

**Association of Urinary Type IV Collagen With GFR Decline in Young Patients  
With Type 1 Diabetes**

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## ABSTRACT

**Background:** Some diabetic patients have advanced diabetic glomerular lesions and progressive kidney function decline even if levels of urinary albumin are normal. Therefore, another prognostic marker for diabetic kidney disease needs to be identified. We aimed to clarify whether urinary type IV collagen is associated with the progression of kidney function decline in patients with type 1 diabetes.

**Study Design:** Hospital-based observational cohort study

**Setting and participants:** 231 normo- and microalbuminuric patients with type 1 diabetes who were less than 40 years old at the start of study.

**Predictor and Measurements:** Urinary type IV collagen, determined using 1-step sandwich enzyme immunoassay

**Outcome:** The primary outcome measurement was the rate of change in estimated glomerular filtration rate (eGFR).

**Result:** Mean ( $\pm$  SD) follow-up period was 7.4  $\pm$  1.3 years. T4C was significantly associated with the rate of change in eGFR in both the univariate ( $r = -0.169$ ,  $p = 0.01$ ) and multivariate regression analyses (standardized estimate =  $-0.131$ ,  $p = 0.03$ ). In the sensitivity analysis limited to patients with normoalbuminuria ( $n = 213$ ), T4C but not urinary albumin to creatinine ratio (ACR) was significantly associated with the rate of change in eGFR (standardized estimate =  $-0.12$ ,  $p = 0.03$ ). The interaction between logarithmically transformed ACR and logarithmically transformed T4C on eGFR decline was not significant ( $P$  interaction = 0.2). We compared adjusted rate of change in eGFR among 4 groups classified according to normal or elevated levels of T4C and ACR, and found that the rate of change in eGFR in patients with elevated T4C and normal ACR was significantly higher than that in patients with normal T4C and normal

ACR (-4.3 and -3.0 ml/min/1.73 m<sup>2</sup>/year, p = 0.004, ANCOVA).

**Limitations:** Study size was relatively small.

**Conclusions:** T4C is associated with the progression of kidney function decline in young patients with type 1 diabetes.

Diabetic kidney disease is a major cause of end-stage renal disease (ESRD) worldwide<sup>1</sup>, and the lifetime risk for ESRD in patients with type 1 diabetes is 25 – 50 %<sup>2,3</sup>. Hence, there is an urgent requirement for a prognostic marker for the decrease of kidney function; such a marker would enable initiation of early intervention. Currently, albuminuria is considered the most sensitive prognostic marker for the progression of diabetic kidney disease<sup>4,5</sup>. However, previous reports have indicated that some patients with normoalbuminuria have advanced diabetic glomerular lesions, decreased glomerular filtration rate (GFR), and progressive kidney function decline<sup>6,7</sup>. Consequently, another prognostic marker for diabetic kidney disease needs to be identified.

Morphologically, diabetic kidney disease is characterized by the thickening of the glomerular basement membrane (GBM), and by the extracellular matrix expansion surrounding mesangial cells<sup>8</sup>. Type IV collagen is the principal component of GBM and mesangial matrix<sup>9</sup>. Urinary type IV collagen is minimally affected by serum levels because of its high molecular weight of approximately 540 kDa<sup>10</sup>, which is too large for filtration through glomeruli from blood. Therefore, its increased shedding from glomeruli is considered to be responsible for elevated urinary type IV collagen. Indeed, increased levels of urinary type IV collagen are known to be associated with decreased kidney function and increased urinary albumin<sup>11,12</sup>; however, previous studies including patients with type 1 diabetes on the relationship between urinary type IV collagen and

decreased kidney function were limited by cross-sectional design. Therefore, we conducted a long follow-up study to determine whether urinary type IV collagen is associated with the kidney function decline in young patients with early-onset type 1 diabetes.

## **RESEARCH DESIGN AND METHODS**

### **Patients**

This study included normo- and microalbuminuric patients who had been diagnosed with type 1 diabetes before the age of 30 years, had been treated as outpatients at the Diabetes Center, Tokyo Women's Medical University School of Medicine from 1998 to 2003, and were < 40 years old at the start of observation. Figure 1 shows the composition of the study population. Overall, 231 patients were enrolled.

### **Methods**

This study was a hospital-based observational cohort study. Baseline evaluation included measurement of height, weight, blood pressure, hemoglobin A1c (HbA1c), lipid profile, serum creatinine (Cr), presence or absence of retinopathy, and use or non-use of antihypertensive drugs or anti-lipid drugs. The diagnosis of type 1 diabetes was defined by both clinical features and laboratory data. All the patients were ketosis-prone, lacked endogenous insulin secretion as judged by urinary C-peptide levels of <3.3 nmol/day, and needed more than four insulin injections per day<sup>13</sup>. To assess diabetic retinopathy, fundoscopic examinations with dilated pupils were performed by ophthalmologists during the baseline year. Diabetic retinopathy was defined as the presence of microaneurysms or dot hemorrhages or new vessels, vitreous

hemorrhage, vitreoretinal traction, or retinal detachment believed to be attributable to diabetic neovascularization.

The study protocol was designed in adherence to the Declaration of Helsinki.

## Measurements

Urinary albumin to creatinine ratio (ACR) and urinary type IV collagen to creatinine ratio (T4C) were measured in the first-voided urine samples collected early in the morning on the day of hospital visit. Urinary albumin was measured using a latex agglutination immunoturbidimetric assay (currently under confirmation, Eiken Kagaku, Tokyo, Japan) to calculate ACR. Urinary type IV collagen was measured using a 1-step sandwich enzyme immunoassay (urinary type IV collagen assay kit; Daiichi Fine Chemical, Toyama, Japan) to calculate the T4C. The intra-assay coefficients of variation for T4C were 6.78, 2.56 and 4.27 % with low, mid-range and high levels of T4C, respectively. The inter-assay coefficients of variation for T4C were 7.42, 6.45 and 5.14 % with low, mid-range and high levels of T4C, respectively.

Levels of T4C were defined as normal T4C ( $\leq 4.0 \mu\text{g/g creatinine}^{14}$ ), and stages of ACR were defined as normoalbuminuria ( $\text{ACR} < 30 \text{ mg/g creatinine}$ ) or microalbuminuria ( $30 \leq \text{ACR} < 300 \text{ mg/g creatinine}^5$ ) based on at least 2 out of 3 measurements.

Total cholesterol (T-Cho) was measured using an enzymatic method. HbA1c was measured by high-performance liquid chromatography (HPLC) (HA8131; Daiichi Kagaku, Kyoto, Japan), which is defined as HbA1c (Japan Diabetes Society, JDS) (%). The value for HbA1c (%) is estimated as the National Glycohemoglobin Standardization Program (NGSP) equivalent value (%) calculated by the formula;

HbA1c (%) = HbA1c (JDS) (%) + 0.4%, considering the relational expression of HbA1c (JDS) (%) measured by the previous Japanese standard substance and measurement methods and HbA1c (NGSP)<sup>15</sup>.

### **Estimated GFR**

Glomerular filtration rate (GFR) for an adult (age  $\geq 18$  years) was estimated using the following equation, originating from the Modification of Diet in Renal Disease (MDRD) study group<sup>16</sup> and refitted for Japanese individuals: estimated GFR (eGFR) =  $194 \times \text{SCr (mg/dl)}^{-1.094} \times \text{Age}^{-0.287}$ ; (if female)  $\times 0.739$ , where SCr = serum creatinine<sup>17</sup>. GFR for children (age  $< 18$  years) was estimated using the following equation: eGFR = Length (cm)  $\times k / \text{serum Cr (mg/dl)}$  ( $k = 0.70$  for males aged  $\geq 13$  years, and  $k = 0.55$  for the others)<sup>18</sup>.

Serum creatinine was measured by Jaffe's method, and the value was calibrated using the following equation, prior to inclusion in the equation: serum creatinine (enzymatic method) =  $0.972 \times \text{serum creatinine (Jaffe's method)} - 0.224$  ( $r = 0.999$ ,  $p < 0.001$ )<sup>19</sup>.

### **Outcome measurement**

The primary outcome measurement of this study was the rate of change in eGFR. For each individual, the rate was determined by parameter estimates using a simple regression analysis, with eGFR as a function of time in years applied to all eGFR values obtained during the follow-up period.

### **Statistical analysis**

Data were expressed as percentages, arithmetic mean  $\pm$  standard deviation (SD) or geometric mean with 95% confidence interval (CI), as appropriate on the basis of the data distribution. Categorical data were compared using Fisher's exact probability test and continuous data were compared using Student t test. Relationships between variables were assessed by Pearson's correlational analysis or multivariate linear regression analysis. Analysis of covariance (ANCOVA) was performed for the comparison among the 4 groups. The following covariates were used as conventional risk factors: age, sex, duration of diabetes, presence of retinopathy, eGFR at baseline, and mean values during the follow-up period of HbA1c, systolic blood pressure, body mass index (BMI), and T-Cho. *PASW Statistics 18* (SPSS Japan Inc, Tokyo, Japan) and *SAS version 9.2* (SAS Institute, Cary, NC) were used for analysis. P values less than 0.05 were considered significant.

## **RESULTS**

### **Demographic and clinical characteristics**

The baseline clinical characteristics and laboratory data are presented in Table 1. We studied 231 patients with type 1 diabetes, including 146 females and 85 males, with a mean age of  $24 \pm 7$  years (range, 5-39). At baseline, 188 patients had normal T4C, and the remaining 43 patients had elevated T4C. Additionally, 213 patients had normoalbuminuria, 18 had microalbuminuria. T4C was significantly associated with ACR ( $n = 231$ ,  $r = 0.220$ ,  $p = 0.001$ ). ACR was associated with eGFR, but T4C was not associated with eGFR at the baseline ( $r = 0.180$ ,  $p = 0.006$ ;  $r = 0.114$ ,  $p = 0.09$ ; respectively).

The mean values of HbA1c, blood pressure (BP), lipid profile, and BMI during

the median ( $\pm$  SD) follow-up period ( $7.4 \pm 1.3$  years; range, 4.4 – 9.3 years) are shown in Table 1.

### **Association between T4C and the rate of change in eGFR**

During the follow-up period, the median number of eGFR determinations was 10 (range 4-19). The mean ( $\pm$  SD) annual rate of change in eGFR in the entire cohort was  $-3.4 \pm 2.6$  ml/min/1.73 m<sup>2</sup>/year.

Patients with elevated urinary type IV collagen have steeper declines in eGFR than those with normal urinary type IV collagen ( $-4.6 \pm 3.2$  and  $-3.1 \pm 2.4$  mL/min/year,  $p = 0.001$ ). The adjusted decline in eGFR in patients with elevated urinary type IV collagen was also significantly higher than those in patients with normal urinary type IV collagen ( $-4.4$  and  $-3.2$  mL/min/1.73 m<sup>2</sup>/year,  $p = 0.004$ , ANCOVA).

In the univariate correlational analysis, T4C was significantly correlated with the rate of change in eGFR ( $r = -0.169$ ,  $p = 0.01$ ) (Fig. 2). To clarify an independent effect, we conducted a multiple linear regression analysis using conventional risk factors as dependent variables. In this analysis, T4C was independently associated with the rate of change in eGFR (standardized estimate =  $-0.131$ ,  $p = 0.02$ ). In addition, we conducted a multivariate regression analysis incorporating ACR, which showed that both T4C and ACR were significantly associated with the rate of change in eGFR (standardized estimate =  $-0.115$  and  $-0.139$ , respectively, and  $p = 0.04$  and  $0.02$ , respectively).

We then did further analysis limited to patients with normoalbuminuria ( $n = 213$ ). In this sensitivity analysis, T4C (standardized estimate =  $-0.12$ ,  $p = 0.03$ ) but not ACR (standardized estimate =  $0.039$ ,  $p = 0.5$ ) was significantly associated with the rate of change in eGFR.

Finally, we conducted analyses to detect the interaction between logarithmically transformed ACR and logarithmically transformed T4C on eGFR decline since the values of urinary type IV collagen for eGFR decline may differ according to levels of urinary albumin. However, no significant interaction was obtained in this analysis (p interaction = 0.2).

### **Comparison of the rate of change in eGFR among 4 groups classified according to normal or elevated levels of T4C and ACR**

We classified the subjects into 4 groups according to normal or elevated levels of T4C and ACR. T4C > 4 µg/g Cr was defined as elevated T4C, and micro- and macroalbuminuria were defined as elevated ACR. The rate of change in eGFR in patients with elevated T4C and normal ACR was significantly higher than those with normal T4C and normal ACR (-4.3 and -3.0 ml/min/1.73 m<sup>2</sup>/year, p = 0.002). The rate of change in eGFR in patients with normal T4C and elevated ACR was also significantly higher than those with normal T4C and normal ACR (-5.3 and -3.0 ml/min/1.73 m<sup>2</sup>/year, p = 0.003) (Fig. 3-A). Similar results were obtained after adjusting for conventional risk factors. The adjusted rate of change in eGFR in patients with elevated T4C and normal ACR was significantly higher than those with normal T4C and normal ACR (-4.3 and -3.0 ml/min/1.73 m<sup>2</sup>/year, p = 0.004). The adjusted rate of change in eGFR in patients with normal T4C and elevated ACR was also significantly higher than those with normal T4C and normal ACR (-5.5 and -3.0 ml/min/1.73 m<sup>2</sup>/year, p <0.001) (Fig. 3-B)

## **DISCUSSION**

In this hospital-based observational cohort study, we have demonstrated that the level of T4C is independently associated with the progression of kidney function decline in young patients with early-onset type 1 diabetes, who are less affected by aging. Furthermore, we have shown that T4C but not ACR is significantly associated with the rate of change in eGFR in patients with normoalbuminuria. Indeed, patients with normoalbuminuria and elevated T4C had a significant higher decline in the rate of change in eGFR than those with normoalbuminuria and normal T4C. These findings suggest that T4C detects kidney function decline which ACR is unable to do. We believe that the measurement of T4C enhances prognostic ability for progression of kidney function decline in patients with type 1 diabetes.

No significant interaction between urinary albumin and urinary type IV collagen on eGFR decline was obtained in this study. We speculated as to several possible reasons for the lack of an interaction. In our cohort, the number of patients with elevated urinary albumin may be too small to detect an interaction, if one exists. Alternatively, these results may suggest that urinary type IV collagen is associated with GFR decline in patients with microalbuminuria, as it is in those with normoalbuminuria. A recent report on patients with type 2 diabetes showed an association of urinary type IV collagen with GFR decline in microalbuminuric patients<sup>20</sup>.

Several cross-sectional studies have reported that an increase in T4C occurs prior to an increase in ACR in diabetic patients<sup>12,21,22</sup>; the normoalbuminuria and elevated T4C levels in our subjects are likely attributable to this. Whereas, a few of our subjects had normal T4C but elevated ACR and these subjects were also at risk for eGFR decline. However, in another study, normal T4C but elevated ACR in type 2 diabetic patients was not a risk for eGFR decline<sup>20</sup>. Further studies are needed to

elucidate whether these states in diabetic subjects are associated with a risk for GFR decline.

Glomerulosclerosis has been demonstrated to be characterized by mesangial expansion, which is strongly correlated with a decline in the GFR<sup>23</sup>. Moreover, a recent study documented that Smad1, which is activated by the stimulation of glycation endproducts (AGE), regulates the production of type IV collagen and also predicted mesangial matrix expansion in an experimental model<sup>24,25</sup>. Therefore, the predictive value of Smad1 in diabetic patients with progressive diabetic nephropathy needs to be further elucidated.

Our study has some limitations. First, no standard cutoff point of T4C has been established. Second, the mean annual rate of change in eGFR in our study was relatively faster than that found in other studies in western countries<sup>26</sup>. However, previous studies of Japanese diabetic patients have shown results similar to ours<sup>6,27</sup>. Therefore, ethnic differences may be factors in the rate of GFR decline in diabetic patients. Finally, our study was based on relatively small cohort, and conducted at a single diabetes specialty center in an area comprised of an ethnically homogenous population<sup>28</sup>; therefore, the subject characteristics may be different from those of the general type 1 diabetes population.

In spite of these limitations, our study had the following advantages. First, the follow-up period of our study was relatively long. Second, analyses were conducted using mean values of HbA1c, systolic BP, BMI, and T-Cho during follow-up period, rather than the baseline values; therefore, our results can be considered highly reliable. Finally, a recent report demonstrated that T4C is associated with the deterioration of kidney function in elderly type 2 diabetic patients, consistent with our results<sup>20</sup>.

However, diabetes duration in patients with type 2 diabetes is ambiguous. In addition, aging kidneys are affected by various factors other than glycemic control, such as hypertension, dyslipidemia and obesity. Therefore, the present study was aimed at young type 1 diabetic patients. We believe that kidney outcomes in this study showed the effect of high glucose alone on kidneys *i.e.* diabetic kidney disease.

In conclusion, this hospital-based observational cohort study provides evidence that urinary type IV collagen is independently associated with GFR decline in young patients with early-onset type 1 diabetes. Measurement of T4C, in addition to ACR, may prove to be useful in preventing the progression of kidney disease.

## References

1. Colhoun HM, Lee ET, Bennett PH, et al. Risk factors for renal failure: the WHO Multinational Study of Vascular Disease in Diabetes. *Diabetologia*. 2001; 44 (suppl 2): S46-S53.
2. Krolewski M, Egger PW, Warram JH. Magnitude of end-stage renal disease in IDDM: A 35 year follow-up study. *Kidney Int*. 1996; 50 (6): 2041–2046.
3. Nordwall M, Bojesting M, Arnqvist HJ, Ludvigsson J. Diabetes Complications Study. Declining incidence of severe retinopathy and persisting decrease of nephropathy in an unselected population of type 1 diabetes: the Linköping diabetes Complications study. *Diabetologia*. 2004; 47 (7): 1266-1272.
4. Mogensen CE. Microalbuminuria, blood pressure and diabetic renal disease: origin and development of ideas. *Diabetologia*. 1999; 42 (3): 263–285.
5. Molitch ME, DeFronzo RA, Franz MJ, et al. Nephropathy in Diabetes. *Diabetes*

- Care*. 2004; 27 (suppl 1): S79-S83.
6. Yokoyama H, Kanno S, Takahashi S, et al. Determinants of decline in glomerular filtration rate in nonproteinuric subjects with or without diabetes and hypertension. *Clin J Am Soc Nephrol*. 2009; 4 (9): 1432-1440.
  7. Caramori ML, Fioretto P, Mauer M. Low Glomerular Filtration Rate in Normoalbuminuric type 1 diabetic patients: an indicator of more advanced glomerular lesions. *Diabetes*. 2003; 52 (4): 1036–1040.
  8. Tsilibary EC. Microvascular basement membranes in diabetes mellitus. *J Pathol*. 2003; 200 (4): 537-546
  9. Yagame M, Kim Y, Zhu D, et al. Differential distribution of type IV collagen chains in patients with diabetic nephropathy in non-insulin-dependent diabetes mellitus. *Nephron*. 1995; 70 (1): 42-48
  10. Takizawa H, Satoh T, Kurusu A, et al. Increase of urinary type IV collagen in normoalbuminuric patients with impaired glucose tolerance. *Nephron*. 1998; 79 (4): 474-475
  11. Cohen MP, Lautenslager GT, Shearman CW. Increased collagen IV excretion in diabetes. A marker of compromised filtration function. *Diabetes Care*. 2001; 24 (5): 914-918.
  12. Tomino Y, Suzuki S, Azushima C, et al. Asian multicenter trials on urinary type IV collagen in patients with diabetic nephropathy. *J Clin Lab Anal*. 2001; 15(4): 188-192.
  13. Kawabata Y, Ikegami H, Kawaguchi Y, et al. Asian-specific HLA haplotypes reveal heterogeneity of the contribution of HLA-DR and –DQ haplotypes to susceptibility

- to type 1 diabetes. *Diabetes*. 2002; 51 (2): 545-551
14. Yokouchi K, Mitsusima T, Izuwa F, Matsuki S. Examination of upper reference values of urinary type IV collagen on healthy subjects. *Journal of Medicine and Pharmaceutical Science (in Japanese)*. 1999; 42 (1): 139-143.
  15. Seino Y, Nanjo k, Tajima N, et al. Report of the committee on the classification and Diagnostic criteria of diabetes mellitus. *Diabetol Int*. 2010; 1 (1): 2-20
  16. Levey AS, Coresh J, Greene T, et al. Chronic Kidney Disease Epidemiology Collaboration.: Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Int Med*. 2006; 145 (4): 247-254.
  17. Imai E, Matsuo S, Makino H, et al. CKD-JAC Study Group.: Chronic kidney disease Japan cohort (CKD-JAC) study: Design and methods. *Hypertens Res*. 2008; 31 (6): 1101-1107.
  18. Schwartz GJ, Haycock GB, Edelmann CM Jr, Spitzer A. A simple estimate of glomerular filtration rate in children derived from body length and plasma creatinine. *Pediatrics*. 1976; 58 (2): 259-263.
  19. Babazono T, Hanai K, Suzuki K, et al. Lower Haemoglobin level and subsequent decline in kidney function in type 2 diabetic adults without clinical albuminuria. *Diabetologia*. 2006; 49 (6): 1387-1393.
  20. Araki S, Haneda M, Koya D, et al. Association between urinary type IV collagen level and deterioration of renal function in type 2 diabetic patients without overt proteinuria. *Diabetes Care*. 2010; 33 (8): 1805-1810.
  21. Kotajima N, Kimura T, Kanda T, et al. Type IV collagen as early marker for

- diabetic nephropathy in non-insulin-dependent diabetes mellitus. *J Diabetes complications*. 2000; 14 (1): 13-17.
22. Kado S, Aoki A, Wada S, et al. Urinary type IV collagen as a marker for early diabetic nephropathy. *Diab Res Clin Prac*. 1996; 31 (1 - 3): 103-108.
23. Mauer SM, Steffes MW, Ellis EN, Sutherland DE, Brown DM, Goetz FC. Structural-functional relationships in diabetic nephropathy. *J Clin Invest*. 1984; 74 (4): 1143–1155.
24. Doi T, Mima A, Matsubara T, Inaga T, Arai H, Abe H. The current clinical problems for early phase of diabetic nephropathy and approach for pathogenesis of diabetic nephropathy. *Diabetes Res Clin Pract*. 2008; 82 (suppl 1): S21-S24
25. Mima A, Arai H, Matsubara T, et al. Urinary Smad1 is a Novel Marker to Predict Later Onset of Mesangial Matrix Expansion in Diabetic Nephropathy. *Diabetes*. 2008; 57 (6): 1712–1722
26. Perkins BA, Ficociello LH, Ostrander BE, et al. Microalbuminuria and the Risk for Early Progressive Renal Function Decline in Type 1 Diabetes. *J Am Soc Nephrol*. 2007; 18 (4): 1353–1361.
27. Babazono T, Nyumura I, Toya K, et al. Higher levels of urinary albumin excretion within the normal range predict faster decline in glomerular filtration rate in diabetic patients. *Diabetes Care*. 2009; 32 (8): 1518-1520.
28. Uchigata Y, Asao K, Matsushima M, et al. Impact on mortality and incidence of end-stage renal disease of education and treatment at a diabetes centre among patients with type 1 diabetes: comparison of two subgroups in the Japanese DERI cohort. *J Diabetes complications*. 2004; 18 (3): 155-159.

## **Titles and legends**

**Figure 1.** Flow diagram of the study population

**Figure 2.** Correlation between urinary type IV collagen and the annual rate of change in eGFR

**Figure 3.** Comparison of the annual rate of change in estimated glomerular filtration rate (eGFR) among the 4 groups classified into normal or elevated levels of type 4 collagen-to-creatinine ratio (T4C) and albumin-to-creatinine ratio (ACR). In multivariate analysis, the annual rate of change in eGFR were adjusted by age, sex, duration of diabetes, presence of retinopathy, eGFR at baseline, and mean values during follow-up period of hemoglobin A1c (HbA1c), systolic blood pressure, body mass index, and total cholesterol (T-Cho) (analysis of covariance, ANCOVA). \*  $p < 0.05$  versus “normal T4C and normal ACR”.

Data are means  $\pm$  standard error.

**Table 1 Clinical and biochemical characteristics of patients with type 1 diabetes**

	Normoalbuminuria	Microalbuminuria
<b>Baseline</b>	N = 213	N = 18
Follow-up period (years)	7.3 ± 1.3	7.6 ± 1.4
Age (years)	23.6 ± 7.4	28.4 ± 5.9*
Male (%)	36.2	44.4
Duration of diabetes(years)	10.0 ± 7.4	18.0 ± 6.9*
BMI (kg/m <sup>2</sup> )	21.9 ± 3.5	23.2 ± 3.0
Systolic blood Pressure (mmHg)	125 ± 14	132 ± 20*
Diastolic blood Pressure (mmHg)	77 ± 10	85 ± 10*
Retinopathy (%)	13.4	72.2*
Use of antihypertensive drugs (%)	4.2	33.3*
CCB / RASB (%)	44.4 / 77.8	50.0 / 50.0
Use of anti-lipid drugs (%)	2.8	27.8*
Laboratory data		
HbA1c (%)	7.3 ± 1.4	9.0 ± 2.2*
T-Cho (mg/dl)	185 ± 33	200 ± 39
eGFR (ml/min/1.73m <sup>2</sup> )	114.0 ± 20.4	115 ± 32
T4C(µg/g Cr)	2.7 (1.5-3.9)	3.3 (2.3-5.3)
ACR (mg/g Cr)	6.0 (4.0-10.6)	86.6 (40.5-158.7)*
<b><u>Mean during the follow up period</u></b>		
BMI (kg/m <sup>2</sup> )	22.8 ± 3.3	23.7 ± 3.2
Systolic blood Pressure (mmHg)	126 ± 12	135 ± 15*
Diastolic blood Pressure (mmHg)	77 ± 8	84 ± 8*
Laboratory data		
HbA1c (%)	7.4 ± 1.1	8.7 ± 1.1*
T-Cho (mg/dl)	185 ± 24	204 ± 21*

Data are mean ± SD for normally distributed variables and median (25th -75th interquartiles) for skewed variables unless otherwise indicated. \*P< 0.05 vs. normoalbuminuria.

CCB : Ca channel blocker, RASB: renin angiotensin system blocker