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Glucometabolic characteristics and higher vascular complication risk in Korean patients with type 2 diabetes with non-albumin proteinuria

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ABSTRACT

Objective: We investigated the clinical relevance of non-albumin proteinuria (NAP) in Korean patients with type 2 diabetes (T2D).
Research design and methods: We enrolled 883 T2D patients who had both their urinary albumin-to-creatinine ratio (uACR) and protein-to-creatinine ratio (uPCR) measured. We classified the patients into non-proteinuria (NP; uPCR <150 mg/g and uACR <30 mg/g), isolated NAP (iNAP; uPCR ≥150 mg/g and uACR <30 mg/g), and albuminuria (uACR ≥30 mg/g) groups. The associations between uPCR, uACR, and several indices of glucose metabolism were investigated.
Results: The glucometabolic pathophysiology of iNAP (96 [10.9%]) group was more associated with a decrease in homeostatic model assessment (HOMA)-beta value (aOR 1.89 [95% CI, 1.21–2,96]) than with an increase in HOMA-insulin resistance (aOR 1.29 [95% CI, 0.83–2.01]). uPCR ≥150 mg/g was also found to have more consistent and stronger association with vascular complications than uACR ≥30 mg/g (aOR 1.44 [95% CI, 1.03–2.02] vs. 1.26 [95% CI, 0.89–1.79]).
Conclusions: The nephropathy of iNAP may be mainly attributed to decreased beta cell function. Furthermore, uPCR might be a more sensitive urinary biomarker than uACR for the detection of vascular complications in T2D patients.

1. Introduction

Diabetic kidney disease (DKD) affects about 40% of diabetic patients, and is a major contributor to the progression of chronic kidney disease (CKD) to end stage renal disease.[1,2] Since albuminuria reflecting impairment of the glomerulus is known to have many advantages in DKD screening and evaluation,[3] urinary albumin-to-creatinine ratio (uACR) of 30 mg/g or more is the standard index that is mainly used for DKD screening. However, in non-diabetic kidney disease, total proteinuria is also often used as indicators.[4] Both albuminuria evaluated by uACR and proteinuria evaluated by protein-to-creatinine ratio (uPCR), which correlate well with 24 hour urine collection,[5] have been found to be closely associated with cardiovascular mortality and CKD progression.[6–8] Recently, there have also been reports that uPCR, which includes non-albumin proteinuria (NAP), can be a more sensitive screening tool for prediction of CKD progression compared to uACR.[9] It has been found that NAP is related to tubulointerstitial pathology, and low urinary albumin-to-total urinary protein ratio (uAPR) has been strongly associated with tubulointerstitial disease in renal biopsies.[10]

Although the diagnostic or screening value of NAP in subjects with type 2 diabetes (T2D) is controversial, NAP is gaining popularity on diabetic complications index, which is difficult to be explained by albuminuria. Subjects with DKD but without albuminuria have been confirmed in several autopsy results.[11] In addition, the number of T2D patients with decreased renal function and without albuminuria has increased, together with a corresponding increase in their relative mortality rates.[12] The improvements in blood glucose and blood pressure control due to the development of effective drugs, and increased use of medicines based on the inhibition of renin-angiotensin aldosterone system (RAAS), may have contributed to these changes. Although these drugs are currently the most effective at preventing glomerular

Abbreviations: uACR, urinary albumin-to-creatinine ratio; AHA, American Heart Association; uAPR, urinary albumin-to-total urinary protein ratio; BMI, body mass index; CKD, chronic kidney disease; DKD, diabetic kidney disease; GA, glycated albumin; eGFR, estimated glomerular filtration rate; HOMA, homeostatic model assessment; HOMA-IR, homeostasis model assessment of insulin resistance; KDIGO, Kidney Disease: Improving Global Outcomes; MDRD, Modification of Diet in Renal Disease Study equation; MS, metabolic syndrome; NAG, *N*-acetyl-β-D-glucosaminidase; NAP, non-albumin proteinuria; iNAP, isolated NAP; NHLBI, National Heart, Lung, and Blood Institute; NP, non-proteinuria; uPCR, urinary protein-to-creatinine ratio; RAAS, renin-angiotensin aldosterone system; WC, waist circumference.

Competing interests: The authors have no potential conflicts of interest to disclose.

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injury, renal tubular disease can eventually lead to the loss of renal function and mortality.

Nonetheless, little is known about the relative distributions of albuminuria and NAP in patients with T2D, as well as the demographic and clinical associations of NAP and its prognostic significance. This study aimed to investigate proteinuria assessments in patients with T2D by determining the clinical importance of NAP without albuminuria.

2. Methods

2.1. Subjects

By reviewing patient documents using electronic medical records at Severance Hospital, we enrolled patients ≥19 years old with T2D who had undergone both mixed meal tolerance test to evaluate for blood glucometabolic parameters and test for urinary markers including *N*-acetyl-β-D-glucosaminidase (NAG), albumin, protein, and creatinine simultaneously from July 2015 to December 2017 in Severance Hospital. T2D patients were defined according to the International Classification of Diseases 10th revision. Patients were excluded if they fulfilled any one of the following criteria: 1) were <19 years of age; 2) had type 1 diabetes; 3) were pregnant; 4) had renal diseases other than DKD; 5) were on renal replacement therapy including renal transplantation; or 6) did not fully satisfy the inclusion criteria.

Age, sex, weight, height, waist circumference (WC), blood pressure, duration of diabetes, and current medications were recorded. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2), and evaluated according to the World Health Organization classification (<25 kg/m^2 = underweight/normal, ≥25 kg/m^2 = overweight/obese). Vascular disease was defined as a positive history of myocardial infarction, proven single- or multi-vessel coronary artery disease with or without symptoms of angina pectoris, proven peripheral artery obstructive disease, and ischemic stroke. History of vascular disease had to be documented by hospital records. The diagnosis of metabolic syndrome (MS) followed the definition of the American Heart Association (AHA) and the National Heart, Lung, and Blood Institute (NHLBI) statements for Asian populations in 2005.[13] Hypertension was defined as a systolic blood pressure ≥130 mmHg and/or a diastolic blood pressure ≥85 mmHg, or current use of antihypertensive medications following the AHA/NHLBI scientific statement. Taking a RAAS inhibitor was defined as treatment with any dose of angiotensin converting enzyme inhibitor or angiotensin receptor blocker for over 3 months.

This study of retrospectively reviewed medical records was approved by the independent institutional review board of Severance Hospital (4-2018-0311), and was waived the requirement of informed consent. This study adhered to the tenets of the Declaration of Helsinki.

3. Materials and methods

Following an overnight fast (≥8 h), spot urine and blood samples before (0 min, designated as fasting) and after (90 min, designated as stimulated) ingestion of two cans (total 400 mL, 400 kcal, 18 g fat, 44 g carbohydrate, and 20 g protein) of a standardized mixed meal (Mediwell Diabetic Meal, Meal Dairies Co, Yeongdong-gun, Chungbuk, Republic of Korea) were obtained to measure HbA1c, glycated albumin (GA), basal and stimulated glucose/insulin/C-peptide, and other chemistry profiles. Pancreatic β-cell function and insulin sensitivity were assessed using homeostasis model assessment (HOMA) and homeostasis model assessment of insulin resistance (HOMA-IR).[14] A low HOMA-beta value was defined as being below the median value of 43.5 in this study. A high HOMA-IR value was defined as being above the median value of 3.0, based on the entire study population. eGFR was calculated using the Modification of Diet in Renal Disease Study equation (MDRD).[15] For the data of enrolled patients, we further collected the data of those who were measured after 1 year of follow-up with laboratory tests focused on eGFR, and calculated the declined percentages of eGFR.

Urinary NAG, albumin, and protein levels were adjusted according to the levels of urinary creatinine, and were expressed as NAG-to-creatinine ratio, uACR, and uPCR, respectively. We defined proteinuria as having a uPCR ≥150 mg/g, according to the most conservative reported normal value for urinary protein excretion of <150 mg/day.[16] Albuminuria was defined as having a uACR ≥30 mg/g, as according to the Kidney Disease: Improving Global Outcomes (KDIGO) recommendation.[17] NAP was indirectly calculated from the difference between uPCR and uACR using the following formula: NAP (mg/g) = uPCR (mg/g) − uACR (mg/g).[18] Isolated NAP (iNAP) and non-proteinuria (NP) were defined as having both a uPCR ≥150 mg/g and a uACR <30 mg/g,[19] and having both a uPCR <150 mg/g and a uACR <30 mg/g, respectively. The urinary albumin-to-protein ratio (uAPR) was calculated as uACR divided by uPCR, and a uAPR <0.4 used as cut-off as it was identified to indicate primary tubulointerstitial disorders.[20] CKD was defined as being under CKD stage 3 (eGFR <60 mL/min per 1.73 m^2).

Data are presented as the mean ± standard deviation (SD) for normally distributed continuous variables, median (interquartile range) for non-normally distributed continuous variables, and as a number or percentage for categorical variables. Spearman correlation coefficient was used for correlating uPCR and uACR. We analyzed participants' characteristics according to the status of proteinuria, using one-way analysis of variance (ANOVA) or Kruskal-Wallis test to compare continuous variables, and $\chi 2$ test to compare categorical variables, followed by post hoc analyses using the Bonferroni procedure for ANOVA and Dunn procedure for Kruskal-Wallis test. In subgroup analysis, Mann-Whitney U or two-sample Student's *t*-tests were used for continuous variables. Multiple logistic regression analysis was performed to evaluate the clinical significance of iNAP group and the role of uPCR for the prediction of vascular complications. Several related factors were calibrated in various adjusted models. Adjusted odds ratios (aORs) and 95% confidence intervals (CIs) were determined.

Statistical analyses were performed using IBM SPSS statistical software for Windows, version 23.0 (IBM, Armonk, NY, USA). A *p*-value <0.05 was considered statistically significant.

4. Results

4.1. Clinical characteristics of patients and prevalence of proteinuria

In this study, we enrolled 883 T2D patients (516 men and 367 women) who had undergone both the mixed meal test and urine test for uACR and uPCR measurements on the same day. The mean age and median duration of diabetes were 60.3 and 2.0 years, respectively. Based on the above-mentioned definitions in the Methods section, we classified the patients into non-proteinuria (NP) (531 (60.1%)), iNAP (96 (10.9%)) and albuminuria (256 (29.0%)) groups (Fig. 1a). With the exclusion of albuminuria group from the total population of the study, iNAP group accounted for 15.3% of the total population (Fig. 1b). The relationship between uPCR and uACR showed a moderately strong linear correlation overall (Spearman correlation, $r = 0.768$, Supplementary Fig. 1a), but only a fair one (Spearman correlation, $r = 0.451$) in non-albuminuria cases (uACR <30 mg/g, Supplementary Fig. 1b).

Table 1 shows the demographic and laboratory characteristics of the participants of our study cohort. Compared to NP group, the groups with iNAP and albuminuria showed differences in the proportion of females, longer duration of diabetes, poorer glucometabolic parameters, decreased eGFR, increased uNAG, uACR, uPCR, and higher prevalence of vascular disease (all $p < 0.05$). Regarding glucometabolic pathophysiology, iNAP group showed a decrease in HOMA-beta value, but no increase in HOMA-IR. However, albuminuria group showed a decrease in HOMA-beta values as well as an increase in HOMA-IR values.

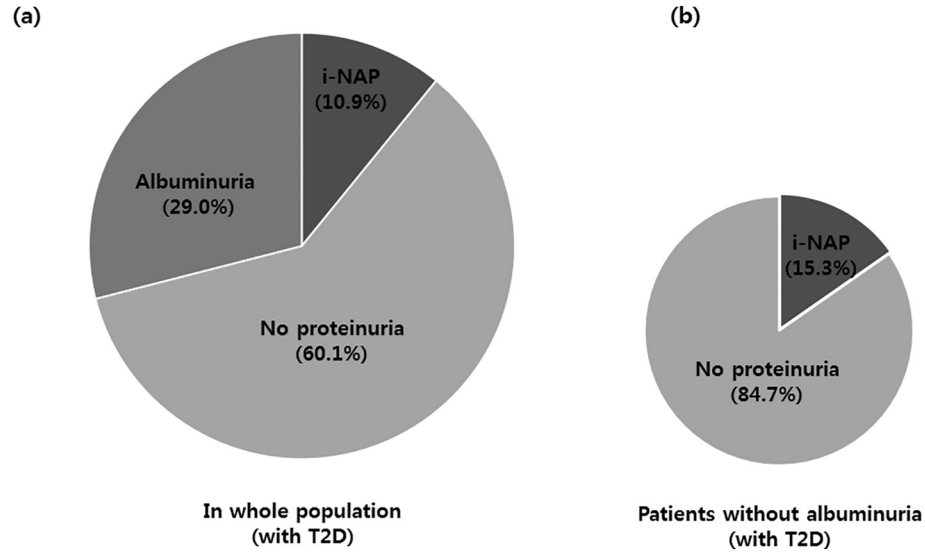

Fig. 1. Proportion of study subjects according to the presence of proteinuria. Among 883 type 2 diabetes patients, we classified the patients into iNAP (96 (10.9%), high or very high uPCR without albuminuria), albuminuria (256 (29.0%), high or very high uACR) and non-proteinuria (NP) (531 (60.1%), normal ranges of uACR and uPCR) groups. The patients of iNAP group increased up to 15.3% after non-albuminuria subgroup analysis.

Compared to NP group, iNAP group showed lower BMI and diastolic blood pressure (all $p < 0.05$).

When comparing iNAP and albuminuria groups, age, BMI, duration of diabetes, history of vascular disease, and glucose parameters including HbA1c, GA, fasting and stimulated glucose were similar (all $p > 0.05$). However, iNAP group had lower HOMA-IR values (3.0 (1.9–4.5) vs 3.4 (1.9–6.3)) and lower HOMA-beta values (35.0 (19.2–71.9) vs. 37.2 (19.6–66.5)) without statistical significance. The iNAP group also showed lower diastolic blood pressure, as well as higher values of eGFR compared to albuminuria group (all $p < 0.05$).

4.2. Deterioration of renal function after 1 year of follow-up

To investigate the changes in renal function, we further collected the data of 642 enrolled patients who were measured after 1 year of follow-up with laboratory tests focused on eGFR and declined percentages of eGFR (Table 2). As a result, patients in both albuminuria group (27.2%) and iNAP group (24.2%) showed high rates of CKD involvement at basal. The most dominant decrease in renal glomerular filtration function was observed in albuminuria group followed by iNAP and NP groups, with decreases in eGFR >10% being most dominant in the group with albuminuria.

4.3. Odds ratios for clinical relevance of iNAP and albuminuria

We performed multivariate logistic regression to investigate the clinical relevance of iNAP and albuminuria groups by comparing them to NP group as a control (Fig. 2). After adjusting for age and sex, both albuminuria and iNAP groups showed higher risk for poor glycemic control. In albuminuria group, increase in insulin resistance (HOMA-IR) and decrease in insulin secretory function (HOMA-beta) were all significantly associated. However, in iNAP group, poor glycemic control might be attributed to a decrease in insulin secretory function (OR 1.89 (95% CI, 1.21–2.96)) rather than insulin resistance (OR 1.29 (95% CI, 0.83–2.01)). Albuminuria group was markedly associated with MS (OR 1.90 (95% CI, 1.34–2.70)), hypertension (OR 2.38 (95% CI, 1.63–3.47)), and declined renal function after 1 year of follow up (OR 2.02 (95% CI, 1.41–2.90), Supplementary Table 1).

4.4. Subgroup analysis of subjects within each group

In subgroup analysis of the subjects within NP group who did not meet the criteria of proteinuria defined in this study, we further divided the NP patients into two subgroups based on the median value of uPCR of 85 mg/g. There were no significant differences in insulin resistance and insulin secretory function index, and we also found no significant differences in the prevalence of diseases such as vascular disease, hypertension, and MS (all $p > 0.05$, Table 3). Although uPCR was not higher than 150 mg/g, a poorer glycemic control pattern was seen in the subgroup with higher uPCR (over 85 mg/g). Urinary NAG and ACR were also markedly higher in the higher uPCR group (over 85 mg/g). In addition, there was a higher risk of renal dysfunction after 1 year of follow up in the higher uPCR (85 mg/g ≤ uPCR < 150 mg/g) group ($p = 0.012$, Table 3).

We performed further subgroup analyses by dividing the albuminuria group into two groups based on the uAPR (ratio of uACR to uPCR) of 0.4, which is one of the indicators of tubulointerstitial disease.[20] In the subgroup with uAPR <0.4, the level of glycated albumin was higher ($p = 0.041$) and it seemed to be related to the decrease in HOMA-beta values ($p = 0.028$) rather than HOMA-IR ($p = 0.392$). The group with uAPR ≥0.4 showed significantly longer duration of diabetes, higher prevalence of hypertension, higher level of triglyceride, decreased eGFR, and higher uACR. In addition, more patients with uAPR ≥0.4 showed decrease in eGFR after 1 year in this group (all $p < 0.05$, Supplementary Table 2).

4.5. Odds ratios for presence of vascular disease by uPCR and uACR

The risk for comorbidity of vascular disease was similarly elevated in both albuminuria and iNAP groups without statistical significance (Supplementary Table 1). Therefore, we performed multivariate logistic regression to investigate the association between accompanied vascular disease and related factors including age, sex, BMI, and history of hypertension according to uPCR ≥150 mg/g and uACR ≥30 mg/g (Table 4). In an unadjusted model (Model 1), both uPCR over 150 mg/g (OR 1.57 (95% CI, 1.14–2.17)) and uACR over 30 mg/g (OR 1.47 (95% CI, 1.47 (1.01–2.05))) were significantly associated with the comorbidity of vascular disease. After sequentially adjusting for age, sex (Model 2), BMI (Model 3), and history of hypertension (Model 4), uPCR over 150 mg/

Table 1
Baseline clinical characteristics of patients.

Total	Non-proteinuria	Isolated NAP	Albuminuria	*p* value
N = 883	*N* = 531	*N* = 96	*N* = 256	
Female, n (%)	**221 (41.6)**	**50 (52.1)**	**96 (37.5)**	**0.047**‡
Age, years	59.3 ± 17.6	61.5 ± 14.5	61.9 ± 13.4	0.083
BMI, kg/m²	**25.8 ± 3.7**	**24.7 ± 5.2***	**25.3 ± 4.4**	**0.021**
WC, cm	89.7 ± 9.5	86.8 ± 9.6	89.9 ± 9.7	0.051
Duration of diabetes, years	**1.0 (0–7.0)**	**4.0 (0–15.0)***	**5.0 (0–14.0)***	**<0.001**
Systolic BP, mmHg	128.1 ± 49.0	120.4 ± 15.5	131.2 ± 16.1	0.076
Diastolic BP, mmHg	**79.1 ± 11.2**	**72.3 ± 10.7***	**79.3 ± 11.5**†	**<0.001**
Hypertension, n (%)	**351 (66.1)**	**61 (63.5)**	**213 (83.2)**	**<0.001**‡
Use of ACEi or ARB, n (%)	**110 (20.7)**	**31 (32.3)**	**86 (33.6)**	**<0.001**‡
Vascular disease, n (%)	**104 (19.6)**	**26 (27.1)**	**71 (27.7)**	**0.022**‡
Metabolic syndrome, n (%)	**349 (65.7)**	**62 (64.6)**	**201 (78.5)**	**0.001**‡
Glucose, fasting, mg/dL	**126.0 (111.0–151.0)**	**141.5 (112.5–181.5)***	**149.0 (120.0–201.0)***	**<0.001**
Glucose, stimulated, mg/dL	**180.0 (143.0–220.0)**	**218.0 (170.8–265.0)***	**223.0 (170.0–276.0)***	**<0.001**
Glycated albumin, %	**16.7 (14.6–19.6)**	**20.0 (16.7–25.8)***	**20.4 (16.4–26.2)***	**<0.001**
HbA1c				**<0.001**
%	**6.8 (6.2–7.7)**	**7.6 (6.5–9.3)***	**7.7 (6.5–9.4)***	
mmol/mol	**50.8 (44.3–60.7)**	**59.6 (47.5–78.1)***	**60.7 (47.5–79.2)***	
HbA1c over 7.0%, n (%)	**241 (45.6)**	**64 (67.4)**	**164 (64.1)**	**<0.001**‡
HbA1c over 9.0%, n (%)	**63 (11.9)**	**29 (30.5)**	**82 (32.0)**	**<0.001**‡
Glycated albumin/HbA1c	**2.5 ± 0.5**	**2.7 ± 0.6**	**2.8 ± 1.7***	**0.001**
HOMA IR	**2.8 (1.7–4.5)**	**3.0 (1.9–4.5)**	**3.4 (1.9–6.3)***	**0.005**
HOMA IR ≥ 3.0 (High), n (%)	243 (45.8)	49 (51.0)	139 (54.3)	0.072
HOMA beta (%)	**47.8 (27.3–76.1)**	**35.0 (19.2–71.9)***	**37.2 (19.6–66.5)***	**<0.001**
HOMA beta (low), n (%)	**235 (44.3)**	**56 (58.3)**	**150 (58.6)**	**<0.001**‡
AST, IU/L	24.6 ± 12.8	23.7 ± 13.3	25.1 ± 15.7	0.668
ALT, IU/L	28.4 ± 21.4	24.5 ± 14.3	26.4 ± 18.3	0.146
Uric acid, mg/dL	**4.9 ± 1.3**	**4.7 ± 1.7**	**5.3 ± 1.7***†	**<0.001**
Albumin, mg/dL	**4.3 ± 0.3**	**4.1 ± 0.4***	**4.2 ± 0.4***	**<0.001**
Total cholesterol, mg/dL	166.0 (140.0–197.0)	154.5 (126.8–203.3)	166.0 (132.0–203.0)	0.216
Triglyceride, mg/dL	**118.0 (87.0–165.0)**	**118.5 (83.5–173.5)**	**128.0 (93.0–193.0)***	**0.030**
HDL-cholesterol, mg/dL	**44.0 (38.0–53.0)**	**44.5 (35.0–57.0)**	**42.0 (36.0–51.0)***	**0.031**
LDL-cholesterol, mg/dL	**92.2 (68.6–120.6)**	**82.3 (60.2–112.4)**	**87.0 (61.0–115.8)**	**0.046**
eGFR CKD-MDRD, mL/min/1.73 m²	**97.3 ± 23.5**	**95.9 ± 37.7**	**82.1 ± 35.9***†	**<0.001**
CKD (eGFR < 60 mL/min/1.73 m²), n (%)	**16 (3.0)**	**20 (20.8)**	**69 (27.0)**	**<0.001**‡
Urinary NAG (U/g creatinine)	**6.5 (4.5–9.5)**	**12.2 (8.7–19.3)***	**12.0 (8.1–22.2)***	**<0.001**
Urinary ACR (mg/g creatinine)	**6.5 (3.2–11.3)**	**14.0 (8.1–22.6)***	**96.4 (48.8–375.6)***†	**<0.001**
Urinary PCR (mg/g creatinine)	**80.0 (70.0–100.0)**	**180.0 (152.5–227.5)***	**340.0 (190.0–817.5)***†	**<0.001**

Data are expressed as mean ± SD for normally distributed continuous variables, median (interquartile range) for non-normally distributed continuous variables, and number (%) for categorical variables.
Abbreviations: NAP, non-albumin proteinuria; BMI, body mass index; WC, waist circumference; BP, blood pressure; ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; HOMA, homeostatic model assessment; IR, insulin resistance; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; eGFR, estimated glomerular filtration rate; CKD, chronic kidney disease; MDRD, modification of diet in renal disease study equation; NAG, *N*-acetyl-beta-D-glucosaminidase; ACR, albumin-to-creatinine ratio.
NOTE. Bold text indicates *p* values <0.05.
* *p* values <0.05 vs. non-proteinuria, by post hoc analyses (Bonferroni tests or Dunn procedure).
† *p* values <0.05 vs. iNAP, by post hoc analyses (Bonferroni tests or Dunn procedure).
‡ Significant chi-square tests, *p* values <0.05.

Table 2
Deterioration of renal function after 1 year of follow-up.

	Non-proteinuria	Isolated NAP	Albuminuria	*p* value
	N = 381	*N* = 66	*N* = 195	
Female, n (%)	155 (40.7)	36 (54.5)	74 (37.9)	0.057
Age, years	59.5 ± 18.7	62.8 ± 13.3	61.5 ± 13.5	0.188
eGFR CKD-MDRD, mL/min/1.73 m²	**96.3 ± 22.8**	**90.8 ± 33.5**	**81.5 ± 36.8***	**<0.001**
CKD (eGFR <60 mL/min/1.73 m²), n (%)	**14 (3.7)**	**16 (24.2)**	**53 (27.2)**	**<0.001**‡
After 1 year				
eGFR CKD-MDRD after 1 year, mL/min/1.73 m²	**92.9 ± 22.5**	**84.9 ± 25.6**	**73.6 ± 33.0***	**<0.001**
CKD (eGFR <60 mL/min/1.73 m²), n (%)	**13 (3.4)**	**10 (15.2)**	**67 (34.4)**	**<0.001**‡
Newly developed CKD (eGFR <60 mL/min/1.73 m²), n (%)	**4 (1.0)**	**1 (1.5)**	**20 (10.3)**	**<0.001**‡
Decline of eGFR after 1 year (%)	**−2.0 ± 18.1**	**−2.5 ± 20.7**	**−8.4 ± 21.8***†	**0.001**
Deteriorated eGFR over 10% after 1 year, n (%)	**115 (30.2)**	**24 (36.4)**	**90 (46.2)**	**0.001**‡

Data are expressed as mean ± SD for continuous variables and number (%) for categorical variables.
Abbreviations: eGFR, estimated glomerular filtration rate; CKD, chronic kidney disease; MDRD, modification of diet in renal disease study equation.
NOTE. Bold text indicates *p* values <0.05.
* *p* values <0.05 vs. non-proteinuria, by post hoc analyses (Bonferroni tests or Dunn procedure).
† *p* values <0.05 vs. iNAP, by post hoc analyses (Bonferroni tests or Dunn procedure).
‡ Significant chi-square tests, *p* values <0.05.

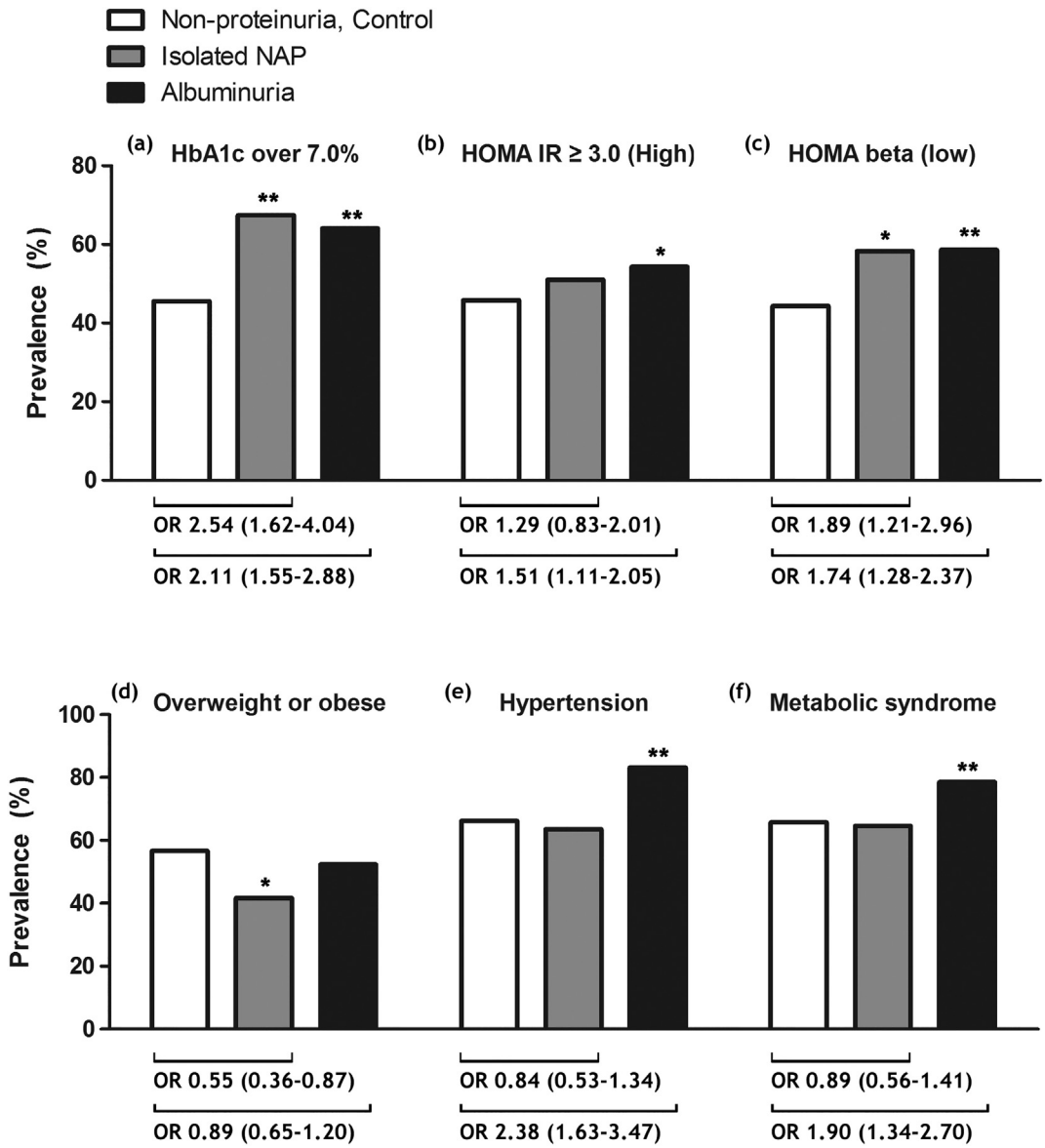

Fig. 2. Prevalence and odds ratios of clinical parameters between iNAP and albuminuria groups. Prevalence of several clinical parameters including (a) HbA1c over 7.0%, (b) HOMA-IR ≥3.0, (c) low HOMA-beta value (compared to median value), (d) overweight/obese (BMI ≥25.0), (e) hypertension, and (f) metabolic syndrome according to proteinuria status. Multiple logistic regression analysis was performed to evaluate the clinical significance of the iNAP group. Age and sex were adjusted. **Abbreviations:** NAP, non-albumin proteinuria; HOMA, homeostatic model assessment; IR, insulin resistance; BMI, body mass index. * p values <0.05; ** p values <0.001.

g was found to have more consistent and stronger association with vascular disease than uACR over 30 mg/g.

5. Discussion

In this study, we found that T2D patients with iNAP comprised 10.9% of all T2D patients. Moreover, the categorized understanding of albuminuria and iNAP might provide additional clinical information for the management of patients with T2D. We have elucidated three main findings in this study. First, the decrease in renal glomerular filtration function was observed not only in albuminuria group but also in iNAP group. Second, despite similar poor glucose parameters between T2D patients with iNAP and those with albuminuria, the metabolic pathophysiology of those with iNAP were less associated with insulin resistance and MS. Third, compared to albuminuria, proteinuria was a more sensitive urinary biomarker in predicting the vascular complications in Korean patients with T2D.

Urinary albumin measurement is widely accepted as a screening test for the detection of CKD, especially in DKD; however, eGFR and albuminuria have some limitations in estimating the risk of DKD progression in the early stages of DKD.[21] Similarly, previous studies demonstrated that the relationship of albuminuria to end-stage renal disease and cardiovascular events begin even below the cutoff of albuminuria (≥30 mg/g) as a continuum.[22] Therefore, a more optimal measurement for estimating the early stage and level of DKD is needed to reflect hidden or subclinical kidney damage in T2D patients. Furthermore, there is accumulating evidence that uPCR is highly sensitive in evaluating and monitoring kidney function and identifying kidney damage, and is not significantly different from uACR in predicting renal outcome and mortality in CKD patients.[9,20] In the present study, 10.9% of T2D patients had either high or very high proteinuria with normo-albuminuria (<30 mg/g). In other words, a simple screening using only uACR failed to identify 10.9% of patients with significant proteinuria who could be identified by additional uPCR measurements. It has been reported that the

Table 3
Clinical characteristics of patients in non-proteinuria group.

	Subgroup1 - Ref NP, uPCR <85	Subgroup 2 NP, uPCR ≥85	*p* value
	N = 265	*N* = 266	
Female, n (%)	**97 (36.6)**	**124 (46.6)**	**0.019**
Age, years	56.2 ± 12.0	62.3 ± 21.4	0.153
BMI, kg/m^2	26.1 ± 3.7	25.6 ± 3.6	0.694
WC, cm	89.9 ± 9.4	89.4 ± 9.7	0.840
Duration of diabetes, years	1.0 (0–6.0)	1.0 (0–7.0)	0.581
Systolic BP, mmHg	125.9 ± 13.4	130.3 ± 67.8	0.184
Diastolic BP, mmHg	79.3 ± 11.5	78.9 ± 11.0	0.767
Hypertension, n (%)	165 (62.3)	186 (69.9)	0.062
Use of ACEi or ARB, n (%)	49 (18.5)	61 (22.9)	0.207
Vascular disease, n (%)	49 (18.5)	55 (20.7)	0.526
Metabolic syndrome, n (%)	170 (64.2)	179 (67.3)	0.446
Glucose, fasting, mg/dL	**124.0 (110.0–146.5)**	**129.0 (111.8–159.0)**	**0.049**
Glucose, stimulated, mg/dL	**175.0 (139.5–203.0)**	**190.5 (151.0–229.0)**	**0.004**
Glycated albumin, %	**16.0 (14.3–18.8)**	**17.4 (14.8–20.3)**	**0.001**
HbA1c, %	**6.7 (6.2–7.3)**	**7.1 (6.3–8.2)**	**<0.001**
HbA1c over 7.0%, n (%)	**97 (36.9)**	**144 (54.3)**	**<0.001**
HbA1c over 9.0%, n (%)	**21 (8.0)**	**42 (15.8)**	**0.005**
Glycated albumin/HbA1c	2.5 ± 0.5	2.5 ± 0.4	0.889
HOMA IR	2.6 (1.6–4.3)	3.0 (1.7–4.7)	0.080
HOMA IR ≥ 3.0 (high), n (%)	113 (42.6)	130 (48.9)	0.150
HOMA beta (%)	47.9 (27.0–74.5)	47.8 (27.5–77.0)	0.870
HOMA beta (low), n (%)	117 (44.2)	118 (44.4)	0.961
eGFR CKD-MDRD, mL/min/1.73 m^2	**94.9 ± 20.5**	**99.6 ± 25.9**	**0.020**
CKD (eGFR <60 mL/min/1.73 m^2), n (%)	9 (3.4)	7 (2.6)	0.606
Deteriorated eGFR over 10% after 1 year, n/total N (%)	**47/193 (24.4)**	**68/188 (36.2)**	**0.012**
Urinary NAG (U/g creatinine)	**5.3 (3.7–7.4)**	**8.1 (5.7–11.7)**	**<0.001**
Urinary ACR (mg/g creatinine)	**4.9 (2.5–7.6)**	**8.9 (5.1–14.8)**	**<0.001**
Urinary PCR (mg/g creatinine)	**70.0 (60.0–80.0)**	**100.0 (90.0–120.0)**	**<0.001**

Data are expressed as mean ± SD for normally distributed continuous variables, median (interquartile range) for non-normally distributed continuous variables, and number (%) for categorical variables.
Abbreviations: NP, non-proteinuria, uPCR, urine protein-to-creatinine ratio, BMI, body mass index, WC, waist circumference, BP, blood pressure, ACEi, angiotensin-converting enzyme inhibitor, ARB, angiotensin receptor blocker, HOMA, homeostatic model assessment, IR, insulin resistance, eGFR, estimated glomerular filtration rate, CKD, chronic kidney disease, MDRD, modification of diet in renal disease study equation, NAG, *N*-acetyl-beta-D-glucosaminidase, ACR, albumin-to-creatinine ratio.
NOTE. Bold text indicates *p* values <0.05.

prevalence of iNAP in patients was 10.1% (15.2% for females and 4.7% for males) in the United States. Moreover, it was increased up to 20.0% (27.3% for females and 10.7% for males) among patients with normal random albumin.[23] These results are similar to those of our study, which included only T2D patients.

Regarding the pathophysiologic relevance of iNAP, a possible explanation might stem from the tubular hypothesis in renal hyperfiltration, which hypothesizes that damage to the proximal tubule may precede damage to the glomerulus,[24] suggesting that increased NAP may occur in the early stages of DKD progression without albuminuria. Urinary NAG, one of the markers of proximal tubule injury, has been reported to be closely associated with glycemic control[25] and diabetic vascular complications[26] in T2D patients. In our study, uNAG increased in iNAP group as well. In addition, even in the group without significant albuminuria or proteinuria, the increase in uNAG was observed in the higher uPCR group (over 85 mg/g).

Table 4
Odds ratios for the presence of vascular disease by urinary PCR and urinary ACR.

	uACR over 30 mg/g	*p* value	uPCR over 150 mg/g	*p* value
Model 1	**1.47 (1.01–2.05)***	**0.025**	**1.57 (1.14–2.17)***	**0.005**
Model 2	1.31 (0.93–1.86)	0.123	**1.43 (1.03–1.99)***	**0.035**
Model 3	1.33 (0.94–1.89)	0.106	**1.48 (1.06–2.07)***	**0.021**
Model 4	1.26 (0.89–1.79)	0.193	**1.44 (1.03–2.02)***	**0.033**

Model 1 unadjusted; Model 2 adjusted for age, sex; Model 3 adjusted for model 2 parameters + BMI; Model 4 adjusted for model 3 parameters + history of hypertension.
Abbreviations: uACR, urinary albumin-to-creatinine ratio; uPCR, urinary total protein-to-creatinine ratio; BMI, body mass index.
NOTE. Bold text indicates *p* values <0.05.
* *p* values <0.05.

Regarding the clinical relevance of iNAP, iNAP group showed poor glycemic control parameters without increased insulin resistance and prevalence of MS. In the development of albuminuria,[27] the role of hypertension is widely known to be important. On the other hand, iNAP group consisted mostly of patients without hypertension. Based on these results, we postulated that the lower insulin resistance and metabolic stress in iNAP group might have less deteriorating effects on the glomerulus. However hyperglycemia caused by dysfunctional insulin secretory function might cause increases in glucose reabsorption in the proximal tubule,[24] resulting in tubulointerstitial hypoxia, increased oxidative stress,[28] and CKD progression.[29] This chronic glucotoxicity and increased oxidative stress caused by long durations of poor glycemic control may cause tubular cell damage in iNAP group.[30] Also, in the present study, high rate of CKD progression was found in iNAP group, without albuminuria.

Recently, various efforts were made to reclassify T2D based on its highly heterogeneous features. In a large-scale data-based study, T2D patients with severe insulin resistance showed the fastest progression to very high albuminuria and CKD, but there was no significant difference in coronary events or stroke outcomes from the other groups without severe insulin resistance.[31] Also, in the present study, the association with short-term prognosis of renal dysfunction could be confirmed more clearly in albuminuria group with severe insulin resistance. However, the risk for comorbidity of vascular disease was similarly elevated in both albuminuria and iNAP groups. In addition, uPCR, which also includes NAP, showed more consistent and stronger association in predicting vascular disease associated with the progression of atherosclerosis. Evidence is accumulating on the roles of renal tubular damage as potential indicators of risk factors for cardiovascular disease.[26] Chronic hyperglycemia in T2D patients may cause the increase in systemic toxic metabolites, and lead to vascular complications.[32] In this regard, the clinical relevance of NAP measurement, which reflects tubulointerstitial pathology, should not be overlooked. Based on these findings, we suggest that the concurrent measurements of uACR and uPCR might offer an advantage in predicting deteriorated eGFR and accompanying vascular complications in Korean patients with T2D, respectively. In addition, the clinical significance of iNAP group was not limited to the early detection of DKD.

The current study had several limitations. First, since this study was a retrospective study, we could not elucidate the causal relationship between the observed findings. Only the patients experienced all of the related complications were examined, so selection bias could have occurred in this process. Second, single rather than repeated measurements of uPCR could have low positive predictive value for the detection and segregation of iNAP group.[19] Third, since the follow-up period was only up to 1 year, we were not able to confirm whether a significant number of patients in iNAP group could develop renal dysfunction after a longer period of follow-up. Considering the possibility that proximal tubule damage may precede glomerular damage, more people may progress to albuminuria or renal dysfunction after >1 year has passed. However, the current study also had several distinguishing strengths. We evaluated the metabolic characteristics of iNAP group in a large number of patients, and this study was carried out in Asians with T2D who are known to have a high risk of developing CKD[29,29] but have not been studied well on NAP issue so far. In addition, we could identify the expected clinical significance of concurrent measurement of uPCR and uACR in patients with T2D.

The identification of NAP by concurrent measurement of uPCR and uACR may provide additional clinical information in certain groups of patients with T2D, particularly in women who have had a long history of diabetes without MS. The importance of the role of tubule, as well as glomerular dysfunction, should be emphasized in the development and progression of DKD. Further research in a larger number of patients with a longer period of observation is needed to more clearly determine the effects on reducing renal function and cardiovascular disease in iNAP group.

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Appendix A. Supplementary data

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