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A Synergistic Effect of Retinopathy and Chronic Kidney Disease on Long-term Mortality in Type 2 Diabetic Inpatients with Normal Urinary Albumin or Protein: A Retrospective Cohort Study

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Running title: DR and CKD in DM with Normoalbuminuria

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Abstract

Objective: Normoalbuminuric chronic kidney disease (NA–CKD) is a distinct phenotype of diabetic kidney disease, but the role of diabetic retinopathy (DR) in predicting long-term mortality among these patients still yet to be elucidated. We aimed to investigate the effects of DR and chronic kidney disease (CKD) on mortality in type 2 diabetic patients with normoalbuminuria.

Design: We conducted this study as a retrospective cohort study.

Setting: We collected clinical information from the medical record in a public medical center in central Taiwan.

Participants: Type 2 diabetic patients (n = 665) hospitalized due to poor glucose control were consecutively enrolled and followed for a median of 6.7 years (interquartile range, 4.1–9.6 years). Patients with either urinary protein excretion >150 mg/day or urine albumin excretion >30 mg/day were excluded.

Primary outcome measure: All-cause mortality served as the follow-up outcome. Mortality data were obtained from the national registry in Taiwan.

Results: The patients with CKD and DR showed the highest mortality rate (log-rank P <0.001). The risks of all-cause mortality (HR: 2.422; 95% CI: 1.652, 3.552) and cardiovascular mortality (HR: 2.550; 95% CI: 1.469, 4.429) were significantly higher in patients with CKD and DR than those without CKD and DR after adjustment for age, gender

diabetes duration, body mass index, systolic blood pressure, total cholesterol, metformin treatment, and insulin treatment.

Conclusions: DR is an independent predictor for all-cause and cardiovascular mortality in type 2 diabetic inpatients with normoalbuminuria. In addition, there is a synergic effect of DR with CKD on all-cause and cardiovascular mortality. Fundoscopy screening can provide additive information on mortality for type 2 diabetes, even with absence of albuminuria.

Strengths and limitations of this study

- Twenty-four hour urine collection during the hospitalization
- A median follow-up period of 6.7 years
- Mortality data obtained from a National Health Insurance registry with a nationwide coverage rate of over 99% in Taiwan
- Not only normoabluminuria but also normoproteinuria included for analyses due to limited case numbers

INTRODUCTION

Diabetes is associated with microvascular complications is the leading cause of end-stage renal disease (ESRD) and blindness.[1-5] Diabetic kidney disease (DKD) is a complex and heterogeneous disease, especially in patients with type 2 diabetes.[6] Urinary albumin is an important biomarker for DKD,[7] and is predictive of all-cause and cardiovascular mortality.[8 9] Extensive resources and efforts have focused on albuminuria, and its prevalence has been significantly reduced; however, the estimated glomerular filtration rate (eGFR) has still increased.[10]

Normoalbuminuria was reported in 36% of type 2 diabetic patients with CKD in the Third National Health and Nutrition Examination Survey (NHANES) 1988–1994,[11] whereas it was reported in 126 (48.1 %) out of 262 diabetic patients with CKD in the NHANES 2005–2008.[10] The prevalence of normoalbuminuria in CKD seems to have increased 15 years later.[10 11] With the recent progress that has been made in the management of diabetic complications, it appears that the paradigm has shifted, with the phenotype of normoalbuminuric chronic kidney disease (NA–CKD) emerging.[6 12] However, the mechanism by which NA–CKD leads to cardiovascular disease and mortality remains an area of research.

Diabetic retinopathy (DR) has been traditionally recognized as an early reflection of general microangiopathy in patients with type 2 diabetes.[3 13 14] Accumulating evidence has shown that DR is a predictor of cardiovascular disease and all-cause mortality in subjects

with type 2 diabetes.[15-18] However, some studies have reported that DR is not predictive of mortality while the presence of DKD,[19 20] and the association between DR and cardiovascular disease becomes non-significant after adjusting for albuminuria.[21-23]

We aimed to investigate the mortality risk among type 2 diabetic patients without albuminuria, and we hypothesized that DR was predictive of long-term mortality in type 2 diabetic patients with NA-CKD.

METHODS

Setting and participants

This retrospective cohort study was conducted at Taichung Veterans General Hospital. Clinical data were obtained by reviewing medical records of diabetic patients hospitalized between August 1996 and August 2007. The inclusion criteria were (1) adult type 2 diabetic inpatients in the Endocrinology and Metabolism section, (2) admission due to a primary diagnosis of poor glucose control, and (3) performance of 24-hour urine collection for albumin or protein during the hospitalization. The exclusion criteria included (1) urinary protein excretion ≥ 150 mg/day or urine albumin excretion ≥ 30 mg/day,[24] (2) death during this hospitalization, (3) systolic blood pressure < 90 mmHg, (4) urine volume < 300 ml/day, and (5) the unavailability of a report documenting an eye fundal examination for retinopathy by an ophthalmologist during the hospitalization. In repeatedly hospitalized patients, data

were only recorded from the last admission during the study period. The study complies with the Declaration of Helsinki and the research protocol was approved by the Institutional Review Board of Taichung Veterans General Hospital.

Variables

Normal urinary albumin was defined as a 24-hour urine albumin excretion < 30mg.[24-26] In those inpatients with only protein detected, however, normal urine protein was defined as 24-hour urine protein < 150 mg.[24] CKD was defined as anestimated glomerular filtration rate (eGFR) < 60 mL/min/1.73m^2. The eGFR was calculated by the Modification of Diet in Renal Disease (MDRD) equation: 186 × [serum creatinine (mg/dL)]$^{-1.154}$ × [age (year)]$^{-0.203}$ (× 0.742, if female) mL/min/1.73m^2.[25]

Diabetic retinopathy (DR) included nonproliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR).[27] DR was screened using fundoscopic examinations by ophthalmologists based on formal consultations. Retinal angiography (CF-60UVi fundus camera, Canon, Japan) was subsequently arranged for confirmation of retinopathy diagnosis if there were abnormal fundoscopic findings. Hypertension was defined as blood pressure higher than 130/80 mmHg or a history of anti-hypertensive medications being prescribed. Mortality data up to December 2011 were provided by the Collaboration Center of Health Information Application, Department of Health, Executive Yuan, Taiwan.

Measurement

Biochemistry was assessed from blood samples collected after overnight fasting during hospitalization. HbA1c was determined using cation-exchange HPLC (NGSP certificated; G8, TOSOH, Tokyo, Japan). Lipids were determined using enzymatic methods (Advia 1800, Siemens, New York, USA). Creatinine was determined using the Jaffé method (Advia 1800, Siemens, New York, USA). Urine protein was determined using the dye-binding assay, and urinealbumin using the immune-turbidimetric method (Advia 1800, Siemens, New York, USA).

Statistical Analysis

Continuous data are presented as mean ± standard deviation (SD). Categorical data are presented as number (n) with percentage (%).The linear correlation of daily excretion between urine protein and albumin was determined by Spearman's rank correlation coefficient. The receiver operating characteristic (ROC) analysis curve was applied to determine the optimal cut-off point of urine protein for normoalbuminuria. The one-way analysis of variance (ANOVA) was used to determine the significance of the differences among groups. Pairwise multiple comparisons were conducted to determine the significance of the differences between two groups if a statistically significant difference was found by the

one-way ANOVA. The Chi-square test was conducted to compare categorical variables across groups. The overall significance of univariate survival analysis was detected by the log-rank test using Kaplan–Meier analysis. Cox proportional hazards regression analyses were conducted to determine the hazard ratios of risk factors. A value of $P < 0.05$ was considered statistically significant. Statistical analysis was performed using SPSS 22.0 (IBM, Armonk, NY, USA).

RESULTS

Comparison of cutoff values for daily urinary protein and albumin excretion

In 2482 diabetic inpatients who had undergone 24-hour urine collection, there were only 245 subjects who had the data for both urine albumin and protein in the same urine sample. The median daily protein excretion was 184 mg (interquartile range, 80–620 mg); and the median daily albumin excretion was 58 mg (interquartile range, 15–362 mg). There was a significant positive correlation between daily urine protein and albumin excretion ($r = 0.884$, $P < 0.001$). Using receiver operating characteristic (ROC) analysis to differentiate a normal urine albumin excretion (<30 mg/day), the area under the curve in ROC analysis was 0.956 (95% confidence interval 0.932 to 0.979, $P < 0.001$; Fig. 1). The optimum diagnostic cutoff value fordaily urine protein was 145 mg, which corresponded to a sensitivity of 93.8% and specificity of 86.5% for normoalbuminuria. Using 150 mg as diagnostic cutoff value of daily

urine protein again also gave a sensitivity of 93.8% and specificity of 86.5% for normoalbuminuria.

Comparison of the outcomes between subjects with and without fundal examination

A total of 665 patients met the criteria for enrollment in the study and were included in these analyses (Fig. 2). In comparison with the 238 patients who were eligible for all the criteria except for lacking a fundal examination for retinopathy, there were no statically significant differences in age (62 ± 14 vs. 63 ± 15 years, P = 0.777), gender (56.5% men vs. 61.3% men, P = 0.226), diabetes duration (8.7 ± 7.7 vs. 8.9 ± 8.6 years, P= 0.789), eGFR (73 ± 29 vs. 71 ± 30 mL/min/1.73m^2, P = 0.642), all-cause mortality incidence (6.6 vs. 6.5 events/100 person-years, log-rank test P = 0.888), and cardiovascular mortality (2.9 vs. 2.9 events/100 person-years, log-rank test P = 0.965).

The risk of long-term mortality in CKD or DR in subjects with normoalbuminuria

There were 229 (34.4%) subjects with CKD and 210 (31.6%) with DR in the 665 enrolled type 2 diabetic inpatients with normoabluminuria. During a median follow-up of 6.7 years (interquartile range between 4.1–9.6years), the all-cause mortality rate was higher in the subjects with CKD than in those without CKD (9.4 vs. 5.4 events/100 person-years, log-rank test P < 0.001); and the rate was also higher in the subjects with DR than those

without DR (9.0 vs. 5.6 events/100 person-years, log-rank test P < 0.001). The cardiovascular mortality rate was higher in subjects with CKD than in those without CKD (4.2 vs. 2.4 events/100 person-years, log-rank test P < 0.001); this rate was also higher in subjects with DR than in those without DR (4.5 vs. 2.2 events/100 person-years, log-rank test P < 0.001).

A synergistic effect of retinopathy and CKD on long-term mortality

The proportion of subjects with DR was not significantly different between the subjects with CKD and those without CKD (34.9% vs. 29.8%, P = 0.207). The 665 patients were separated into four groups based on CKD and DR, including (1) patients without CKD or DR in the CKD(–)DR(–) group, (2) patients with DR but not CKD in the CKD(–)DR(+) group, (3) patients with CKD but not DR in the CKD(+)DR(–) group, and (4) patients with both CKD and DR in the CKD(+)DR(+) group. Table 1 shows all the clinical characteristics of patients among these four groups. Figure 3 shows that survival rates were significantly different cross these four groups (log-rank test P < 0.001) by Kaplan–Meier analysis. The highest mortality incidence (12.4 events/100 person-years) was observed in the CKD(+)DR(+) group, which was significantly higher than the 8.1 events/100 person-years in the CKD(+)DR(–) group (P = 0.010), the 7.4 events/100 person-years in the CKD(–)DR(+) group (P = 0.004), and the 4.6 events/100 person-years in the CKD(–)DR(–) group (P < 0.001). The incidences of mortality in the CKD(+)DR(–) and CKD(–)DR(+) groups were also significantly higher than those in

the CKD(–)DR(–) group (P < 0.001 and P = 0.003, respectively). However, there was no significant difference in the mortality incidence between the CKD(+)DR(–) and CKD(–)DR(+) groups (P = 0.479).

Cox regression analysis for all-cause and cardiovascular mortalities

To identify the predictive factors for long-term mortality, univariate Cox regression analysis was conducted for all the enrolled subjects. In addition to the different groups categorized by CKD and DR, age, gender, diabetes duration, body mass index (BMI), systolic blood pressure, total cholesterol, metformin tratment and insulin treatment were significantly associated with total mortality. Using multivariate Cox regression analysis, patients with CKD and DR have the highest hazard ratio (2.422, 95% CI: 1.652–3.552) for all-cause mortality in comparison with the ones without CKD or DR after adjustment for age, gender, diabetes duration, BMI, systolic blood pressure, total cholesterol, metformin treatment and insulin treatment (Table 2A); the patients with CKD and DR also had the highest hazard ratio (2.550, 95% CI: 1.469 – 4.429) for cardiovascular mortality in comparison to the ones without CKD or DR after adjustment for age, gender, diabetes duration, BMI and systolic blood pressure (Table 2B).

DISCUSSION

In this retrospective cohort study, we found that patients with NA–CKD have a higher risk of all-cause mortality compared with patients without CKD. Furthermore, the presence of DR imposed a higher mortality risk in patients with NA-CKD.

The mortality risk associated with NA-CKD has been debated. In the Casale Monferrato study, which had an 11-year follow-up, eGFR showed a significantly inverse trend with long-term mortality only in type 2 diabetic patients with macroalbuminuria, but not in those with microalbuminuria or normoalbuminuria.[28] Conversely, several studies reported that low eGFR was significantly associated with a higher all-cause mortality risk, independent of albuminuria.[29-33] The magnitude of the impact on all-cause mortality varied. Rigalleau et al.[34] reported that NA-CKD was associated with a very low risk of dialysis or mortality in comparison with albuminuric CKD in diabetic patients during a 38-month follow-up study in France. In an Asian study with a 44-month follow-up, albuminuria was associated with a significantly higher risk of renal events, but not cardiovascular events in diabetic patients with CKD.[35] In the present study, we found that NA-CKD is associated with an approximately 1.8-fold increase in either all-cause mortality or cardiovascular disease, compared with type 2 diabetic patients without CKD. In line with our findings, Hsieh et al.[36] reported that eGFR was inversely related to risk of cardiovascular events in type 2 diabetic outpatients with normoalbuminuria during a 4-year follow-up study.

We postulated that heterogeneity in the pathogenesis of NA-CKD might contribute to

the differences in results. Different natural courses have been observed in the NA-CKD phenotype: initial albuminuria with regression to normoalbuminuria with intensive renin-angiotensin-aldosterone system (RAAS) inhibitor use in some diabetic patients, but eGFR loss might be the only manifestation of renal involvement in others.[12 37] Based on renal biopsy, tubular-interstitial lesions and arterial hyalinosis were the predominant findings in NA–CKD rather than the typical glomerulosclerotic lesions seen with albuminuria in diabetic patients.[38 39] With albuminuria regression due to the widespread use of RAAS inhibitors in recent decades, NA-CKD is being found in majority of DKD patients. The number of patients with normoalbuminuria has been found to be great than those with albuminuria in several large studies of type 2 diabetic patients with CKD.[40-42] There is an urgent need to identify a predictor of mortality among this distinct population.[43]

Although there is a high prevalence of DR in type 2 diabetic patients with albuminuria,[20] the concordance between DR and CKD was lower in patients with normoalbuminuria than in those with albuminuria.[44, 45] In the present study, we also found that the prevalence of retinopathy was not significantly different between subjects with and without CKD. However, our results showed a synergistic effect of CKD and DR on all-cause mortality and cardiovascular mortality. Compared with the NEHANS III population, in which the synergistic effect of CKD and retinopathy on morality was also observed,[46] our findings showed the evidence in type 2 diabetic inpatients even without albuminuria. Although similar

risk factors and cardiovascular effects for DR and CKD have been reported,[30 47 48] the exact mechanism for the superimposed DR effect on mortality in type 2 diabetic patients with NA–CKD still needs further investigation.

In the present study, both isolated CKD, i.e. CKD(+)DR(–), and isolated DR, i.e. CKD(–)DR(+), showed significantly higher mortality risks than those with neither CKD nor DR in univariate analyses; however, the significant difference seemed to attenuate in isolated CKD after adjustment for other traditional CV risk factors. NA-CKD has been reported to be highly associated with cardiovascular risk factors.[36 42] Therefore, the attenuation of the mortality prediction after adjustment for cardiovascular risk factors might be more obvious in isolated CKD in comparison with isolated DR. Furthermore, it is notable that a higher BMI showed a protective effect for all-cause and cardiovascular mortality in the present study. Although this seems somewhat contradictory to traditional concepts, we were not the only one to report this paradoxical effect of BMI on mortality in subjects with diabetes.[49] Cea Soriano et al.[50] reported that BMI $\geq$ 25 kg/m^2 predicted a lower mortality risk than BMI < 25 kg/m^2 in type 2 diabetic patients with CKD.

We acknowledge several limitations in our study. First, we enrolled subjects having not only normoabluminuria, but also normoproteinuria due to limited case numbers. Second, we only included type 2 diabetic inpatients who were admitted to the hospital with the primary diagnosis of poor glucose control. Third, we assessed the subjects only at baseline, but not in

follow-up. Therefore, treatment might have confounded the results after patients discharge.

In conclusion, DR is a significant predictor for all-cause and cardiovascular mortality in type 2 diabetic inpatients with normoalbuminuria. Presence of DR also showed a synergistic impact on mortality for type 2 diabetic inpatients with NA-CKD. Screening for DR and eGFR may help identify those who harbor a high mortality risk after discharge in type 2 diabetic inpatients hospitalized due to poor glucose control, even with normoalbuminuria.

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Data sharing statement: No additional data are available.

Author contributions

YL, WHS and IL contributed to the study design. YL and IL participated in the data collection. YL and IL participated in the analysis and interpretation of the data. YL drafted the manuscript. IL had full access to the data in the study. IL is the guarantor. All the authors performed a critical revision of the manuscript for important intellectual content.

FIGURE LEGENDS

Figure 1. Receiver operating characteristic (ROC) analysis curves for differentiating normoalbuminuria based on daily urinary protein excretion.

Figure 2. Flow diagram of enrollment of study subjects with normoalbuminuria.

Figure 3. Kaplan-Meier curves showing survival rates grouped based on chronic kidney disease (CKD) and diabetic retinopathy (DR) in type 2 diabetic inpatients with normoalbuminuria.

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Table 1. The clinical data of patients according to the presence CKD and DR

	CKD(–)DR(–) (n = 306)	CKD(–)DR(+) (n = 130)	CKD(+)DR(–) (n = 149)	CKD(+)DR(+) (n = 80)	P-value
Age (years)	57 ± 15	62 ± 13	69 ± 10	70 ± 9	<0.001
Male, n (%)	189 (61.8%)	63 (48.5%)	82 (55.0%)	42 (52.5%)	0.057
BMI (kg/m^2)	23.7 ± 4.5	23.3 ± 4.0	24.2 ± 4.1	24.2 ± 4.3	0.367
Systolic blood pressure (mmHg)	124 ± 14	129 ± 15	127 ± 15	128 ± 14	0.004
Diastolic blood pressure (mmHg)	74 ± 11	75 ± 10	71 ± 9	72 ± 10	0.003
Diabetes duration (years)	6.7 ± 6.7	11.4 ± 7.4	7.9 ± 7.7	13.2 ± 8.4	<0.001
Current smoker, n (%)	96 (31.4%)	36 (27.7%)	31 (20.8%)	13 (16.3%)	0.014
White blood cell count (10^6/L)	7836 ± 5193	7210 ± 2421	8258 ± 3504	8358 ± 3285	0.227
HbA1c (%)	11.5 ± 2.9	10.6 ± 2.3	10.4 ± 3.3	9.3 ± 2.6	<0.001
Total cholesterol (mmol/L)	4.8 ± 1.3	5.0 ± 1.3	4.6 ± 1.3	4.7 ± 1.5	0.197
Triglyceride (mmol/L)	1.9 ± 1.9	1.9 ± 2.2	2.0 ± 2.2	1.8 ± 1.3	0.882
HDL cholesterol (mmol/L)	1.1 ± 0.4	1.1 ± 0.4	1.0 ± 0.3	1.0 ± 0.4	0.149
eGFR (mL/min/1.73m^2)	88 ± 23	87 ± 20	45 ± 13	42 ± 15	<0.001
Hypertension, n (%)	171 (55.9%)	93 (71.5%)	113 (75.8%)	63 (78.8%)	<0.001
Antihypertensive agents, n (%)	91 (29.7%)	63 (48.5%)	90 (60.4%)	47 (58.8%)	<0.001
Oral antihyperglycemic drugs, n (%)	151 (49.3%)	65 (50.0%)	53 (35.6%)	27 (33.8%)	0.005
Insulin secretagogues, n (%)	127 (41.5%)	56 (43.1%)	46 (30.9%)	23 (28.8%)	0.027
Metformin, n (%)	95 (31.0%)	41 (31.5%)	26 (17.4%)	17 (21.3%)	0.007
Thiazolidinediones, n (%)	6 (2.0%)	5 (3.8%)	2 (1.3%)	0 (0.0%)	0.230
α-glucosidase inhibitor, n (%)	10 (3.3%)	5 (3.8%)	3 (2.0%)	2 (2.5%)	0.811
Insulin therapy, n (%)	231 (75.5%)	80 (61.5%)	70 (47.0%)	38 (47.5%)	<0.001
Statins, n (%)	28 (9.2%)	12 (9.2%)	17 (11.4%)	12 (15.0%)	0.442

BMI= body mass index, CKD= chronic kidney disease, DR= diabetic retinopathy, HbA1c= glycated hemoglobin, HDL= high-density lipoprotein.

Table 2. Results of Cox regression analysis for the effects of risk factors on (A) all-cause and (B) cardiovascular mortality

(A) All-cause mortality

	Univariate model			Multivariate model					
	Crude			Model 1			Model 2		
	HR	95%CI	P-value	HR	95%CI	P-value	HR	95%CI	P-value
CKD(-)DR(-)	1.000			1.000			1.000		
CKD(-)DR(+)	1.590	(1.179, 2.145)	0.002	1.556	(1.148, 2.108)	0.004	1.674	(1.181, 2.373)	0.004
CKD(+)DR(-)	1.842	(1.377, 2.463)	<0.001	1.416	(1.042, 1.924)	0.026	1.299	(0.910, 1.855)	0.149
CKD(+)DR(+)	2.791	(2.016, 3.866)	<0.001	2.209	(1.573, 3.101)	<0.001	2.422	(1.652, 3.552)	<0.001
Age (every 10 years)	1.455	(1.318, 1.607)	<0.001	1.328	(1.197, 1.472)	<0.001	1.313	(1.161, 1.485)	<0.001
Gender (male)	1.763	(1.394, 2.229)	<0.001	1.763	(1.388, 2.239)	<0.001	1.471	(1.111, 1.947)	0.007
Current smoker (yes/no)	1.223	(0.958, 1.561)	0.107						
Diabetes duration (every 1 year)	1.014	(1.000, 1.029)	0.046				1.005	(0.987, 1.024)	0.597
BMI (every 1 kg/m^2)	0.959	(0.931, 0.987)	0.004				0.957	(0.927, 0.989)	0.008
Systolic BP (every 10 mmHg)	1.081	(1.002, 1.167)	0.045				1.018	(0.929, 1.116)	0.697
HbA1c (every 1%)	0.969	(0.927, 1.013)	0.163						
Total cholesterol (every 1 mmol/L)	0.847	(0.769, 0.933)	<0.001				0.892	(0.801, 0.994)	0.039
Metformin (yes/no)	0.776	(0.604, 0.998)	0.049				0.806	(0.582, 1.117)	0.196
Insulin secretagogues (yes/no)	0.851	(0.677, 1.070)	0.167						
Insulin therapy (yes/no)	0.777	(0.619, 0.974)	0.029				0.959	(0.702, 1.310)	0.792
Statins (yes/no)	1.090	(0.763, 1.557)	0.635						

BMI= body mass index, BP= blood pressure, HbA1c= glycated hemoglobin, CI = confidence interval, HR = hazard ratio

(B) Cardiovascular mortality

	Univariate model			Multivariate model					
	Crude			Model 1			Model 2		
	HR	95%CI	P-value	HR	95%CI	P-value	HR	95%CI	P-value
CKD(-)DR(-)	1.000			1.000			1.000		
CKD(-)DR(+)	2.092	(1.340, 3.267)	0.001	2.039	(1.297, 3.203)	0.002	1.965	(1.201, 3.215)	0.007
CKD(+)DR(-)	1.937	(1.221, 3.072)	0.005	1.387	(0.855, 2.251)	0.185	1.393	(0.826, 2.349)	0.214
CKD(+)DR(+)	3.601	(2.215, 5.854)	<0.001	2.710	(1.634, 4.493)	<0.001	2.550	(1.469, 4.429)	<0.001
Age (every 10 years)	1.615	(1.377, 1.894)	<0.001	1.445	(1.224, 1.706)	<0.001	1.430	(1.184, 1.726)	<0.001
Gender (male)	2.124	(1.472, 3.066)	<0.001	2.132	(1.465, 3.102)	<0.001	2.089	(1.364, 3.200)	<0.001
Current smoker (yes/no)	1.326	(0.922, 1.908)	0.128						
Diabetes duration (every 1 year)	1.023	(1.002, 1.044)	0.029				1.004	(0.977, 1.032)	0.772
BMI (every 1 kg/m^2)	0.885	(0.843, 0.928)	<0.001				0.877	(0.836, 0.921)	<0.001
Systolic BP (every 10 mmHg)	1.157	(1.034, 1.295)	0.011				1.047	(0.927, 1.184)	0.459
HbA1c (every 1%)	0.977	(0.914, 1.045)	0.503						
Total cholesterol (every 1 mmol/L)	0.899	(0.780, 1.035)	0.138						
Metformin (yes/no)	0.819	(0.562, 1.193)	0.297						
Insulin secretagogues (yes/no)	0.950	(0.675, 1.339)	0.771						
Insulin therapy (yes/no)	0.900	(0.635, 1.276)	0.554						
Statins (yes/no)	0.788	(0.425, 1.460)	0.448						

BMI= body mass index, BP= blood pressure, HbA1c= glycated hemoglobin, CI = confidence interval, HR = hazard ratio

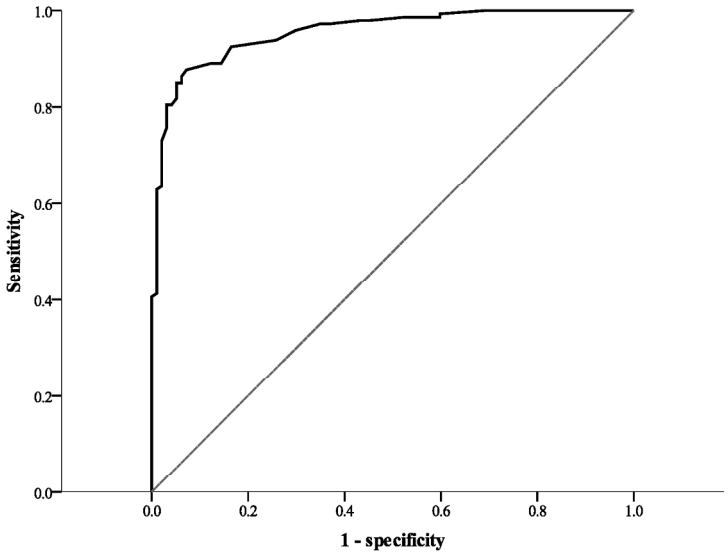
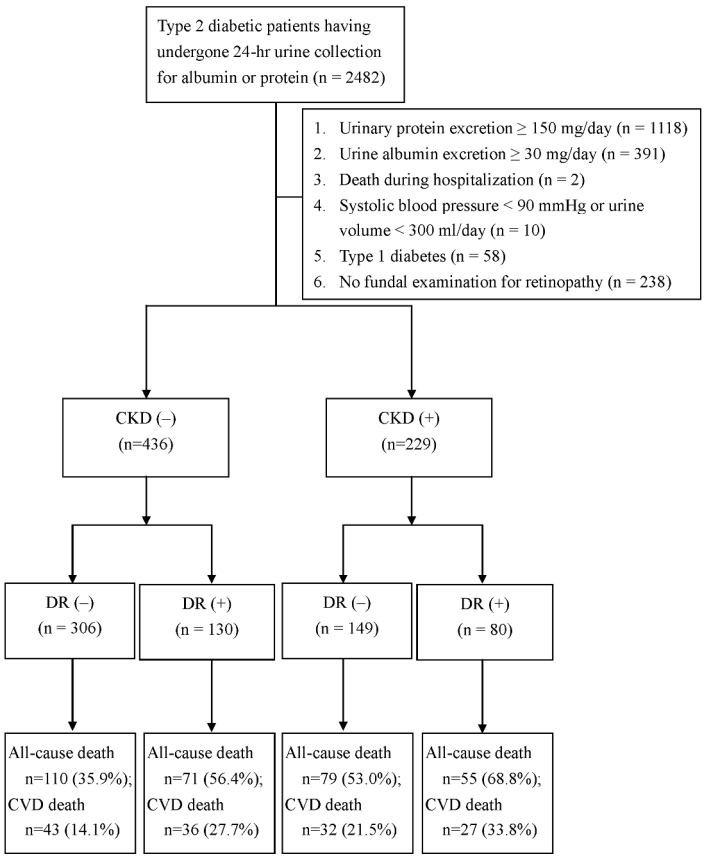


Figure 1

196x133mm (300 x 300 DPI)



CKD = chronic kidney disease, CVD = cardiovascular disease, DR = diabetic retinopathy

1

Figure 2

210x297mm (300 x 300 DPI)

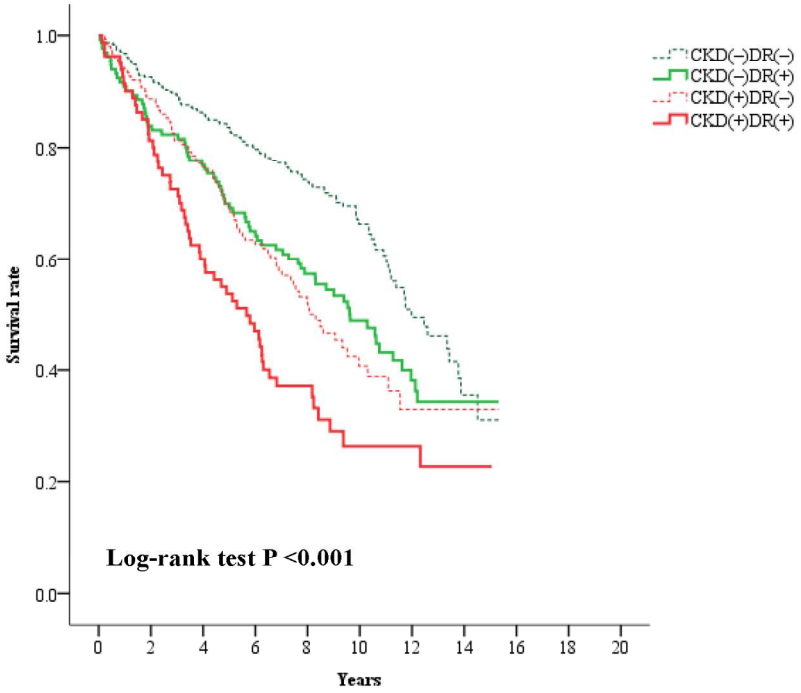


Figure 3

164x129mm (300 x 300 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1,2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2,3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4,5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*7*
Bias	9	Describe any efforts to address potential sources of bias	14,15
Study size	10	Explain how the study size was arrived at	8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7,8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7,8
		(*b*) Describe any methods used to examine subgroups and interactions	10
		(*c*) Explain how missing data were addressed	NA
		(*d*) If applicable, explain how loss to follow-up was addressed	NA
		(*e*) Describe any sensitivity analyses	8,9
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9, Figure 2
		(b) Give reasons for non-participation at each stage	Figure 2
		(c) Consider use of a flow diagram	Figure 2
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) Summarise follow-up time (eg, average and total amount)	9
Outcome data	15*	Report numbers of outcome events or summary measures over time	9,10, Figure 2
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Table 2
		(*b*) Report category boundaries when continuous variables were categorized	11
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-14
Generalisability	21	Discuss the generalisability (external validity) of the study results	12-14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

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A Synergistic Effect of Retinopathy and Chronic Kidney Disease on Long-term Mortality in Type 2 Diabetic Inpatients with Normal Urinary Albumin or Protein: A Retrospective Cohort Study

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Running title: DR and CKD in DM with Normoalbuminuria

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Abstract

Objective: Normoalbuminuric chronic kidney disease (NA-CKD) is recognized as a distinct phenotype of diabetic kidney disease, but the role of diabetic retinopathy (DR) in predicting long-term mortality among these patients remains unclear. Here, we aimed to investigate the effects of DR and chronic kidney disease (CKD) on mortality in type 2 diabetic patients with normoalbuminuria.

Design: We conducted this study as a retrospective cohort study.

Setting: We collected clinical information from the medical records of a public medical center in central Taiwan.

Participants: Patients with type 2 diabetes (n = 665) who were hospitalized due to poor glucose control were consecutively enrolled and followed for a median of 6.7 years (interquartile range, 4.1–9.6 years). Patients with either urinary protein excretion >150 mg/day or urine albumin excretion >30 mg/day were excluded.

Primary outcome measure: All-cause mortality served as the primary follow-up outcome, and the mortality data were obtained from the national registry in Taiwan.

Results: The patients with CKD and DR showed the highest mortality rate (log-rank P < 0.001). The risks of all-cause mortality (hazard ratio [HR], 2.263; 95% confidence interval [CI], 1.551–3.302) and cardiovascular mortality (HR, 2.471; 95% CI, 1.421–4.297) were significantly greater in patients with CKD and DR than in those without CKD or DR, after

adjusting for the associated risk factors.

Conclusions: DR is an independent predictor for all-cause and cardiovascular mortality in type 2 diabetic inpatients with normoalbuminuria. Moreover, DR with CKD exerts a synergistic effect on all-cause and cardiovascular mortality. Fundoscopy screening can provide additive information on mortality in patients with type 2 diabetes, even among those with NA-CKD.

Strengths and limitations of this study

- Twenty-four hour urine collection during the hospitalization period
- A median follow-up period of 6.7 years
- Mortality data obtained from a National Health Insurance registry with a nationwide coverage rate of over 99% in Taiwan
- In addition to normoabluminuria, normoproteinuria was also included in the analyses due to limited case numbers

INTRODUCTION

Diabetes is associated with microvascular complications, and is the leading cause of both end-stage renal disease (ESRD) and blindness.[1-5] Diabetic kidney disease (DKD) is a complex and heterogeneous disease, particularly among patients with type 2 diabetes.[6] The urinary albumin level is an important biomarker for DKD,[7] and is predictive of all-cause and cardiovascular mortality.[8 9] Extensive resources and efforts have focused on understanding and preventing albuminuria, and its prevalence has consequently been significantly reduced; however, the prevalence of low estimated glomerular filtration rate (eGFR) has still increased among patients with diabetes.[10]

Normoalbuminuria was reported in 36% of type 2 diabetic patients with CKD in the Third National Health and Nutrition Examination Survey (NHANES) 1988–1994,[11] and was reported in 48.1 % of diabetic patients with CKD in the NHANES 2005–2008.[10] The prevalence of normoalbuminuria in CKD appears to have increased over the 15 years.[10 11] With the recent progress in the management of diabetic complications, it appears that this paradigm has shifted, and the phenotype of normoalbuminuric chronic kidney disease (NA-CKD) has emerged.[6 12] Hence, the mechanism by which NA-CKD leads to cardiovascular disease and mortality remains a popular area of research.

Diabetic retinopathy (DR) has been traditionally recognized as an early reflection of general microangiopathy in patients with type 2 diabetes.[3 13 14] Accumulating evidence has

shown that DR is a predictor of cardiovascular disease and all-cause mortality in subjects with type 2 diabetes.[15-18] However, some studies have reported that DR is not predictive of mortality in the presence of DKD,[19 20] and the association between DR and cardiovascular disease becomes non-significant after adjusting for the albuminuria.[21-23]

In the present study, we aimed to investigate the mortality risk among type 2 diabetic patients without albuminuria, and we hypothesized that DR was predictive of long-term mortality in type 2 diabetic patients with NA-CKD or without NA-CKD.

METHODS

Setting and participants

This retrospective cohort study was conducted at Taichung Veterans General Hospital. Clinical data were obtained by reviewing the medical records of diabetic patients hospitalized between August 1996 and August 2007. The inclusion criteria were (1) adult diabetic inpatients based on the clinical diagnosis, (2) admission to the Endocrinology and Metabolism section due to a primary diagnosis of poor glucose control, (3) availability of eGFR data, and (4) performance of 24-hour urine collection for the determination of albumin or protein levels during the hospitalization period. The exclusion criteria included (1) urinary protein excretion ≥ 150 mg/day or urine albumin excretion ≥ 30 mg/day,[24] (2) death during this hospitalization period, (3) systolic blood pressure < 90 mmHg, (4) urine volume < 300

ml/day, (5) diagnosis of diabetes other than type 2, and (6) the unavailability of reports documenting eye fundal examinations for retinopathy by an ophthalmologist during the hospitalization period. In repeatedly hospitalized patients, data recorded from the last admission during the study period were used. The research protocol was approved by the Institutional Review Board of Taichung Veterans General Hospital.

Patient and Public Involvement

All-cause mortality served as the primary outcome in this retrospective cohort study, and the mortality data were obtained from the national registry in Taiwan. We collected clinical information from the medical records at Taichung Veterans General Hospital. The Institutional Review Board waived the need for informed consent before reviewing the medical records.

Variables

A normal urinary albumin level was defined as 24-hour urine albumin excretion < 30mg.[24-26] In those inpatients with only protein detected, however, normal urine protein was defined as 24-hour urine protein < 150 mg.[24] CKD was defined as an eGFR < 60 mL/min/1.73m^2. The eGFR was calculated by the Modification of Diet in Renal Disease (MDRD) equation: 186 × [serum creatinine (mg/dL)]$^{-1.154}$ × [age (year)]$^{-0.203}$ (× 0.742, if

female) mL/min/1.73m^2.[25]

DR includes nonproliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR).[27] DR was screened using fundoscopic examinations by ophthalmologists based on formal consultations. Retinal angiography (CF-60UVi fundus camera, Canon, Japan) was subsequently arranged for the confirmation of retinopathy diagnosis in cases with abnormal fundoscopic findings. Hypertension was defined as blood pressure higher than 130/80 mmHg or a history of anti-hypertensive medications use. After medical information was collected from our hospital, we also obtained the mortality data up to December 2011 from the Collaboration Center of Health Information Application, Department of Health, Executive Yuan, Taiwan. The causes of death were categorized according to the International Classification of Disease (ICD), 9th Revision, Clinical Modification diagnostic criteria before 2008 and according to the ICD-10 after 01 January, 2008.

Measurement

Biochemistry was assessed from blood samples collected after overnight fasting during the hospitalization period. HbA1c levels were determined using cation-exchange HPLC (NGSP certificated; G8, TOSOH, Tokyo, Japan). Lipid levels were determined using enzymatic methods (Advia 1800, Siemens, New York, USA). Creatinine levels were determined using the Jaffé method (Advia 1800, Siemens, New York, USA). Urine protein

levels were determined using the dye-binding assay, and urine albumin levels were assessed using the immune-turbidimetric method (Advia 1800, Siemens, New York, USA).

Comparison of cutoff values of daily urinary protein excretion for normoalbuminuria

Among the 2482 diabetic inpatients who had undergone 24-hour urine collection, only 245 had the data for both urine albumin and protein levels in the same urine sample. The median level of daily protein excretion was 184 mg (interquartile range, 80–620 mg) and the median level of daily albumin excretion was 58 mg (interquartile range, 15–362 mg). There was a significant positive correlation between daily urine protein and albumin excretion (r = 0.884, $P < 0.001$). Receiver operating characteristic (ROC) curves were used to differentiate normal urine albumin excretion (<30 mg/day), and we found that the area under the curve was 0.956 (95% confidence interval [CI], 0.932–0.979; $P < 0.001$; supplementary figure). The optimal diagnostic cutoff value for the daily urine protein level was 145 mg, which corresponded to a sensitivity of 93.8% and specificity of 86.5% for normoalbuminuria. Using 150 mg as diagnostic cutoff value of daily urine protein also gave a sensitivity of 93.8% and specificity of 86.5% for normoalbuminuria.

Statistical analysis

Continuous data are presented as mean ± standard deviation (SD), whereas categorical

data are presented as number (n) with percentage (%). The linear correlation of daily excretion between urine protein and albumin was determined using Spearman's rank correlation coefficient. The ROC curve was used to determine the optimal cut-off value of daily urine protein for normoalbuminuria. One-way analysis of variance (ANOVA) was used to determine the significance of the differences among groups. Pairwise multiple comparisons were conducted to determine the significance of the differences between two groups, if a statistically significant difference was detected via one-way ANOVA; however, Kruskal-Wallis tests were conducted to determine the significance of the differences in the duration of diabetes, triglycerides levels and eGFR values among groups due to the presence of a skewed distribution in these variables. The Chi-square test was used to compare categorical variables across groups. The overall significance of univariate survival analysis was determined by the log-rank test using Kaplan-Meier analysis. Cox proportional hazards regression analyses were conducted to determine the hazard ratios of risk factors. The risk factors in the univariate model were selected based on the findings in Table 1, whereas the risk factors in model 2 were selected based on the statistical significance indicated in the univariate model. Except the inclusion and exclusion criteria, the mean imputation method was used for missing data. A value of $P < 0.05$ was considered statistically significant. Statistical analysis was performed using SPSS 22.0 (IBM, Armonk, NY, USA).

RESULTS

Comparison of the outcomes between patients with and without fundal examination

A total of 665 patients with a median diabetes duration of 7 years (interquartile range, 2–12 years) met the criteria for enrollment in the study and were included in these analyses (figure 1). In comparison with the 238 patients who were eligible for all the criteria except for lacking a fundal examination for retinopathy, there were no statically significant differences in age (62 ± 14 vs. 63 ± 15 years, P = 0.777), gender (56.5% men vs. 61.3% men, P = 0.226), diabetes duration (8.7 ± 7.7 vs. 8.9 ± 8.6 years, P= 0.789), eGFR (73 ± 29 vs. 71 ± 30 mL/min/1.73m^2, P = 0.642), all-cause mortality incidence (6.6 vs. 6.5 events/100 person-years, log-rank test P = 0.888), and cardiovascular mortality (2.9 vs. 2.9 events/100 person-years, log-rank test P = 0.965).

Risk of long-term mortality in CKD or DR in patients with normoalbuminuria

There were 229 (34.4%) patients with CKD and 210 (31.6%) with DR in the 665 enrolled type 2 diabetic inpatients with normoabluminuria. During a median follow-up of 6.7 years (interquartile range, 4.1–9.6years), 315 (47.4%) patients died from any cause; in particular, 138 patients died of cardiovascular disease (figure 1). The all-cause mortality rate was higher in the patients with CKD than in those without CKD (9.4 vs. 5.4 events/100 person-years, log-rank test P < 0.001); and the all-cause mortality rate was also higher in the

patients with DR than those without DR (9.0 vs. 5.6 events/100 person-years, log-rank test P < 0.001). Moreover, the cardiovascular mortality rate was higher in patients with CKD than in those without CKD (4.2 vs. 2.4 events/100 person-years, log-rank test P < 0.001); this rate was also higher in patients with DR than in those without DR (4.5 vs. 2.2 events/100 person-years, log-rank test P < 0.001).

A synergistic effect of retinopathy and CKD on long-term mortality

The proportion of patients with DR was not significantly different between the patients with CKD and those without CKD (34.9% vs. 29.8%, P = 0.207). The 665 patients were separated into four groups based on the presence of CKD and DR, including (1) patients without CKD or DR in the CKD(–)DR(–) group, (2) patients with DR but not CKD in the CKD(–)DR(+) group, (3) patients with CKD but not DR in the CKD(+)DR(–) group, and (4) patients with both CKD and DR in the CKD(+)DR(+) group. Table 1 shows all the clinical characteristics of patients among these four groups. Figure 2 shows that survival rates were significantly different across these four groups (log-rank test P < 0.001), as demonstrated by Kaplan–Meier analysis. The highest mortality incidence (12.4 events/100 person-years) was observed in the CKD(+)DR(+) group; this value was significantly higher than the 8.1 events/100 person-years in the CKD(+)DR(–) group (P = 0.010), the 7.4 events/100 person-years in the CKD(–)DR(+) group (P = 0.004), and the 4.6 events/100 person-years in

the CKD(–)DR(–) group (P < 0.001). The incidences of mortality in the CKD(+)DR(–) and CKD(–)DR(+) groups were also significantly higher than that in the CKD(–)DR(–) group (P < 0.001 and P = 0.003, respectively). However, there was no significant difference in the mortality incidence between the CKD(+)DR(–) and CKD(–)DR(+) groups (P = 0.479).

Cox regression analysis for all-cause and cardiovascular mortality

To identify the predictive factors for long-term mortality, univariate Cox regression analysis was conducted for all the enrolled patients. In addition to the different groups categorized based on CKD and DR, age, gender, diabetes duration, body mass index (BMI), systolic blood pressure, metformin treatment, insulin treatment, and diuretic treatment were significantly associated with total mortality. Using multivariate Cox regression analysis, patients with CKD and DR have the highest hazard ratio (2.263; 95% CI, 1.551–3.302) for all-cause mortality in comparison with the ones without CKD or DR after adjustment for age, gender, diabetes duration, BMI, systolic blood pressure, metformin treatment, insulin treatment and diuretics treatment (Table 2A). Moreover, patients with CKD and DR also had the highest hazard ratio (2.471; 95% CI, 1.421–4.297) for cardiovascular mortality in comparison to the ones without CKD or DR after adjustment for age, gender, diabetes duration, BMI, systolic blood pressure and angiotensin-converting enzyme (ACE) inhibitor or angiotensin II receptor antagonist (ARB) treatment (Table 2B).

DISCUSSION

In this retrospective cohort study, we found that patients with NA-CKD have a higher risk of all-cause mortality compared with patients without CKD. Furthermore, the presence of DR imposed a higher mortality risk in patients with NA-CKD.

The mortality risk associated with NA-CKD remains controversial. In the Casale Monferrato study, which had an 11-year follow-up, eGFR showed a significantly inverse trend with long-term mortality only in type 2 diabetic patients with macroalbuminuria, but not in those with microalbuminuria or normoalbuminuria.[28] Conversely, several studies reported that low eGFR was significantly associated with a higher all-cause mortality risk, independent of albuminuria.[29-33] The magnitude of the impact on all-cause mortality varied. Rigalleau et al.[34] reported that NA-CKD was associated with a very low risk of dialysis or mortality in comparison with albuminuric CKD in diabetic patients during a 38-month follow-up study in France. In an Asian study with a 44-month follow-up, albuminuria was associated with a significantly higher risk of renal events, but not cardiovascular events in diabetic patients with CKD.[35] In the present study, we found that NA-CKD is associated with an approximately 1.8-fold increase in either all-cause mortality or cardiovascular disease, compared with type 2 diabetic patients without CKD. In line with our findings, Hsieh et al.[36] reported that eGFR was inversely related to risk of cardiovascular events in type 2 diabetic

outpatients with normoalbuminuria during a 4-year follow-up study.

We postulated that heterogeneity in the pathogenesis of NA-CKD might contribute to the differences in results. Different natural courses have been observed in the NA-CKD phenotype: initial albuminuria with regression to normoalbuminuria with intensive renin-angiotensin-aldosterone system (RAAS) inhibitor use in some diabetic patients, but eGFR loss might be the only manifestation of renal involvement in others.[12 37] Based on renal biopsy, tubular-interstitial lesions and arterial hyalinosis were the predominant findings in NA-CKD rather than the typical glomerulosclerotic lesions seen with albuminuria in diabetic patients.[38 39] With albuminuria regression due to the widespread use of RAAS inhibitors in recent decades, NA-CKD is being found in majority of DKD patients. The number of patients with normoalbuminuria has been found to be great than those with albuminuria in several large studies of type 2 diabetic patients with CKD.[40-42] Hence, there is an urgent need to identify the predictors of mortality among this distinct population.[43]

In the present study, patients using ACE inhibitors or ARBs showed a higher risk of cardiovascular mortality in the univariate model, and this finding may result from the higher proportion of CKD patients using these drugs at baseline. In the multivariate model, the use of ACE inhibitors or ARBs at baseline was not significantly associated with cardiovascular mortality. Consistent with these findings, early ARB treatment was not found to significantly improve eGFR in type 2 diabetic patients with urine albumin-to-creatinine ratio <300 mg/g.[44]

Although there is a high prevalence of DR in type 2 diabetic patients with albuminuria,[20] the concordance between DR and CKD was lower in patients with normoalbuminuria than in those with albuminuria.[45 46]. In the present study, we found that the prevalence of retinopathy was not significantly different between patients with and without CKD. However, our results showed a synergistic effect of CKD and DR on all-cause mortality and cardiovascular mortality. Compared with the NEHANS III population, in which the synergistic effect of CKD and retinopathy on morality was also observed,[47] our findings showed the evidence in type 2 diabetic inpatients even without albuminuria. Although similar risk factors and cardiovascular effects for DR and CKD have been reported,[30 48 49] the exact mechanism underlying the superimposed DR effect on mortality in type 2 diabetic patients with NA-CKD requires further investigation.

In the present study, both isolated CKD, i.e. CKD(+)DR(–), and isolated DR, i.e. CKD(–)DR(+), showed significantly higher mortality risks than those with neither CKD nor DR in univariate analyses; however, the significant difference seemed to attenuate in isolated CKD after adjustment for other traditional cardiovascular risk factors. NA-CKD has been reported to be highly associated with cardiovascular risk factors.[36 42] Therefore, the attenuation of the mortality prediction after adjustment for cardiovascular risk factors might be more obvious in isolated CKD in comparison with isolated DR. Furthermore, it is notable that a higher BMI showed a protective effect for all-cause and cardiovascular mortality in the

present study. Although this seems somewhat contradictory to traditional concepts, we were not the only one to report this paradoxical effect of BMI on mortality in patients with diabetes.[50] Cea Soriano et al.[51] reported that BMI ≥ 25 kg/m^2 predicted a lower mortality risk than BMI < 25 kg/m^2 in type 2 diabetic patients with CKD.

Among type 2 diabetic patients with normoalbumiuria, high urine albumin excretion (10–29 mg/day) was reportedly associated with multiple cardiovascular risk factors, as compared to low urine albumin excretion (< 10 mg/day).[52] Recently, the highest tertile of the urine albumin excretion rate was found to be associated with diabetic retinopathy and arterial stiffness, in comparison to the lowest tertile among type 2 diabetic patients with normoalbuminuria.[53 54] In the present study, 257 patients without CKD had available urine albumin data, including 108 patients with low normoalbuminuria (< 10 mg/day) and 149 patients with high normoalbuminuria (10–29 mg/day). However, there was no significant difference in DR prevalence ($P > 0.05$) or all-cause and cardiovascular mortality (both log-rank test $P > 0.05$). Further investigations with a large number of cases will be needed to evaluate the association between high normoalbuminuria and mortality.

We acknowledge several limitations in our study. First, we enrolled patients with not only normoabluminuria, but also normoproteinuria, due to the limited numbers of cases overall. Second, we used the 24-hour urine data instead of spot-urine data, since the latter has been well reported in type 2 outpatients previously.[55] Third, we only included type 2 diabetic

inpatients who were admitted to the hospital with a primary diagnosis of poor glucose control. Fourth, we calculated eGFR using the MDRD equation instead of the CKD Epidemiology Collaboration (EPI) equation as consensus had not been reached regarding the use of the CKD-EPI equation in the Taiwanese population.[56] Finally, we assessed the patients only at baseline, but not during the follow-up. Therefore, treatment might have confounded the results following patients discharged.

In conclusion, DR is a significant predictor for all-cause and cardiovascular mortality in type 2 diabetic inpatients with normoalbuminuria. Presence of DR also showed a synergistic impact on mortality for type 2 diabetic inpatients with NA-CKD. Screening for DR and eGFR may help identify those who harbor a high mortality risk after discharge in type 2 diabetic inpatients hospitalized due to poor glucose control, even with normoalbuminuria.

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Author contributions

YL, WHS and IL contributed to the study design. YL and IL participated in the data collection. YL and IL participated in the analysis and interpretation of the data. YL drafted the manuscript. IL had full access to the study data. IL is the guarantor. All the authors performed a critical revision of the manuscript for important intellectual content.

FIGURE LEGENDS

Figure 1. Flow diagram of enrollment of study subjects with normoalbuminuria.

Figure 2. Kaplan-Meier curves showing survival rates categorized according to chronic kidney disease (CKD) and diabetic retinopathy (DR) in type 2 diabetic inpatients with normoalbuminuria.

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Table 1. The clinical data of patients according to the presence of CKD and DR

	CKD(–)DR(–) (n = 306)	CKD(–)DR(+) (n = 130)	CKD(+)DR(–) (n = 149)	CKD(+)DR(+) (n = 80)	P value
Age (years)	57 ± 15	62 ± 13	69 ± 10	70 ± 9	<0.001
Male, n (%)	189 (61.8%)	63 (48.5%)	82 (55.0%)	42 (52.5%)	0.057
BMI (kg/m^2)	23.7 ± 4.5	23.3 ± 4.0	24.2 ± 4.1	24.2 ± 4.3	0.367
Systolic blood pressure (mmHg)	124 ± 14	129 ± 15	127 ± 15	128 ± 14	0.004
Diastolic blood pressure (mmHg)	74 ± 11	75 ± 10	71 ± 9	72 ± 10	0.003
Diabetes duration (years)[#]	6.7 ± 6.7	11.4 ± 7.4	7.9 ± 7.7	13.2 ± 8.4	<0.001
Current smoker, n (%)	96 (31.4%)	36 (27.7%)	31 (20.8%)	13 (16.3%)	0.014
White blood cell count (10^6/L)	7836 ± 5193	7210 ± 2421	8258 ± 3504	8358 ± 3285	0.227
HbA1c (%)	11.5 ± 2.9	10.6 ± 2.3	10.4 ± 3.3	9.3 ± 2.6	<0.001
Total cholesterol (mmol/L)	4.8 ± 1.3	5.0 ± 1.3	4.6 ± 1.3	4.7 ± 1.5	0.197
Triglyceride (mmol/L)[#]	1.9 ± 1.9	1.9 ± 2.2	2.0 ± 2.2	1.8 ± 1.3	0.574
HDL cholesterol (mmol/L)	1.1 ± 0.4	1.1 ± 0.4	1.0 ± 0.3	1.0 ± 0.4	0.149
eGFR (mL/min/1.73m^2)[#]	88 ± 23	87 ± 20	45 ± 13	42 ± 15	<0.001
Hypertension, n (%)	171 (55.9%)	93 (71.5%)	113 (75.8%)	63 (78.8%)	<0.001
Antihypertensive agents, n (%)	91 (29.7%)	63 (48.5%)	90 (60.4%)	47 (58.8%)	<0.001
ACE inhibitor or ARB, n (%)	58 (19.0%)	44 (33.8%)	59 (39.6%)	32 (40.0%)	<0.001
α-blocker, n (%)	20 (6.5%)	14 (10.8%)	20 (13.4%)	10 (12.5%)	0.079
β-blocker, n (%)	20 (6.5%)	9 (6.9%)	3 (2.0%)	6 (7.5%)	0.172
Calcium channel blocker, n (%)	43 (14.1%)	28 (21.5%)	37 (24.8%)	20 (25.0%)	0.015
Diuretics, n (%)	8 (2.6%)	6 (4.6%)	11 (7.4%)	16 (20.0%)	<0.001
Oral antihyperglycemic drugs, n (%)	151 (49.3%)	65 (50.0%)	53 (35.6%)	27 (33.8%)	0.005
Insulin secretagogues, n (%)	127 (41.5%)	56 (43.1%)	46 (30.9%)	23 (28.8%)	0.027
Metformin, n (%)	95 (31.0%)	41 (31.5%)	26 (17.4%)	17 (21.3%)	0.007

Thiazolidinediones, n (%)	6 (2.0%)	5 (3.8%)	2 (1.3%)	0 (0.0%)	0.230
α-glucosidase inhibitor, n (%)	10 (3.3%)	5 (3.8%)	3 (2.0%)	2 (2.5%)	0.811
Insulin therapy, n (%)	231 (75.5%)	80 (61.5%)	70 (47.0%)	38 (47.5%)	<0.001
Statins, n (%)	28 (9.2%)	12 (9.2%)	17 (11.4%)	12 (15.0%)	0.442

ACE = angiotensin-converting enzyme, ARB = angiotensin II receptor antagonists, BMI = body mass index, CKD = chronic kidney disease, DR = diabetic retinopathy, HbA1c = glycated hemoglobin, HDL = high-density lipoprotein.

[#] Kruskal-Wallis tests to determine the significance of the differences due to skewed distribution in diabetic duration, triglycerides and eGFR

Table 2. Results of Cox regression analysis for the effects of risk factors on (A) all-cause and (B) cardiovascular mortality

(A) All-cause mortality

	Univariate model			Multivariate model					
	Crude			Model 1			Model 2		
	HR	95%CI	P value	HR	95%CI	P value	HR	95%CI	P value
CKD(-)DR(-)*	1.000			1.000			1.000		
CKD(-)DR(+)	1.590	(1.179, 2.145)	0.002	1.556	(1.148, 2.108)	0.004	1.686	(1.202, 2.364)	0.002
CKD(+)DR(-)	1.842	(1.377, 2.463)	<0.001	1.416	(1.042, 1.924)	0.026	1.381	(0.984, 1.939)	0.062
CKD(+)DR(+)	2.791	(2.016, 3.866)	<0.001	2.209	(1.573, 3.101)	<0.001	2.263	(1.551, 3.302)	<0.001
Age (every 10 years)	1.455	(1.318, 1.607)	<0.001	1.328	(1.197, 1.472)	<0.001	1.325	(1.177, 1.491)	<0.001
Gender (male)	1.763	(1.394, 2.229)	<0.001	1.763	(1.388, 2.239)	<0.001	1.692	(1.298, 2.206)	<0.001
Current smoker (yes/no)	1.223	(0.958, 1.561)	0.107						
Diabetes duration >7 years (yes/no)	1.271	(1.012, 1.596)	0.039				1.084	(0.832, 1.412)	0.550
BMI (every 1 kg/m^2)	0.959	(0.931, 0.987)	0.004				0.954	(0.925, 0.984)	0.003
Systolic BP (every 10 mmHg)	1.081	(1.002, 1.167)	0.045				1.017	(0.932, 1.111)	0.701
HbA1c (every 1%)	0.969	(0.927, 1.013)	0.163						
Metformin (yes/no)	0.776	(0.604, 0.998)	0.049				0.751	(0.549, 1.028)	0.074
Insulin secretagogues (yes/no)	0.851	(0.677, 1.070)	0.167						
Insulin therapy (yes/no)	0.777	(0.619, 0.974)	0.029				1.094	(0.807, 1.483)	0.562
Statins (yes/no)	1.090	(0.763, 1.557)	0.635						
ACE inhibitor or ARB (yes/no)	1.144	(0.900, 1.454)	0.271						
Calcium channel blocker (yes/no)	1.237	(0.942, 1.625)	0.125						
Diuretics (yes/no)	2.517	(1.714, 3.696)	<0.001				1.765	(1.115, 2.793)	0.015

ACE = angiotensin-converting enzyme, ARB = angiotensin II receptor antagonists, BMI = body mass index, BP = blood pressure, HbA1c = glycated hemoglobin, CI = confidence interval, HR = hazard ratio

The risk factors in the univariate model were selected based on the findings in Table 1.

The risk factors in model 2 were selected based on the statistical significance indicated in the univariate model.

*the overall P value <0.001 among the CKD(-)DR(-), CKD(-)DR(+), CKD(+)DR(-), CKD(+)DR(+) groups

(B) Cardiovascular mortality

	Univariate model			Multivariate model					
	Crude			Model 1			Model 2		
	HR	95%CI	P value	HR	95%CI	P value	HR	95%CI	P value
CKD(-)DR(-)*	1.000			1.000			1.000		
CKD(-)DR(+)	2.092	(1.340, 3.267)	0.001	2.039	(1.297, 3.203)	0.002	1.896	(1.153, 3.115)	0.012
CKD(+)DR(-)	1.937	(1.221, 3.072)	0.005	1.387	(0.855, 2.251)	0.185	1.376	(0.815, 2.322)	0.232
CKD(+)DR(+)	3.601	(2.215, 5.854)	<0.001	2.710	(1.634, 4.493)	<0.001	2.471	(1.421, 4.297)	0.001
Age (every 10 years)	1.615	(1.377, 1.894)	<0.001	1.445	(1.224, 1.706)	<0.001	1.389	(1.152, 1.674)	<0.001
Gender (male)	2.124	(1.472, 3.066)	<0.001	2.132	(1.465, 3.102)	<0.001	2.126	(1.391, 3.250)	<0.001
Current smoker (yes/no)	1.326	(0.922, 1.908)	0.128						
Diabetes duration >7 years (yes/no)	1.775	(1.237, 2.546)	0.002				1.196	(0.788, 1.815)	0.401
BMI (every 1 kg/m^2)	0.885	(0.843, 0.928)	<0.001				0.877	(0.836, 0.921)	<0.001
Systolic BP (every 10 mmHg)	1.157	(1.034, 1.295)	0.011				1.035	(0.912, 1.175)	0.593
HbA1c (every 1%)	0.977	(0.914, 1.045)	0.503						
Metformin (yes/no)	0.819	(0.562, 1.193)	0.297						
Insulin secretagogues (yes/no)	0.950	(0.675, 1.339)	0.771						
Insulin therapy (yes/no)	0.900	(0.635, 1.276)	0.554						
Statins (yes/no)	0.788	(0.425, 1.460)	0.448						
ACE inhibitor or ARB (yes/no)	1.572	(1.113, 2.221)	0.010				1.159	(0.765, 1.756)	0.487
Calcium channel blocker (yes/no)	1.366	(0.914, 2.041)	0.128						
Diuretics (yes/no)	2.387	(1.316, 4.330)	0.004				1.321	(0.618, 2.821)	0.473

ACE = angiotensin-converting enzyme, ARB = angiotensin II receptor antagonists, BMI = body mass index, BP = blood pressure, HbA1c = glycated hemoglobin, CI = confidence interval, HR = hazard ratio

The risk factors in the univariate model were selected based on the findings in Table 1.

The risk factors in model 2 were selected based on the statistical significance indicated in the univariate model.

*the overall P value <0.001 among the CKD(-)DR(-), CKD(-)DR(+), CKD(+)DR(-), CKD(+)DR(+) groups

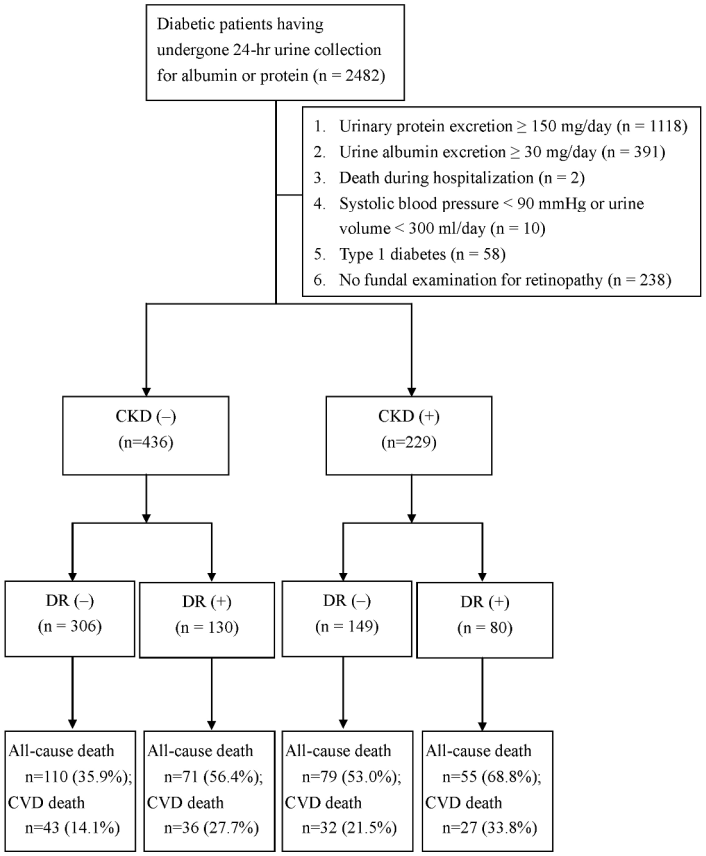


Figure 1.
Flow diagram of enrollment of study subjects with normoalbuminuria.

210x297mm (300 x 300 DPI)

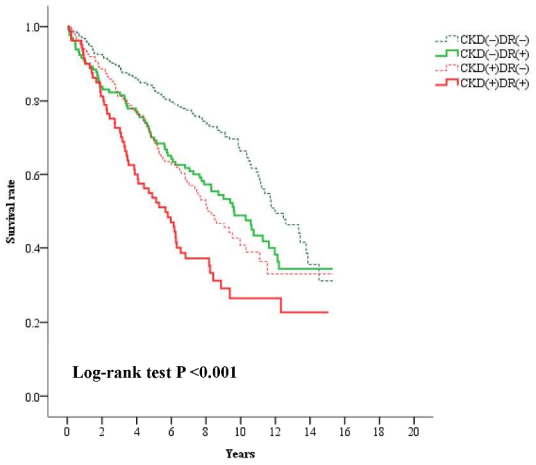
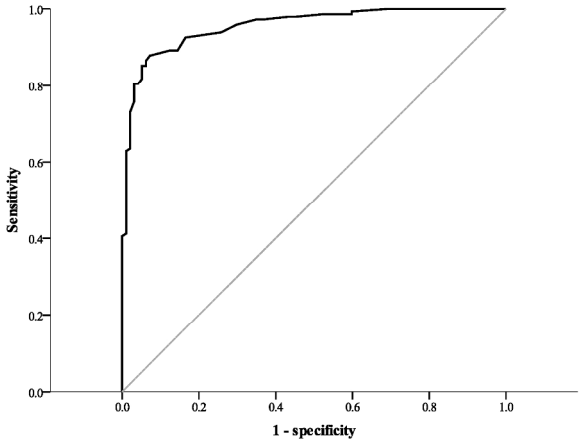


Figure 2.
Kaplan-Meier curves showing survival rates categorized according to chronic kidney disease (CKD) and diabetic retinopathy (DR) in type 2 diabetic inpatients with normoalbuminuria.

210x297mm (300 x 300 DPI)



Supplementary figure.
Receiver operating characteristic (ROC) analysis curves for differentiating normoalbuminuria (urine albumin excretion < 30 mg/day) based on daily urinary protein excretion.

210x297mm (300 x 300 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1,2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2,3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4,5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*7*
Bias	9	Describe any efforts to address potential sources of bias	14,15
Study size	10	Explain how the study size was arrived at	8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7,8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7,8
		(*b*) Describe any methods used to examine subgroups and interactions	10
		(*c*) Explain how missing data were addressed	NA
		(*d*) If applicable, explain how loss to follow-up was addressed	NA
		(*e*) Describe any sensitivity analyses	8,9
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9, Figure 2
		(b) Give reasons for non-participation at each stage	Figure 2
		(c) Consider use of a flow diagram	Figure 2
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) Summarise follow-up time (eg, average and total amount)	9
Outcome data	15*	Report numbers of outcome events or summary measures over time	9,10, Figure 2
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Table 2
		(*b*) Report category boundaries when continuous variables were categorized	11
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-14
Generalisability	21	Discuss the generalisability (external validity) of the study results	12-14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Effects of Retinopathy and Chronic Kidney Disease on Long-term Mortality in Type 2 Diabetic Inpatients with Normal Urinary Albumin or Protein: A Retrospective Cohort Study

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Running title: DR and CKD in DM with Normoalbuminuria

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Abstract

Objective: Normoalbuminuric chronic kidney disease (NA-CKD) is recognized as a distinct phenotype of diabetic kidney disease, but the role of diabetic retinopathy (DR) in predicting long-term mortality among these patients remains unclear. Here, we aimed to investigate the effects of DR and chronic kidney disease (CKD) on mortality in type 2 diabetic patients with normoalbuminuria.

Design: We conducted this study as a retrospective cohort study.

Setting: We collected clinical information from the medical records of a public medical center in central Taiwan.

Participants: Patients with type 2 diabetes (n = 665) who were hospitalized due to poor glucose control were consecutively enrolled and followed for a median of 6.7 years (interquartile range, 4.1–9.6 years). Patients with either urinary protein excretion >150 mg/day or urine albumin excretion >30 mg/day were excluded.

Primary outcome measure: All-cause mortality served as the primary follow-up outcome, and the mortality data were obtained from the national registry in Taiwan.

Results: The patients with CKD and DR showed the highest mortality rate (log-rank P < 0.001). The risks of all-cause mortality (hazard ratio [HR], 2.263; 95% confidence interval [CI], 1.551–3.302) and cardiovascular mortality (HR, 2.471; 95% CI, 1.421–4.297) were significantly greater in patients with CKD and DR than in those without CKD or DR, after

adjusting for the associated risk factors.

Conclusions: DR is an independent predictor for all-cause and cardiovascular mortality in type 2 diabetic inpatients with normoalbuminuria. Moreover, DR with CKD shows the highest risks of all-cause and cardiovascular mortality among these patients. Fundoscopy screening can provide additive information on mortality in patients with type 2 diabetes, even among those with NA-CKD.

Strengths and limitations of this study

- Twenty-four hour urine collection during the hospitalization period
- A median follow-up period of 6.7 years
- Mortality data obtained from a National Health Insurance registry with a nationwide coverage rate of over 99% in Taiwan
- In addition to normoabluminuria, normoproteinuria was also included in the analyses due to limited case numbers

INTRODUCTION

Diabetes is associated with microvascular complications, and is the leading cause of both end-stage renal disease (ESRD) and blindness.[1-5] Diabetic kidney disease (DKD) is a complex and heterogeneous disease, particularly among patients with type 2 diabetes.[6] The urinary albumin level is an important biomarker for DKD,[7] and is predictive of all-cause and cardiovascular mortality.[8 9] Extensive resources and efforts have focused on understanding and preventing albuminuria, and its prevalence has consequently been significantly reduced; however, the prevalence of low estimated glomerular filtration rate (eGFR) has still increased among patients with diabetes.[10]

Normoalbuminuria was reported in 36% of type 2 diabetic patients with CKD in the Third National Health and Nutrition Examination Survey (NHANES) 1988–1994,[11] and was reported in 48.1 % of diabetic patients with CKD in the NHANES 2005–2008.[10] The prevalence of normoalbuminuria in CKD appears to have increased over the 15 years.[10 11] With the recent progress in the management of diabetic complications, it appears that this paradigm has shifted, and the phenotype of normoalbuminuric chronic kidney disease (NA-CKD) has emerged.[6 12] Hence, the mechanism by which NA-CKD leads to cardiovascular disease and mortality remains a popular area of research.

Diabetic retinopathy (DR) has been traditionally recognized as an early reflection of general microangiopathy in patients with type 2 diabetes.[3 13 14] Accumulating evidence has

shown that DR is a predictor of cardiovascular disease and all-cause mortality in subjects with type 2 diabetes.[15-18] However, some studies have reported that DR is not predictive of mortality in the presence of DKD,[19 20] and the association between DR and cardiovascular disease becomes non-significant after adjusting for the albuminuria.[21-23]

In the present study, we aimed to investigate the mortality risk among type 2 diabetic patients without albuminuria, and we hypothesized that DR was predictive of long-term mortality in type 2 diabetic patients with NA-CKD or without NA-CKD.

METHODS

Setting and participants

This retrospective cohort study was conducted at Taichung Veterans General Hospital. Clinical data were obtained by reviewing the medical records of diabetic patients hospitalized between August 1996 and August 2007. The inclusion criteria were (1) adult diabetic inpatients based on the clinical diagnosis, (2) admission to the Endocrinology and Metabolism section due to a primary diagnosis of poor glucose control, (3) availability of eGFR data, and (4) performance of 24-hour urine collection for the determination of albumin or protein levels during the hospitalization period. The exclusion criteria included (1) urinary protein excretion ≥ 150 mg/day or urine albumin excretion ≥ 30 mg/day,[24] (2) death during this hospitalization period, (3) systolic blood pressure < 90 mmHg, (4) urine volume < 300

ml/day, (5) diagnosis of diabetes other than type 2, and (6) the unavailability of reports documenting eye fundal examinations for retinopathy by an ophthalmologist during the hospitalization period. In repeatedly hospitalized patients, data recorded from the last admission during the study period were used. The research protocol was approved by the Institutional Review Board of Taichung Veterans General Hospital.

Patient and Public Involvement

All-cause mortality served as the primary outcome in this retrospective cohort study, and the mortality data were obtained from the national registry in Taiwan. We collected clinical information from the medical records at Taichung Veterans General Hospital. The Institutional Review Board waived the need for informed consent before reviewing the medical records.

Variables

A normal urinary albumin level was defined as 24-hour urine albumin excretion < 30mg.[24-26] In those inpatients with only protein detected, however, normal urine protein was defined as 24-hour urine protein < 150 mg.[24] CKD was defined as an eGFR < 60 mL/min/1.73m^2. The eGFR was calculated by the Modification of Diet in Renal Disease (MDRD) equation: 186 × [serum creatinine (mg/dL)]$^{-1.154}$ × [age (year)]$^{-0.203}$ (× 0.742, if

female) mL/min/1.73m^2.[25]

DR includes nonproliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR).[27] DR was screened using fundoscopic examinations by ophthalmologists based on formal consultations. Retinal angiography (CF-60UVi fundus camera, Canon, Japan) was subsequently arranged for the confirmation of retinopathy diagnosis in cases with abnormal fundoscopic findings. Hypertension was defined as blood pressure higher than 130/80 mmHg or a history of anti-hypertensive medications use. After medical information was collected from our hospital, we also obtained the mortality data up to December 2011 from the Collaboration Center of Health Information Application, Department of Health, Executive Yuan, Taiwan. The causes of death were categorized according to the International Classification of Disease (ICD), 9th Revision, Clinical Modification diagnostic criteria before 2008 and according to the ICD-10 after 01 January, 2008.

Measurement

Biochemistry was assessed from blood samples collected after overnight fasting during the hospitalization period. HbA1c levels were determined using cation-exchange HPLC (NGSP certificated; G8, TOSOH, Tokyo, Japan). Lipid levels were determined using enzymatic methods (Advia 1800, Siemens, New York, USA). Creatinine levels were determined using the Jaffé method (Advia 1800, Siemens, New York, USA). Urine protein

levels were determined using the dye-binding assay, and urine albumin levels were assessed using the immune-turbidimetric method (Advia 1800, Siemens, New York, USA).

Comparison of cutoff values of daily urinary protein excretion for normoalbuminuria

Among the 2482 diabetic inpatients who had undergone 24-hour urine collection, only 245 had the data for both urine albumin and protein levels in the same urine sample. The median level of daily protein excretion was 184 mg (interquartile range, 80–620 mg) and the median level of daily albumin excretion was 58 mg (interquartile range, 15–362 mg). There was a significant positive correlation between daily urine protein and albumin excretion (r = 0.884, $P < 0.001$). Receiver operating characteristic (ROC) curves were used to differentiate normal urine albumin excretion (<30 mg/day), and we found that the area under the curve was 0.956 (95% confidence interval [CI], 0.932–0.979; $P < 0.001$; supplementary figure). The optimal diagnostic cutoff value for the daily urine protein level was 145 mg, which corresponded to a sensitivity of 93.8% and specificity of 86.5% for normoalbuminuria. Using 150 mg as diagnostic cutoff value of daily urine protein also gave a sensitivity of 93.8% and specificity of 86.5% for normoalbuminuria.

Statistical analysis

Continuous data are presented as mean ± standard deviation (SD), whereas categorical

data are presented as number (n) with percentage (%). The linear correlation of daily excretion between urine protein and albumin was determined using Spearman's rank correlation coefficient. The ROC curve was used to determine the optimal cut-off value of daily urine protein for normoalbuminuria. One-way analysis of variance (ANOVA) was used to determine the significance of the differences among groups. Pairwise multiple comparisons were conducted to determine the significance of the differences between two groups, if a statistically significant difference was detected via one-way ANOVA; however, Kruskal-Wallis tests were conducted to determine the significance of the differences in the duration of diabetes, triglycerides levels and eGFR values among groups due to the presence of a skewed distribution in these variables. The Chi-square test was used to compare categorical variables across groups. The overall significance of univariate survival analysis was determined by the log-rank test using Kaplan-Meier analysis. Cox proportional hazards regression analyses were conducted to determine the hazard ratios of risk factors. The risk factors in the univariate model were selected based on the findings in Table 1, whereas the risk factors in model 2 were selected based on the statistical significance indicated in the univariate model. Except the inclusion and exclusion criteria, the mean imputation method was used for missing data. A value of $P < 0.05$ was considered statistically significant. Statistical analysis was performed using SPSS 22.0 (IBM, Armonk, NY, USA).

RESULTS

Comparison of the outcomes between patients with and without fundal examination

A total of 665 patients with a median diabetes duration of 7 years (interquartile range, 2–12 years) met the criteria for enrollment in the study and were included in these analyses (figure 1). In comparison with the 238 patients who were eligible for all the criteria except for lacking a fundal examination for retinopathy, there were no statically significant differences in age (62 ± 14 vs. 63 ± 15 years, P = 0.777), gender (56.5% men vs. 61.3% men, P = 0.226), diabetes duration (8.7 ± 7.7 vs. 8.9 ± 8.6 years, P= 0.789), eGFR (73 ± 29 vs. 71 ± 30 mL/min/1.73m^2, P = 0.642), all-cause mortality incidence (6.6 vs. 6.5 events/100 person-years, log-rank test P = 0.888), and cardiovascular mortality (2.9 vs. 2.9 events/100 person-years, log-rank test P = 0.965).

Risk of long-term mortality in CKD or DR in patients with normoalbuminuria

There were 229 (34.4%) patients with CKD and 210 (31.6%) with DR in the 665 enrolled type 2 diabetic inpatients with normoabluminuria. During a median follow-up of 6.7 years (interquartile range, 4.1–9.6years), 315 (47.4%) patients died from any cause; in particular, 138 patients died of cardiovascular disease (figure 1). The all-cause mortality rate was higher in the patients with CKD than in those without CKD (9.4 vs. 5.4 events/100 person-years, log-rank test P < 0.001); and the all-cause mortality rate was also higher in the

patients with DR than those without DR (9.0 vs. 5.6 events/100 person-years, log-rank test P < 0.001). Moreover, the cardiovascular mortality rate was higher in patients with CKD than in those without CKD (4.2 vs. 2.4 events/100 person-years, log-rank test P < 0.001); this rate was also higher in patients with DR than in those without DR (4.5 vs. 2.2 events/100 person-years, log-rank test P < 0.001).

A combined effect of retinopathy and CKD on long-term mortality

The proportion of patients with DR was not significantly different between the patients with CKD and those without CKD (34.9% vs. 29.8%, P = 0.207). The 665 patients were separated into four groups based on the presence of CKD and DR, including (1) patients without CKD or DR in the CKD(–)DR(–) group, (2) patients with DR but not CKD in the CKD(–)DR(+) group, (3) patients with CKD but not DR in the CKD(+)DR(–) group, and (4) patients with both CKD and DR in the CKD(+)DR(+) group. Table 1 shows all the clinical characteristics of patients among these four groups. Figure 2 shows that survival rates were significantly different across these four groups (log-rank test P < 0.001), as demonstrated by Kaplan–Meier analysis. The highest mortality incidence (12.4 events/100 person-years) was observed in the CKD(+)DR(+) group; this value was significantly higher than the 8.1 events/100 person-years in the CKD(+)DR(–) group (P = 0.010), the 7.4 events/100 person-years in the CKD(–)DR(+) group (P = 0.004), and the 4.6 events/100 person-years in

the CKD(–)DR(–) group (P < 0.001). The incidences of mortality in the CKD(+)DR(–) and CKD(–)DR(+) groups were also significantly higher than that in the CKD(–)DR(–) group (P < 0.001 and P = 0.003, respectively). However, there was no significant difference in the mortality incidence between the CKD(+)DR(–) and CKD(–)DR(+) groups (P = 0.479).

Cox regression analysis for all-cause and cardiovascular mortality

To identify the predictive factors for long-term mortality, univariate Cox regression analysis was conducted for all the enrolled patients. In addition to the different groups categorized based on CKD and DR, age, gender, diabetes duration, body mass index (BMI), systolic blood pressure, metformin treatment, insulin treatment, and diuretic treatment were significantly associated with total mortality. Using multivariate Cox regression analysis, patients with CKD and DR have the highest hazard ratio (2.263; 95% CI, 1.551–3.302) for all-cause mortality in comparison with the ones without CKD or DR after adjustment for age, gender, diabetes duration, BMI, systolic blood pressure, metformin treatment, insulin treatment and diuretics treatment (Table 2A). Moreover, patients with CKD and DR also had the highest hazard ratio (2.471; 95% CI, 1.421–4.297) for cardiovascular mortality in comparison to the ones without CKD or DR after adjustment for age, gender, diabetes duration, BMI, systolic blood pressure and angiotensin-converting enzyme (ACE) inhibitor or angiotensin II receptor antagonist (ARB) treatment (Table 2B).

DISCUSSION

In this retrospective cohort study, we found that patients with NA-CKD have a higher risk of all-cause mortality compared with patients without CKD. Furthermore, the presence of DR imposed a higher mortality risk in patients with NA-CKD.

The mortality risk associated with NA-CKD remains controversial. In the Casale Monferrato study, which had an 11-year follow-up, eGFR showed a significantly inverse trend with long-term mortality only in type 2 diabetic patients with macroalbuminuria, but not in those with microalbuminuria or normoalbuminuria.[28] Conversely, several studies reported that low eGFR was significantly associated with a higher all-cause mortality risk, independent of albuminuria.[29-33] The magnitude of the impact on all-cause mortality varied. Rigalleau et al.[34] reported that NA-CKD was associated with a very low risk of dialysis or mortality in comparison with albuminuric CKD in diabetic patients during a 38-month follow-up study in France. In an Asian study with a 44-month follow-up, albuminuria was associated with a significantly higher risk of renal events, but not cardiovascular events in diabetic patients with CKD.[35] In the present study, we found that NA-CKD is associated with an approximately 1.8-fold increase in either all-cause mortality or cardiovascular disease, compared with type 2 diabetic patients without CKD. In line with our findings, Hsieh et al.[36] reported that eGFR was inversely related to risk of cardiovascular events in type 2 diabetic

outpatients with normoalbuminuria during a 4-year follow-up study.

We postulated that heterogeneity in the pathogenesis of NA-CKD might contribute to the differences in results. Different natural courses have been observed in the NA-CKD phenotype: initial albuminuria with regression to normoalbuminuria with intensive renin-angiotensin-aldosterone system (RAAS) inhibitor use in some diabetic patients, but eGFR loss might be the only manifestation of renal involvement in others.[12 37] Based on renal biopsy, tubular-interstitial lesions and arterial hyalinosis were the predominant findings in NA-CKD rather than the typical glomerulosclerotic lesions seen with albuminuria in diabetic patients.[38 39] With albuminuria regression due to the widespread use of RAAS inhibitors in recent decades, NA-CKD is being found in majority of DKD patients. The number of patients with normoalbuminuria has been found to be great than those with albuminuria in several large studies of type 2 diabetic patients with CKD.[40-42] Hence, there is an urgent need to identify the predictors of mortality among this distinct population.[43]

In the present study, patients using ACE inhibitors or ARBs showed a higher risk of cardiovascular mortality in the univariate model, and this finding may result from the higher proportion of CKD patients using these drugs at baseline. In the multivariate model, the use of ACE inhibitors or ARBs at baseline was not significantly associated with cardiovascular mortality. Consistent with these findings, early ARB treatment was not found to significantly improve eGFR in type 2 diabetic patients with urine albumin-to-creatinine ratio <300 mg/g.[44]

Although there is a high prevalence of DR in type 2 diabetic patients with albuminuria,[20] the concordance between DR and CKD was lower in patients with normoalbuminuria than in those with albuminuria.[45 46]. In the present study, we found that the prevalence of retinopathy was not significantly different between patients with and without CKD. However, the highest risks of all-cause mortality and cardiovascular mortality were shown in the patients with CKD and DR. Compared with the NEHANS III population, in which the synergistic effect of CKD and retinopathy on morality was also observed,[47] our findings showed the evidence in type 2 diabetic inpatients even without albuminuria. Although similar risk factors and cardiovascular effects for DR and CKD have been reported,[30 48 49] the exact mechanism underlying the superimposed DR effect on mortality in type 2 diabetic patients with NA-CKD requires further investigation.

In the present study, both isolated CKD, i.e. CKD(+)DR(–), and isolated DR, i.e. CKD(–)DR(+), showed significantly higher mortality risks than those with neither CKD nor DR in univariate analyses; however, the significant difference seemed to attenuate in isolated CKD after adjustment for other traditional cardiovascular risk factors. NA-CKD has been reported to be highly associated with cardiovascular risk factors.[36 42] Therefore, the attenuation of the mortality prediction after adjustment for cardiovascular risk factors might be more obvious in isolated CKD in comparison with isolated DR. Furthermore, it is notable that a higher BMI showed a protective effect for all-cause and cardiovascular mortality in the

present study. Although this seems somewhat contradictory to traditional concepts, we were not the only one to report this paradoxical effect of BMI on mortality in patients with diabetes.[50] Cea Soriano et al.[51] reported that BMI ≥ 25 kg/m^2 predicted a lower mortality risk than BMI < 25 kg/m^2 in type 2 diabetic patients with CKD.

Among type 2 diabetic patients with normoalbumiuria, high urine albumin excretion (10–29 mg/day) was reportedly associated with multiple cardiovascular risk factors, as compared to low urine albumin excretion (< 10 mg/day).[52] Recently, the highest tertile of the urine albumin excretion rate was found to be associated with diabetic retinopathy and arterial stiffness, in comparison to the lowest tertile among type 2 diabetic patients with normoalbuminuria.[53 54] In the present study, 257 patients without CKD had available urine albumin data, including 108 patients with low normoalbuminuria (< 10 mg/day) and 149 patients with high normoalbuminuria (10–29 mg/day). However, there was no significant difference in DR prevalence (P > 0.05) or all-cause and cardiovascular mortality (both log-rank test P > 0.05). Further investigations with a large number of cases will be needed to evaluate the association between high normoalbuminuria and mortality.

We acknowledge several limitations in our study. First, we enrolled patients with not only normoabluminuria, but also normoproteinuria, due to the limited numbers of cases overall. Second, we used the 24-hour urine data instead of spot-urine data, since the latter has been well reported in type 2 outpatients previously.[55] Third, we only included type 2 diabetic

inpatients who were admitted to the hospital with a primary diagnosis of poor glucose control. Fourth, we calculated eGFR using the MDRD equation instead of the CKD Epidemiology Collaboration (EPI) equation as consensus had not been reached regarding the use of the CKD-EPI equation in the Taiwanese population.[56] Finally, we assessed the patients only at baseline, but not during the follow-up. Therefore, treatment might have confounded the results following patients discharged.

In conclusion, DR is a significant predictor for all-cause and cardiovascular mortality in type 2 diabetic inpatients with normoalbuminuria. Presence of DR also showed an impact on mortality for type 2 diabetic inpatients with NA-CKD. Screening for DR and eGFR may help identify those who harbor a high mortality risk after discharge in type 2 diabetic inpatients hospitalized due to poor glucose control, even with normoalbuminuria.

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Data sharing statement: No additional data are available.

Author contributions

YL, WHS and IL contributed to the study design. YL and IL participated in the data collection. YL and IL participated in the analysis and interpretation of the data. YL drafted the manuscript. IL had full access to the study data. IL is the guarantor. All the authors performed a critical revision of the manuscript for important intellectual content.

FIGURE LEGENDS

Figure 1. Flow diagram of enrollment of study subjects with normoalbuminuria.

Figure 2. Kaplan-Meier curves showing survival rates categorized according to chronic kidney disease (CKD) and diabetic retinopathy (DR) in type 2 diabetic inpatients with normoalbuminuria.

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Table 1. The clinical data of patients according to the presence of CKD and DR

	CKD(–)DR(–) (n = 306)	CKD(–)DR(+) (n = 130)	CKD(+)DR(–) (n = 149)	CKD(+)DR(+) (n = 80)	P value
Age (years)	57 ± 15	62 ± 13	69 ± 10	70 ± 9	<0.001
Male, n (%)	189 (61.8%)	63 (48.5%)	82 (55.0%)	42 (52.5%)	0.057
BMI (kg/m^2)	23.7 ± 4.5	23.3 ± 4.0	24.2 ± 4.1	24.2 ± 4.3	0.367
Systolic blood pressure (mmHg)	124 ± 14	129 ± 15	127 ± 15	128 ± 14	0.004
Diastolic blood pressure (mmHg)	74 ± 11	75 ± 10	71 ± 9	72 ± 10	0.003
Diabetes duration (years)[#]	6.7 ± 6.7	11.4 ± 7.4	7.9 ± 7.7	13.2 ± 8.4	<0.001
Current smoker, n (%)	96 (31.4%)	36 (27.7%)	31 (20.8%)	13 (16.3%)	0.014
White blood cell count (10^6/L)	7836 ± 5193	7210 ± 2421	8258 ± 3504	8358 ± 3285	0.227
HbA1c (%)	11.5 ± 2.9	10.6 ± 2.3	10.4 ± 3.3	9.3 ± 2.6	<0.001
Total cholesterol (mmol/L)	4.8 ± 1.3	5.0 ± 1.3	4.6 ± 1.3	4.7 ± 1.5	0.197
Triglyceride (mmol/L)[#]	1.9 ± 1.9	1.9 ± 2.2	2.0 ± 2.2	1.8 ± 1.3	0.574
HDL cholesterol (mmol/L)	1.1 ± 0.4	1.1 ± 0.4	1.0 ± 0.3	1.0 ± 0.4	0.149
eGFR (mL/min/1.73m^2)[#]	88 ± 23	87 ± 20	45 ± 13	42 ± 15	<0.001
Hypertension, n (%)	171 (55.9%)	93 (71.5%)	113 (75.8%)	63 (78.8%)	<0.001
Antihypertensive agents, n (%)	91 (29.7%)	63 (48.5%)	90 (60.4%)	47 (58.8%)	<0.001
ACE inhibitor or ARB, n (%)	58 (19.0%)	44 (33.8%)	59 (39.6%)	32 (40.0%)	<0.001
α-blocker, n (%)	20 (6.5%)	14 (10.8%)	20 (13.4%)	10 (12.5%)	0.079
β-blocker, n (%)	20 (6.5%)	9 (6.9%)	3 (2.0%)	6 (7.5%)	0.172
Calcium channel blocker, n (%)	43 (14.1%)	28 (21.5%)	37 (24.8%)	20 (25.0%)	0.015
Diuretics, n (%)	8 (2.6%)	6 (4.6%)	11 (7.4%)	16 (20.0%)	<0.001
Oral antihyperglycemic drugs, n (%)	151 (49.3%)	65 (50.0%)	53 (35.6%)	27 (33.8%)	0.005
Insulin secretagogues, n (%)	127 (41.5%)	56 (43.1%)	46 (30.9%)	23 (28.8%)	0.027
Metformin, n (%)	95 (31.0%)	41 (31.5%)	26 (17.4%)	17 (21.3%)	0.007

Thiazolidinediones, n (%)	6 (2.0%)	5 (3.8%)	2 (1.3%)	0 (0.0%)	0.230
α-glucosidase inhibitor, n (%)	10 (3.3%)	5 (3.8%)	3 (2.0%)	2 (2.5%)	0.811
Insulin therapy, n (%)	231 (75.5%)	80 (61.5%)	70 (47.0%)	38 (47.5%)	<0.001
Statins, n (%)	28 (9.2%)	12 (9.2%)	17 (11.4%)	12 (15.0%)	0.442

ACE = angiotensin-converting enzyme, ARB = angiotensin II receptor antagonists, BMI = body mass index, CKD = chronic kidney disease, DR = diabetic retinopathy, HbA1c = glycated hemoglobin, HDL = high-density lipoprotein.

[#] Kruskal-Wallis tests to determine the significance of the differences due to skewed distribution in diabetic duration, triglycerides and eGFR

Table 2. Results of Cox regression analysis for the effects of risk factors on (A) all-cause and (B) cardiovascular mortality

(A) All-cause mortality

	Univariate model			Multivariate model					
	Crude			Model 1			Model 2		
	HR	95%CI	P value	HR	95%CI	P value	HR	95%CI	P value
CKD(-)DR(-)*	1.000			1.000			1.000		
CKD(-)DR(+)	1.590	(1.179, 2.145)	0.002	1.556	(1.148, 2.108)	0.004	1.686	(1.202, 2.364)	0.002
CKD(+)DR(-)	1.842	(1.377, 2.463)	<0.001	1.416	(1.042, 1.924)	0.026	1.381	(0.984, 1.939)	0.062
CKD(+)DR(+)	2.791	(2.016, 3.866)	<0.001	2.209	(1.573, 3.101)	<0.001	2.263	(1.551, 3.302)	<0.001
Age (every 10 years)	1.455	(1.318, 1.607)	<0.001	1.328	(1.197, 1.472)	<0.001	1.325	(1.177, 1.491)	<0.001
Gender (male)	1.763	(1.394, 2.229)	<0.001	1.763	(1.388, 2.239)	<0.001	1.692	(1.298, 2.206)	<0.001
Current smoker (yes/no)	1.223	(0.958, 1.561)	0.107						
Diabetes duration >7 years (yes/no)	1.271	(1.012, 1.596)	0.039				1.084	(0.832, 1.412)	0.550
BMI (every 1 kg/m^2)	0.959	(0.931, 0.987)	0.004				0.954	(0.925, 0.984)	0.003
Systolic BP (every 10 mmHg)	1.081	(1.002, 1.167)	0.045				1.017	(0.932, 1.111)	0.701
HbA1c (every 1%)	0.969	(0.927, 1.013)	0.163						
Metformin (yes/no)	0.776	(0.604, 0.998)	0.049				0.751	(0.549, 1.028)	0.074
Insulin secretagogues (yes/no)	0.851	(0.677, 1.070)	0.167						
Insulin therapy (yes/no)	0.777	(0.619, 0.974)	0.029				1.094	(0.807, 1.483)	0.562
Statins (yes/no)	1.090	(0.763, 1.557)	0.635						
ACE inhibitor or ARB (yes/no)	1.144	(0.900, 1.454)	0.271						
Calcium channel blocker (yes/no)	1.237	(0.942, 1.625)	0.125						
Diuretics (yes/no)	2.517	(1.714, 3.696)	<0.001				1.765	(1.115, 2.793)	0.015

ACE = angiotensin-converting enzyme, ARB = angiotensin II receptor antagonists, BMI = body mass index, BP = blood pressure, HbA1c = glycated hemoglobin, CI = confidence interval, HR = hazard ratio

The risk factors in the univariate model were selected based on the findings in Table 1.

The risk factors in model 2 were selected based on the statistical significance indicated in the univariate model.

*the overall P value <0.001 among the CKD(-)DR(-), CKD(-)DR(+), CKD(+)DR(-), CKD(+)DR(+) groups

(B) Cardiovascular mortality

	Univariate model			Multivariate model					
	Crude			Model 1			Model 2		
	HR	95%CI	P value	HR	95%CI	P value	HR	95%CI	P value
CKD(-)DR(-)*	1.000			1.000			1.000		
CKD(-)DR(+)	2.092	(1.340, 3.267)	0.001	2.039	(1.297, 3.203)	0.002	1.896	(1.153, 3.115)	0.012
CKD(+)DR(-)	1.937	(1.221, 3.072)	0.005	1.387	(0.855, 2.251)	0.185	1.376	(0.815, 2.322)	0.232
CKD(+)DR(+)	3.601	(2.215, 5.854)	<0.001	2.710	(1.634, 4.493)	<0.001	2.471	(1.421, 4.297)	0.001
Age (every 10 years)	1.615	(1.377, 1.894)	<0.001	1.445	(1.224, 1.706)	<0.001	1.389	(1.152, 1.674)	<0.001
Gender (male)	2.124	(1.472, 3.066)	<0.001	2.132	(1.465, 3.102)	<0.001	2.126	(1.391, 3.250)	<0.001
Current smoker (yes/no)	1.326	(0.922, 1.908)	0.128						
Diabetes duration >7 years (yes/no)	1.775	(1.237, 2.546)	0.002				1.196	(0.788, 1.815)	0.401
BMI (every 1 kg/m^2)	0.885	(0.843, 0.928)	<0.001				0.877	(0.836, 0.921)	<0.001
Systolic BP (every 10 mmHg)	1.157	(1.034, 1.295)	0.011				1.035	(0.912, 1.175)	0.593
HbA1c (every 1%)	0.977	(0.914, 1.045)	0.503						
Metformin (yes/no)	0.819	(0.562, 1.193)	0.297						
Insulin secretagogues (yes/no)	0.950	(0.675, 1.339)	0.771						
Insulin therapy (yes/no)	0.900	(0.635, 1.276)	0.554						
Statins (yes/no)	0.788	(0.425, 1.460)	0.448						
ACE inhibitor or ARB (yes/no)	1.572	(1.113, 2.221)	0.010				1.159	(0.765, 1.756)	0.487
Calcium channel blocker (yes/no)	1.366	(0.914, 2.041)	0.128						
Diuretics (yes/no)	2.387	(1.316, 4.330)	0.004				1.321	(0.618, 2.821)	0.473

ACE = angiotensin-converting enzyme, ARB = angiotensin II receptor antagonists, BMI = body mass index, BP = blood pressure, HbA1c = glycated hemoglobin, CI = confidence interval, HR = hazard ratio

The risk factors in the univariate model were selected based on the findings in Table 1.

The risk factors in model 2 were selected based on the statistical significance indicated in the univariate model.

*the overall P value <0.001 among the CKD(-)DR(-), CKD(-)DR(+), CKD(+)DR(-), CKD(+)DR(+) groups

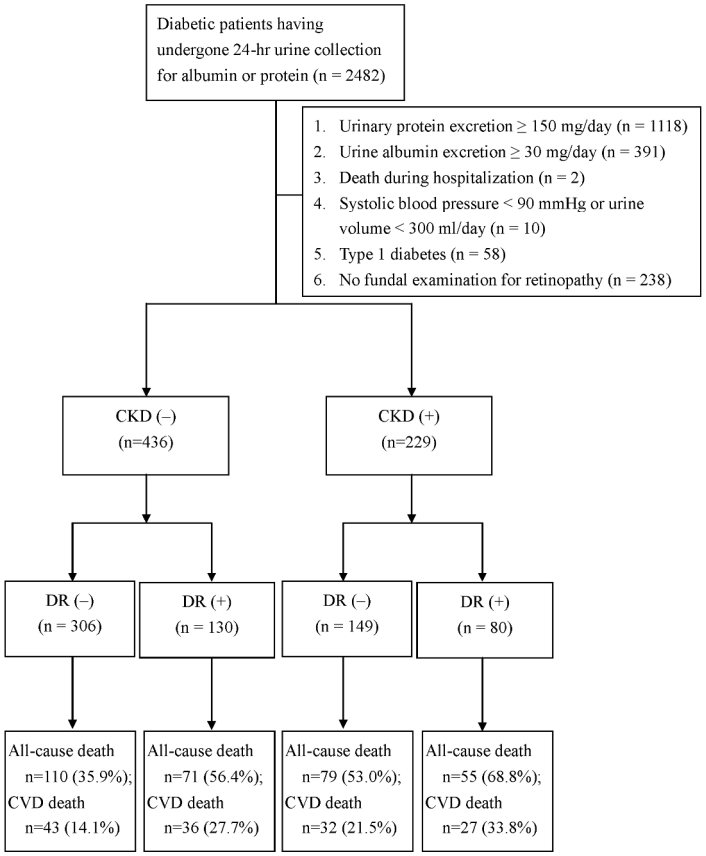


Figure 1.
Flow diagram of enrollment of study subjects with normoalbuminuria.

210x297mm (300 x 300 DPI)

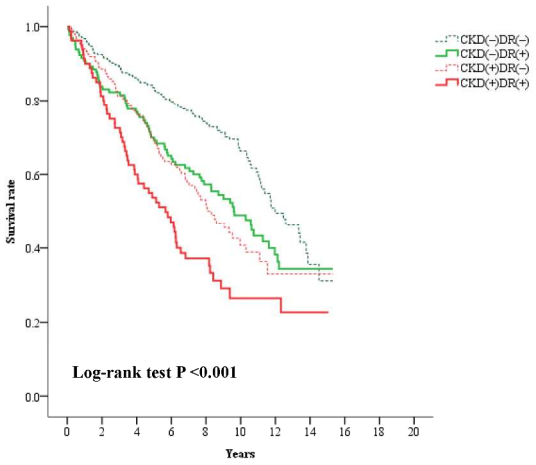
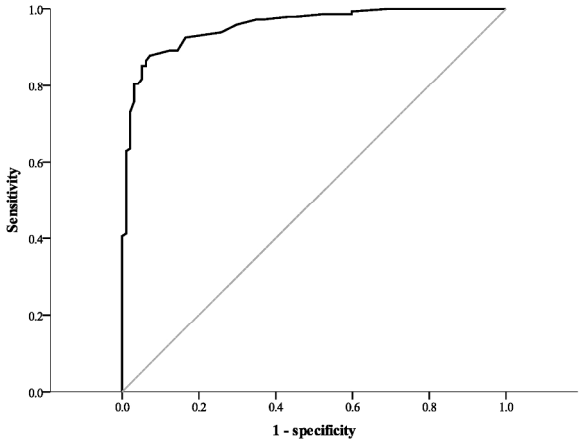


Figure 2.
Kaplan-Meier curves showing survival rates categorized according to chronic kidney disease (CKD) and diabetic retinopathy (DR) in type 2 diabetic inpatients with normoalbuminuria.

210x297mm (300 x 300 DPI)



Supplementary figure.
Receiver operating characteristic (ROC) analysis curves for differentiating normoalbuminuria (urine albumin excretion < 30 mg/day) based on daily urinary protein excretion.

210x297mm (300 x 300 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1,2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2,3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4,5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*7*
Bias	9	Describe any efforts to address potential sources of bias	14,15
Study size	10	Explain how the study size was arrived at	8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7,8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7,8
		(*b*) Describe any methods used to examine subgroups and interactions	10
		(*c*) Explain how missing data were addressed	NA
		(*d*) If applicable, explain how loss to follow-up was addressed	NA
		(*e*) Describe any sensitivity analyses	8,9
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9, Figure 2
		(b) Give reasons for non-participation at each stage	Figure 2
		(c) Consider use of a flow diagram	Figure 2
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) Summarise follow-up time (eg, average and total amount)	9
Outcome data	15*	Report numbers of outcome events or summary measures over time	9,10, Figure 2
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Table 2
		(*b*) Report category boundaries when continuous variables were categorized	11
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-14
Generalisability	21	Discuss the generalisability (external validity) of the study results	12-14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.