Research Article

Plasma and urinary matrix metalloproteinase-9 as a marker for detection of nephropathy in type 2 diabetic patients

K Balu Mahendran[1], S Sethupathy[1], KK Perumal[2], R Inmozhi[1], K Santha[1]

[1]Department of Biochemistry, Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalainagar, Tamil Nadu, India.
[2]Department of Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalainagar, Tamil Nadu, India.
Correspondence to: K Santha, E-mail: santhakarunanithi@gmail.com

Received April 9, 2015. Accepted April 28, 2015

Abstract

Background: Diabetic nephropathy (DN) is a serious complication in diabetes and now it has become the most common cause of end-stage renal disease (ESRD). Development of diabetic nephropathy is characterized by a thickening of the glomerular basement membrane and expansion of extracellular matrix proteins in the mesangial and tubulointerstitial areas, followed ultimately by progression to glomerular sclerosis and tubulointerstitial atrophy and fibrosis associated with renal dysfunction. Several growth factors, signaling pathways, along with hyperglycemia affect ECM synthesis and turnover in DN.

Objective: The aim of this study was to evaluate the significance of plasma and urinary matrix metalloproteinase-9 (MMP-9) in type 2 diabetic patients with microalbuminuria.

Materials and Methods: Fifty type 2 diabetic patients with more than 5 year diabetic duration in the age group of 35–60 years were selected for this study and 25 age-matched healthy individuals were selected as a control group. Plasma and urinary levels of MMP-9 were assessed by ELISA method and microalbumin by turbilatex method.

Results: The plasma and urinary MMP-9 levels are significantly elevated in type 2 DM with microalbuminuria compared to normoalbuminuric type 2DM and also there is significant elevation observed in normoalbuminuric type 2 DM compared to controls.

Conclusion: Plasma and urinary MMP-9 might be useful to detect early stages of nephropathy in T2DM patients. Hence, measurement of plasma and urinary MMP-9 could be useful diagnostic markers for the assessment of renal changes in type 2 diabetic patients even before the appearance of microalbuminuria.

KEY WORDS: Diabetic nephropathy, matrix metalloproteinase-9 (MMP-9), microalbuminuria

Introduction

Diabetic nephropathy (DN) is a serious complication of diabetes mellitus, and can eventually progress to end-stage renal disease. There are several distinct phases of development of DN. Functional changes occur in the nephron at the level of the glomerulus, including glomerular hyperfiltration and hyperperfusion, before the onset of any measurable clinical changes.[1] Subsequently, development of diabetic nephropathy mesangial expansion and changes in the matrix of glomerular and tubular basement membranes take place.[2,3] Hyperglycemia is associated with an increase in mesangial cell proliferation and hypertrophy, as well as increased matrix production and basement membrane thickening.[4,5] Mesangial cells are crucial for maintenance of glomerular capillary structure and for the modulation of glomerular filtration via smooth-muscle activity. In vitro studies have demonstrated that hyperglycemia is associated with increased mesangial cell matrix production and mesangial cell apoptosis.[6,7] The extracellular matrix (ECM) in the basement membrane of the kidney glomeruli is of particular importance for the filtration properties. Structural changes in mesangial and basement matrix are related to proteinuria and thus

Access this article online	
Website: http://www.ijmsph.com	Quick Response Code:
DOI: 10.5455/ijmsph.2015.09042015292	

the progression of clinical diabetic nephropathy and kidney failure.[8]

The matrix metalloproteinases or matrixins (MMPs) are members of the large metzincin superfamily such as the astacins, serralysins, reprolysins, and adamalysins or disintegrin metalloproteinases (ADAMs). In the classical view, MMPs are collectively capable of degrading all components of the ECM and basement membrane, restricting their functions to tissue remodeling and maintenance; they are now recognized as being responsible for mediating crucial functions in a variety of processes, particularly related to immunity and repair, such as cell migration, leukocyte activation, antimicrobial defense, and chemokine processing.[9–11] MMP-9 (Gelatinase B) is released by neutrophils, monocytes, macrophages, and eosinophils in the course of inflammatory response. MMP-9 is also involved in cytokine cleavage, growth factor mobilization, and tissue remodeling.[12] MMPs have been shown to be increased in several diseases. MMP-2 (Gelatinase A) and MMP-9 (Gelatinase B) are the most important MMPs in normal kidneys and basement membrane homeostasis.[13,14] So the objective of this study was to evaluate plasma and urinary MMP-9 levels in type 2 diabetic patients with normoalbuminuria and microalbuminuria and find out association with albumin-to-creatinine ratio (ACR).

Materials and Methods

A total of 50 type 2 diabetic patients of both sexes with more than 5 year diabetic duration, aged between 35 and 60 years on oral hypoglycemic drugs, attending diabetic out-patient department of Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalainagar, Tamil Nadu, India, were selected for our study. The included diabetic patients were categorized into two groups based on ACR. Groups were divided as follows: 25 patients with normoalbuminuria (<30 mg/g creatinine), 25 patients with microalbuminuria (30–299 mg/g creatinine). We excluded the patients based on the following criteria: patients on insulin, smokers, alcoholics, tobacco chewers, abnormal urinary sediment, urinary tract infection, history of other renal disease and active or chronic persistent infection or inflammatory disorders, neoplastic disorders, uncontrolled thyroid disorders, severe liver dysfunction, history of acute myocardial infarction, stroke, and occlusive peripheral vascular disease. Twenty-five healthy individual age, sex-matched subjects were selected as control. The informed consent was obtained from all the study subjects and the study was approved by the Institutional Human Ethics Committee (IHEC). Experiments were done in accordance with Helsinki declaration of 1975.

Biochemical Analysis

A fasting blood and urine samples were obtained from the subjects immediately after enrollment. Blood samples were centrifuged at 2000×g for 10 min. Samples were analyzed for routine investigations blood sugar, lipid profile (total cholesterol, HDL, triglycerides), glycosylated hemoglobin (HbA1C) and urine microalbumin, urinary creatinine. Plasma and urinary MMP-9, insulin assessed by ELISA, and the 2 h post-prandial venous plasma glucose (PPBS) estimation was also done.

Statistical analysis

Statistical analysis were carried out with SPSS 20.0. Values were expressed as mean ± standard deviation, and *p*-value < 0.05 was considered statistically significant. Normally distributed data were analyzed by using one-way ANOVA. The Pearson's correlation test was used for correlation analysis.

Results

The plasma and urinary MMP-9 levels are significantly elevated in type 2 DM with microalbuminuria compared to normoalbuminuric type 2DM, and also there is significant elevation observed in normoalbuminuric type 2 DM compared to controls [Tables 1–4].

Table 1: Baseline and biochemical parameters in control and type 2 diabetic subjects

Parameters	Control (*n* = 25)	Normoalbuminuria (*n* = 25)	Microalbuminuria (*n* = 25)
Age	47.6 ± 4.3	48.3 ± 6.5	50.8 ± 5.5b*
Body mass index	25.4 ± 1.5	26.8 ± 3.7	25.8 ± 3.2
Waist/hip ratio	0.90 ± 0.04	0.92 ± 0.06	0.92 ± 0.04
DM duration (years)	—	8.2 ± 2.1	8.9 ± 2.8
Systolic BP (mm Hg)	114.1 ± 7.1	124.5 ± 16.2a*	127 ± 13.1 b**
Diastolic (mm Hg)	73.8 ± 3.3	79.1 ± 7.9a*	78.7 ± 7.6b*
Urine albumin-to-creatinine ratio (mg/gm of creatinine)	18.3 ± 2.6	23.4 ± 3.5a**	161.8 ± 70.7b,c**
Serum urea (mg/dl)	24.3 ± 4.6	28.1 ± 5.4a*	33.4 ± 12.2b**
Serum creatinine (mg/dl)	0.6 ± 0.1	0.7 ± 0.2	0.9 ± 0.3b**

Data are expressed as mean ± SD; **$p < 0.001$; *$p < 0.05$ was considered statistically significant.

[a]Comparison between normal and type 2 diabetic patients with normoalbuminuria.

[b]Comparison between normal and type 2 diabetic patients with microalbuminuria.

[c]Comparison between type 2 diabetic patients with normoalbuminuria and microalbuminuria.

Table 2: FBS, PPBS, HbA1C, insulin, lipid profile and matrix metalloproteinase-9 (MMP-9) levels in control and type 2 diabetic subjects

Parameters	Control ($n = 25$)	Normoalbuminuria ($n = 25$)	Microalbuminuria ($n = 25$)
FBS (mg/dl)	81.9 ± 5.9	128.3 ± 40.1a**	145.9 ± 53.6b**
PPBS (mg/dl)	108.1 ± 9.8	191.7 ± 56a**	221 ± 82.1b**
HbA1C	5.4 ± 0.4	7.2 ± 0.8a**	8.0 ± 1.1b**,c*
Serum cholesterol (mg/dl)	168.8 ± 9.0	186.8 ± 20.4a**	193.8 ± 21.8b**
Serum triglycerides (mg/dl)	95.5 ± 7.4	130.5 ± 39.3a**	141.8 ± 38.1b**
HDL cholesterol (mg/dl)	43.7 ± 2.4	39.4 ± 3.0a**	38.3 ± 2.3b**
LDL cholesterol (mg/dl)	105.9 ± 9.1	121.3 ± 16.5a**	127.1 ± 20.8b**
Insulin (μIU/mL)	6.5 ± 0.7	10.9 ± 4.1a**	13.2 ± 5.0b**
HOMA-IR	1.3 ± 0.17	3.4 ± 1.6a**	4.6 ± 2.2b**,c*
Plasma MMP-9 (ng/ml)	5.8 ± 1.2	10.4 ± 2.0a**	14.0 ± 3.2b**,c**
Urine MMP-9 (ng/mg of creatinine)	2.6 ± 0.6	6.1 ± 2.1a**	11.6 ± 6.7b**,c**

Data are expressed as mean ± SD; **$p < 0.001$; *$p < 0.05$ was considered statistically significant.

[a]Comparison between normal and type 2 diabetic patients with normoalbuminuria.

[b]Comparison between normal and type 2 diabetic patients with microalbuminuria.

[c]Comparison between type 2 diabetic patients with normoalbuminuria and microalbuminuria.

Table 3: Correlation between plasma MMP-9 and measured parameters

Parameters	Correlation coefficient (r)
Albumin-to-creatinine ratio	0.706**
Urine MMP-9	0.758**
FBS	0.428**
PPBS	0.447**
HbA1C	0.654**
HOMA-IR	0.503**
Cholesterol	0.434**
TGL	0.496**
HDL	–0.597**
LDL	0.402**

**Correlation is significant at the 0.01 level (two-tailed).

Table 4: Correlation between urinary MMP-9 and measured parameters

Parameters	Correlation coefficient (r)
Albumin-to-creatinine ratio	0.755**
FBS	0.216
PPBS	0.227
HbA1C	0.488**
HOMA-IR	0.280*
Cholesterol	0.387**
TGL	0.374**
HDL	–0.456**
LDL	0.372

*Correlation is significant at the 0.05 level (two-tailed).

**Correlation is significant at the 0.01 level (two-tailed).

Discussion

Diabetic nephropathy is characterized by excessive deposition of ECM proteins in the mesangium and basement membrane of the glomerulus and in renal tubulointerstitium. The basement membrane changes, accompanied by glomerular hyper filtration, and increased glomerular hydrostatic pressure leading to microalbuminuria.[15,16] However, the mesangial changes appear to be the main cause of declining renal function in DN. Declining glomerular function correlates well with the extent of these changes in both types of diabetes.[17,18] The major physiologic regulators of ECM degradation in the glomerulus are matrix metalloproteinases (MMPs).[19,20]

In this study, we observed that plasma and urinary levels of MMP-9 were significantly increased in diabetic patients with normoalbuminuria compared to control subjects, and significantly increased in microalbuminuria patients compared to normoalbuminuria type 2 diabetic patients. It has been suggested that in type2 diabetic patients, the development of microalbuminuria was preceded by a significant increase in plasma MMP-9 concentration.[21–23] Hao and Yu,[24] Uemura et al.[25] have found that hyperglycemia induces activity and expression of MMP-2 and -9 in rat aortic smooth muscle cells and mouse vascular tissue and plasma. Chronic incubation with high glucose increased MMP-9 promoter activity, mRNA and protein expression, and gelatinase activity in bovine aortic endothelial cells, and also MMP-9 known to be activated in a cellular environment under oxidant stress and with reduced NO bioavailability.[26] Endothelial NOS (eNOS) dysfunction, coupled with activation of NAD(P)H-dependent oxidase, is largely responsible for enhanced superoxide production in human diabetic vascular tissue.[27] Therefore,

oxidative stress and limited NO bioavailability could be a cause elevation of MMP-9 in type2 DM.

In addition, we observed that plasma and urinary MMP-9 levels showed strong positive correlation with ACR, HbA1C, and HOMA-IR. Chronic hyperglycemia produces reactive oxygen species (ROS), protein glycation reactions that lead to the formation of advanced glycation end products (AGEs).[28] MMP-9 is induced or repressed by a variety of soluble factors such as cytokines and growth factors. Endogenous tissue inhibitors of MMPs (TIMPs) regulate their activation, and TIMP-1 shows greater preference for MMP-9 than any other.[29,30] High glucose as well as glycated albumin and AGE induce Transforming growth factor-β (TGF-β) over expression in mesangial cells in culture[31–33] and is a key regulator of ECM synthesis in renal cells. Three mammalian TGF-β isoforms, TGF-β1, -β2, and -β3, are recognized, of which TGF-β1 is the most potent promoter of ECM accumulation. Expression of TGF-β isoforms occurs in different patterns in renal fibrosis.[34,35] Several studies have implicated TGF-β1 in renal fibrosis because this cytokine both stimulates ECM deposition and inhibits matrix degradation.[36] The TGF-β1 gene product is translated in a latent form that may be activated by MMP-9 mediated proteolytic cleavage when MMP-9 interacts with CD44, a receptor for the extracellular glycosaminoglycan hyaluronan (HA), at the cell surface.[37,38] Studies have shown that increased monocyte activation and differentiation activated macrophages that can further induce inflammatory cytokines, resulting in increased activation and expression of matrix metalloproteinase (MMPs), particularly MMP-9.[39–41] A balance between ECM synthesis and degradation is required for maintaining the structural and functional integrity of the glomerulus. Any changes in MMP expression or activity will directly alter the ECM turnover, which may lead to glomerular scarring and a decline in renal function.[42,43] Therefore, evaluation of plasma and urinary MMP-9 has been regarded as an index of progressive renal damage in diabetic patients.

Conclusion

In conclusion, plasma and urinary MMP-9 might be useful to detect early stages of nephropathy in T2DM patients. Hence, measurement of plasma and urinary MMP-9 could be useful diagnostic markers for the assessment of renal changes in type 2 diabetic patients even before the appearance of microalbuminuria. Further large-scale studies are needed to confirm it.

References

1. Dronavalli S, Duka I, Bakris GL. The pathogenesis of diabetic nephropathy. Nat Rev Endocrinol 2008;4:444–52.
2. Bangstad HJ, Osterby R, Rudberg S, Hartmann A, Brabrand K, Hanssen KF: Kidney function and glomerulopathy over 8 years in young patients with Type I (insulin-dependent) diabetes mellitus and microalbuminuria. Diabetologia 2002;45(2):253–61.
3. Osterby R, Hartmann A, Bangstad HJ: Structural changes in renal arterioles in Type I diabetic patients. Diabetologia 2002;45(4):542–9.
4. Harris RD, Steffes MW, Bilous RW, Sutherland DE, Mauer SM. Global glomerular sclerosis and glomerular arteriolar hyalinosis in insulin dependent diabetes. Kidney Int 1991;40:107–14.
5. Heilig CW, Concepcion LA, Riser BL, Freytag SO, Zhu M, Cortes P. Overexpression of glucose transporters in rat mesangial cells cultured in a normal glucose milieu mimics the diabetic phenotype. J Clin Invest 1995;96:1802–14.
6. Mishra R, Emancipator SN, Kern T, Simonson MS. High glucose evokes an intrinsic proapoptotic signaling pathway in mesangial cells. Kidney Int 2005;67:82–93.
7. Lin CL, Wang JY, Huang YT, Kuo YH, Surendran K, Wang FS. Wnt/beta-catenin signaling modulates survival of high glucose-stressed mesangial cells. J Am Soc Nephrol 2006;17:2812–20.
8. Svennevig K, Kolset SO, Bangstad HJ: Increased syndecan-1 in serum is related to early nephropathy in type I diabetes mellitus patients. Diabetologia 2006;49(9):2214–6.
9. Manicone AM, McGuire JK. Matrix metalloproteinases as modulators of inflammation. Semin. Cell Dev Biol 2008;19:34–41.
10. Parks WC, Wilson CL, López-Boado YS. Matrix metalloproteinases as modulators of inflammation and innate immunity. Nat Rev Immunol 2004;4:617–629.
11. Gill SE, Parks WC, Metalloproteinases and their inhibitors: regulators of wound healing. Int J Biochem Cell Biol 2008;40:1334–47.
12. Lelongt B, Legallicier B, Piedagnel R, Ronco PM. Do matrix metalloproteinases MMP-2 and MMP-9 (gelatinases) play a role in renal development, physiology and glomerular diseases? Curr Opin Nephrol Hyperten 2001;10:7–12.
13. Lenz O, Elliot SJ, Stetler-Stevenson WG: Matrix metalloproteinases in renal development and disease. J Am Soc Nephrol 2000;11(3):574–81.
14. Osterby R. Glomerular structural changes in type 1 (insulin-dependent) diabetes mellitus: Causes, consequences and prevention. Diabetologia. 1992; 35: 803-812.
15. Osterby R, Gall MA, Schmitz A, Nielsen FS, Nyberg G, Parving HH: Glomerular structure and function in proteinuric type 2 (non-insulin-dependent) diabetic patients. Diabetologia 1993;36:1064–70.
16. Steffes MW, Osterby R, Chavers B, Mauer SM: Mesangial expansion as a central mechanism for loss of kidney function in diabetic patients. Diabetes 1989;38:1077–81.
17. Osterby R, Tapia J, Nyberg G, Tencer J, Willner J, Rippe B, Torffvit O. Renal structures in type 2 diabetic patients with elevated albumin excretion rate. APMIS 2001;109:751–61.
18. Osterby R, Hartmann A, Nyengaard JR, Bangstad HJ. Development of renal structural lesions in type 1 diabetic patients with microalbuminuria. Observations by light microscopy in 8-year follow-up biopsies. Virchows Arch 2002;440:94–101.
19. Woessner JF Jr. Matrix metalloproteinases and their inhibitors in connective tissue remodeling. FASEB J 1991;5:2145–54.
20. Lenz O, Elliot SJ, Stetler-Stevenson WG. Matrix metalloproteinases in renal development and disease. JASN 2000;11(3):574–81.
21. Ebihara I, Nakamura T, Shimada N, Koide H. Increased plasma metalloproteinase-9 concentrations precede development of microalbuminuria in non-insulin-dependent diabetes mellitus. Am J Kidney Dis 1998;32:544–50.
22. Vande Wal RM, van der Harst P, Gerritsen WB, van der Horst F, Plokker TH, Gansevoort RT, van Gilst WH, Voors AA.

Plasma matrix metalloproteinase-9 and ACE-inhibitor-induced improvement of urinary albumin excretion in non-diabetic, microalbuminuric subjects. J Renin Angiotensin Aldosterone Syst 2007;8(4):177–80.
23. Naidele S, Shetty S, Rao AV. To study serum Mmp-9 levels in early diabetic nephropathy. IJPSI 2014;3(2):46–50.
24. Hao F, Yu JD. High glucose enhance expression of matrix metalloproteinase-2 in smooth muscle cells. Acta Pharmacol Sin 2003;24:534–8.
25. Uemura S, Matsushita H, Li W, Glassford AJ, Asagami T, Lee KH, Harrison DG, Tsao PS. Diabetes mellitus enhances vascular matrix metalloproteinase activity: role of oxidative stress. Circ Res 2001;88:1291–8.
26. Upchurch GR Jr, Ford JW, Weiss SJ, Knipp BS, Peterson DA, Thompson RW, Eagleton MJ, Broady AJ, Proctor MC, Stanley JC. Nitric oxide inhibition increases matrix metalloproteinase-9 expression by rat aortic smooth muscle cells in vitro. J Vasc Surg 2001; 34:76–83.
27. Guzik TJ, Mussa S, Gastaldi D, Sadowski J, Ratnatunga C, Pillai R, Channon KM. Mechanisms of increased vascular superoxide production in human diabetes mellitus: role of NAD(P)H oxidase and endothelial nitric oxide synthase. Circulation 2002;105:1656–62.
28. Ryan A, Murphy M, Godson C, Hickey FB. Diabetes mellitus and apoptosis: inflammatory cells. Apoptosis 2009;14:1435–50.
29. Van den Steen PE, Dubois B, Nelissen I, Rudd PM, Dwek RA, Opdenakker G. Biochemistry and molecular biology of gelatinase B or matrix metalloproteinase-9 (MMP-9). Crit Rev Biochem Mol Biol 2002;37:375–536.
30. Brew K, Dinakarpandian D, Nagase H. Tissue inhibitors of metalloproteinases: evolution, structure and function. Biochim Biophys Acta 2000;1477:267–83.
31. Ziyadeh FN, Han DC, Cohen JA, Guo J, Cohen MP. Glycated albumin stimulates fibronectin gene expression in glomerular mesangial cells: involvement of the transforming growth factor-beta system. Kidney Int 1998;53:631–8.
32. Kim YS, Kim BC, Song CY, Hong HK, Moon KC, Lee HS. Advanced glycosylation end products stimulate collagen mRNA synthesis in mesangial cells mediated by protein kinase C and transforming growth factor-beta. J Lab Clin Med 2001; 138:59–68.
33. Lee HS, Moon KC, Song CY, Kim BC, Wang S, Hong HK. Glycated albumin activates PAI-1 transcription through Smad DNA binding sites in mesangial cells. Am J Physiol Renal Physiol 2004;287:F665–72.
34. Shankland SJ, Pippin J, Pichler RH, Gordon KL, Friedman S, Gold LI, Johnson RJ, Couser WG. Differential expression of transforming growth factor-beta isoforms and receptors in experimental membranous nephropathy. Kidney Int 1996;50:116–24.
35. Iglesias-de la Cruz MC, Ziyadeh FN, Isono M, Kouahou M, Han DC, Kalluri R, Mundel P, Chen S. Effects of high glucose and TGF-beta1 on the expression of collagen IV and vascular endothelial growth factor in mouse podocytes. Kidney Int 2002;62:901–13.
36. Ziyadeh FN. Mediators of diabetic renal disease: the case for TGF- β as the major mediator. J Am Soc Nephrol 2004;15:S55–7.
37. Keski-Oja J, Koli K, von Melchner H. TGF- β activation by traction? Trends Cell Biol 2004;14:657–9.
38. Yu Q, Stamenkovic I. Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis. Genes Dev 2000;14:163–76.
39. Chana RS, Martin J, Rahman EU, Wheeler DC. Monocyte adhesion to mesangial matrix modulates cytokine and metalloproteinase production. Kidney Int 2003;63:889–98.
40. Vos C, Gartner S, Ransohoff R, McArthur J, Wahl L, Sjulson L, Hunter E, Conant K. Matrix metalloprotease-9 release from monocytes increases as a function of differentiation: implications for neuroinflammation and neurodegeneration. J Neuroimmunol 2000;109:221–7.
41. Min D, Lyons JG, Bonner J, Twigg SM, Yue DK, McLennan SV. Mesangial cell-derived factors alter monocyte activation and function through inflammatory pathways: possible pathogenic role in diabetic nephropathy. Am J Physiol Renal Physiol 2009;297:F1229–37.
42. Turck J, Pollock AS, Lovett DH: Gelatinase A is a glomerular mesangial cell growth and differentiation factor. Kidney Int 1997;51:1397–400.
43. Giannelli G, Falk-Marzillier J, Schiraldi O, Stetler-Stevenson WG, Quaranta V. Induction of cell migration by matrix metalloprotease-2 cleavage of laminin-5. Science 1997;277:225–8.

How to cite this article: Mahendran KB, Sethupathy S, Perumal KK, Inmozhi R, Santha K. Plasma and urinary matrix metalloproteinase-9 as a marker for detection of nephropathy in type 2 diabetic patients. Int J Med Sci Public Health 2015;4:1409-1413

Source of Support: Nil, **Conflict of Interest:** None declared.