Plasma and Urinary Type IV Collagen Levels for Early Detection of Nephropathy in Type 2 Diabetes Mellitus Patients

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Abstract

Background: Diabetic nephropathy is a major complication with high morbidity and mortality, and leads to end stage renal disease (ESRD). Type IV collagen is the main component of the glomerular basement membrane (GBM) and the extracellular matrix. The thickening of the GBM is due to accumulation of type IV collagen and alterations in its structure and composition.

Aim: The aim of this study was to evaluate the association of plasma and urine type IV collagen with albuminuria status and to determine the clinical implications of type IV collagen as a marker in the early stage of diabetic nephropathy.

Materials and methods: A total of 150 type 2 diabetes mellitus patients with more than 5 year diabetic duration in the age group of 35 to 60 years were selected for this study and 50 age and sex matched healthy individuals were selected as control group. Type IV collagen (Plasma and urine), Insulin were analyzed by ELISA method and micro albumin was analyzed by turbilatex method. Routine investigations fasting plasma glucose, post prandial glucose, lipid profile parameters, serum urea and creatinine were analyzed by using Auto analyzer.

Results: The plasma and urinary type IV collagen levels were significantly higher in the normoalbuminuric group with diabetes than in the control group, and increased with increasing severity of albuminuria among diabetics. Both plasma and urine type IV collagen levels showed positive correlation with albumin creatinine ratio (ACR) and regression analysis showed significant influence with ACR and also positive significant correlation of ACR with FPG, PPG, HbA1C ,HOMA-IR , negative correlation with HDL cholesterol was observed.

Conclusion: Plasma and urinary type IV collagen can be helpful in the prediction of the subsequent development of albuminuria in type 2 diabetic patients.

Key words: Diabetic nephropathy, Microalbuminuria, Type IV collagen.

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Introduction

Diabetic nephropathy (DN), a microvascular complication occurring in approximately 20-40% of patients with type 2 diabetes mellitus (T2DM), is characterized by the progressive impairment of glomerular filtration and the development of Kimmelstiel-Wilson lesions leading to end-stage renal disease (ESRD). [1] Microalbuminuria is usually viewed as the earliest putative diagnostic sign of renal damage in type 2 diabetic patients. [2] Indeed, microalbuminuria grossly correlates with the complex histopathological picture of glomerular and tubular damage, thus probably representing a nonspecific indicator of ongoing renal injury. [3] Moreover, patients with type 2 diabetes can progress to a significant degree of renal impairment even if they remain normoalbuminuric, and this occurrence may reflect renal parenchymal diseases other than classic diabetic glomerulosclerosis. [4-6] Consequently, it is necessary to develop more sensitive markers for detecting the early stage of nephropathy in diabetic patients. Type IV collagen, the major collagenous component of glomerular basement membrane (GBM) and mesangial matrix, exists as a triple helix of α (IV) and α (IV) chains with a noncollagenous (NC) globular domain at its carboxyl terminus [7] and measurement of the concentration of this extracellular matrix protein in biologic fluids might be useful indicator of early diabetic nephropathy. [8-11] So, the aim of this study was to evaluate the association of plasma and urine type IV collagen with albuminuria and to determine the clinical implications of type IV collagen as a marker in the early stage of diabetic nephropathy.

Materials and Methods

The study groups comprised of 150 type 2 diabetic patients of both sexes with more than 5 years duration aged between 35-60 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 the present study. The exclusion criteria of this study are patients on insulin, hypertension, 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, thyroid disorders, liver dysfunction, history of acute myocardial infarction, stroke, and occlusive peripheral vascular disease. The included diabetic patients were categorized into three groups based on albumin creatinine ratio (ACR). The groups were divided as follows: 50 patients with normoalbuminuria (<30 mg/g creatinine), 50 patients with microalbuminuria (30–299 mg/g creatinine), and 50 patients with macroalbuminuria (≥300 mg/g creatinine). Fifty healthy age and sex matched subjects were selected as controls. The informed consent was obtained from all the 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 random spot urine and fasting blood samples were obtained from the subjects immediately after enrolment. Blood samples were centrifuged at 3000X g for 10 min. Samples were analyzed for fasting plasma glucose (FPG-GOD-POD method), lipid profile (Total Cholesterol- CHOD/ POD method, HDL- Direct Enzymatic colorimetric method, Triglycerides- GPO-POD method), by using Auto analyzer. HbA1C was estimated by Ion exchange resin method, type IV collagen (Sincere Biotech; Ltd, Beijing, China) and insulin were assessed by ELISA method. Urine samples were analyzed for microalbumin, creatinine by using auto analyzer. Post prandial blood sample was collected for plasma glucose (PPG) analysis.

Statistical analysis:

Statistical analysis were carried out with SPSS 20.0. Values were expressed as mean ± standard deviation, p value < 0.05 was considered statistically significant. Normally distributed data were analyzed by using one-way ANOVA. The Pearson correlation test was used for correlation analysis and multiple regression analysis used to identify the variables which influence the level of ACR.

Results

Table 1: Baseline data of control and type 2 diabetic patients differentiated according to albuminuria status

Parameters	Controls (n=50)	Normoalbuminuria T 2DM (n=50)	Microalbuminuria T 2 DM (n=50)	Macro albuminuria T2 DM (n=50)
Age (years)	47.1±3.9	47.7±6.3	48.8±4.2	48.4±4.2
Body mass index (BMI- Kg/M^2)	24.5±1.8	27.5±3.2 [a*]	26.9±3.3 [a*]	26.8±2.4[a*]
Waist/Hip ratio	0.91±0.04	0.90±0.05	0.91±0.03	0.91±0.04
Systolic BP (mmHg)	114.2±6.6	122.8±14.8[a*]	126.8±11.5 [a*]	131.2±9.0 [a*,b*,c#]
Diastolic BP (mm Hg)	74.2±3.4	78.6±7.8 [a*]	78.5±6.1 [a*]	79.8±6.5 [a*]
Duration DM (years)	-	8.1±2.0	9.2±3.0	9.9±2.3 [b#]

Data are expressed as mean ±SD, P<0.05 was considered statistically significant.
a - Controls versus Normoalbuminuria T 2DM, Microalbuminuria T 2 DM, Macro albuminuria T2 DM.
b -Normoalbuminuria T 2DM versus Microalbuminuria T 2 DM, Macro albuminuria T2 DM.
c- Microalbuminuria T 2 DM versus Macro albuminuria T2 DM.
*   p value <0.001, # p value <0.05

Table 2: Clinical parameters of control and type 2 diabetic patients differentiated according to albuminuria status

Parameters	Control (n=50)	Normoalbuminuria T2DM (n=50)	Microalbuminuria T2DM (n=50)	Macroalbuminuria T2DM (n=50)
Urine Albumin Creatinine ratio (mg/gm. of creatinine)	18.5±2.7	23.0±3.2[a*]	129.3±40.4 [a*,b*]	395.3±54.7 [a*,b*,c*]
FPG(mg/dl)	82.1±5.4	121.6±21.9 [a*]	135.9±33.0 [a*]	240.8±28.1 [a*,b*,c*]
PPG(mg/dl)	106.9±8.4	170.1±0.8 [a*]	206.2±41.1 [a*,b*]	318.1±29.4 [a*,b*,c*]
HbA1C (%)	5.5±0.4	7.1±0.8 [a*]	8.8±1.0 [a*,b*]	10.3±0.8 [a*,b*,c*]
Insulin (µIU/mL)	6.4±0.7	10.4±2.6 [a*]	15.0±3.0 [a*,b*]	18.9±2.2 [a*,b*,c*]
HOMA-IR	1.29±0.17	3.1±0.9 [a*]	5.0±1.5 [a*,b*]	11.2±1.8 [a*,b*,c*]
Serum cholesterol (mg/dl)	169.7±8.7	185.0±17.4 [a*]	192.6±21.6 [a*,b#]	208.2±23.2 [a*,b*,c*]
Serum Triglycerides (mg/dl)	96.1±7.0	130.3±31.3 [a*]	139.4±36.0 [a*]	164.0±21.4 [a*,b*,c*]
HDL cholesterol (mg/dl)	44.3±2.3	39.0±2.8 [a*]	38.5±2.5 [a*]	37.9±2.6 [a*]
LDL cholesterol (mg/dl)	106.2±8.9	119.9±14.5 [a*]	126.2±20.5 [a*]	137.4±21.6 [a*,b*]
Serum urea (mg/dl)	24.9±3.9	27.9±4.0 [a*]	31.6±4.7 [a*,b*]	32.0±2.6 [a*,b*]

Serum creatinine (mg/dl)	0.7±0.18	0.80±0.27	1.0±0.3 [a*,b*]	1.2±0.3 [a*,b*,c*]
Plasma type IV collagen (ng/ml)	3.8±0.6	7.6±2.4 [a*]	10.5±2.7 [a*,b*]	20.4±4.0 [a*,b*,c*]
Urine type IV collagen (ng/mg of creatinine)	1.0±0.2	1.8±0.6 [a*]	4.2±2.2 [a*,b*]	11.2±4.0 [a*,b*,c*]

Data are expressed as mean ±SD, P<0.05 was considered statistically significant.
a - Controls versus Normoalbuminuria T 2DM, Microalbuminuria T 2 DM, Macro albuminuria T2 DM.
b -Normoalbuminuria T 2DM versus Microalbuminuria T 2 DM, Macro albuminuria T2 DM.
c- Microalbuminuria T 2 DM versus Macro albuminuria T2 DM.
*   p value <0.001, # p value <0.05

Table 3. Multiple regression analysis between ACR and type IV collagen in Micro albuminuria T2DM group

Model	Unstandardized Coefficients		Standardized Coefficients	t value	p value
	B	Std. Error	Beta		
(Constant)	38.025	18.800		2.023	0.049
Plasma Type IV collagen ng/ml	8.659	1.726	.586	5.016	0.000
Urine Type IV collagen ng/mg cr	14.472	1.611	.792	8.984	0.000
a. Dependent Variable: ACR					

Table 4.Multiple regression analysis between ACR and type IV collagen in Macro albuminuria T2DM group

Model	Unstandardized Coefficients		Standardized Coefficients	t value	p value
	B	Std. Error	Beta		
(Constant)	148.201	19.230		7.707	0.000
Plasma Type IV collagen ng/ml	12.070	.922	.884	13.093	0.000
Urine Type IV collagen ng/mg cr	10.730	1.19a1	.793	9.007	0.000
a. Dependent Variable: ACR					

Table 5: Correlation between ACR &measured parameters in type 2 diabetic patients

Parameters	Correlation Coefficient (r)
FPG	0.863**
PPG	0.869**
HbA1C	0.821**
HOMA-IR	0.912**
Cholesterol	0.448**
TGL	0.472**
HDL	-0.191*
LDL	0.375**
Plasma type IV collagen	0.927**
Urinary type IV collagen	0.911**

*Correlation is significant at the 0.05 level (2-tailed).

**Correlation is significant at the 0.01 level (2-tailed).

Base line characteristics of diabetic patients and the control subjects are summarized in Table 1. Body mass index and systolic blood pressure was significantly elevated in diabetic patients when compared to healthy controls.

Clinical parameters of diabetic patients and control subjects are summarized in Table 2. Plasma and urinary type IV collagen levels were significantly higher in normoalbuminuric patients with diabetes than those in control subjects. Furthermore, their levels were increased with increasing categories of albuminuria in patients with diabetic groups (p< 0.001).

Table 3 and 4 shows multiple regression analysis of plasma and urinary type IV collagen levels with ACR in microalbuminuric and macroalbuminuric diabetic patients. Plasma and urinary type IV collagen levels were significantly associated with ACR.

Table 5 shows positive and significant correlation of ACR with FPG, PPG, HbA1C, HOMA-IR, type IV collagen (plasma &urine) and negative correlation with HDL cholesterol.

Discussion

Diabetic nephropathy is a serious complication of diabetes mellitus, and can eventually progress to end-stage renal disease. Although established therapeutic strategies, such as appropriate blood glucose control, blood pressure control with renin–angiotensin system blockade, and lipid lowering with statins, are used to treat diabetes, the contribution of diabetic end-stage renal disease to the total number of cases requiring hemodialysis has increased tremendously in the past two decades. [12] Type IV collagen is the main constituent of both glomerular and tubular basement membranes as well as mesangial matrix [13-15] and a small amount of type IV collagen is excreted into the urine in healthy subjects, although its main origin is not clear. [16] Moreover, urinary type IV collagen excretion is less susceptible to physiological changes than urinary albumin. [17]

In the present study, we observed that plasma and urinary type IV collagen levels were significantly increased in type 2 diabetic patients compared with healthy controls and there was also significant difference observed in macro and microalbuminuric diabetic patients compared with normoalbuminuric diabetic patients. High glucose stimulates type IV collagen production by activating the cellular TGF-β system. [18] Specifically, high glucose increases the secretion of endogenous TGF-β1 that then acts upon the cell in autocrine fashion to stimulate the expression of collagen IV and other extracellular matrix proteins. [19] Hyperglycemia activates various inflammatory pathways both directly and via gene transcription factors which induce oxidative stress, TGF-β, renin-angiotensin system (RAS), monocyte chemo attractant protein - 1 (MCP). This leads to podocyte injury, malfunction, apoptosis and deposition of proteins in extracellular matrix of the nephron with albumin leak. [20, 21]

The present study also confirmed that ACR is positively correlated with FPG, PPG, HbA1C and HOMA-IR, plasma, urinary type IV collagen, and also negative correlation was

observed with HDL cholesterol. In particular, longstanding diabetes with chronic hyperglycemia causes quantitative and qualitative changes in basement membrane structure of retinal and renal capillaries. [22, 23] Immunohistochemical studies of diabetic kidneys with diffuse glomerulosclerosis showed increased type IV collagen deposition in the mesangial matrix and decreased heparan sulfate proteoglycan content in the mesangial matrix and glomerular basement membrane. [24,25] In nodular glomerulosclerosis normal basement membrane components are decreased or absent while the occurrence of collagen type III in this stage has been interpreted as an irreversible alteration of the glomerular structure. These changes seem to be the underlying cause for the alterations in renal function like persistent albuminuria and proteinuria. [26]

In addition the multiple regression analysis showed both plasma and urinary type IV collagen levels were significantly correlating with ACR in micro and macroalbuminuric diabetic patients. This implies that plasma and urinary type IV collagen could be useful as an early diagnostic marker for detection of nephropathy even before the onset of microalbuminuria. Hence estimation of plasma and urinary type IV collagen can be helpful in the prediction of the subsequent development of albuminuria in diabetic patients.

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