

The association of serum prolidase activity and erectile dysfunction

Savas M¹, Yeni E¹, Celik H², Ciftci H¹, Utangac M¹, Oncel H¹, Altunkol A¹, Verit A¹

Departments of ¹Urology and ²Clinical Biochemistry, Medicine School of Harran University,
Sanliurfa, Turkey.

Corresponding Adress

Murat Savas, MD

Urology Department, Medicine School of Harran University,

63100, Sanliurfa, TURKEY

Phone: +904143168821 Fax:+904143168821

E-Mail: mrtsvs@yahoo.com

Running title: Serum prolidase activity in ED

Abstract

Prolidase is a cytosolic exopeptidase that cleaves iminodipeptides with carboxy-terminal proline or hydroxyproline and plays major role in collagen turnover. Collagen is the essential content playing a key role in the stability/instability of atherosclerotic plaque and progression of endothelial dysfunction that may have a role in the pathogenesis of erectile dysfunction (ED). Consequently, in this study we sought to determine serum prolidase activity and markers of oxidative stress such as lipid hydroperoxide and total free sulfhydryl in vasculogenic ED. We have evaluated 92 patients with vasculogenic ED and 50 control cases with clinical and laboratory investigation. We have measured serum prolidase activity and serum total free sulfhydryl levels spectrophotometrically. Serum lipid hydroperoxide levels were determined with ferrous ion oxidation-xylenol orange method. We assessed the association of serum prolidase activity with the presence and severity of vasculogenic ED and clinical characteristics, and laboratory parameters. We also assessed the association of serum prolidase activity with the variables according to the vascular status of patients with vasculogenic ED. The comparison included 92 vasculogenic ED patients grouped into three categories according to the vascular status of patients with ED; arterial insufficiency (n=26), veno-occlusive dysfunction (n=37), mixed type impotence (n=29) besides 50 controls. Receiver operator characteristics (ROC) were analyzed to find a cut-off value with best sensitivity and lowest false positive rate. Serum prolidase activity (53.5 ± 5.5 vs. 45.7 ± 4.9 U/l, respectively, $P < 0.001$) and serum lipid hydroperoxide levels were significantly increased in patients with vasculogenic ED compared with control cases whereas, serum total free sulfhydryl levels were significantly decreased in patients with vasculogenic ED compared with control cases ($P < 0.001$). The lowest and highest mean serum prolidase activities were detected in control participants and in patients with arterial insufficiency, respectively (ANOVA $P < 0.001$). The overall findings of this study support the predictive accuracy of the serum prolidase activity in our cohort with a statistically significant ROC value of 0.78. Findings of this study have shown that serum prolidase activity is significantly associated with the presence and severity of vasculogenic ED, and elevated serum prolidase activity might be an independent predictor of erectile dysfunction.

Keywords: erectile dysfunction, collagen turnover, extracellular matrix, oxidative stress, prolidase activity

Introduction

Prolidase is a manganese-dependent cytosolic exopeptidase that cleaves iminodipeptides containing carboxyterminal proline or hydroxyproline, and plays an important role in collagen metabolism, matrix remodeling, and cell growth (Palka et al, 1997). Its activity has been documented in plasma, erythrocytes, leukocytes, dermal fibroblasts, and in various organs, such as kidney, brain, heart, thymus, uterus, liver, small intestine, stomach, spleen, lung, and pancreas (Zanaboni et al, 1994; Liu et al, 2007). Prolidase activity has been investigated in various disorders, such as chronic liver disease (Myra et al, 1984), osteoporosis (Erbagci et al, 2002), osteoarthritis (Altindag et al, 2007), uremia (Gejyo et al, 1983), hypertension (Demirbag et al, 2007) and presence and severity of coronary artery disease (Yildiz et al, 2008).

Erectile dysfunction (ED) is defined as the consistent inability to obtain or maintain an erection for satisfactory sexual intercourse. Basic science research on erectile physiology has been devoted to investigating the pathogenesis of ED and has led to the conclusion that ED is predominately a disease of vascular origin. Patients who had a vascular evaluation had vascular pathology can be classified as arteriogenic ED, venogenic ED and mixed vasculogenic ED (Tal et al, 2009). The incidence of ED dramatically increases in men with diabetes mellitus, hypercholesterolemia, and cardiovascular disease. Loss of the functional integrity of the endothelium and subsequent endothelial dysfunction plays an integral role in the occurrence of ED in this cohort of men. ED is highly prevalent in men with cardiovascular disease, and because cardiovascular disease is well known to be associated with endothelial dysfunction, one can infer that endothelial dysfunction of the penile vascular tree may contribute to impairments in erectile function. Therefore, it has been hypothesized that endothelial dysfunction can result in ED (Maas et al, 2002; Solomon et al, 2003). Atherosclerotic plaques initially consist of fatty streaks that develop into fibro-proliferative lesions. A mature lesion consists mainly of foam cells, smooth muscle cells, a necrotic core, and a fibrous cap containing extracellular matrix components. The principal matrix proteins in plaques are type I and III collagens, proteoglycans, and elastin, with collagens accounting for up to 60% of the total protein content (Watanabe et al, 2003). Measurement of circulating levels of extracellular matrix turnover biomarkers such as the matrix metalloproteinases (MMP) and the tissue inhibitors of metalloproteinases (TIMP) have long been used in the evaluation of atherosclerosis (Gensini et al, 1983; Myra et al, 1982). Accordingly, we have hypothesized that the serum level of prolidase activity would increase in ED, as increased extracellular matrix turnover is a pathophysiologic mechanism in the progression of atherosclerosis collagen biosynthesis and endothelial dysfunction. Therefore, this study was mainly planned to evaluate the

association between the presence and severity of ED and the serum prolidase activity and serum levels of oxidative stress markers such as total free sulfhydryl (–SH) and lipid hydroperoxide (LOOH).

Methods

Ninety-two consecutive patients with ED (mean age 52.05 ± 8.90 , range 37 to 72 years) and 50 control cases that have no ED (mean age 53.07 ± 7.60 range 39 to 73 years) were included to the study after giving informed consent for participation in the study. The study protocol conforms to the principles of the Declaration of Helsinki and was approved by the institutional ethics review board. Past medical history and current medications were recorded, in addition to detailed physical examination in all cases. Criteria for inclusion of all patients were a minimum 3-month history of ED, a stable monogamous relationship with a female partner and at least one attempt of sexual intercourse over the last 4 weeks. All patients were evaluated with a detailed sexual history, physical examination, blood chemistry and endocrine assay, and color Doppler ultrasonography (CDU) during pharmacologically induced (intracavernosal injection of 60 mg of papaverine) and sexually stimulated erection. Patients with angina during intercourse, unstable angina or any other evidence of recently diagnosed coronary artery disease, poorly controlled blood pressure or orthostatic hypotension, congestive heart failure, arrhythmia, significant renal or hepatic dysfunction, and anemia were also excluded. Patients taking antioxidant agents and any drugs that might affect collagen turnover (angiotensin converting enzyme inhibitors, angiotensin receptor blockers, aldosterone antagonists, and statins) were also excluded from the study. Also ineligible were men who failed to achieve an erection after radical prostatectomy or pelvic surgery; those who had penile implants, clinically noteworthy penile deformities, a history of stroke or spinal-cord trauma within 6 months of study onset; and those who were receiving nitrates, anti-androgens, or cancer chemotherapy.

Erectile function was evaluated by the erectile function domain of the International Index of Erectile Function (IIEF-EF) a validated 15-item self-administered questionnaire. Erectile function is specifically addressed by six questions that form the so called ‘erectile function domain’ of the questionnaire. Each question is scored 0 to 5. ED is defined as any value less than 26 points. ED severity is classified into three categories based on the IIEF-5: Group I, severe (5 to 7 points), Group II, moderate (8 to 16 points), Group III, mild (17 to 26 points). Height and weight were measured according to a standardized protocol. Body mass index (BMI) was calculated by dividing weight in kilograms by height in meters squared (kg/m^2).

Blood sample collection

Blood samples were obtained after an overnight fasting state. Samples were withdrawn from a cubital vein into blood tubes and serum was immediately separated from the cells by centrifugation at 3000g for 10 min, stored at -70°C , and then analyzed.

Measurement of serum prolidase activity (U/l)

Serum was diluted 40-fold with 2.5 mmol/l Mn^{2+} and 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 pre-incubation serum in 1 ml was incubated at 37°C for 30 min. The supernatant was used for measurement of proline by the method proposed by Myara et al, (1982) which is a modification of Chinard's method (Chinard et al, 1952). Intra-assay coefficient of variation (CV) of the assay was 3.8 %.

Measurement of serum lipid hydroperoxide ($\mu\text{mol tBLOOH/l}$)

Serum LOOH ($\mu\text{mol tBLOOH/l}$) levels were determined by the ferrous ion oxidation-xyleneol orange method as previously described (Arab et al, 2004). The method is based on a known principle of the oxidation of Fe II to Fe III by LOOHs under acidic conditions. CV for measurement of serum LOOH levels was 3.1%.

Measurement of total free sulfhydryl groups

Serum -SH (mmol/l) levels were assayed according to the method of Ellman et al (1959) as modified by Hu et al. (1993). Briefly, 1 ml of buffer containing 0.1M Tris, 10 mmol/l EDTA, pH 8.2, and 50 μl serum was added to cuvettes, followed by 50 μl of 10 mmol/l 5,5-dithiobis 2-nitrobenzoic acid in methanol. Blanks were run for each sample as a test. After incubation for 15 min at room temperature, sample absorbance was interpreted at 412 nm on a Cecil 3000 spectrophotometer (Cecil Instruments, Cambridge, UK). Sample and reagent blanks were subtracted. The concentration of -SH groups was calculated using reduced glutathione as the free -SH group standard and the results were expressed as millimolars/liter. CV for measurement of serum -SH levels were %3.6.

Measurement of other laboratory markers

The serum levels of uric acid, creatinine, triglyceride, total cholesterol, high-density lipoprotein (HDL) cholesterol, low density lipoprotein cholesterol (LDL), and fasting glucose were determined using commercially available assay kits (Abbott, Illinois, USA) with Abbott Aeroset auto-analyzer (Abbott).

Statistical analysis

All analyses were conducted using SPSS 11.5 (SPSS Inc., Chicago, Illinois, USA). Continuous variables were expressed as mean \pm SD and categorical variables were expressed as percentages. Comparison of categorical and continuous variables between the ED and control groups was performed using the χ^2 test and independent samples t test, respectively. Comparison of laboratory variables between the groups categorized according to the severity of ED was performed using one way analysis of variance (ANOVA) with least significant difference post-hoc test. The correlation between serum prolidase activity, IIEF-EF score, and clinical and laboratory parameters was assessed by the Pearson's correlation test. To determine independent predictors of the presence of ED, multiple logistic regression analysis was performed by including the parameters that were significantly different between ED and control groups. Multiple linear regression analysis was performed to identify the independent predictors of serum prolidase activity and IIEF-EF score by including the parameters that were correlated with serum prolidase activity and IIEF-EF score, respectively, in bivariate analysis. Standardized β -regression coefficients and their significance from multiple linear regression analysis were reported. Comparison of laboratory variables between the groups categorized according to the vascular status of ED was performed using one way analysis of variance (ANOVA) with least significant difference post-hoc test. Receiver operator curve (ROC) characteristics of serum prolidase levels were examined to identify a cut-off value in order to predict ED. A two tailed $P < 0.05$ was considered statistically significant.

Results

Clinical characteristics of the study population the clinical characteristics and hemodynamic and laboratory parameters of ED and control groups are presented in Table 1. Serum triglyceride, urea, creatinine, and LOOH levels, serum prolidase activity were significantly higher in the ED group than in the control group, whereas serum HDL cholesterol and $-SH$ levels were significantly lower in cases with ED compared with the controls (Table 1). Independent predictor(s) of the presence of ED were determined with multiple logistic regression analysis by including serum triglyceride, urea, creatinine, LOOH, $-SH$, and HDL cholesterol levels, and serum prolidase activity into the model. Serum triglyceride ($\chi^2 = 4.277$, $\beta = 0.681$, $P = 0.059$), urea ($\chi^2 = 9.111$, $\beta = 0.089$, $P = 0.03$), $-SH$ ($\chi^2 = 25.988$, $\beta = -30.473$, $P = 0.018$) levels, and serum prolidase activity ($\chi^2 = 76.533$, $\beta = 0.221$, $P = 0.004$) but not serum creatinine, HDL cholesterol, and LOOH levels, were independent predictors of the presence of ED. Comparisons of variables according to severity of ED are shown in Table 2. The comparison included 92 ED patients grouped into three categories according to the IIEF-EF scores; mild disease ($n=37$), moderate disease ($n=30$), and severe disease ($n=25$) besides 50 controls. The lowest and highest mean serum prolidase activities were detected in control participants and in patients with severe ED disease,

respectively, and we have shown gradual increase in mean serum prolidase activity with increasing severity of ED (ANOVA $P < 0.001$). Serum –SH levels of patients with either moderate or severe disease were significantly lower than either control cases or patients with mild disease (ANOVA $P < 0.001$). Serum LOOH levels were significantly increased in patients with moderate or severe disease compared with control cases (ANOVA $P = 0.021$). Relationship between serum prolidase activity and clinical characteristics and laboratory data is presented in Table 3. Serum prolidase activity was positively correlated with age, presence of hypertension, fasting blood glucose, serum urea, creatinine, and LOOH levels, and severity of ED (serum prolidase activity was inversely correlated with IIEF-EF score) ($P < 0.05$ for all, Table 3). Additionally, serum prolidase activity was inversely correlated with serum HDL cholesterol and –SH levels in bivariate analysis ($P < 0.05$ for all, Table 3). To determine independent predictors of serum prolidase activity, a stepwise linear regression analysis was performed by including parameters that were correlated with serum prolidase activity in bivariate analysis. Serum HDL cholesterol ($\beta = -0.140$, $P = 0.024$) and urea levels ($\beta = 0.145$, $P = 0.039$) and IIEF-EF score ($\beta = -0.320$, $P < 0.001$) were independent predictors of serum prolidase activity (Table 3). Relationship between IIEF-EF score and clinical characteristics and laboratory parameters data is presented in Table 4. IIEF-EF score was inversely correlated with age, serum triglyceride and creatinine levels, and serum prolidase activity ($P < 0.05$ for all, Table 4). Additionally, IIEF-EF score was positively correlated with serum HDL cholesterol and –SH levels in bivariate analysis ($P < 0.05$ for all, Table 4). To determine independent predictors of IIEF-EF score, a stepwise linear regression analysis was performed by including parameters that were correlated with IIEF-EF score in bivariate analysis. Serum triglyceride ($\beta = 0.171$, $P = 0.002$), HDL cholesterol ($\beta = 0.176$, $P = 0.002$), and –SH levels ($\beta = 0.266$, $P < 0.001$), and serum prolidase activity ($\beta = -0.270$, $P < 0.001$) were independent predictors of IIEF-EF score (Table 4). CDU values according to the vascular status of patients with ED are shown in Table 5. Comparisons of variables according to the vascular status of patients with ED are shown in Table 6. The lowest and highest mean serum prolidase activities were detected in control participants and in patients with arterial insufficiency, respectively (ANOVA $P < 0.001$). Serum –SH levels of patients with vasculogenic ED were significantly lower than control cases (ANOVA $P < 0.001$). Serum LOOH levels were significantly higher in patients with vasculogenic ED compared with control cases (ANOVA $P < 0.001$). Additionally, serum prolidase activity was inversely correlated with peak systolic blood flow velocity and resistance index in vasculogenic ED ($P < 0.05$). The ROC characteristics of serum prolidase activity to predict ED is presented in Figure 1. Area under the curve (AUC) was 0.78. The serum prolidase ROC curve analysis showed a sensitivity of 96 percent (95% CI 79.6-99.3) and a specificity of 71.4 percent (95% CI 49.2-95.1) for the detection of vasculogenic ED, with serum prolidase activity 53.4 U/l used as the cut point. The positive predictive

value (PPV) was 86 percent (95% CI 65-100) and the negative predictive value (NPV) was 90 percent (95% CI 36-100).

Discussion

Prolidase, a cytosolic exopeptidase cleaving carboxyterminal proline and hydroxyproline of iminodipeptides, is recognized as an important regulator of endogenous protein synthesis, especially collagen, by providing endogenous proline. Hyperglycemia is the defining characteristic of type 1 and 2 diabetes. Glucose is known to bind nonenzymatically to free amino acids on proteins or lipids. Through a series of oxidative and nonoxidative reactions, advanced glycation end products (AGEs) are formed irreversibly and accumulated in tissues over time, in particular endothelial and vascular smooth muscle cells (Singh et al, 2001). A common consequence of AGE formation is the pathologic cross-linking of collagen, which leads to vascular thickening with loss of elasticity, endothelial dysfunction, and ultimately atherosclerosis of the vascular tree. Prolidase is a homodimeric enzyme whose activity is affected by oxidative stress, and plays an important role in the recycling of proline for collagen biosynthesis. Collagen, basically type I and III collagens, is the main component of fibrous cap of atherosclerotic plaque (Lijnen et al, 2003) and is responsible for the toughness and the stability of the plaque with firm structure (Shah et al, 1998; Newby et al, 1994). Increased prolidase activity in patients with vasculogenic ED compared with control cases, and the significant relationship between serum prolidase activity and IIEF-EF score revealed in this study suggest the presence of increased collagen turnover in vasculogenic ED. Although prolidase activity was revealed to be related to collagen metabolism, the mechanism and end-points by which this enzyme is regulated remain unknown. Prolidase is phosphotyrosine protein and contains at least two potential sites for tyrosine phosphorylation (Surazynski et al, 2005). Previously it has been shown that prolidase activity in normal fibroblasts is regulated by the interaction of extracellular matrix proteins, mainly type I collagen with $\beta 1$ integrin receptor (Palka et al, 1997) and $\beta 1$ -integrin-dependent signaling (Surazynski et al, 2005). Previous clinical studies have shown increased serum prolidase activity, which indicates increased collagen turnover, in chronic liver disease (Myra et al, 1984), osteoporosis (Erbagci et al, 2002), osteoarthritis (Altindag et al, 2007), Helicobacter pylori gastritis (Aslan et al, 2007), breast cancer (Cechowska et al, 2006), wound healing (Senboshi et al, 1996) and keloid formation (Duong et al, 2006). Serum prolidase activity was reported to be increased in patients with hypertension (Demirbag et al, 2007). Conversely deficient/reduced serum prolidase activity was reported to be associated with lupus-like syndrome characterized by non-healing skin ulcers owing to defective collagen turnover (Shrinath et al, 1997). Reduced serum prolidase activity was also reported in renal insufficiency (Evrenkaya et al, 2006) and uremia (Gejyo et al, 1983), as the kidney is the main source of prolidase (Liu et al, 2007).

LOOH is a well-known marker of oxidative stress formed from unsaturated phospholipids, glycol-lipids, and cholesterol by peroxidative reactions under oxidative stress. A considerable body of evidence implicates oxidative stress, in particular the reaction of NO and superoxide anion, as an important pathogenic element in the development of endothelial dysfunction in vascular diseases such as diabetes, hypertension, arteriosclerosis, and hypercholesterolemia. Increased inactivation of NO by superoxide anion in conditions of increased oxidative stress creates an imbalance that leads to a deficit of endothelial-derived NO acutely and ultimate development of endothelial dysfunction. Oxidized low-density lipoprotein (LDL), besides membrane-bound cholesterol-derived hydroperoxides, is the main form of LOOH to be responsible for the development of oxidative stress-related atherosclerosis and adverse cardiovascular events (Girotti et al, 1998). LDL peroxidation contributes to the development of atherosclerosis, and injuries to endothelial cells have a principal role in the progression of atherosclerotic lesions (Rubbo et al, 2002). Oxidized LDL has been shown to impair endothelium-dependent relaxation in the penis and may also contribute to endothelial dysfunction observed in hypercholesterolemia through an increased production of superoxide anion via uncoupling of e-NOS or a reduction in the e-NOS cofactor BH4 (Tetrahydrobiopterin) (Ahn et al, 1999).

Fibrosis of the corpora cavernosa and the media of penile arteries, involving loss of smooth muscle cells (SMC), is a highly prevalent process that underlies most cases of vasculogenic ED (Gonzales, 2009). The concept that a progressive fibrosis of the smooth muscle (SM) tissue within the penile corpora cavernosa is responsible for the vasculogenic erectile dysfunction (ED) associated with diabetes, ageing, heavy smoking, and pelvic surgery, has gained support over the last decade (Kovanecz et al, 2009). Histologically, this fibrotic process is characterized by the excessive deposition of collagen fibers and extracellular matrix, loss of SM cells (SMCs), presumably due to a combination of a higher rate of SMC apoptosis with a reduced rate of cell proliferation, and an increase in profibrotic factors such as TGF- β 1 and reactive oxygen species (Schwartz et al, 2004). From a functional perspective, this fibrotic process leads to a decrease in the compliance of the corporal tissue after stimulation by the nitric oxide (NO)/c-GMP system. This inability of the corporal tissue to relax sufficiently enough to occlude the aggressing subtunical veins occurs in most patients with ED (Luo et al, 2007; Rajfer et al, 1988; Metro et al, 1999; Nehra et al, 1996; Lue, 1996) and is termed venous leakage or corporal veno-occlusive dysfunction (CVOD). Therefore, without disregarding the potential role of endothelial dysfunction, the progressive damage of the SM tissue in the corpora cavernosa by either an acute and/or chronic process is probably the major single factor impairing erectile function in patients with ED. As the penis is considered to be an extension of the vascular system, it is not surprising that many changes that occur within the corporal tissue are also reflected in the cardiovascular system. This helps to explain why there is a strong association between ED and various cardiovascular disorders such as hypertension, heart

disease, etc. Indeed, with ageing, the fibrotic changes seen in the penile corpora cavernosa resemble those seen within the arterial wall, and it has been suggested that this relative fibrosis of the media of the arterial tree (arteriosclerosis) is pathophysiologically the same disorder as CVOD, where both tissues have lost their SMCs, together with an increase in fibrosis within that part of the tissue where the SMCs are located (Wang et al, 2004; Najjar et al, 2005; Aronson, 2003, Diez, 2007; Izzo, 2007). In general terms, ageing related changes as represented by other markers of fibrosis and oxidative stress were similar in the arterial media and the corpora cavernosa. Therefore, the study of fibrosis may provide a unifying view on the vasculogenic disorders affecting the penis. Profibrotic factors, the excessive deposit of collagen fibers and other extracellular matrix, the appearance of a synthetic cell phenotype in smooth muscle cells or the onset of a fibroblast-myofibroblast transition, and in the case of the corporal or penile arterial tissue the reduction of the smooth muscle cellular compartment underlies vasculogenic ED. This histopathology leads either to localized plaques or nodules in penile tissues, or to the diffuse fibrosis causing impairment of tissue compliance that underlies CVOD and arteriogenic ED. The anti-fibrotic role of the sustained stimulation of the nitric oxide/cyclic guanosine monophosphate pathway in the penis and its possible relevance to exogenous and endogenous stem cell differentiation may be interpreted to the collagen biosynthesis. The relationship between collagen and prolidase activity was observed during fibrotic processes, where an increase in prolidase activity was accompanied by increase in tissue collagen deposition (Verit et al, 2006). The negative effect of free radicals is mediated by degradative agents such as proteolytic enzymes and the last step of collagen degradation is mediated by prolidase (Altindag et al, 2007). Surazynski et al pointed out that prolidase may also have a possible role in angiogenesis depending on the fact that prolidase deficiency is associated with angiopathy (Surazynski et al, 2008). Oxidative stress resulted in collagen degradation and this process is mediated by prolidase (Altindag et al, 2007). Moreover the degree of severity of oxidative stress is directly correlated with the inhibition of collagen production, and prolidase is supposed to be the target enzyme of this process (Sienkiewicz et al, 2007). Yildiz et al. showed that serum prolidase activity in an increasing manner was significantly associated with the presence and severity of coronary artery disease (Yildiz et al, 2008). In addition, it was suggested that hypertension and its duration is associated with increased serum prolidase activity and it may be a marker for the follow up of hypertensive patients (Demirbag et al, 2007).

Our results revealed that serum prolidase activity can be assessed as a predictor of vasculogenic ED. Our study is the first to address a cut-off value for serum prolidase level in vasculogenic ED. We found that serum prolidase level of 53.4 U/l [sensitivity of 96 percent (95% CI 79.6-99.3) and a specificity of 71.4 percent (95% CI 49.2-95.1)] can be used to predict vasculogenic ED.

Several limitations of this study should be considered. One potential limitation of this study is the cross-sectional study design. Beyond the findings of our study, assessing serum prolidase activity in atherosclerotic plaque and in endothelial cells, and evaluating the association of serum prolidase activity and the presence and the extent of atherosclerosis in other territories of arterial system, which remain to be evaluated, would better clarify the pathophysiological role of prolidase activity in atherosclerotic process and endothelial dysfunction. Evaluating the association of serum prolidase activity and the extent of endothelial ischemia would identify the role of endothelial ischemia in increased collagen turnover in patients with vasculogenic ED. Measuring serum and urine levels of proline or hydroxyproline would add to the value of this study, however, we did not have the opportunity to perform these measurements.

In conclusion, with the data of this study, we have shown an independent relationship between increased serum prolidase activity and the presence and severity of vasculogenic ED, which may be interpreted as evidence of increased collagen turnover in vasculogenic ED. Serum prolidase activity appears to be a sensitive and specific predictor of vasculogenic ED and for this reason, it may be used in early prediction of vasculogenic ED in male population. However, further clinical studies are needed to clarify the pathophysiological role of serum prolidase activity in vasculogenic ED.

References

- Ahn TY, Gomez-Coronado D, Martinez V, Cuevas P, Goldstein I, Saenz de Tejada I. Enhanced contractility of rabbit corpus cavernosum smooth muscle by oxidized low density lipoproteins. *Int J Impot Res.* 1999; 11:9–14.
- Altindag O, Erel O, Aksoy N, Selek S, Celik H, Karaoglanoglu M. Increased oxidative stress and its relation with collagen metabolism in knee osteoarthritis. *Rheumatol Int* 2007; 27:339–344.
- Arab K, Steghens JP. Plasma lipid hydroperoxides measurement by an automated xylenol orange method. *Anal Biochem* 2004; 325:158–163.
- Aronson D. Cross-linking of glycated collagen in the pathogenesis of arterial and myocardial stiffening of aging and diabetes. *J Hypertens* 2003; 21: 3–12.
- Aslan M, Nazligul Y, Horoz M, Bolukbas C, Bolukbas FF, Aksoy N, et al. Serum prolidase activity and oxidative status in *Helicobacter pylori* infection. *Clin Biochem* 2007; 40:37–40.
- Bivalacqua TJ, Usta MF, Champion HC, Kadowitz PJ, Hellstrom WJ. Endothelial dysfunction in erectile dysfunction: role of the endothelium in erectile physiology and disease. *J Androl* 2003; 24(6 Suppl):S17-37.
- Cechowska-Pasko M, Palka J, Wojtukiewicz MZ. Enhanced prolidase activity and decreased collagen content in breast cancer tissue. *Int J Exp Pathol* 2006; 87:289–296.
- Chinard FP. Photometric estimation of proline and ornithine. *J Biol Chem* 1952; 199:91–95.
- Demirbag R, Yildiz A, Gur M, Yilmaz R, Elci K, Aksoy N. Serum prolidase activity in patients with hypertension and its relation with left ventricular hypertrophy. *Clin Biochem* 2007; 40:1020–1025.
- Díez J. Arterial stiffness and extracellular matrix. *Adv Cardiol* 2007; 44: 76–95.
- Duong HS, Zhang QZ, Le AD, Kelly AP, Kamdar R, Messadi DV. Elevated prolidase activity in keloids: correlation with type I collagen turnover. *Br J Dermatol* 2006; 154:820–828.
- Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959; 82:70–77.
- Erbagci AB, Araz M, Erbagci A, Tarakcioglu M, Namiduru ES. Serum prolidase activity as a marker of osteoporosis in type 2 diabetes mellitus. *Clin Biochem* 2002; 35:263–268.

- Evrenkaya TR, Atasoyu EM, Kara M, Unver S, Gultepe M. The role of prolidase activity in the diagnosis of uremic bone disease. *Ren Fail* 2006; 28:271–274.
- Gejyo F, Kishore BK, Arakawa M. Prolidase and prolinase activities in the erythrocytes of patients with chronic uremia. *Nephron* 1983; 35:58–61.
- Gensini GG. A more meaningful scoring system for determining the severity of coronary heart disease. *Am J Cardiol* 1983; 51:606–607.
- Girotti AW. Lipid hydroperoxide generation, turnover, and effector action in biological systems. *J Lipid Res* 1998; 39:1529–1542.
- Gonzalez-Cadavid NF. Mechanisms of penile fibrosis. *J Sex Med.* 2009 Mar;6 Suppl 3:353-62.
- Gur M, Aslan M, Yildiz A, Demirbag R, Yilmaz R, Selek S, et al. Paraoxonase and arylesterase activities in coronary artery disease. *Eur J Clin Invest* 2006; 36:779–787.
- Hu ML, Louie S, Cross CE, Motchnik P, Halliwell B. Antioxidant protection against hypochlorous acid in human plasma. *J Lab Clin Med* 1993; 121:257–262.
- Izzo JL Jr, Mitchell GF. Aging and arterial structure-function relations. *Adv Cardiol* 2007; 44: 19–34.
- Kovanecz I, Nolzco G, Ferrini MG, Toblli JE, Heydarkhan S, Vernet D, Rajfer J, Gonzalez-Cadavid NF. Early onset of fibrosis within the arterial media in a rat model of type 2 diabetes mellitus with erectile dysfunction. *BJU Int.* 2009 May;103(10):1396-404.
- Lijnen HR. Metalloproteinases in development and progression of vascular disease. *Pathophysiol Haemost Thromb* 2003–2004; 33:275–281.
- Liu G, Nakayama K, Awata S, Tang S, Kitaoka N, Manabe M, et al. Prolidase isoenzymes in the rat: their organ distribution, developmental change and specific inhibitors. *Pediatr Res* 2007; 62:54–59.
- Lubos E, Schnabel R, Rupprecht HJ, Bickel C, Messow CM, Prigge S, et al. Prognostic value of tissue inhibitor of metalloproteinase-1 for cardiovascular death among patients with cardiovascular disease: results from the AtheroGene study. *Eur Heart J* 2006; 27:150–156.
- Luo H, Goldstein I, Udelson D. A three-dimensional theoretical model of the relationship between cavernosal expandability and percent cavernosal smooth muscle. *J Sex Med* 2007; 4:644–5

- Lue TF. Veno-occlusive dysfunction of corpora cavernosa: comparison of diagnostic methods. *J Urol* 1996; 155:786–7.
- Maas R, Schwedhelm E, Albsmeier J, Boger RH. The pathophysiology of erectile dysfunction related to endothelial dysfunction and mediators of vascular function. *Vasc Med.* 2002; 7:213–225.
- Metro MJ, Broderick GA. Diabetes and vascular impotence: does insulin dependence increase the relative severity? *Int J Impot Res* 1999; 11: 87–9.
- Myara I, Charpentier C, Lemonnier A. Optimal conditions for prolidase assay by proline colorimetric determination: application to imminodipeptiduria. *Clin Chim Acta* 1982; 125:193–205.
- Myara I, Myara A, Mangeot M, Fabre M, Charpentier C, Lemonnier A. Plasma prolidase activity: a possible index of collagen catabolism in chronic liver disease. *Clin Chem* 1984; 30:211–215. Najjar SS, Scuteri A, Lakatta EG. Arterial aging: is it an immutable cardiovascular risk factor? *Hypertension* 2005; 46: 454–62.
- Nehra A, Goldstein I, Pabby A et al. Mechanisms of venous leakage: a prospective clinicopathological correlation of corporeal function and structure. *J Urol* 1996; 156: 1320–9.
- Newby AC, Southgate KM, Davies M. Extracellular matrix degrading metalloproteinases in the pathogenesis of arteriosclerosis. *Basic Res Cardiol* 1994; 89:59–70.
- Palka JA, Phang JM. Prolidase activity in fibroblasts is regulated by interaction of extracellular matrix with cell surface integrin receptors. *J Cell Biochem* 1997; 67:166–175.
- Rajfer J, Rosciszewski A, Mehringer M. Prevalence of corporal venous leakage in impotent men. *J Urol* 1988; 140: 69–71
- Rubbo H, Trostchansky A, Botti H, Batthyany C. Interactions of nitric oxide and peroxynitrite with low-density lipoprotein. *Biol Chem.* 2002;383:547–552.
- Schwartz EJ, Wong P, Graydon RJ. Sildenafil preserves intracorporeal smooth muscle after radical retropubic prostatectomy. *J Urol* 2004; 171: 771–4.
- Senboshi Y, Oono T, Arata J. Localization of prolidase gene expression in scar tissue using in situ hybridization. *J Dermatol Sci* 1996; 12:163–171.

- Shah PK. Role of inflammation and metalloproteinases in plaque disruption and thrombosis. *Vasc Med* 1998; 3:199–206.
- Shrinath M, Walter JH, Haeney M, Couriel JM, Lewis MA, Herrick AL. Prolidase deficiency and systemic lupus erythematosus. *Arch Dis Child* 1997; 76:441–444.
- Sienkiewicz P, Pałka M, Pałka J. Oxidative stress induces IGF-I receptor signaling disturbances in cultured human dermal fibroblasts. A possible mechanism for collagen biosynthesis inhibition. *Cell Mol Biol Lett* 2004;9:643-50.
- Singh R, Barden A, Mori T, Beilin L. Advanced glycation end-products: a review. *Diabetologia*. 2001; 44:129–146.
- Solomon H, Man JW, Jackson G. Erectile dysfunction and the cardiovascular patient: endothelial dysfunction is the common denominator. *Heart*. 2003; 89:251–253.
- Surazynski A, Palka J, Wolczynski S. Phosphorylation of prolidase increases the enzyme activity. *Mol Cell Biochem* 2001; 220:95–101.
- Surazynski A, Sienkiewicz P, Wolczynski S, Palka J. Differential effects of echistatin and thrombin on collagen production and prolidase activity in human dermal fibroblasts and their possible implication in beta1-integrin-mediated signaling. *Pharmacol Res* 2005; 51:217–221.
- Surazynski A, Donald SP, Cooper SK, Whiteside MA, Salnikow K, Liu Y, Phang JM. Extracellular matrix and HIF-1 signaling: the role of prolidase. *Int J Cancer* 2008;122:1435-40.
- Tal R, Voelzke BB, Land S, Motarjem P, Munarriz R, Goldstein I, Mulhall JP. Vasculogenic erectile dysfunction in teenagers: a 5-year multi-institutional experience. *BJU Int*. 2009 Mar;103(5):646-50.
- Verit FF, Geyikli I, Yazgan P, Celik A. Correlations of serum prolidase activity between bone turnover markers and mineral density in postmenopausal osteoporosis. *Arch Gynecol Obstet*. 2006;274:133-7.
- Wang YX, Fitch RM. Vascular stiffness: measurements, mechanisms and implications. *Curr Vasc Pharmacol* 2004; 2: 379–84.
- Watanabe N, Ikeda U. Matrix metalloproteinases and atherosclerosis. *Curr Atheroscler Rep* 2004; 6:112–120.

Yildiz A, Demirbag R, Yilmaz R, Gur M, Altiparmak IH, Akyol S, Aksoy N, Ocak AR, Erel O. The association of serum prolidase activity with the presence and severity of coronary artery disease. *Coron Artery Dis* 2008;19:319-25.

Zanaboni G, Dyne KM, Rossi A, Monafò V, Cetta G. Prolidase deficiency: biochemical study of erythrocyte and skin fibroblast prolidase activity in Italian patients. *Haematologica* 1994; 79:13–18.

Table 1: Baseline clinical and laboratory characteristics of ED and control groups.

	Control group	ED group	P value
	n=50	n=92	
Clinical and hemodynamic data			
Age (years)	54.07 ± 7.60	56.05 ± 8.90	0.08
BMI (kg/m ²)	27.1 ± 5.6	26.7 ± 4.1	0.30
Hypertension (n %)	23 (46.0%)	48(52.1%)	0.38
Diabetes mellitus (n %)	18(36.0%)	39(42.4%)	0.53
Cigarettes smoking (n %)	26(52.0%)	52(56.5%)	0.39
Biochemical data			
Total cholesterol (mmol/l)	48.9 ± 1.2	5.3 ± 1.3	0.13
LDL cholesterol (mmol/l)	3.2 ± 0.8	3.3 ± 1.0	0.18
HDL cholesterol (mmol/l)	1.2 ± 0.4	1.0 ± 0.1	0.003
Triglyceride (mmol/l)	1.8 ± 1.3	2.3 ± 1.3	0.037
Fasting glucose (mg/dl)	116.7 ± 50.6	123 ± 52.7	0.78
Uric acid (mg/dl)	4.8 ± 1.6	4.9 ± 1.4	0.47
Urea (mg/dl)	35.8 ± 11.9	42 ± 16.8	0.004
Creatinine (mg/dl)	0.91 ± 0.19	1.1 ± 0.33	<0.001
-SH (mmol/l)	0.29 ± 0.03	0.27 ± 0.04	<0.001
LOOH (μmol tBLOOH/l)	4.8 ± 0.8	4.9 ± 0.8	0.005
Prolidase activity (U/I)	45.7 ± 4.9	53.5 ± 5.5	<0.001

BMI, body mass index; ED, erectile dysfunction; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LOOH, lipid hydroperoxide; -SH, free sulfhydryl groups; tBLOOH, tert butyl hydroperoxide.

Table 2: Comparison of variables according to severity of ED.

	Severity of ED				ANOVA P
	Control	Mild	Moderate	Severe	
	n=50	n=37	n=30	n=25	
Prolidase activity (U/l)	45.7 ± 4.9*	50.3 ± 4.9**	52.2 ± 5.6***	55.0 ± 5.2	<0.001
-SH (mmol/l)	0.29 ± 0.03†	0.27 ± 0.04††	0.25 ± 0.04	0.25 ± 0.03	<0.001
LOOOH (μmol tBLOOH/l)	4.8 ± 0.8†††	4.76 ± 0.84	4.90 ± 0.74	4.99 ± 0.80	0.021

ANOVA, analysis of variance; LOOH, lipid hydroperoxide; -SH, free sulfhydryl groups; tBLOOH, tert butyl hydroperoxide.

*P < 0.001 versus mild, moderate, and severe disease.

**P = 0.035 versus moderate, P < 0.001 versus severe disease.

***P = 0.008 versus severe disease.

†P = 0.297 versus mild, <0.001 versus moderate, and severe disease.

††P = 0.004 versus moderate, P < 0.001 versus severe disease.

†††P = 0.021 versus moderate, P = 0.005 versus severe disease.

Table 3: Relationship between serum prolidase activity and clinical charecteristics and laboratory parameters.

	Pearson's coefficient	P value	β regression coefficient^a	P value
Age (years)	0.290	<0.001	0.092	0.177
BMI (kg/m ²)	-0.008	0.093		
Hypertension	0.216	<0.001	0.084	0.203
Diabetes mellitus	0.062	0.285		
Cigarette smoking	-0.105	0.073		
Total cholesterol (mmol/l)	0.095	0.140		
LDL cholesterol (mmol/l)	0.114	0.070		
HDL cholesterol (mmol/l)	-0.160	0.013	-0.140	0.024
Triglyceride (mmol/l)	0.115	0.072		
Fasting glucose (mg/dl)	0.121	0.039	0.045	0.422
Uric acid (mg/dl)	0.040	0.680		
Urea (mg/dl)	0.220	<0.001	0.145	0.039
Creatinine (mg/dl)	0.188	0.002	0.066	0.360
-SH (mmol/l)	-0.115	0.040	0.061	0.334
LOOH (μ mol BLOOH/l)	0.170	0.002	0.103	0.0082
IIEF-EF score	-0.392	<0.001	-0.320	<0.001
PSV	-0.352	0.062	-0.213	<0.001
RI	-0.109	0.56	-0.145	<0.001

BMI, body mass index; HDL, high-density lipoprotein; IIEF-EF, erectile function domain of the international index of erectile function; LDL, low-density lipoprotein; LOOH, lipid hydroperoxide; -SH, free sulfhydryl groups; tBLOOH, tert butyl hydroperoxide; PSV: peak systolic blood flow velocity (cm/s); RI: resistance index

^aFrom multiple linear regression analysis.

Table 4: Relationship between serum IIEF-EF score and clinical characteristics and laboratory parameters.

	Pearson's coefficient	P value	β regression coefficient^a	P value
Age (years)	-0.210	< 0.001	-0.093	0.115
BMI (kg/m ²)	0.054	0.430		
Hypertension	0.040	0.490		
Diabetes mellitus	0.006	0.920		
Cigarette smoking	0.025	0.640		
Total cholesterol (mmol/l)	0.087	0.171		
LDL cholesterol (mmol/l)	0.063	0.339		
HDL cholesterol (mmol/l)	0.215	0.001	0.176	0.003
Triglyceride (mmol/l)	-0.191	0.004	0.171	0.002
Fasting glucose (mg/dl)	-0.035	0.522		
Uric acid (mg/dl)	0.082	0.470		
Urea (mg/dl)	0.087	0.138		
Creatinine (mg/dl)	-0.143	0.014	-0.051	0.374
-SH (mmol/l)	0.288	< 0.001	0.266	< 0.001
LOOH (μ mol BLOOH/l)	0.106	0.058		
Prolidase activity (U/I)	-0.392	< 0.001	-0.270	<0.001

BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LOOH, lipid hydroperoxide; -SH, free sulfhydryl groups; tBLOOH, tert butyl hydroperoxide;.

^aFrom multiple linear regression analysis

Table 5: Colour Doppler ultrasonography values according to the vascular status of patients with erectile dysfunction.

		Arterial insufficiency	Veno-occlusive dysfunction	Mixed type impotence	<i>P</i> value
N		26	37	29	
PSV	<i>Right</i>	24.0 ± 6.2	44.1 ± 14.9	21.9 ± 7.2	< 0.001
	<i>Left</i>	23.9 ± 3.5	43.4 ± 15.5	29.2 ± 9.9	< 0.001
EDV	<i>Right</i>	-2.4 ± 2.5	7.7 ± 3.8	6.9 ± 3.6	< 0.001
	<i>Left</i>	-2.3 ± 2.6	5.9 ± 4.5	6.6 ± 2.9	< 0.001
RI	<i>Right</i>	1.05 ± 0.08	0.83 ± 0.10	0.77 ± 0.08	< 0.001
	<i>Left</i>	1.04 ± 0.07	0.84 ± 0.08	0.73 ± 0.07	< 0.001

The measurements were given as the mean value±SD; PSV: peak systolic blood flow velocity (cm/s); EDV: end systolic blood flow velocity (cm/s); RI: resistance index

Table 6: Comparisons of variables according to the vascular status of patients with erectile dysfunction.

	Control n=50	Type of ED			ANOVA P
		Arterial insufficiency n=26	Veno-occlusive dysfunction n=37	Mixed type impotence n=29	
		Prolidase activity (U/l)	45.7 ± 4.9*	56.3 ± 5.7**	
-SH (mmol/l)	0.29 ± 0.03†	0.27 ± 0.04	0.28 ± 0.04	0.27 ± 0.03	<0.001
LOOOH (µmol tBLOOH/l)	4.8 ± 0.8††	5.12 ± 0.43	4.89 ± 0.69	4.96 ± 0.73	<0.001

ANOVA, analysis of variance; LOOH, lipid hydroperoxide; -SH, free sulfhydryl groups; tBLOOH, tert butyl hydroperoxide.

*P < 0.001 versus arterial insufficiency, veno-occlusive dysfunction, mixed type impotence.

**P < 0.001 versus veno-occlusive dysfunction, P < 0.001 >versus mixed type impotence.

***P < 0.001 versus mixed type impotence.

†P < 0.001 versus arterial insufficiency, veno-occlusive dysfunction, mixed type impotence.

††P < 0.001 versus arterial insufficiency, versus mixed type impotence.

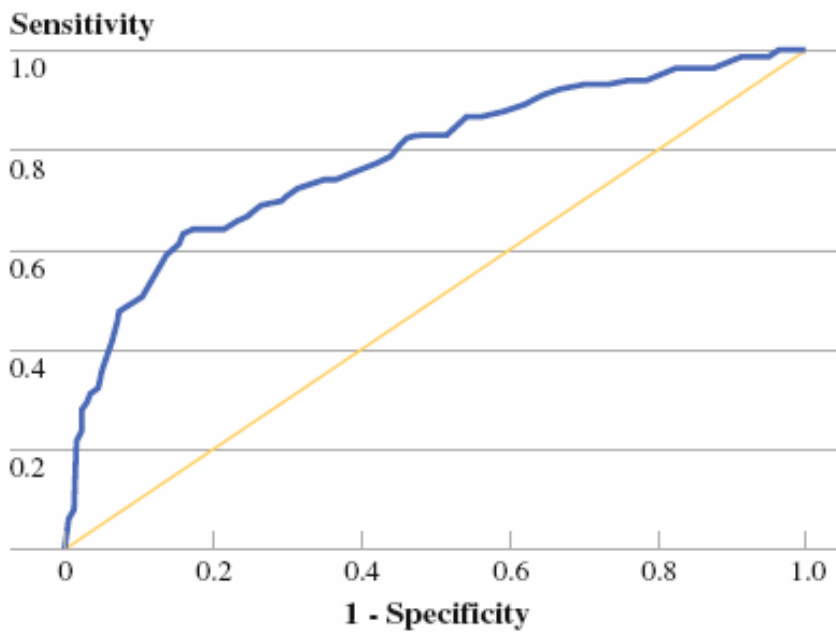


Figure 1 Receiver–operating characteristic (ROC) curve calculation for the serum prolidase activity in erectile dysfunction.