

ORIGINAL ARTICLE

Urinary type IV collagen levels in diabetes mellitus

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Abstract

Type IV collagen is the principal component of glomerular basement membrane and messangial matrix. Studies have shown increased levels of urinary type IV collagen (uIV) in diabetic patients compared to healthy controls. The concentration of uIV increases gradually as diabetic nephropathy progresses. *Aim and method:* This study was carried out to determine whether urinary type IV collagen (uIV) can serve as an indicator of diabetic nephropathy. Using a sandwich enzyme immunoassay technique, uIV excretion was determined in 30 type 2 diabetic patients with normoalbuminuria and 20 patients with microalbuminuria. *Results:* uIV excretion was significantly increased in type 2 diabetics, in both normoalbuminuric and microalbuminuric patients, compared with healthy controls. The increase in urinary type IV collagen was well correlated with the amount of urinary albumin but not with HbA_{1c}. *Conclusion:* Our findings that uIV is higher in those with microalbuminuria and correlates with albuminuria, support uIV as an indicator of diabetic nephropathy. Whether the increased uIV excretion would predict the impending renal failure needs further confirmation.

Keywords: Type IV collagen, type 2 diabetes mellitus, urine microalbumin

INTRODUCTION

Diabetes mellitus is the leading cause of end-stage renal disease. Early detection of diabetic nephropathy relies upon tests for urinary excretion of microalbumin. Microalbuminuria is defined as excretion of 30-300 mg of albumin per 24 hours or 30-300 µg/mg creatinine on two of three urinary collections.¹ Sequential monitoring of such markers serves as guide to monitor progressive renal injury. Experimental studies suggest that newer markers like urinary N-acetyl glucosaminidase, urinary β₂-microglobulin, urinary transferrin and serum and urinary type III and type IV collagen may serve as early indicators of renal injury.^{2,3}

Type IV collagen is the principal component of glomerular basement membrane and mesangial matrix.⁴ Studies in animal models have documented that the glomerular extracellular matrix expansion responsible for the encroachment of the filtration surface area is associated with the overproduction of type IV collagen.⁵ Clinical trials have shown increased levels of urinary type IV collagen (uIV) in diabetic patients compared to those in healthy controls.⁶ The concentration of uIV increases gradually as diabetic nephropathy progresses.⁶ The aim of the present study was to

determine whether uIV can be used as an early indicator of diabetic nephropathy.

METHODS AND MATERIALS

Patients & controls

The study group consisted of 50 patients with type 2 diabetes in whom the duration of diabetes varied from 2 to 24 years. The study population was further divided into two groups. Group 1 had urinary excretion of microalbumin levels less than 29 mg/day and group 2 had microalbuminuria ranging from 30 to 299 mg/day. Measurements of HbA_{1c} levels, fasting glucose and serum creatinine were also performed for all these patients.

The control group consisted of 20 healthy volunteers who had neither renal and cardiac problem nor hypertension.

Urinary type IV collagen measurement

Twelve-hour urine samples were collected from each subject. Urinary type IV collagen was measured by solid phase enzyme immunoassay. (Fuji Chemical Industries co Ltd, Takaka, Toyama, Japan) based on a one-step sandwich immunoreaction.

TABLE 1: Urinary Type IV collagen levels in the study groups

	Control	Group 1	Group II
Number	20	30	20
Age	37.75 ± 6.8	52.60 ± 7.42	54.15 ± 6.40
Glucose in mmol/L	4.42 ± 0.87	7.78 ± 2.23	7.95 ± 2.00
Creatinine in μmol	86.35 ± 13.7	80.83 ± 17.51	79.80 ± 18.04
HbA _{1c} %	4.56 ± 0.44	7.08 ± 1.15	7.41 ± 1.53
Urine albumin in mg/day	11.05 ± 2.65	18.86 ± 5.26	93.85 ± 63.69
Type IV in $\mu\text{g/g}$ of creatinine	1.63 ± 0.29	2.23 ± 0.59	6.18 ± 2.75

Statistical analysis

Statistical analysis was performed using unpaired t-tests to compare the means. Correlations were determined by linear regression analysis.

RESULTS

Table 1 shows the results of control and study groups. Groups 1 and 2 did not differ in age, glycaemic control and serum creatinine levels. Group 1 had urine microalbumin excretion less than 30 mg/day and group 2 had microalbuminuria more than 30 but less than 300mg/day.

Urinary excretion of type IV collagen in control was noted to be $1.63 \pm 0.29 \mu\text{g/g}$ of creatinine. uIV excretion ranged from 0.6 to 4.2 $\mu\text{g/g}$ of creatinine in normoalbuminuric type 2 diabetes mellitus patients (mean $2.02 \mu\text{g/g}$ of

creatinine). But in microalbuminuric patients it ranged from 2.8 to 12.44 $\mu\text{g/g}$ of creatinine (mean $6.17 \mu\text{g/g}$ of creatinine) (Figure 1).

Type IV collagen excretion was higher in both group 1 and group 2 diabetes mellitus patients compared with that of the control group ($p < 0.05$ and < 0.0001 respectively). uIV levels were significantly higher in group 2 compared to that of group 1 ($p < 0.0001$).

uIV excretion showed a significant correlation with albumin excretion in group 1 ($r = 0.52$, $p < 0.01$) (Figure 2). A very good correlation was observed between albumin excretion and type IV collagen in group 2 diabetes mellitus patients ($r = 0.95$, $p < 0.0001$) (Figure 3). No correlation was observed between glycaemic control indicator (HbA_{1c}) and uIV both in groups 1 and 2 ($p > 0.05$).

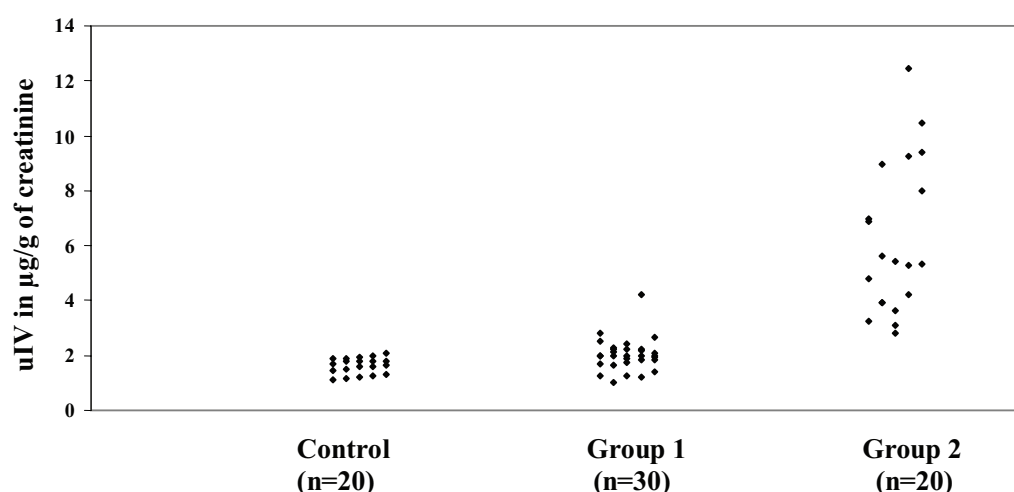


FIG. 1: Urine type IV collagen levels in controls and diabetes mellitus

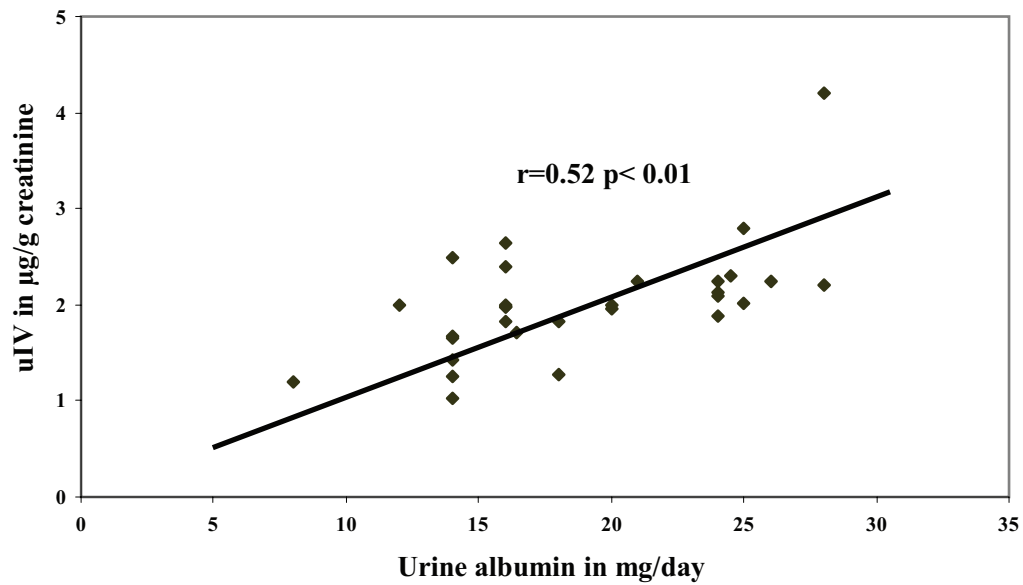


FIG. 2: Correlation between urine albumin and type IV collagen in group I (normoalbuminuric) diabetes mellitus patients

DISCUSSION

It is well known that the characteristic pathological changes of diabetic nephropathy are the accumulation of extracellular matrix and widening of the basement membrane. Type IV collagen, the main component of the glomerular basement membrane and the extracellular matrix, has emerged as a new indicator of renal injury.

Phillips *et al*⁷ noted that proximal tubular cells produce excess amounts of collagen when cultured under hyperglycaemic condition. Animal models of diabetes exhibit increase in type IV collagen excretion, which manifest soon after the onset of hyperglycaemia and coincided with the histopathological evidence of glomerular matrix accumulation and the onset of declining glomerular filtration rate.⁸ These results

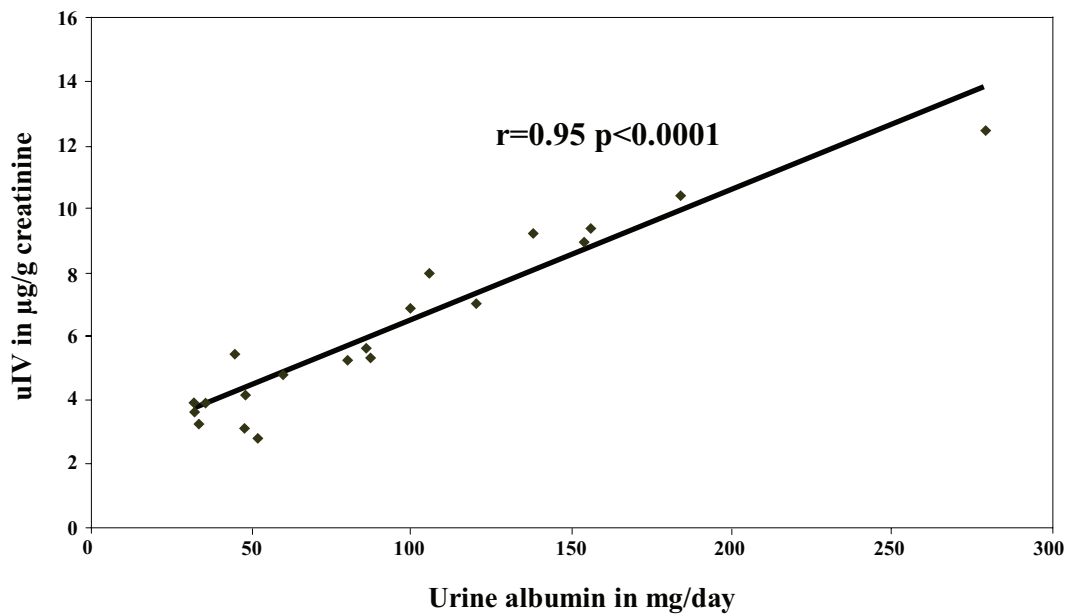


FIG. 3: Correlation between urine albumin and type IV collagen in group II (microalbuminuric) diabetes mellitus patients

suggest that an increase in type IV collagen excretion accompanies the overproduction of the extracellular matrix protein. As the expansion of the extracellular matrix encroaches on the glomerular basement membrane, filtration function begins to decline. The renal biopsies of subjects with increased type IV collagen showed typical features of diabetic nephropathy.⁹

The main findings in our study were that the excretion of uIV was increased in diabetic patients with microalbuminuria and there was a significant correlation between albumin excretion and collagen excretion ($p < 0.0001$). Kikkawa *et al*⁴ had reported a similar observation. In their study they noted that type IV collagen excretion increased in accordance with the increase in urine albumin excretion.

Higher levels of uIV had also been reported even in normoalbuminuric individuals.^{10,11} A recent study by Takamatsu *et al*¹² observed abnormal excretion of type IV collagen in diabetic individuals with normal estimated glomerular filtration rate (≥ 90 mL/min per 1.73 m²). We also noted significantly higher levels of uIV in group 1 of the study population than that of controls. The concentration of uIV in group 1 patients was higher ($2.02 \pm 0.59 \mu\text{g/g}$ of creatinine) than normal adults ($1.63 \pm 0.29 \mu\text{g/g}$ of creatinine). Even though the normal controls were younger than the study group, in the adult population there is not much difference in excretion of type IV collagen.¹² The percentage of patients with an abnormal excretion of type IV collagen in the urine, defined as values over a mean ± 2 SD of the healthy group increased from 63% in the normoalbuminuric to 100% in the microalbuminuric individuals, thus confirming the finding of Tomino *et al*⁶ who had reported that uIV levels increased gradually due to progression of the clinical stage of diabetic nephropathy.

Haneda *et al*¹³ have shown that glucose enhances type IV collagen production in cultured rat glomerular mesangial cells. Increased serum type IV collagen concentrations have been observed in patients with increased blood glucose levels.¹⁴ These data suggest that a significant relationship should exist between glycaemic control and concentration of uIV. While our diabetes mellitus patients showed a significantly higher level of HbA_{1c}, no significant correlation was observed between HbA_{1c} and uIV. The implication of this negative finding is that increased excretion of uIV must reflect other factors in addition to hyperglycemia. Enhancement of increased excretion of uIV in

diabetic subjects with hypertension supports this idea.¹⁰ The study by Ellis *et al*¹⁵ also support this. In their study they observed increased excretion of the transforming growth factor (TGF- β_1) which is a marker of extracellular matrix synthesis or remodeling in microalbuminuric diabetic patients. They reported that the modest increase in TGF- β_1 excretion in patients with microalbuminuria or overt proteinuria did not correlate with the marked increase in the excretion of collagen. Hence they suggested that factors other than TGF- β_1 may play a role in the enhanced matrix production.

Cohen *et al*¹⁶ observed a negative correlation between collagen excretion and creatinine clearance. Such a relationship suggests that renal function in patients with high collagen excretion might be deteriorating gradually. Mogensen¹⁷ reported that 80% of patients with type 1 diabetes mellitus and 20% of patients with type 2 diabetes mellitus with microalbuminuria develop overt proteinuria in 10 years. Although microalbuminuria is the established predictor of risk for subsequent development of diabetic nephropathy, not all patients with microalbuminuria progress to overt nephropathy. This discrepancy is particularly notable in type 2 diabetes mellitus.

In our study, type 2 diabetes mellitus patients have increased levels of uIV. Excretion of type IV collagen, possibly reflecting increased production may be a useful indicator of diabetic nephropathy. Whether increased excretion of uIV predicts impending renal failure needs further confirmation.

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