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Association of Urinary and Plasma Type IV Collagen Levels with Albumin Creatinine Ratio in Type 2 Diabetic Patients

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Abstract:

Background: *Diabetic nephropathy (DN) is a chronic disease characterized by proteinuria with loss of renal function. Chronic hyperglycemia has generally considered as the key initiator of nephropathy due to dysregulation of metabolic pathways. Currently microalbuminuria is a marker for the diagnosis of DN. Consequently, it is necessary to develop more sensitive markers for detecting the early stage of nephropathy in diabetic patients.*

Aim: *The aim of this study was to assess the significance of plasma type IV collagen (p IV) and urinary type IV collagen (u IV) 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 to 60 years were selected for this study and 20 age matched healthy individuals were selected as a control group. Plasma and urinary levels of type IV collagen were assessed by ELISA method and microalbumin by turbilatex method.*

Results: *The p IV and u IV levels were significantly higher in the normoalbuminuric group with diabetes than in the control group, and significantly increased microalbuminuria patients compared to patients without microalbuminuria. The p IV and u IV levels were positively correlated with the albumin creatinine ratio (ACR), glycated hemoglobin (HbA1C) and insulin resistance.*

Conclusion: *The p IV and u IV levels were elevated in type 2 diabetic patients and its elevation in normoalbuminuric patients indicates that it could be an early marker of diabetic nephropathy. Hence u IV might be useful as a noninvasive marker for the early detection of diabetic nephropathy.*

Keywords: *Diabetic nephropathy, Type IV collagen, Insulin resistance*

I. Introduction

Diabetic nephropathy (DN) is a major complication in diabetic patients and is a leading cause of end stage renal disease. Morphologically, the most prominent feature of diabetic nephropathy is characterized by hypertrophy of both glomerular and tubular elements, thickening of the glomerular and tubular basement membranes, progressive accumulation of extracellular matrix (ECM) components in the glomerular mesangium, and tubulointerstitial fibrosis [1,2]. Currently, available best noninvasive marker for the diagnosis of DN is microalbumin [3]. However, many patients, who were normoalbuminuric and just recently had positive test for microalbuminuria, have advanced renal histopathological changes, decreased glomerular filtration rate (GFR) and progressive loss of kidney function [4- 8]. Consequently, it is necessary to develop more sensitive markers for detecting the early stage of nephropathy in diabetic patients. Since type IV collagen is the principal component of glomerular basement membrane and mesangial matrix, the plasma and urinary levels may reflect the rate of matrix turnover in diseased kidney [9]. Therefore the objective of the present study was to assess p IV and u IV collagen levels in type 2 diabetic patients with microalbuminuria and its usefulness as early marker for the detection of diabetic nephropathy.

II. Materials And Methods

The study groups comprised of 50 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. We excluded the patients based on the following criteria: 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, uncontrolled thyroid disorders, severe liver dysfunction, history of acute myocardial infarction, stroke, and

occlusive peripheral vascular disease. The included diabetic patients were categorized into two groups according to the presence or absence of microalbuminuria. Twenty healthy age, sex matched subjects were selected as a controls. 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 random spot urine and fasting blood samples were obtained from the subjects immediately after enrolment. Blood samples were centrifuged at 2000×g for 10 min. Samples were analyzed for fasting blood glucose, lipid Profile(Total Cholesterol, HDL, Triglycerides), by using Auto analyzer. HbA1C estimated by Ion exchange resin method and type IV collagen, insulin assessed by ELISA. Urine samples were analyzed for microalbumin, creatinine by using auto analyzer.

Statistical analysis:

Statistical analyses 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.

III. Results

Table 1: Baseline data of controls and type 2 diabetic patients differentiated according to microalbuminuria

Parameters	Controls (n=20)	Normoalbuminuria (n=25)	Microalbuminuria (n=25)	p value
Age	46.9±4.1	48.3±6.5	50.8±5.5	0.067
Body mass index	25.2±1.3	26.8±3.7	25.8±3.2	0.210
Waist/Hip ratio	0.90±0.04	0.92±0.06	0.92±0.04	0.338
DM duration (years)	-	8.2±2.1	8.9±2.8	0.298
Systolic BP (mm Hg)	114.4±6.9	124.5±16.2	127±13.1	0.005
Diastolic (mm Hg)	73.5±3.2	79.1±7.9	78.7±7.6	0.011

Data are expressed as mean ±SD, p<0.05 was considered statistically significant.

Table 2: Clinical data of control and type 2 diabetic patients differentiated according to level of microalbuminuria

Parameters	Control (n=20)	Normoalbuminuria (n=25)	Microalbuminuria (n=25)	Control Vs Normoalbumiuria p value	Normoalbuminuria Vs microalbuminuria p value
Urine albumin Creatinine ratio (mg/gm. of creatinine)	18.8±2.6	23.4±3.5	161.8±70.7	0.001	0.001
FBS(mg/dl)	81.6±6.1	128.3±40.1	145.9±53.6	0.001	0.195
PPBS(mg/dl)	107.4±10.3	191.7±56	221±82.1	0.001	0.135
HbA1C	5.4±0.5	7.2±0.8	8.0±1.1	0.001	0.007
Serum urea(mg/dl)	24.2±4.7	28.1±5.4	33.4±12.2	0.014	0.056
Serum creatinine(mg/dl)	0.6±0.2	0.7±0.2	0.9±0.3	0.189	0.082
Serum cholesterol (mg/dl)	168.4±8.6	186.8±20.4	193.8±21.8	0.001	0.248
Serum Triglycerides (mg/dl)	96.7±7.6	130.5±39.3	141.8±38.1	0.001	0.306
HDL cholesterol (mg/dl)	43.1±2.2	39.4±3.0	38.3±2.3	0.001	0.182
LDL cholesterol (mg/dl)	105.9±8.7	121.3±16.5	127.1±20.8	0.001	0.285
Insulin (µIU/mL)	6.6±0.69	10.9±4.1	13.2±5.0	0.001	0.081
HOMA-IR	1.3±0.1	3.4±1.6	4.6±2.2	0.001	0.036
Plasma type IV collagen (ng/ml)	4.0±0.65	7.1±2.4	9.9±2.9	0.001	0.001
Urine type IV collagen (ng/mg of creatinine)	1.0±0.27	1.7±0.63	4.0±2.3	0.001	0.001

Data are expressed as mean ±SD, p<0.05 was considered statistically significant.

Table 3: Correlation between p IV collagen &measured parameters

Parameters	Correlation Coefficient(r)	p value
Albumin Creatinine Ratio	0.593**	0.001
Urine type IV collagen	0.773**	0.001
FBS	0.420**	0.001
PPBS	0.422**	0.001
HbA1C	0.566**	0.001
HOMA-IR	0.565**	0.001
Cholesterol	0.288*	0.015
TGL	0.342**	0.004
HDL	-0.469**	0.001
LDL	0.270*	0.024

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

Table 4: Correlation between u IV collagen &measured parameters

Parameters	Correlation Coefficient(r)	p value
Albumin Creatinine Ratio	0.712**	0.001
FBS	0.357**	0.002
PPBS	0.357**	0.002
HbA1C	0.518**	0.001
HOMA-IR	0.483**	0.001
Cholesterol	0.297*	0.013
TGL	0.303*	0.011
HDL	-0.399**	0.001
LDL	0.283*	0.018

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

Table 5: Correlation between ACR &measured parameters

Parameters	Correlation Coefficient(r)	p value
FBS	0.286*	0.017
PPBS	0.342**	0.004
HbA1C	0.491**	0.001
HOMA-IR	0.284*	0.017
Cholesterol	0.392**	0.001
TGL	0.426**	0.001
HDL	-0.344**	0.004
LDL	0.331**	0.005

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

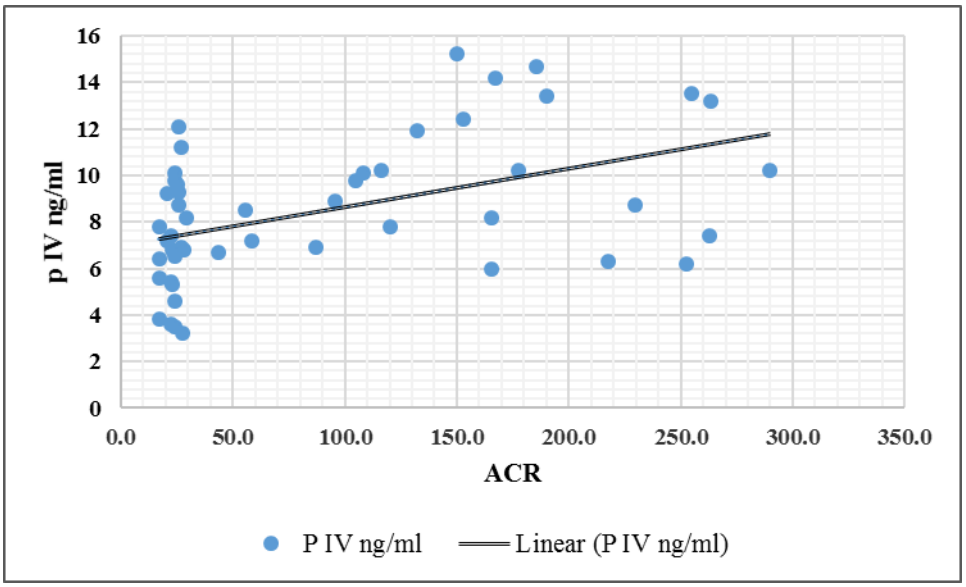

Figure 1: Correlation between ACR and p IV in type 2 diabetic patients

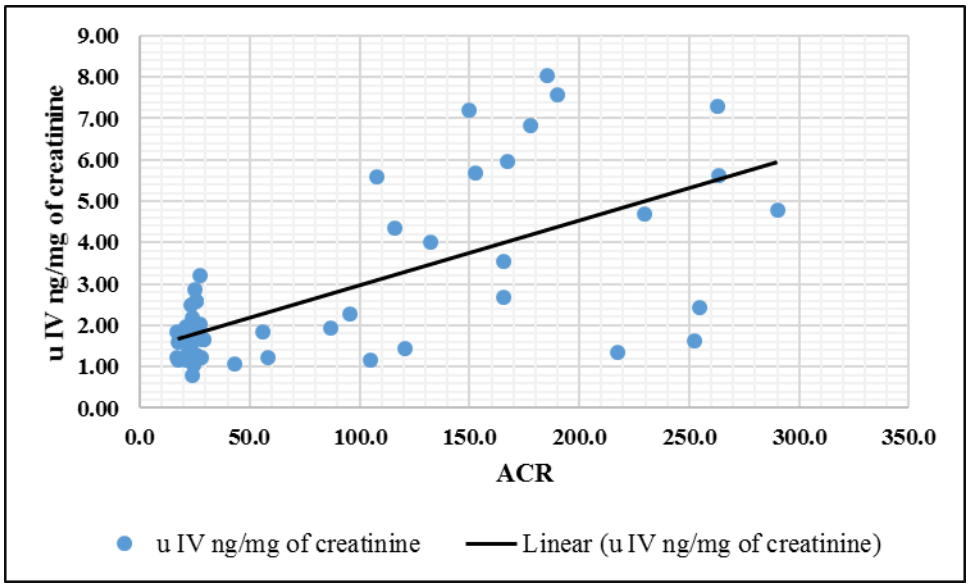

Figure 2: Correlation between ACR and u IV in type 2 diabetic patients

IV. Discussion

Diabetic nephropathy is characterized by thickening of the glomerular basement membrane (GBM) and expansion of the mesangial matrix [10, 11]. In early stages of diabetic, glomerular basement membrane (GBM) changes may produce microalbuminuria, subsequently overt proteinuria, and end stage renal disease (ESRD) in uncontrolled diabetes [12, 13]. Type IV collagen is the main component of the glomerular basement membrane and the extracellular matrix. The thickening of the GBM in diabetes is due to accumulation of type IV collagen and alterations in its architecture and composition [14]. The measurement of this extracellular matrix proteins in biologic fluids might be useful for early detection of nephropathy in diabetic patients [15-17].

In the present study, we observed that p IV and u IV levels were significantly increased in type 2 diabetic patients as reported earlier [18]. It also positively correlated with ACR indicating its relevance for diagnosis of diabetic nephropathy. P IV and u IV levels were significantly elevated in normoalbuminuric type 2 diabetics when compared with controls. Its rise in diabetics even before the appearance of microalbuminuric state suggests that it can be an earlier marker of diabetic nephropathy. Haneda et al reported that glucose enhances type IV collagen production in cultured rat glomerular mesangial cells [19]. Pavai sthaneshwar et.al [20] and Kikkawa et al [21] had reported that u IV excretion increased in microalbuminuric diabetic patients. Kotajima N et.al has reported that u IV could be used as an early marker of diabetic nephropathy [17].

In addition, p IV and u IV levels showed strong positive correlation with HbA1C and HOMA-IR , and also a strong negative correlation was observed between HDL cholesterol and p IV, u IV, ACR .T2DM is associated with a dysregulation in HDL metabolism and dysfunctional properties of HDL particles including HDL-mediated reverse cholesterol transport [22]. Chronic hyperglycemia produces reactive oxygen species (ROS), protein glycation reactions which leads to the formation of advanced glycation end products (AGEs) leading to inflammatory changes [23]. Susumu Ogawa et.al [24] reported that higher excretion of u IV was due to oxidative stress, inflammation and could be a predictor of change in the ACR. Since there is positive correlation of p IV and u IV with both ACR and HbA1c, its estimation could predict the nephropathy changes induced by chronic hyperglycemia. Studies have revealed that elevated glucose concentration in glomerular mesangial cells and proximal tubular cells stimulates the biosynthesis of collagen and other extracellular matrix constituents [10, 25-27].

It has been shown that AGEs trigger the activation of nuclear factor kappa B (NF-κB) by interaction with Receptor for AGE (RAGE). Moreover, the AGE-RAGE interaction activates TGF-β1 signaling pathways and subsequently induces mesangial cell hypertrophy and glomerular sclerosis by ECM synthesis [28]. Increased ECM synthesis is mainly supported by epithelial to mesenchymal transition which describes the trans differentiation of an epithelial cell to a cell with myofibroblast-like features. Endothelial to mesenchymal transition has been described as a potential source of activated myofibroblasts causing fibrosis in the kidney [29, 30]. The fibrosis is characterized by an increased glycation and oxidation of type IV collagen together with increased accumulation of extracellular matrix components in kidney diseases [31]. Therefore, accumulation of type IV collagen in the kidney has been regarded as an index of progressive renal damage in diabetic patients. This implies that p IV and u IV could be useful as an early diagnostic marker for detection of nephropathy even before the onset of microalbuminuric state.

In conclusion, p IV and u IV levels were elevated in type 2 diabetic patients and its elevation in normoalbuminuric patients indicates that it could be an early marker of diabetic nephropathy. Further studies are needed to correlate the renal functional status and the u IV levels.

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