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Association Between Matrix Metalloproteinase-3 Activity and Glomerular Filtration Rate and Albuminuria Status in Patients With Type 2 Diabetes Mellitus

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Original Paper

Introduction. Diabetic nephropathy is pictured as matrix accumulation and thickening of glomerular basal membrane. Matrix metalloproteinases (MMPs) are major proteases involved in extracellular matrix degradation. Moreover, plasminogen activator inhibitor-1 (PAI-1) primarily regulates plasmin dependent proteolysis. It plays a role in renal fibrosis causing extracellular matrix accumulation through inhibition of plasmin-dependent extracellular matrix degradation. This study investigated PAI-1 serum level and MMP-3 activity and their correlation with glomerular filtration rate in patients with diabetes mellitus.

Materials and Methods. In a case-control design, serum PAI-1 concentrations and MMP-3 activity were measured in 80 patients with normoalbuminuria, microalbuminuria, and macroalbuminuria. Receiver operating characteristics curve analysis was used to assess the diagnostic accuracy of MMP-3 activity in discriminating albuminuria.

Results. In the patients with microalbuminuria, serum PAI-1 levels were higher compared with macroalbuminuric patients ($P < .001$). The patients with macroalbuminuria exhibited a significantly lower MMP-3 activity than the patients with microalbuminuria and normoalbuminuria ($P < .001$). No significant correlation was found between serum MMP-3 activity and serum PAI-1 levels in those with normoalbuminuria, microalbuminuria, and macroalbuminuria. The MMP-3 activity had a strong positive correlation with estimated glomerular filtration ($r = 0.853$, $P < .001$).

Conclusions. We found that there was a positive correlation between glomerular filtration rate and MMP-3 activity in diabetic patients. This concludes that MMP-3 may have a role in the pathogenesis of diabetic nephropathy progressions towards macroalbuminuria, and therefore, MMP-3 activity may be used in evaluating albuminuria status.

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INTRODUCTION

Diabetic nephropathy (DN) is deemed one of the major health issues in developed countries.[1] Generally, patients with diabetes mellitus (DM) who progress to end-stage renal disease may not be able to afford renal replacement therapy. Therefore, it seems adopting strategies to prevent the onset of nephropathy and its progression

is absolutely essentiall.[2] Due to the complex mechanism involved in DN, its exact pathogenesis has not been understood completely.[3] Some noticeable early renal alterations in DM include glomerular hyperfiltration, renal hypertrophy, and microalbuminuria. However, it is believed that a key factor in DN is changes in extracellular matrix (ECM).[4]

Accumulation of mesangial matrix and thickening of glomerular basement membrane are the most frequent changes found in DN.[4] Furthermore, the pathological complications in the mesangial expansion are including an increase in type IV and V collagens, laminin, and fibronectin as the components of the mesangial matrices, and other components of ECM which usually might have not been seen in normal glomeruli.[5] In fact, matrix accumulation leads to the expansion of mesangial matrix and decreases the available capillary surface area for filtration, which can correlate with protein excretion that has an important role in the progression of renal insufficiency.[6]

Two major classes of enzymes are in the core of ECM degradation: matrix metalloproteinases (MMPs) and serine proteases.[4] Matrix metalloproteinases are considered one of the subfamilies of zinc- and calcium-dependent enzymes. They are members of metzincin superfamily that are responsible for degradation of most ECM proteins, including collagens.[7] It is demonstrated that the major component in renal ECM is collagen type IV. In comparison of patients suffering from microalbuminuria with those with normoalbuminuria, it has been documented that urinary levels of type IV collagen in microalbuminuria is significantly higher.[8] In patients with type 2 DM, higher levels of urinary type IV collagen has been introduced as an indicator for DN in early stages, because urinary levels of type IV collagen correlate with albuminuria.[3] Matrix metalloproteinase-3, with specific substrates including collagens, laminin, fibronectin, and proteoglycans, has a role in ECM degradation. These mesangial matrices and components of glomerular basement membrane are also increased in DN.[5] Almost all MMPs, particularly MMP-3, except for MMP-2, is activated by the actions of plasmin. As a matter of the fact, plasmin activity is declined in DM, in which mesangial cells are exposed to high glucose concentration, retarding the activation of MMPs and contributing to decreasing matrix degradation.[9,10] Ebihara and colleagues have shown that MMP-9 concentration increases before the beginning of microalbuminuria in type 2 diabetic patients.[11] Likewise, Diamant and coworkers have found that the activity of urinary MMP-2 and MMP-9 were elevated in patients with DN. Consequently, they suggested that MMP-2 and MMP-9 could be used as sensitive biomarkers for assessing the extent of kidney disease.[12]

Plasminogen activator inhibitor-1 (PAI-1) is one of serine-protease inhibitors secreted primarily by adipocytes, endothelial cells, and hepatocytes. It acts as a main negative regulator of fibrinolysis through its role as the primary inhibitor of tissue plasminogen activator.[13] It is well-documented that PAI-1 could inhibit activation of tissue plasminogen activator and also urokinase plasminogen activator. These factors function result in conversion of plasminogen to plasmin, as a strong proteolytic enzyme. Consequently, PAI-1 could primarily regulate plasmin-driven proteolysis in negative feedback manner. Regarding abnormal deposition of ECM as the hallmark of DN, it is to be concluded that changes in MMP expression or its activation may be involved in DN and beginning of renal hypertrophy.[14] Plasminogen activator inhibitor-1 may also have a role in renal fibrosis, because of its involvement in matrix accumulation through inhibition of plasmin-dependent ECM degradation.[15,16]

The evidence hypothesizes that matrix accumulation in DN may be resulted from decreased degradation of extracellular matrix caused by a decrease in the MMPs, especially MMP-3, which is mainly expressed in the kidney. In the present study, the levels of PAI-1 and MMP-3 activity in patients with normoalbuminuria, microalbuminuria, and macroalbuminuria and their correlation with estimated glomerular filtration rate (GFR) and the extent of nephropathy were investigated.

MATERIALS AND METHODS

Study Design and Participants

Designed as a case-control study, 80 patients suffering from type 2 DM, aged 45 to 65 years old, including 40 with macroalbuminuria, 20 with microalbuminuria, and 20 with normoalbuminuria were enrolled in the study. Glucose cutoff values were used for diagnosis of DM based on instruction

of the American Diabetes Association. The presence of microalbuminuria and overt albuminuria were considered as the indicator for diagnosis of DN. Excretion of 30 mg/24 h to 300 mg/24 h of urinary albumin was considered as microalbuminuria, and an excretion greater than 300 mg/24 h was regarded as macroalbuminuria.[17] Exclusion criteria were history of heart diseases, malignancies, acute or chronic inflammatory and infectious diseases, and primary hypertension; insulin therapy; treatment with hemodialysis or kidney transplantation; smoking; and administration of beta-blockers. Most of the participants received oral hypoglycemic agents such as metformin and other drugs based on their clinical condition. All the patients received furosemide against hypertension and gemfibrozil or cholestyramine against hyperlipidemia. The study protocol was approved by the Ethics Committee of Tabriz University of Medical Sciences. Written informed consent was obtained from all participants.

Laboratory Assessments

Biochemical analyses and anthropometric measurements were done on each participant. Serum specimens were separated and stored at -70°C until analysis. The PAI-1 levels in sera were determined using a PAI-1 enzyme-linked immunosorbent assay kit (Abcam, Cambridge, MA, USA). The MMP-3 activity was quantified by spectrofluorometry assay using an MMP-3 activity fluorometric assay kit (BioVision, Milpitas, CA, USA), according to the manufacturer instructions. Fasting blood glucose, total cholesterol, triglyceride, creatinine, total protein, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and urea were analyzed by enzymatic colorimetric methods (Pars-Azmoon, Tehran, Iran). Urinary albumin excretion was measured by turbidimetry. Body mass index (BMI), blood pressure (BP), heart rate, waist, and hip were measured.

For each patient, blood pressure measurement was carried out 3 times on the same arm following the seating and resting. Body mass index was obtained as body weight (kg) divided by height squared (m^2). Waist circumference was measured on the standing position. Estimated GFR was calculated using the simplified Modification of Diet in Renal Disease (MDRD) equation,[18] and percentage of patients within the different CKD stages (1 to 4) was stratified based on the estimated GFR[19]:

$$GFR\ (mL/min/1.73\ m^2) = 186.3 \times (\text{serum creatinine})^{-1.154} \times age^{-0.203} \times (0.742 \text{ if female})$$

Statistical Analysis

Descriptive statistics were expressed as mean ± standard deviation. The 1-sample Kolmogorov-Smirnov test was used for evaluating of normality of distributions. The differences between the three groups were assessed using the 1-way analysis of variance and the Welch tests.The Pearson correlation coefficient was used for evaluating the associations between the variables. The receiver operating characteristics curve analysis was used for usefulness of MMP-3 activity in discriminating albuminuria. All statistical analyses were performed using the SPSS software (Statistical Package for the Social Sciences, version 16.0, SPSS Inc, Chicago, IL, USA). A $P < .05$ was considered significant.

RESULTS

Characteristics of the patients among those with macroalbuminuria (n = 40), microalbuminuria (n = 20), and normoalbuminuria (n = 20) are listed in Table 1. The PAI-1 levels and MMP-3 activity in the three groups are demonstrated in Figure 1. Patients with macroalbuminuria exhibited

Table 1. Characteristics of Patients With Macroalbuminuria, Microalbuminuria, and Normoalbuminuria*

Characteristic	Patients With Diabetic Nephropathy			*P*
	Normoalbuminuria (n = 20)	Microalbuminuria (n = 20)	Macroalbuminuria (n = 40)	
Sex				
Male	10	10	20	
Female	10	10	20	…
Age, y	57.55 ± 6.31	56.35 ± 6.01	57.85 ± 5.63	.65
Duration of diabetes, y	7.45 ± 3.05	6.40 ± 3.20	9.90 ± 5.60	.01
Body mass index, kg/m^2	27.33 ± 4.21	29.22 ± 5.55	29.02 ± 3.51	.29
Waist-hip ratio	0.94 ± 0.05	0.94 ± 0.04	0.94 ± 0.05	.98

*Values are mean ± standard deviation, except for sex, which is frequency.

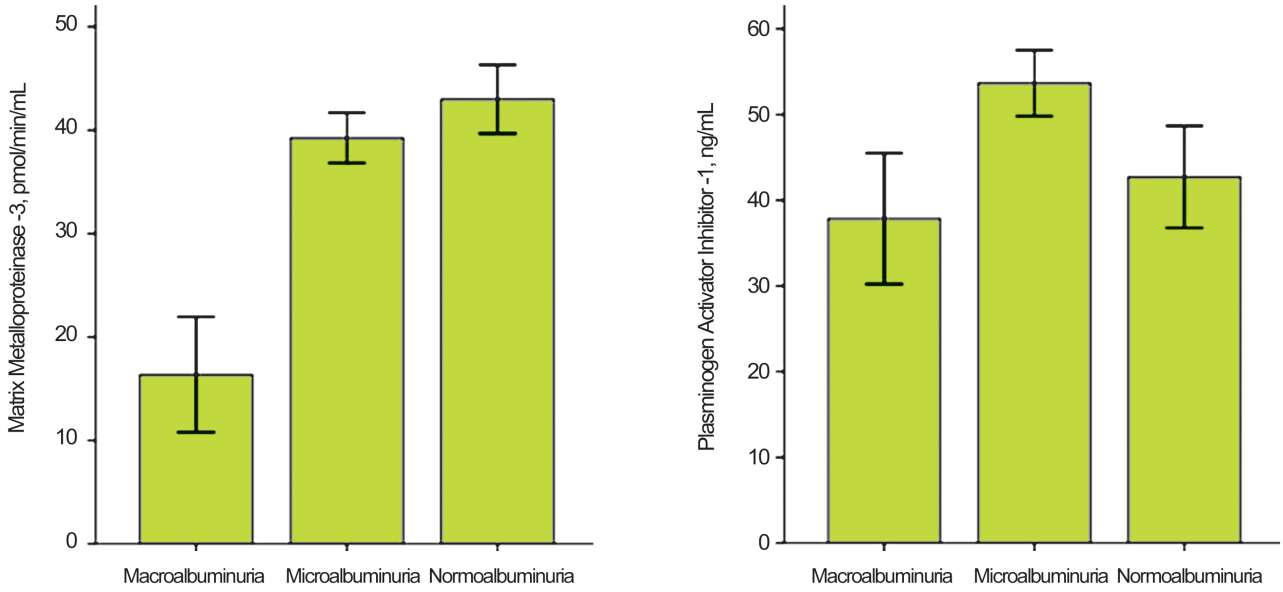

Figure 1. Matrix metalloproteinase-3 and plasminogen activator inhibitor-1 levels in the three groups diabetic patients with normoalbuminuria, microalbuminuria, and macroalbuminuria.

a significantly lower MMP-3 activity than the patients with microalbuminuria (16.37 ± 5.58 pmol/min/mL versus 39.26 ± 2.42 pmol/min/mL, respectively, $P < .001$) and normoalbuminuria (43.00 ± 3.31 pmol/min/mL; $P < .001$; Table 2). There was no correlation between serum MMP-3 activity levels and other parameters. Serum PAI-1 levels were significantly increased in the patients with microalbuminuria (53.67 ± 3.85 ng/mL; $P < .001$) and were significantly decreased in macroalbuminuria (37.8 ± 7.64 85 ng/mL; $P < .001$) in comparison with those with normoalbuminuria (42.72 ± 5.96 ng/mL).

Patients with normoalbuminuria showed serum PAI-1 levels correlated positively with systolic BP (r = 0.481, $P = .03$), and in the macroalbuminuria patients, PAI-1 levels correlated negatively with systolic BP and diastolic BP (r = -0.435, $P = .005$ and r = -0.392, $P = .01$, respectively; Figure 2). Serum total protein in macroalbuminuria patients were significantly lower than in the microalbuminuria and normoalbuminuria patients (Table 2). A significant difference was observed in the estimated GFR between the three groups ($P < .001$; Table 2). Interestingly, there was a strong positive correlation between MMP-3 activity and estimated GFR in

Table 2. Clinical, Laboratory, and Biochemical Parameters of Patient With Diabetic Nephropathy

Parameter	Patients With Diabetic Nephropathy			*P*
	Normoalbuminuria (n = 20)	Microalbuminuria (n = 20)	Macroalbuminuria (n = 40)	
Creatinine, mg/dL	2.57 ± 0.71	1.07 ± 0.17	0.84 ± 0.17	< .001
Urea , mg/dL	49.44 ± 3.35	40.98 ± 2.92	38.51 ± 2.74	< .001
High-density lipoprotein cholesterol, mg/dL	47.40 ± 8.33	47.15 ± 8.73	44.35 ± 8.19	.40
Low-density lipoprotein cholesterol, mg/dL	111.82 ± 24.04	117.25 ± 22.48	123.50 ± 24.04	.20
Total protein , g/dL	5.62 ± 1.04	7.57 ± 0.55	7.82 ± 0.83	< .001
Cholesterol, mg/dL	193.52 ± 28.87	188.60 ± 35.43	187.85 ± 28.47	.74
Glucose , mg/dL	148.88 ± 25.62	154.10 ± 34.19	162.30 ± 46.59	.46
Systolic blood pressure, mm Hg	130.0 ± 11.54	131.50 ± 10.40	125.00 ± 70.60	.12
Diastolic blood pressure, mm Hg	83.00 ± 10.90	84.00 ± 10.95	80.00 ± 9.17	.45
Matrix metalloproteinase -3, pmol/min/mL	16.37 ± 5.58	39.26 ± 2.42	43.00 ± 3.31	< .001
Plasminogen activator inhibitor -1, ng/mL	37.86 ± 7.64	53.67 ± 3.85	42.72 ± 5.96	< .001
Triglyceride, mg/dL	206.55 ± 41.30	201.60 ± 34.05	197.70 ± 32.96	.68
Estimated glomerular filtration rate, min/mL/1.73 m^2	26.12 ± 8.89	66.55 ± 5.58	90.75 ± 14.68	< .001
Heart rate, /min	75.05 ± 9.99	79.40 ± 4.12	77.30 ± 5.25	.06

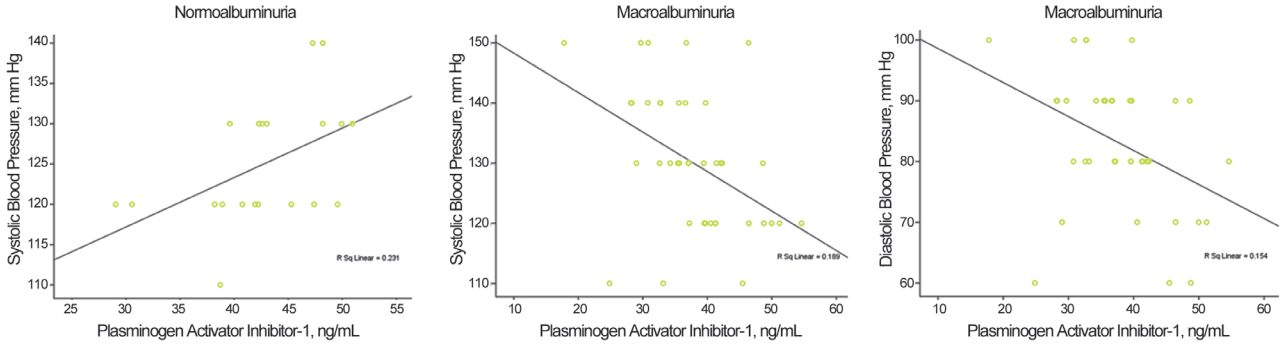

Figure 2. Correlation of plasminogen activator inhibitor-1 with systolic blood pressure in patients with normoalbuminuria and macroalbuminuria and with diastolic blood pressure in those with macroalbuminuria.

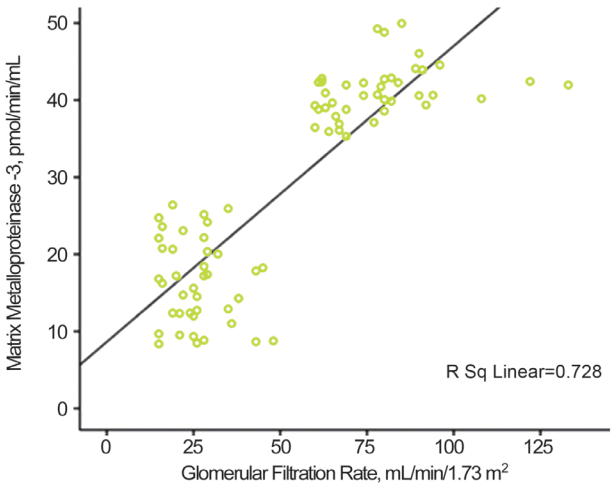

Figure 3. Correlation between matrix metalloproteinase-3 activity and estimated glomerular filtration rate.

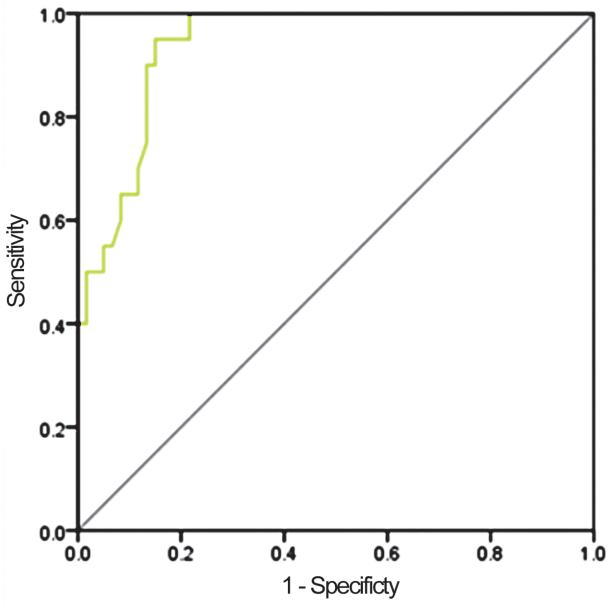

Figure 4. Receiver operating characteristic curve analysis of serum matrix metalloproteinase-3 activity for prediction of microalbuminuria and macroalbuminuria.

all of the patients (r = 0.853; $P < .001$; Figure 3). Figure 4 depicts the ROC analysis of MMP-3 activity for the discrimination of normoalbuminuria from microalbuminuria and macroalbuminuria. The area under the curve was 0.938 (95% confidence interval, 0.888 to 0.987; $P < .001$) with an optimal cutoff value of 39.33 pmol/min/mL (sensitivity, 95%; specificity, 85%).

DISCUSSION

This study found that serum level of PAI-1 in patients with microalbuminuria was higher than those with normoalbuminuria. However, it was shown that PAI-1 level was declined in macroalbuminuria patients, even less than those with normoalbuminuria. Our findings were in agreement with Shirakawa and colleagues,[15] who described a higher level of PAI-1 in patients with microalbuminuria in comparison with patients with normoalbuminuria.

Plasminogen activator inhibitor-1, as a glycoprotein with a molecular weight of approximately 50 kDa, is considered a member of the serine protease inhibitor super-family,[20] and it has been revealed that the liver and adipose tissue are mainly the primary sources of plasma PAI-1.[21-23] There is a delicate balance between synthesis and degradation of ECM, which is mainly regulated by PAI-1. Therefore, increase in expression of PAI-1 could lead to ECM accumulation as the trigger of nephropathy.[24] However, because of the lower molecular weight of PAI-1 compared with albumin molecular weight (65 kDa), lower levels of PAI-1 in patients with macroalbuminuria may be due to renal excretion, which was also reported by Torii and colleagues in 2004.[16] Furthermore, concerning that total protein in macroalbuminuria was lower

than normoalbuminuria and microalbuminuria patients; consequently, it can be concluded that it may be due to exertion of protein and lower levels of PAI-1 in patient with macroalbuminuria may be originated from urinary protein excretion (total protein levels in patients with macroalbuminuria, microalbuminuria, and normoalbuminuria were 5.62 ± 1.04 mg/dL, 7.57 ± 0.55 mg/dL, and 7.82 ± 0.83 mg/dL, respectively).

In a study by Shirakawa and colleagues,[15] a relationship was reported between PA1-1 and BMI, blood pressure, and plasma triglyceride level. In the present study, among the patients with normoalbuminuria, we found a direct significant relationship between PAI-1 and blood pressure; conversely, in the macroalbuminuria patients, this significant relationship was indirect, which may be due in large extent to renal excretion of PAI-1. However, in the present study, PAI-1 did not show any relationship with triglyceride and BMI.

In terms of medication interference in the study, all of the patients with hypertension received furosemide, which cannot decrease the serum PAI-1.[25] Therefore, declined level of PAI-1 in the macroalbuminuria patients could not be related to antihypertensive drugs. Moreover, the patients who received statins or beta-blockers were excluded from the study, owing to their interference with PAI-1 and MMP-3 levels.[26-28] However, regarding the relationship between blood pressure or drugs such as statins and PAI-1, these can interfere with PAI-1 serum levels, and considering renal excretion of PAI-1 in patients with macroalbuminuria, PAI-1 cannot be a good candidate for evaluation of kidney function in end stages, and it may be deemed a good factor for early evaluation of nephropathy. MMPs have been introduced as the major physiological regulators of glomerular ECM degeneration. It has been reported that in patients with microalbuminuria, urinary type-IV collagen levels are is significantly higher than those of patients with normoalbuminuria.[29,30] Matrix metalloproteinase-3 is a neutral proteinase (50 kDa) with a broad substrate range including many components of the extracellular matrix such as laminin, fibronectin, cartilage aggrecan, and types II, IV, IX, and XI collagen.[31] It also has been reported that in patients suffering from DM, the MMP-7 expression is decreased in the kidney, and its level is elevated in serum although the mechanisms involved is not exactly realized.[9] Furthermore, Tashiro and coworkers[8] found that MMPs expression may decrease in glomerular cells excretion of MMPs may increase in urine. They concluded that decreased degradation of type IV collagen by these MMPs may cause matrix accumulation in DN. The present study showed that by the progression of nephropathy, MMP-3 levels in serum were declined, which was in accordance with the previous studies; in patients with macroalbuminuria and microalbuminuria, there was a significant decrease in MMP-3 activity compared with normoalbuminuria. In fact, although there was no relationship between MMP-3 and other factors investigated, there was a positive correlation between estimated GFR and MMP-3. As shown in Figure 3 (Left), MMP-3 has a direct correlation with estimated GFR, and by decreasing the GFR, MMP-3 has been declined, which is independent of even probable protein excretion in macroalbuminuria. Therefore, it can be speculated that measurement of MMP-3 activity can directly reflect the kidney condition, in contrast with PAI-1 level, which are affected by macroalbuminuria, and it cannot reveal the disease progression. Moreover, even considering decrease of MMP-3 activity in patients with macroalbuminuria may be due to a decrease in MMP-3 expression and an increase in renal excretion, it can be postulated that MMP-3 activity has a correlation with disease progression towards end stages.

Although it has been suggested that several proteolytic enzymes are involved in renal response regulation to injury, the exact role of specific proteinases such as MMP-3 is unknown.[32] Our finding suggests that MMP-3 activity may contribute to progression of nephropathy, and it can be a potential marker for investigating the severity of diabetic nephropathy. It should be mentioned that the small sample size was one of our major limitations and further studies with larger sample sizes will be warranted.

CONCLUSIONS

In summary, we found that the serum activity of MMP-3, as an independent factor, was declined significantly by the progression of DN. The results of this study showed that PAI-1 levels in patients with macroalbuminuria were lower than those with normoalbuminuria and microalbuminuria.

Finally, it can be concluded that both MMP-3 and PAI-1 may have a role in the pathogenesis of DN and its progressions towards macroalbuminuria. Prospective in vivo and in vitro studies will be necessary to explore the exact mechanisms of MMP-3 and PAI-1 in the pathogenesis of DN.

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CONFLICT OF INTEREST

None declared.

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