Serum Prolidase Activity in Idiopathic and Ischemic Cardiomyopathy Patients

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Idiopathic and ischemic dilated cardiomyopathies (DCM) are the most common types of DCM, and both exhibit the same histopathological feature of fibrosis. Prolidase is an enzyme that serves a rate-limiting function in collagen turnover. Several studies have shown increased prolidase activity in fibrosis, though controversy persists. In this study, we measured prolidase enzyme activity in patients with idiopathic or ischemic DCM and in healthy controls, making this, to our knowledge, the first study to do so. What we found is that serum

prolidase activity was significantly lower in both DCM groups relative to healthy volunteers and lower in ischemic DCM than idiopathic. These intriguing results could be attributed either to decreased collagen turnover in the heart tissues in which DCM develops, a result of diminished functional heart tissue, or to decreased physical activity levels among DCM patients stemming from their heart failure. Either way, further studies are needed to verify and clarify our results. J. Clin. Lab. Anal. 24:213–218, 2010. © 2010 Wiley-Liss, Inc.

Key words: serum prolidase activity; idiopathic dilated cardiomyopathy; ischemic dilated cardiomyopathy

INTRODUCTION

Idiopathic dilated cardiomyopathy (DCM) is defined as a primary abnormality of myocardial tissue in the absence of occlusive coronary, valvular, or systemic disease, and is characterized by ventricular dilation, especially the left ventricle, and a decrease particularly in systolic function (1). The term *idiopathic* originally was intended to identify that subset of patients with dilated cardiomyopathy in whom there was no known cause of ventricular dilation and depressed myocardial contractility was apparent. However, with increasing sophistication in diagnostic testing, clinicians have become aware that many cases of so-called idiopathic DCM may result from inherited or spontaneous mutations of genes that regulate cardiac structure and function, such as the genes for cytoskeleton proteins or as a consequence of undiagnosed hypertension, autoimmune disease, viral illness, or toxin exposure (1,2). In general, the term *idiopathic DCM* refers to cases in which the cause remains unknown after thorough diagnostic testing.

Ischemic DCM is more common than idiopathic. It is secondary to ischemia, hibernation, and/or infarction of myocardium, and is characterized by a prominent decrease in systolic function and varying degrees of resultant symptoms. The histopathology is similar in these two clinical entities (ischemic and idiopathic DCM), the prevailing feature being widespread fibrosis. However, in some microscopic fields, fibrous tissue patches without myocardial cells can be seen in ischemic DCM owing to cell death (1).

Prolidase is an exopeptidase that is important in collagen turnover, being the main regulatory enzyme in the metabolism of proline and hydroxyproline, which constitute 20% of all collagen in the human body (3,4). Prolidase mediates the final step of collagen degradation

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and is a rate-limiting factor in collagen biosynthesis (5). Serum prolylase also has been shown to be a useful marker in the assessment of liver fibrosis (6–8). On the other hand, with cardiac biopsies, the main histopathological indicator of cardiomyopathy is fibrosis (1); moreover, we are not aware that prolylase activity has been studied in this setting, at least to date. With all this as a backdrop, we sought to measure serum prolylase activity (SPA) in patients with either idiopathic or ischemic DCM, and in healthy controls, hypothesizing (1) that SPA should be increased in DCM because of the underlying fibrosis, and (3) that levels should be similar in these two disease subtypes.

MATERIAL AND METHODS

Subjects

This study was conducted at the Harran University School of Medicine. Before initiating subject recruitment, this study was approved by the local ethics committee of the university, in accordance with the ethical principles for human investigators, as outlined in the second Declaration of Helsinki. All subjects provided written informed consent before participating. All DCM patients referred for angiography between 2008 and 2009 were recruited. Ultimately, we enlisted the participation of 28 patients with ischemic DCM (21 males and 7 females; mean age: 56.6 years), 29 patients with idiopathic DCM (21 males and 8 females; mean age: 54.9), and 50 healthy volunteers (32 males and 18 females; mean age: 56.7). The diagnosis of idiopathic DCM was made on the basis of an end-diastolic volume below 55, a left ventricle ejection fraction below 35%, and the absence of any identifiable etiology, such as coronary artery disease, valvular heart disease, or hypertension. The diagnosis of ischemic DCM was made if the end-diastolic volume was >55 mm and serious atherosclerosis was present to explain it. Exclusion criteria were: atrial fibrillation, acute coronary syndrome, severe primary valvular heart disease, chronic liver or renal disease, gastritis or peptic ulcer, diabetes mellitus, any sort of malignancy, or any acute or chronic infectious or inflammatory disease.

Echocardiographic Imaging

Echocardiographic evaluations were conducted from views along the parasternal long and short axis and from apical spaces 2, 4, and 5, using an Aloka SSD 5000 ultrasound device (Aloka Inc., Tokyo, Japan) with a 2.5 MHz transducer. All measurements were generated in accordance with the basic recommendations of the American Echocardiography Association. Along the parasternal long axis with M-mode and two-dimensional

views, we recorded left ventricle end-diastolic and end-systolic volume, and interventricular septal and back wall thickness. Ejection fraction was calculated by means of a modified Teichholz method (9). Coronary angiographies were performed with Toshiba Infinix Csi (Toshiba Corporation, Japan) in various planes.

Assays

All blood samples were obtained following an overnight fast, drawn from a large antecubital vein without interruption of venous flow, using a 19-gauge butterfly needle connected to a plastic syringe. Twenty milliliters of blood was drawn, with the first few milliliters discarded. Ten milliliters were used for baseline routine laboratories and erythrocyte sedimentation rate. The residual content of the syringe was transferred immediately to polypropylene tubes, which then were centrifuged at 3,000g for 10 min at 10–18°C. Supernatant plasma samples were stored in plastic tubes at –80°C until assayed.

Serum Prolidase Activity

Serum was diluted 40-fold with 2.5 mmol/l Mn²⁺ and 40 mmol/l trizma HCl buffer (pH: 8.0) and preincubated at 37°C for 2 hr. 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. This supernatant was used for the measurement of proline, according to the method proposed by Myara et al., which is a modification of Chinard's method. The intra-assay coefficient of variation was 3.8% (4,10).

Other Variables

Serum sodium, potassium, urea, creatinine, fasting blood sugar, aspartate amino transferase, alanine amino transferase, triglyceride, total cholesterol, HDL-cholesterol, and LDL-cholesterol levels were determined using commercially available assay kits (Abbott®, Illinois, USA) with an auto-analyzer (Aeroset®, Germany). CRP was measured by means of nephelometric analysis (NA Latex CRP reagent: Behring Institute, Marburg, Germany).

Statistical Analysis

All data were analyzed using SPSS version 11.5 (SPSS Inc., Chicago, IL). Continuous variables were expressed as means ± SD and categorical variables as percentages. Between-group analyses were assessed by independent Student's *t*-tests and one-way analysis of variance for continuous variables, and by Pearson's chi-square analysis for categorical variables. Post hoc

comparisons were performed using Tukey's procedure. For examining the impact of independent variables on SPA, multivariate linear regression analysis was conducted. Only differences at $P < 0.05$ were interpreted as statistically significant.

RESULTS

The demographic and clinical characteristics of each of the three study groups are shown in Table 1. Only the prevalence of hypertension was statistically different between the groups, the rate being highest in those with ischemic DCM and lowest in those with idiopathic DCM.

The echocardiographic parameters and SPA levels of each group are shown in Table 2. Echocardiographic variables were similar between the two DCM groups. SPA was significantly decreased in both DCM groups relative to controls; however, the decrease was greater in the ischemic DCM group. Otherwise, SPA was significantly lower in the ischemic vs. idiopathic DCM group (Fig. 1).

Linear regression analysis was performed to identify factors that appear to exert some independent influence on SPA level. As independent variables, left ventricle systolic and diastolic diameter, left ventricle ejection fraction, hypertension, and the presence of cardiomyopathy were included. Of these, only the presence of cardiomyopathy was found to effect SPA ($R^2 = 0.178$, $\beta = -0.331$, $P = 0.001$).

DISCUSSION

As an important marker of collagen turnover, SPA has been evaluated in various diseases. Yildiz et al. assessed the relationship between prolidase activity and the presence and severity of coronary artery disease, based upon the observation that matrix proteins (type 1

TABLE 2. Serum Prolidase Activity and Echocardiographic Parameters of the Study Population

	Idiopathic DCM	Ischemic DCM	Controls	P-value
SPA (U/L)	$686 \pm 22^{a,b}$	$665 \pm 33^{a,c}$	$695 \pm 35^{b,c}$	0.001
LVDd (cm)	$6.2 \pm 0.8^{d,e}$	$6 \pm 0.5^{d,f}$	$4.9 \pm 0.6^{e,f}$	0.0002
LVSd (cm)	$4.9 \pm 0.8^{g,h}$	$4.8 \pm 0.8^{g,i}$	$2.9 \pm 0.2^{h,i}$	0.0002
LVEF (%)	$27 \pm 6^{j,k}$	$26 \pm 6^{j,l}$	$67 \pm 4^{k,l}$	0.0004

By one-way analysis of variance. Values are mean $\pm$ SD or percentage (%). Significant differences are shown in bold. DCM, dilated cardiomyopathy; LVDd, left ventricle diastolic diameter; LVEF, left ventricle ejection fraction; LVSd, left ventricle systolic diameter; SPA, serum prolidase activity.

^aIdiopathic vs. Ischemic DCM, $P < 0.05$.

^bIdiopathic DCM vs. Controls, $P < 0.05$.

^cIschemic DCM vs. Controls, $P < 0.05$.

^dIdiopathic vs. Ischemic DCM, $P > 0.05$.

^eIdiopathic DCM vs. Controls, $P < 0.05$.

^fIschemic DCM vs. Controls, $P < 0.05$.

^gIdiopathic vs. Ischemic DCM, $P > 0.05$.

^hIdiopathic DCM vs. Controls, $P < 0.05$.

ⁱIschemic DCM vs. Controls, $P < 0.05$.

^jIdiopathic vs. Ischemic DCM, $P > 0.05$.

^kIdiopathic DCM vs. Controls, $P < 0.05$.

^lIschemic DCM vs. Controls, $P < 0.05$.

TABLE 1. Demographic Characteristics and Laboratory Parameters of Study Population

	Idiopathic DCM (n = 29)	Ischemic DCM (n = 28)	Controls (n = 50)	P-value
Sex (M/F)	21/8	21/7	32/18	0.093
Hypertension (%)	0 ^a	43 ^a	12 ^a	0.001
Diabetes mellitus (%)	45	46	30	0.251
Dyslipidemia (%)	24	21	24	0.961
Smoking (%)	24	18	26	0.712
Age (year)	55 ± 16	57 ± 11	56 ± 11	0.877
Height (cm)	166 ± 13	159 ± 35	161 ± 7	0.592
Weight (kg)	84 ± 27	79 ± 6	75 ± 15	0.227
HR (beats/min)	80 ± 9	78 ± 11	79 ± 13	0.791
Systolic BP (mmHg)	134 ± 20	132 ± 31	133 ± 25	0.972
Diastolic BP (mmHg)	78 ± 15	83 ± 15	83 ± 13	0.385
Urea (mg/dl)	52 ± 26	49 ± 16	45 ± 20	0.506
Creatinine (mg/dl)	1.2 ± 0.28	1.07 ± 0.29	1.06 ± 0.29	0.350
FBG (mg/dl)	172 ± 134	121 ± 70	137 ± 79	0.411
Total Cholesterol (mg/dl)	219 ± 76	183 ± 54	194 ± 50	0.095
LDL Cholesterol (mg/dl)	142 ± 50	127 ± 55	111 ± 46	0.104
HDL Cholesterol (mg/dl)	41 ± 11	36 ± 6	42 ± 11	0.334
Triglyceride (mg/dl)	173 ± 176	161 ± 61	179 ± 121	0.850

Values are mean $\pm$ SD or percentage (%). Significant differences are shown in bold.

BP, blood pressure; DCM, dilated cardiomyopathy; FBG, fasting blood glucose; HDL, high density lipoprotein; HR, heart rate; LDL, low density lipoprotein; M/F, male/female.

^aIdiopathic vs. ischemic DCM, $P < 0.001$; idiopathic DCM vs. controls, $P < 0.03$; ischemic DCM vs. controls $P < 0.05$.

Fig. 1. Graph showing SPA in patients with idiopathic and ischemic dilated cardiomyopathies, and in healthy controls reveals lowest and highest SPA in patients with ischemic dilated cardiomyopathy and in healthy controls, respectively. IdDCM, idiopathic dilated cardiomyopathy; IsDCM, ischemic dilated cardiomyopathy; SPA, serum prolidase activity.

and 3 collagen, proteoglycans, and elastins) account for 60% of the total protein in atherosclerotic plaques. They found that SPA was higher in CAD (coronary artery disease) than in controls (11). Demirbag et al. also identified higher serum prolidase levels in hypertensive patients relative to controls, and attributed this discovery to the proliferation of smooth muscle cells, to the increased extracellular matrix of arterial wall structures, and to left ventricular hypertrophy as a secondary response to chronic hypertension (12). In addition, Camuzcuoglu et al. have studied and identified elevated prolidase activity in epithelial ovarian cancer (13), Brosset et al. in chronic liver disease (6), Karna et al. in lung cancer (14), and Wolanska et al. in uterine leiomyomas (15). Further studies documenting increased prolidase activity in a wide variety of diseases can be cited. However, if we combined all the above-mentioned results together, they suggest that any significant increase in collagen turnover leads to enhanced prolidase activity, presumably because it is a rate-limiting factor of collagen turnover, in the final step of collagen degradation (16). This presumption was the rationale behind this study, before which we hypothesized that SPA should be elevated in DCM because of the underlying fibrosis that is prominent in both forms of cardiomyopathy.

In actuality, however, our results are quite contrary to what we expected. Rather than elevated, we found

prolidase activity to be lower in patients with DCM than in healthy controls. There might be several explanations for this result. First, it may be that prolidase enzyme deficiency is part of the underlying pathophysiology of DCM. Prolidase enzyme deficiency has been documented to be present with a variety of clinical manifestations and associated with several diseases (17). Therefore, it might be hypothesized that the low prolidase activity we identified in our DCM patients is the result of serum prolidase deficiency, causing disturbed fibrosis within myocardium. Prolidase enzyme deficiency, if it does exist in DCM, also might explain why not all patients with ischemic cardiac disease develop DCM. However, this theory does not extend beyond the conjectural phase at this time; and, in truth, we believe that this explanation is unlikely to be true, given that prolidase deficiency generally is considered to be a rare autosomal recessive disorder, with fewer than a 100 cases reported in the literature (18). Moreover, prolidase deficiency is a multisystemic disorder with skin as its primary target organ. A wide variety of clinical presentations have been defined in prolidase deficiency, such as delayed wound healing, chronic skin lesions, splenomegaly, developmental delay, recurrent dermatitis, diarrhea, respiratory dysfunction, and disturbed psychomotor development (18). The onset of clinical symptoms generally is before the age of 12 years. In our patients, none of these clinical features were present; therefore, prolidase deficiency is not likely to have been prominent, if at all present, in either of our two DCM study groups (19).

A more reasonable explanation for the reduced prolidase enzyme activity in both our DCM groups is related to collagen turnover. As mentioned above, SPA has been found to be increased in several diseases (6,11–15). However, conflicting results also have been published. In a recent article published in this journal, Cakmak et al. reported that SPA concentrations were lower in asthma patients than in controls (20). Similarly, Myara et al. found higher plasma prolidase levels in only 5 of 27 cirrhosis patients; they also failed to identify any correlation between the degrees of fibrosis and prolidase activity. However, they did detect a significant association between prolidase activity and early stages of cirrhosis (21). We feel that the main explanation for our results underlies this point: the conflicting results that have been published in the scientific literature may have stemmed from prolidase activity being affected by the degree of collagen turnover, rather than by the extent of fibrosis. In the late stages of cirrhosis, for example, nearly all liver tissue already has fibrosed. Consequently, in end-stage cirrhosis, there may be insufficient collagen turnover to increase serum prolidase levels, meaning that collagen turnover become lower than normal, possibly

leading to lower prolidase activity. We, in fact, believe that this is the underlying reason we detected lower prolidase levels in our DCM subjects.

As mentioned above, in ischemic and idiopathic DCM, fibrosis is the main pathophysiological event. As time passes and fibrosis progresses, there comes a time when the rate of collagen turnover starts to decline, secondary to there being less residual functional myocardium. In a healthy body, a certain amount of collagen production and degradation takes place on an ongoing basis, and heart tissue is one of the main production sites of prolidase (22). If the quantity of functional heart tissue lessens, collagen turnover also may decrease. In idiopathic DCM, fibrosis is an ongoing process, for reasons that have yet to be elucidated, whereas fibrosis occurs abruptly in ischemic DCM resulting in substantial myocardium loss. Accordingly, long-lasting chronic fibrotic process, which is frequently encountered in idiopathic DCM rather than in ischemic DCM, might be the source of fairly higher levels of SPA in idiopathic DCM compared with ischemic DCM. Why prolidase activity was higher in healthy patients may have been simply because of the increased quantity of functional myocardium healthy people have relative to their DCM counterparts.

A final explanation for lower prolidase activity in DCM might be related to the reduced physical activity levels that these patients tend to exhibit. It is clear that physical activity levels decline in patients with cardiac failure. In addition, collagen turnover appears to be positively correlated to the degree of exercise (23,24). To date, no published data exist regarding the relationship between prolidase activity and exercise. However, in a very recent unpublished study conducted by our research team, we detected a significant relationship between prolidase activity and exercise. To clarify whether any relationship between prolidase activity and DCM exists, we already have launched a new study, the results of which are due to be released in the near future.

In conclusion, we would like to emphasize several points. First, to the best of our knowledge, this study is the first to investigate SPA levels in DCM. Second, serum lower prolidase activity appears to be lower in patients with DCM than in healthy controls, and this appears to be an independent effect, relative to other clinical parameters. This probably stems from both decreased collagen turnover in DCM, which in turn is secondary to decreased functional heart tissue and to the decreased physical activity that DCM patients exhibit because of their failing heart. Nonetheless, further studies are necessary to verify our findings and clarify the mechanisms behind them.

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