 4.6 U/L, respectively; P < 0.001). But there was no statistically significant difference between Group I and Group II (39.20 ± 4.4 U/L and 41.47 ± 4.7 U/L, respectively; P = 0.721) (Figure 1).

Relationships between prolidase activity and other variables in patients with RHVD and in patients with DHVD are shown in Table 2. The prolidase activity was not correlated with the clinic and laboratory variables (all P > 0.05) in bivariate analysis.

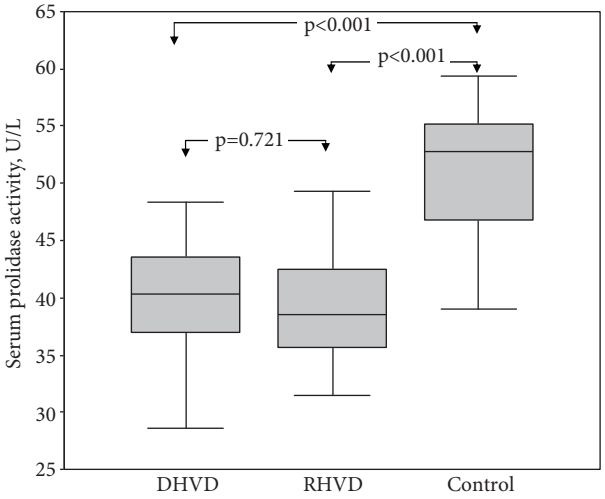

Figure 1. Comparison of groups according to serum prolidase activity (DHVD: degenerative heart valve disease, RHVD: rheumatic heart valve disease).

Discussion

In the present study, we found that prolidase activity was significantly lower in patients than control subjects. Serum prolidase levels were similar between patients with RHVD and patients with DHVD. Prolidase activity was not correlated with severity of valve diseases.

One of our recent studies has reported that the prolidase activity was associated with the presence of

Table 2. Bivariate correlation analysis of the serum prolidase activity with other variables.

	Pearson correlation coefficient	P value
Age	-0.024	0.871
Body mass index	-0.078	0.653
Systolic blood pressure	-0.095	0.511
Diastolic blood pressure	-0.010	0.943
ACEI/ARB use	0.050	0.730
Beta-blocker use	0.139	0.336
Diuretic use	0.037	0.796
C-reactive protein	-0.200	0.289
Leukocytes	0.065	0.700
Left ventricular end-diastolic diameter	-0.179	0.296
Left ventricular end-systolic diameter	-0.222	0.194
Left atrial diameter	0.011	0.952
Left ventricular ejection fraction	0.256	0.173
Intraventricular septum	-0.171	0.304
Mean gradient of mitral stenosis	0.102	0.470
Mean gradient of aortic stenosis	0.130	0.350

ACEI: angiotensin-converting enzyme inhibitor; ARB: angiotensin II receptor blocker.

hypertension, and patients with hypertension had higher levels of prolidase activity when compared to healthy subjects (17). In the present study, we found that the diastolic blood pressure was lower in patients with RHVD and DHVD than in control subjects. Serum prolidase activity was not associated with the diastolic blood pressure.

Collagen Type I and Type III are found in the valve, cordae tendon and myocardial interstitium of rheumatic heart disease (24, 25). It was known that patients with rheumatic valves had elevated collagen synthesis (26), and there was a relationship between acquired valvular heart diseases and increased serum and urine levels of hydroxyproline (27). Degenerative valve lesions have many features characteristic of an active pathobiological process, including chronic inflammation (28), lipoprotein deposition (5), active calcification (29) and renin–angiotensin system activation (30). The linkage of collagen metabolism to prolidase activity is also important in wound healing (31), chronic liver diseases (14) and osteoporosis (15) and to rheumatic and degenerative valve diseases as suggested in our study results.

There are two limitations of this study. First, the sample size was small and this limits the ability to apply our findings to a wider range of patients with cardiovascular disease. Second, collagen type I, type II and type III were not studied in patients with RHVD and DHVD.

This study demonstrates that prolidase activity is higher in healthy subjects than in patients with RHVD and DHVD, which supports the role of prolidase activity as a marker of valve diseases.

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