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ORIGINAL ARTICLE

# The association between cigarette smoking and diabetic nephropathy in Chinese male patients

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## Abstract

**Aims** To investigate the association between cigarette smoking and the clinicopathological features and renal prognosis of type 2 diabetic mellitus (T2DM) patients with diabetic nephropathy (DN).

**Methods** A total of 223 T2DM male patients with biopsy-proven DN who received follow-up for at least 1 year were recruited. The patients were divided into two groups based on smoking status: smoking group and non-smoking group. Clinicopathologic differences were analyzed between the two groups. In addition, smokers were divided into two groups of binary analysis based on smoking amounts and two groups of former smokers and current smokers, and subgroups analysis based on age and DR, respectively. The influence of smoking on estimated glomerular filtration rate (eGFR) was estimated using logistic regression analysis and Cox regression on renal outcomes. Renal outcomes were defined by progression to end-stage renal disease (ESRD) or doubling of serum creatinine (D-SCr) level.

**Results** Compared with nonsmokers, smoking patients had more moderate decline eGFR ($p=0.032$) and tubular atrophy and interstitial fibrosis ($p=0.033$). The adjusted logistic regression analysis suggested cigarette smoking was negatively associated with more severe decline eGFR ($p=0.015$), especially for patients with DR ($p=0.010$) and patients of age $\leq 50$ years ($p=0.012$) in the subgroup analysis. In the prognosis analysis, no obvious significant risk factor was shown about smoking. Interestingly, it was observed that former smokers had lower levels of plasma glucose and triglycerides than current smokers (both $p<0.05$), while smokers with small smoking amounts had lower levels of triglycerides than those with large smoking amounts ($p<0.05$).

**Conclusion** Cigarette smoking patients with T2DM and DN had more moderate decline eGFR, especially for DN patients with DR, and milder IFTA lesions, although an obviously significant risk factor was not shown about smoking for DN.

**Keywords** Diabetic nephropathy · Cigarette smoking · eGFR · Diabetic retinopathy · Tubular atrophy and interstitial fibrosis (IFTA)

## Introduction

The world is undergoing global burden of diabetes and its impact will continue to grow [1], the latest nationwide survey reported that the overall prevalence of diabetes among adults in China in 2013 was 10.9% [2], and it is 12.3% in the USA in 2011–2012 [3]. Diabetic nephropathy (DN) is one of the important microvascular complications in patients with diabetic mellitus (DM), ranking as the leading cause of end-stage renal disease (ESRD) [4, 5]. It is reported that, in the USA, the incidence of DN in renal biopsy patients has dramatically increased from 5.5% in the decade 1986–1995 to 19.1% in the decade 2006–2015 [6]. The widespread

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**Electronic supplementary material** The online version of this article (https://doi.org/10.1007/s00592-018-1197-9) contains supplementary material, which is available to authorized users.

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incidence craved our in-depth exploration to reduce socio-economic burden of DN.

DN is characterized by glomerular basement membrane thickening, mesangial expansion, glomerulosclerosis, albuminuria and progressive reduction in kidney function [4]. The development of DN is genetically predisposed and influenced by many factors, such as prolonged high glucose, metabolic and hemodynamic factors. Cigarette smoking is an established risk factor for cardiovascular disease. Some clinical researchers [7, 8] had emphasized that smoking aggravated the occurrence of macroalbuminuria and ESRD both in type 1 and type 2 DM patients with chronic kidney disease. But, the mechanism of cigarette smoking affecting the kidney is relatively complex. Recently, studies in physical examination individuals have suggested that smoking was associated with an increased risk of glomerular hyperfiltration, which is an early marker of kidney disease and proteinuria [9, 10]. And one study on T2DM patients with albuminuria found that compared with nonsmokers, current smoking patients had higher glomerular filtration rate [11]. But, the diagnosis of diabetic nephropathy (DN) was based on clinical manifestations, and patients with non-diabetic renal disease may have been misdiagnosed with DN, potentially making the results less convincing [12, 13]. Moreover, the association between smoking and renal prognosis in T2DM patients with DN remains poorly stated.

Our present study intended to investigate the association between cigarette smoking and the clinicopathological features and renal prognosis of T2DM patients with biopsy-proven DN.

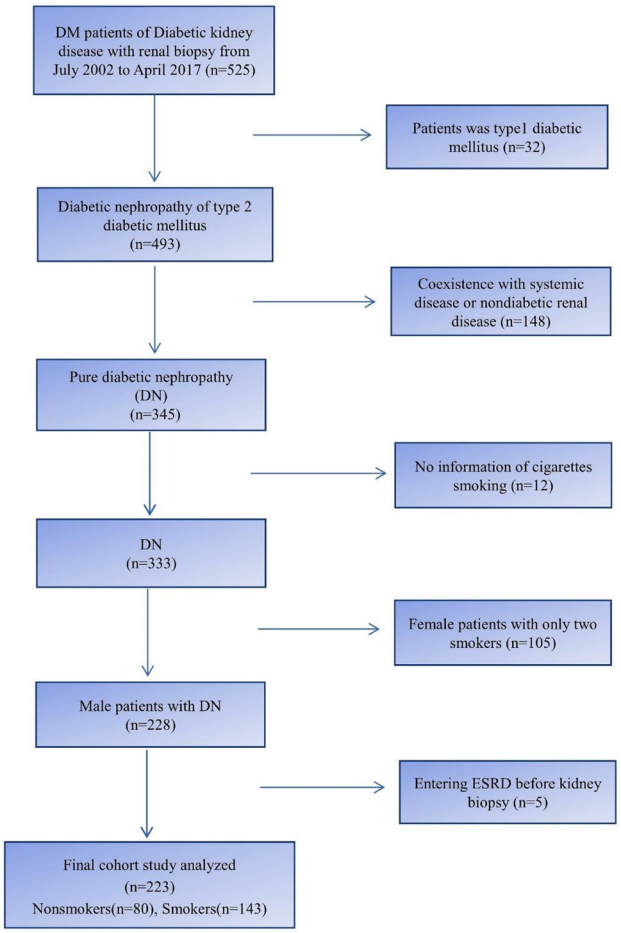


**Fig. 1** Flowchart of study participants

## Materials and methods

### Patients

A total of 525 patients with diabetes mellitus who underwent renal biopsy at West China Hospital of Sichuan University from July 2002 to April 2017 were reviewed. The inclusion criteria were as follows: T2DM with biopsy-proven DN, cigarette smoking status with the available amounts collected at the time of renal biopsy. Exclusion criteria were: coexistent systemic or other renal diseases, other types of DM, no cigarette smoking registration. And 105 female patients were excluded to reduce the effects of gender and data distribution because of only two female smokers. A total of 223 male patients were considered eligible and were enrolled in this study (Fig. 1). The diagnosis of T2DM was based on the American Diabetes Association criteria [14]. The general indications for kidney biopsy of patients with diabetes in our hospital were patients with diabetes with kidney damage who lacked absolute contraindications, especially those without diabetic retinopathy, those with obvious glomerular hematuria and/or sudden onset overt proteinuria, or patients with short diabetic duration (< 5 years) [15, 16]. DN was diagnosed by at least two renal pathologists and/or nephrologists, and the diagnosis was re-evaluated according to Tervaert's classification [12, 17]. All the included patients were divided into two groups according to smoking status until biopsy: smokers and nonsmokers. Nonsmokers were defined as individuals who had never used tobacco, smokers had ever established chronic cigarette smokers. In the subgroup analysis, the patients were divided into two groups: (a) age ≤ 50 years and age > 50 years; (b) DN patients without DR and DN patients with DR. And in the supplement study: (a) smokers were divided into two groups of binary analysis based on smoking amounts; (b) smokers were divided into former smokers and current smokers, former smokers were those who quit smoking at least one months prior to registration. During the observation period from July 2002 to April 2018, the patients in this study were regularly followed up for at least one year at West China Hospital and their renal function and proteinuria were evaluated, presence of renal endpoint events, death or loss to follow-up was the end of follow-up. The composite endpoint of kidney was the

doubling of baseline serum creatinine (D-SCr) level and/or progression to ESRD, which was defined by e-GFR < 15 mL/min/1.73 $m^2$ or commencing the renal replacing therapy.

### Clinical and pathological characteristics

Baseline clinical and biochemical parameters were registered for each patient included at the time of renal biopsy: smoking status, age, gender, weight, height, blood pressure, duration of diabetes, diabetic retinopathy (DR). DR was defined as present if any of the following lesions was detected: microaneurysms, retinal hemorrhages, soft exudates, hard exudates, or vitreous hemorrhage. Fasting plasma glucose, blood lipids, blood urea nitrogen, serum creatinine and serum albumin were measured using routine laboratory methods with a Hitachi 7600 (Hitachi, Tokyo, Japan). Simultaneously, 24-h proteinuria was obtained using a biochemistry autoanalyser (CobasIntera 400 Plus, Roche, Basel, Switzerland). The estimated glomerular filtration rate (eGFR, mL/min/1.73 $m^2$) was computed according to the Modification of Diet in Renal Disease equation. All renal biopsies were performed with patients' consent. Tissue was obtained by needle biopsy and the specimens were then processed for light microscopy, immunofluorescence, and electron microscopy routinely to detect renal pathological changes. All patients were categorized based on the pathologic classification of the Renal Pathology Society in 2010 [17].

### Statistical analysis

The data were analyzed using SPSS 22.0 software. The clinical and pathological characteristics as baseline were compared between the two groups. Numeric values were presented as mean ± standard deviation and range, differences in means were compared using Student's *t* test or the Mann–Whitney test, as appropriate. Categorical values were presented as number (%) and the differences in proportions were analyzed using the Chi-square test. The influence of cigarette smoking on eGFR was evaluated by logistic regression analysis with or without adjusted other influence factors. The relationship between cigarette smoking and composite endpoint was assessed using Cox regression. A two-sided $p$ value < 0.05 was considered to be statistically significant.

## Results

### Baseline clinical and pathological characteristics

Of the 223 patients recruited in this study, there were 80 (35.87%) nonsmokers and 143 (64.13%) smokers. At baseline, the mean age was 51.45 ± 9.20 years, the median duration of T2DM was 7.33 years (range 0–30 years). The median 24-proteinuria was 5.67 g/day (range 0.04-27 g/day), the median serum creatinine level was 147.18 ± 86.87 μmol/L, and the median eGFR was 68.92 ± 34.41 mL/min/1.73 $m^2$ at the time of biopsy. The median follow-up period was 16 months (12–79 months). During follow-up, 109 patients (48.88%) progression to composite endpoint including 88 patients (39.46%) progressed to ESRD from the time of renal biopsy. In terms of the IFTA [12], 10 patients (4.48%) were in grade 0, 105 (47.09%) in grade 1, 88 (39.46%) in grade 2, and 20 (8.97%) in grade 3. In addition, 97.09% patients had thickened glomerular basement membrane (GBM) with mean thickness of 864.81 nm. 97 (43.50%) patients had nodular glomerulosclerosis and 31(13.90%) patients had > 50% global sclerosis. The immune deposits in immunofluorescence were found in 87 patients (39.01%) and IgM deposit was the most common pattern (26.91%). Clinical characteristics of patients with different smoking status are shown in Table 1, and pathological characteristics are shown in Table 2.

### Correlation between cigarette smoking and the clinicopathological features

Compared with patients of non-smoking, the patients of smoking had higher levels of eGFR (62.28 ± 32.71 vs. 72.58 ± 34.88, $p = 0.032$), lower levels of serum creatinine and blood urea nitrogen ($p < 0.05$). There were no significant differences in age, duration of diabetes, hypertension, blood lipid or 24-h proteinuria. As shown in Table 2, further analysis revealed that patients of smoking had more moderate IFTA score compared with patients of non-smoking ($p = 0.033$). But there were no differences in glomerular lesions, interstitial inflammation score or arteriolar hyalinosis between the two groups. And no differences were shown in immunofluorescence features or mean GBM thickness.

### Cigarette smoking and eGFR in cross-sectional study

The risk for eGFR < 60 mL/min/1.73 $m^2$ were determined by logistic regression analysis, as shown in Fig. 2. Unadjusted analyses indicated that age, serum creatine and urine protein were positively associated with the severe decline eGFR, cigarette smoking and hemoglobin were negatively associated with severe decline eGFR. Duration of diabetes ≥ 5 to < 10 years was significantly associated with mild decline eGFR compared with duration < 5 years or duration ≥ 10 years. Adjusted for age and risk factors of kidney, such as blood pressure, blood glucose and blood lipids, cigarette smoking was still significantly negatively associated with eGFR < 60 mL/min/1.73 $m^2$ [odds ratio (OR) = 0.394, 95% confidence interval (CI) = 0.187–0.830,

**Table 1** Characteristics of study participants at baseline according to smoking habits

| | All | Smoking habits | | |
|---|---|---|---|---|
| | | Nonsmokers | Smokers | *p* value |
| Number | 223 | 80 (35.87%) | 143 (64.13%) | N/A |
| Age (years) | 51.45 ± 9.20 | 51.15 ± 9.59 | 51.62 ± 9.01 | 0.714 |
| Body mass index (kg/m$^2$) | 25.88 ± 4.18 | 25.62 ± 4.76 | 26.01 ± 3.90 | 0.676 |
| Diabetic retinopathy (%) | 101 | 36 (45%) | 65 (45.45) | 0.948 |
| Systolic blood pressure (mmHg) | 142.99 ± 21.36 | 146.03 ± 23.69 | 141.28 ± 19.82 | 0.112 |
| Diastolic blood pressure (mmHg) | 86.05 ± 13.03 | 88.11 ± 15.39 | 84.89 ± 11.37 | 0.104 |
| Hypertension (%) | 216 (96.86) | 76 (95) | 140 (97.90) | 0.233 |
| Duration of diabetes (years) | 7.33 (0–30) | 7.58 (0–30) | 7.25 (0–25) | 0.687 |
| Fasting plasma glucose (mmol/L) | 8.04 ± 4.03 | 7.62 ± 3.61 | 8.29 ± 4.25 | 0.217 |
| HbAlc (%) | 7.53 ± 1.97 | 7.66 ± 1.87 | 7.45 ± 2.03 | 0.530 |
| Total Cholesterol (mmol/L) | 5.14 ± 1.71 | 5.18 ± 1.43 | 5.11 ± 1.85 | 0.777 |
| Triglycerides (mmol/L) | 2.09 ± 1.63 | 1.89 ± 1.21 | 2.20 ± 1.82 | 0.174 |
| Serum albumin (g/L) | 33.56 ± 7.90 | 34.14 ± 7.02 | 33.25 ± 8.34 | 0.434 |
| Hemoglobin (g/L) | 124.74 ± 27.93 | 123.62 ± 30.99 | 125.36 ± 26.17 | 0.658 |
| Uricemia (mmol/L) | 393.52 ± 84.82 | 395.44 ± 392.44 | 83.78 ± 85.68 | 0.800 |
| Estimated GFR (mL/min per 1.73 m$^2$) | 68.92 ± 34.41 | 62.28 ± 32.71 | 72.58 ± 34.88 | 0.032 |
| Serum creatinine (μmol/L) | 147.18 ± 86.87 | 164.62 ± 100.23 | 137.43 ± 77.09 | 0.025 |
| Blood urea nitrogen (mmol/L) | 9.36 ± 5.29 | 10.55 ± 6.91 | 8.69 ± 3.99 | 0.011 |
| Urine protein (g/24 h) | 5.67 (0.04–27) | 5.57 (0.08–27) | 5.72 (0.04–22.5) | 0.825 |
| Oral hypoglycemic agents (%) | 98 (43.95) | 37 (46.25) | 61 (42.66) | 0.604 |
| Insulin therapy (%) | 155 (69.51) | 55 (68.75) | 100 (69.93) | 0.854 |
| Antihypertensive medication (%) | 212 (95.07) | 76 (92.68) | 136 (95.10) | 0.972 |
| RAAS inhibitors (%) | 174 (78.03) | 61 (76.25) | 113 (79.02) | 0.736 |
| Calcium antagonists (%) | 147 (65.92) | 53 (66.25) | 94 (65.73) | 1.00 |
| β-blocker (%) | 48 (21.52) | 18 (22.50) | 30 (20.98) | 0.865 |
| α-blocker (%) | 56 (25.11) | 26 (32.50) | 30 (20.98) | 0.076 |
| Number of cigarettes smoked per day | N/A | 0 | 22.72 (1–80) | N/A |
| Cumulative amount of smoking (pack-years) | N/A | 0 | 29.70 (0.5–160) | N/A |
| Progression to ESRD (%) | 88 (39.46%) | 27 (33.75%) | 61 (42.66%) | 0.192 |
| Progression to composite endpoint (%) | 109 (48.88%) | 36 (45%) | 73 (51.05%) | 0.386 |

Values are expressed as mean ± standard, counts and percentages or median with range in parentheses. N/A, not available

Hypertension was defined as systolic blood pressure 140 mmHg or greater, diastolic blood pressure 90 mmHg or greater, or if the participant was receiving antihypertensive medications

$p = 0.014$]. In the subgroup analysis of age ≤ 50 years, cigarette smoking was still significantly negatively associated with eGFR < 60 mL/min/1.73 m$^2$ (OR 0.391, 95% CI 0.171–0.893, $p = 0.026$) in the univariate analysis and (OR 0.263, 95% CI 0.076–0.731, $p = 0.012$) in the multivariate analysis adjusted for other risk factors of kidney. But no significant association between cigarette smoking and eGFR < 60 mL/min/1.73 m$^2$ was shown in the patients of age > 50 years (as shown in Fig. 3). In the subgroup analysis of DN patients with DR, we still found significant negative association between cigarette smoking and eGFR < 60 mL/min/1.73 m$^2$ (OR 0.279, 95% CI 0.113–0.687, p = 0.006) in the univariate analysis and (OR 0.192, 95% CI 0.054–0.677, p = 0.010) in the multivariate analysis adjusted for other risk factors. But no significant association was found between cigarette smoking and eGFR < 60 mL/min/1.73 m$^2$ in the DN patients without DR, the results are shown in Fig. 4.

Furthermore, the comparison between former smokers and current smokers suggested that the levels of fasting plasma glucose (7.23 ± 2.92 vs. 8.81 ± 4.69, $p = 0.016$) and the levels of triglycerides (1.67 ± 0.69 vs. 2.46 ± 2.12, $p = 0.001$) were lower vs. those in current smokers. Binary analysis based on smoking amounts revealed that patients with more smoking amounts bore higher levels of triglycerides (1.87 ± 1.24 vs. 2.54 ± 2.22, $p = 0.030$) (Supplement Table 1).

**Table 2** Pathological findings according to smoking habits

| Light microscopy features [*n*] | All<br>*N* = 223 | Nonsmokers<br>*N* = 80 | Smokers<br>*N* = 143 | *p* value |
|---|---|---|---|---|
| Glomerular lesions | | | | 0.768 |
| I (GBM thickening) | 15 | 6 | 9 | |
| IIa (mild mesangial hyperplasia) | 48 | 16 | 32 | 0.736* |
| IIb (severe mesangial hyperplasia) | 32 | 13 | 19 | 0.555* |
| III (nodular glomerulosclerosis) | 97 | 31 | 66 | 0.325* |
| IV (> 50% global sclerosis) | 31 | 14 | 17 | 0.313* |
| Interstitial fibrosis/tubular atrophy (IFTA) (*n*) | | | | 0.033 |
| Absent (0) | 10 | 3 | 7 | |
| Mild (< 25%, 1+) | 105 | 32 | 73 | |
| Moderate (25–50%, 2+) | 88 | 33 | 55 | |
| Severe (> 50%, 3+) | 20 | 12 | 8 | |
| Interstitial inflammation (*n*) | | | | 0.941 |
| Absent (0) | 21 | 6 | 15 | |
| Moderate (only in relation to IFTA, 1+) | 159 | 60 | 99 | |
| Severe (in areas without IFTA, 2+) | 42 | 14 | 28 | |
| Arteriolar sclerosis/hyalinosis (*n*) | | | | 0.764 |
| Absent (0) | 31 | 13 | 18 | |
| Moderate (only one area, 1+) | 107 | 36 | 71 | |
| Severe (more than one area, 2+) | 84 | 30 | 54 | |
| Immunofluorescence features [n (%)] | All<br>*N* = 223 | Nonsmokers<br>*N* = 80 | Smokers<br>*N* = 143 | *p* value |
| Immune deposits | 87 (39.01) | 27 (33.75) | 60 (41.96) | 0.254 |
| C1q | 40 (17.94) | 13 (16.25) | 27 (18.88) | 0.717 |
| IgG | 45 (20.18) | 17 (21.25) | 28 (19.58) | 0.862 |
| IgM | 60 (26.91) | 20 (25.00) | 40 (27.97) | 0.753 |
| IgA | 21 (9.42) | 6 (7.50) | 15 (10.49) | 0.633 |
| C3 | 47 (21.08) | 19 (23.75) | 28 (19.58) | 0.496 |
| C4 | 28 (12.56) | 10 (12.50) | 18 (12.59) | 0.985 |
| Electron microscopy features [*n* (%)] | All<br>*N* = 172 | Nonsmokers<br>*N* = 61 | Smokers<br>*N* = 111 | *p* value |
| GBM thickening (450 nm in males) | 167 (97.09) | 61 (100) | 106 (95.50) | 0.162 |
| Mean GBM (nm) | 344–2183 | 454–2183 | 344–2069 | 0.714 |

Wilcoxon rank-sum test. A two-tailed $p < 0.05$ was considered statistically significant. Values are expressed as counts and percentages or range. Glomerular lesions were categorized based on the pathologic classification of the Renal Pathology Society in 2010 (the former grade is not up to the latter)

*IFTA* interstitial fibrosis and tubular atrophy, *GBM* glomerular basement membrane

*$p$ value of mesangial hyperplasia, nodular glomerulosclerosis and global sclerosis in the two groups

## Cigarette smoking and renal outcome in longitudinal study

Survival curves of the composited endpoint and progression to ESRD are presented in Fig. 5. The Kaplan–Meier survival analysis (log-rank test) indicated that the overall 1-year renal survival rate was 72.1% in smokers and 55.6% in nonsmokers for ESRD and 69.9% in smokers and 55.6% in nonsmokers for composite endpoint. The risks for composite endpoint were determined by Cox proportional hazards model. As shown in Fig. 6, univariate analysis indicated that lower levels of serum albumin (HR 0.967, 95% CI 0.943–0.992, $p = 0.011$), lower levels of hemoglobin (HR 0.987, 95% CI 0.978–0.995, $p = 0.002$), lower baseline eGFR (HR 0.980, 95% CI 0.972–0.988, $p < 0.01$), more severe urine protein (HR 1.059, 95% CI 1.020–1.100, $p = 0.003$), higher levels of serum creatinine (HR 1.005, 95% CI 1.003–1.007, $p < 0.01$) and higher glomerular class

| Factors | OR | 95%CI | P |
|---|---|---|---|
| **Univariate** | | | |
| Cigarettes smoking | 0.504 | 0.289-0.880 | 0.016 |
| Age | 1.049 | 1.017-1.081 | 0.002 |
| Systolic blood pressure | 1.012 | 0.999-1.025 | 0.065 |
| Diastolic blood pressure | 0.992 | 0.972-1.012 | 0.443 |
| Triglycerides | 0.889 | 0.743-1.064 | 0.200 |
| Total Cholesterol | 1.064 | 0.910-1.244 | 0.436 |
| Duration of diabetes | 1.002 | 0.998-1.006 | 0.297 |
| Compared with duration<5years | | | |
| Duration ≥5-<10years | 0.454 | 0.219-0.941 | 0.034 |
| Duration≥10years | 1.277 | 0.691-2.359 | 0.435 |
| Compared with duration≥10years | | | |
| Duration ≥5-<10years | 0.356 | 0.171-0.738 | 0.006 |
| Fasting plasma glucose | 0.969 | 0.906-1.037 | 0.364 |
| HbAlc | 0.874 | 0.744-1.027 | 0.101 |
| Blood urea nitrogen | 1.596 | 1.400-1.819 | <0.01 |
| Serum albumin | 0.948 | 0.915-0.983 | 0.004 |
| Urine protein | 1.152 | 1.073-1.236 | <0.01 |
| Hemoglobin | 0.953 | 0.940-0.967 | <0.01 |
| **Multivariate** | | | |
| **Model 1** | | | |
| Cigarettes smoking | 0.475 | 0.267-0.843 | 0.011 |
| Age | 1.051 | 1.019-1.084 | 0.002 |
| **Model 2** | | | |
| Cigarettes smoking | 0.394 | 0.187-0.830 | 0.014 |
| Age | 1.032 | 0.992-1.073 | 0.114 |
| Systolic blood pressure | 1.022 | 0.999-1.046 | 0.059 |
| Diastolic blood pressure | 0.955 | 0.916-0.995 | 0.028 |
| Duration of diabetes | 0.999 | 0.994-1.004 | 0.723 |
| Fasting plasma glucose | 1.015 | 0.929-1.110 | 0.735 |
| HbAlc | 0.865 | 0.699-1.070 | 0.180 |
| Triglycerides | 0.889 | 0.696-1.137 | 0.350 |
| Total Cholesterol | 1.157 | 0.912-1.469 | 0.231 |
| **Model 3** | | | |
| Cigarettes smoking | 0.640 | 0.273-1.502 | 0.305 |
| Age | 1.026 | 0.984-1.069 | 0.236 |
| Blood urea nitrogen | 1.510 | 1.271-1.794 | <0.001 |
| Serum albumin | 1.047 | 0.982-1.117 | 0.159 |
| Urine protein | 1.187 | 1.049-1.343 | 0.007 |
| Hemoglobin | 0.963 | 0.944-0.983 | <0.001 |

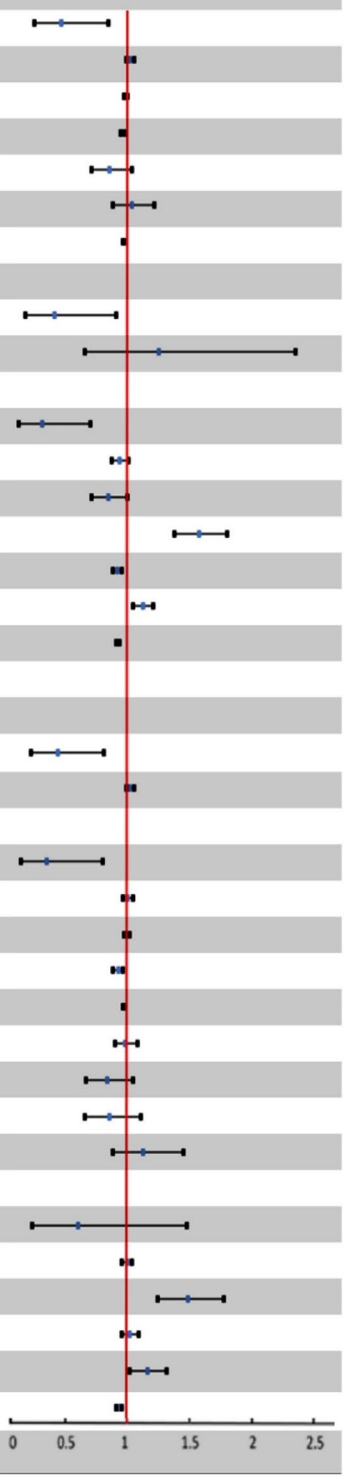


**Fig. 2** Risk factors for eGFR < 60 mL/min per 1.73 $m^2$ identified by univariate and multivariate logistic regression analysis in T2DM patients with DN

| | Age ≤50years (28-50years) (n=104) | | | Age > 50 years (51-72 years) (n=119) | | |
|---|---|---|---|---|---|---|
| Factors | OR | 95%CI | P | OR | 95%CI | P |
| Univariate | | | | | | |
| Cigarettes smoking | 0.391 | 0.171-0.893 | 0.026 | 0.592 | 0.272-1.288 | 0.186 |
| Systolic blood pressure | 1.019 | 1.000-1.039 | 0.054 | 1.006 | 0.989-1.023 | 0.509 |
| Diastolic blood pressure | 1.004 | 0.973-1.036 | 0.809 | 0.990 | 0.963-1.018 | 0.494 |
| Triglycerides | 0.874 | 0.669-1.143 | 0.326 | 0.934 | 0.721-1.210 | 0.605 |
| Total Cholesterol | 0.937 | 0.727-1.207 | 0.614 | 1.167 | 0.934-1.459 | 0.175 |
| Duration of diabetes | 1.002 | 0.996-1.008 | 0.489 | 1.001 | 0.996-1.006 | 0.689 |
| Fasting plasma glucose | 0.971 | 0.859-1.098 | 0.643 | 0.962 | 0.886-1.044 | 0.348 |
| HbAlc | 0.942 | 0.773-1.147 | 0.552 | 0.780 | 0.587-1.036 | 0.086 |
| Multivariate | | | | | | |
| Cigarettes smoking | 0.236 | 0.076-0.731 | 0.012 | 0.489 | 0.172-1.438 | 0.197 |
| Systolic blood pressure | 1.049 | 1.009-1.090 | 0.015 | 1.016 | 0.985-1.047 | 0.312 |
| Diastolic blood pressure | 0.931 | 0.871-0.995 | 0.036 | 0.951 | 0.896-1.008 | 0.090 |
| Duration of diabetes | 1.002 | 0.994-1.011 | 0.619 | 1.000 | 0.993-1.006 | 0.921 |
| Fasting plasma glucose | 1.050 | 0.850-1.297 | 0.650 | 1.030 | 0.924-1.149 | 0.592 |
| HbAlc | 1.003 | 0.757-1.329 | 0.983 | 0.671 | 0.441-1.019 | 0.061 |
| Triglycerides | 0.883 | 0.605-1.289 | 0.519 | 0.868 | 0.590-1.277 | 0.471 |
| Total Cholesterol | 0.868 | 0.591-1.273 | 0.468 | 1.481 | 1.027-2.136 | 0.036 |

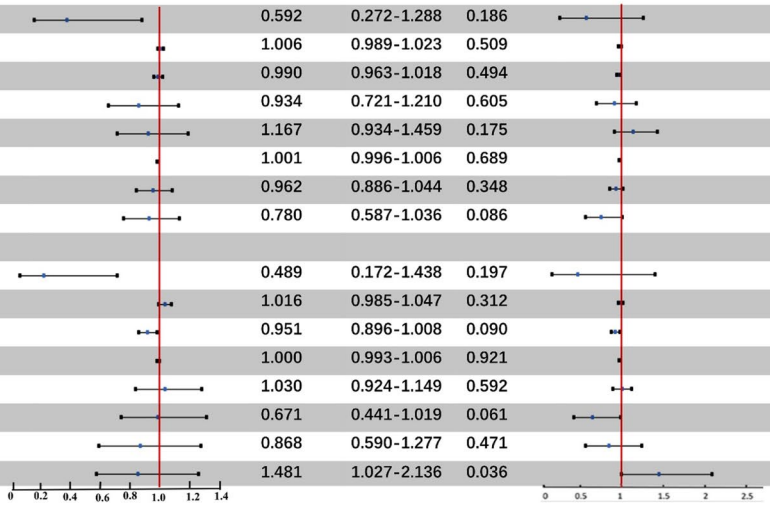


**Fig. 3** Risk factors for eGFR < 60 mL/min per 1.73 $m^2$ identified by univariate and multivariate logistic regression analysis in age-based subgroups analysis of T2DM patients with DN

| | DN without DR (n=122) | | | DN with DR (n=101) | | |
|---|---|---|---|---|---|---|
| Factors | OR | 95%CI | P | OR | 95%CI | P |
| Univariate | | | | | | |
| Cigarettes smoking | 0.750 | 0.355-1.585 | 0.451 | 0.279 | 0.113-0.687 | 0.006 |
| Age | 1.053 | 1.013-1.094 | 0.009 | 1.057 | 1.002-1.115 | 0.041 |
| Systolic blood pressure | 1.012 | 0.995-1.030 | 0.174 | 1.013 | 0.994-1.032 | 0.180 |
| Diastolic blood pressure | 0.981 | 0.955-1.008 | 0.165 | 1.011 | 0.978-1.046 | 0.512 |
| Triglycerides | 0.922 | 0.709-1.198 | 0.542 | 0.873 | 0.683-1.116 | 0.279 |
| Total Cholesterol | 0.965 | 0.781-1.193 | 0.742 | 1.199 | 0.928-1.550 | 0.166 |
| Duration of diabetes | 1.003 | 0.997-1.008 | 0.327 | 1.000 | 0.994-1.006 | 0.925 |
| Fasting plasma glucose | 0.852 | 0.741-0.979 | 0.024 | 1.030 | 0.943-1.124 | 0.515 |
| HbAlc | 0.734 | 0.563-0.957 | 0.022 | 1.007 | 0.809-1.255 | 0.947 |
| Multivariate | | | | | | |
| Cigarettes smoking | 0.595 | 0.205-1.731 | 0.341 | 0.192 | 0.054-0.677 | 0.010 |
| Age | 1.031 | 0.977-1.088 | 0.260 | 1.034 | 0.965-1.107 | 0.343 |
| Systolic blood pressure | 1.032 | 1.000-1.065 | 0.052 | 1.015 | 0.975-1.056 | 0.477 |
| Diastolic blood pressure | 0.946 | 0.895-0.998 | 0.043 | 0.950 | 0.878-1.028 | 0.206 |
| Duration of diabetes | 1.000 | 0.992-1.007 | 0.940 | 0.996 | 0.988-1.004 | 0.335 |
| Fasting plasma glucose | 0.880 | 0.731-1.058 | 0.174 | 1.051 | 0.903-1.224 | 0.518 |
| HbAlc | 0.799 | 0.566-1.126 | 0.199 | 0.953 | 0.684-1.329 | 0.777 |
| Triglycerides | 1.090 | 0.714-1.662 | 0.690 | 0.789 | 0.553-1.125 | 0.190 |
| Total Cholesterol | 0.979 | 0.683-1.403 | 0.908 | 1.391 | 0.897-2.156 | 0.140 |

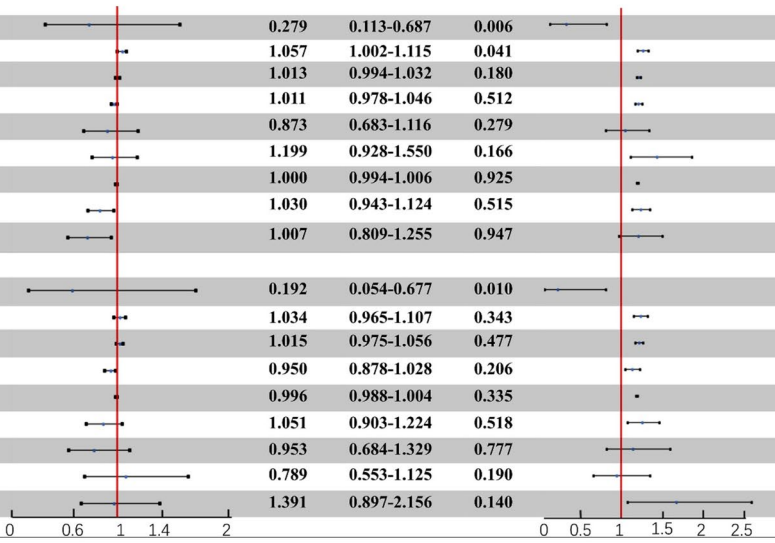


**Fig. 4** Risk factors for eGFR < 60 mL/min per 1.73 $m^2$ identified by univariate and multivariate logistic regression analysis in DR-based subgroups analysis of T2DM patients with DN. *DR* diabetic retinopathy

(HR 1.349, 95% CI 1.064–1.711, $p = 0.013$) were all significantly associated with a poorer prognosis. However, cigarette smoking was not a risk factor for the renal outcomes (HR 1.013, 95% CI 0.673–1.525, $p = 0.950$) either in univariate analysis or (HR 1.187, 95% CI 0.764–1.844, $p = 0.446$) in multivariate analysis.

## Discussion

In this study, we investigated the association between cigarette smoking and renal clinicopathological characteristics and renal prognosis in a large sample of patients with renal

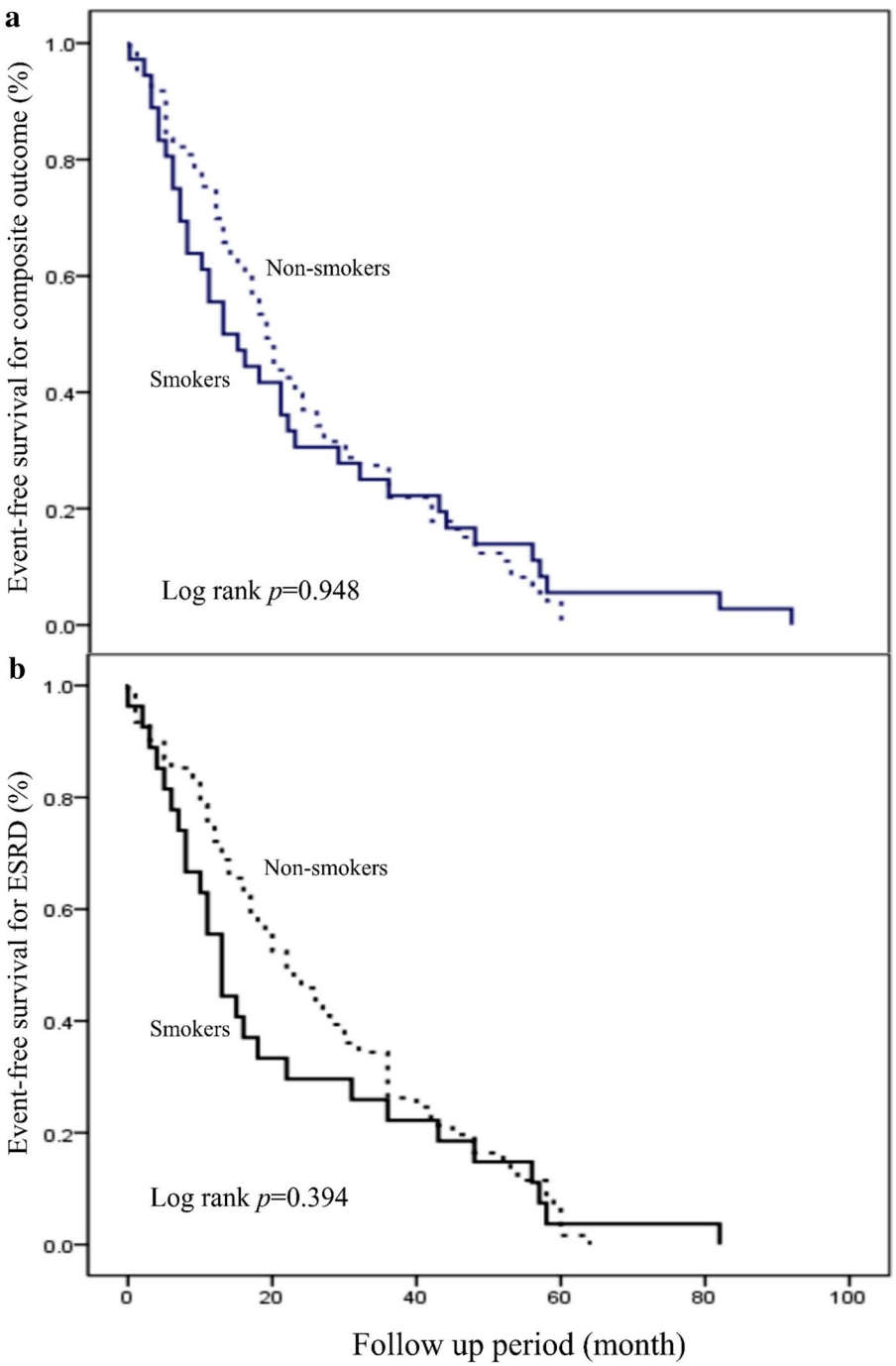


**Fig. 5** Renal survival rate between smokers and non-smokers of DN patients in Kaplan–Meier survival analysis. **a** The event-free survival for composite endpoints; **b** the event-free survival for ESRD

biopsy-proven T2DM-associated DN. The results revealed that the smoking patients had more moderate decline GFR and IFTA lesions than non-smoking patients, while no differences were shown in otherwise clinicopathological features. Moreover, the adjusted logistic regression analysis suggested cigarette smoking was negatively associated with more severe decline eGFR, especially for DN patients with DR and young patients (age ≤ 50 years) as shown in subgroup analysis. Interestingly, it was observed that former smokers had lower levels of plasma glucose and triglycerides than current smokers, while smokers with small smoking amounts had lower levels of triglycerides than those with large smoking amounts.

These findings give further insights into the potential association between smoking and kidney function in male patients with T2DM and DN. Several studies have reported that smoking was associated with hyperfiltration in general population [9, 10]. And a longitudinal study showed that the decline of eGFR in cigarette smokers were smaller than those in nonsmokers, indicating that cigarette

| Factors | HR | 95%CI | P value |
|---|---|---|---|
| **Univariate** | | | |
| Cigarettes smoking | 1.013 | 0.673-1.525 | 0.950 |
| Fasting plasma glucose | 0.981 | 0.932-1.032 | 0.449 |
| HbAlc (%) | 0.946 | 0.839-1.068 | 0.370 |
| Hypertension | 0.411 | 0.100-1.684 | 0.217 |
| Serum albumin | 0.967 | 0.943-0.992 | 0.011 |
| Hemoglobin (g/l) | 0.987 | 0.978-0.995 | 0.002 |
| Estimated GFR | 0.980 | 0.972-0.988 | <0.01 |
| Serum creatinine | 1.005 | 1.003-1.007 | <0.01 |
| Blood urea nitrogen | 1.068 | 1.024-1.114 | 0.002 |
| Urine protein for 24h | 1.059 | 1.020-1.100 | 0.003 |
| Glomerular class | 1.349 | 1.064-1.711 | 0.013 |
| IFTA | 1.306 | 0.994-1.716 | 0.055 |
| Interstitial inflammation | 1.757 | 1.148-2.687 | 0.009 |
| Arteriolar hyalinosis | 0.964 | 0.737-1.262 | 0.792 |
| **Multivariate** | | | |
| Cigarettes smoking | 1.187 | 0.764-1.844 | 0.446 |
| Estimated GFR | 0.986 | 0.976-0.995 | 0.003 |
| Urine protein for 24h | 1.034 | 0.991-1.079 | 0.127 |
| Hemoglobin | 0.995 | 0.985-1.005 | 0.324 |
| IFTA | 1.226 | 0.902-1.665 | 0.193 |

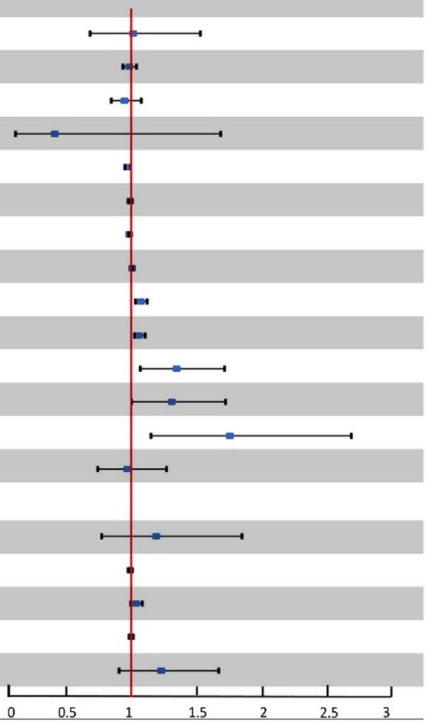


**Fig. 6** Risks for composite endpoint determined by univariate/multivariate COX hazard analysis. Composite endpoint was defined as the doubling of baseline serum creatinine (D-SCr) level or progression to ESRD ($n = 109$). *SD* standard deviation, *CI* confidence interval

smoking might have a modifiable impact on eGFR [18]. Similarly, in this study, we found that smoking patients of T2DM with DN had more moderate decline eGFR and milder IFTA lesions, nonetheless, the levels of proteinuria was comparable in the two groups. One previous study in T2DM patients with albuminuria was performed by Baggio et al. [11], they had indicated that smokers had higher GFR than nonsmokers but no significant results in the index of interstitial fibrosis was found, probably because of a limited sample size of 96 cases. In our study, we enrolled 223 biopsy-proven DN patients and made a more convincing evaluation of the association between smoking and renal clinicopathological characteristics. Otherwise, we first evaluated renal prognosis, and an obviously significant risk factor was not shown about smoking for DN. It was observed that smoking cessation or less smoking had protective effect for hyperlipidemia and hyperglycemia in our study. As hyperlipidemia and hyperglycemia were all risk factors for the development of DN [19, 20], limited cigarette smoking might be suggested for DN patients. These interesting findings in our study based on the association between cigarette smoking and moderate decline eGFR in DN patients might imply that for DN patients at early stage cigarette smoking was a risk factor, on the contrary, cigarette smoking might increase renal perfusion and improve renal ischemia and hypoxia via higher eGFR in late stage of DN.

Nicotine is distilled from burning tobacco, an average tobacco rod contains 10–14 mg of nicotine and about 1–1.5 mg of nicotine is absorbed through mouth or small airways and alveoli of the lung systemically during smoking [21–24]. In a recent study, the average nicotine concentration after smoking a cigarette was 10.9 ng/mL in smokers [25]. Nicotine is extensively metabolized to a number of metabolites, in humans, about 70–80% of nicotine is converted to cotinine. Based on human autopsy samples from smokers, most attractive organs for nicotine are lung, liver, spleen, and kidney. Liver is the major metabolic organ of nicotine and it is excreted by glomerular filtration and tubular secretion depended on urinary pH [26].

The mechanism of how cigarette smoking influence glomerular filtration rate is unclear. Insulin resistance [27, 28] and vascular endothelial growth factor (VEGF) synthesis and activation [29] caused by smoking might contribute to glomerular hyperfiltration in early stage kidney disease. Some interesting reports on the acute effect of smoking on GFR showed that GFR and renal plasma flow fell in healthy volunteers after smoking two cigarettes, but, smoking three cigarettes per hour during a 5.5-h period induced no variation of GFR in type 1 diabetic patients

who had been smoking for several years [30, 31]. Similar effects of 4 mg of nicotine gum on BP and GFR were observed by Halimi et al. [32]. Early experiments in mammals demonstrated that low doses of nicotine increased the glomerular filtration rate (GFR), urine volume, and sodium excretion because of nicotine-induced catecholamine release [33], but higher doses resulted in increased arterial BP and decreased GFR [34].

On the other hand, chronic exposure to nicotine might be associated with structural modifications of kidney architecture involving vascularization, extracellular matrix, and inflammation mediated through nicotinic receptor activation. Renal endothelial cells express nicotinic acetylcholine receptors (nAChR), and it is reported that nicotine stimulates angiogenesis and that this is mediated through activation of the α7nAChR [35]. Diabetic nodular sclerosis is a unique pathological type related to endothelial disease, and nicotine might be such an angiogenic factor responsible for nodular neovascularization [36]. In addition to angiogenesis, nicotine increases expression of matrix metalloproteinases [37] and decreases inflammation through activation of α7nAChR [38] and in such a way may potentially ameliorate some forms of glomerulosclerosis.

In the recent years, epidemiological studies concluded that smoking is an important risk factor for development of proteinuria and diabetic nephropathy [39, 40] and evolution of chronic kidney disease to ESRD [41, 42]. Associated mechanisms included hemodynamic and non-hemodynamic factors, such as inflammation, reactive oxygen species (ROSs) production and angiogenesis. It has been stressed that the effects of cigarette smoking in the progression of chronic kidney disease were mediated by activations of specific nAChRs [43]. KARL et al. figured out that cigarette smoking exhibits its deleterious effect on the kidneys primarily through damage of small interlobular arteries [44]. And a review [45] stated that cigarette smoking induced morphological alterations of the microcirculation at different vascular levels, which can cause severe and widespread damage such as atherosclerotic lesion and microvascular damage. But, at present, there is a lack of prospective studies evaluating the role of nicotine abuse as a risk factor in patients with primary renal disease [46]. It is important to consider the fact that cigarette smoke contains several harmful compounds, including nicotine, and hence some of the adverse effects noted in human subjects may not be completely attributable to nicotine. On the contrary, Agarwal et al. argued that long-term oral treatment with nicotine preserves renal function and reduces inflammation in a rat model of progressive kidney disease [47]. Currently, the treatment of diabetic nephropathy is relatively limited with RAS blockers, and we cannot afford to disregard any approach that may appear promising to slow the progression of kidney disease.

Some certain limitations of this study should be noted. First, we did not find any significant differences in renal function between former smokers and current smokers, possibly because of limited sample size of smoking patients. Second, no significant results were shown in prognosis analysis, and the follow-up data of eGFR is not well recorded to define the outcomes as a 30% decline of eGFR to < 60 mL/min/1.73 $m^2$, future research still needs to be done. Third, we did not study female patients because of their limited number of smokers. Fourth, we did not analyze the effects of smoking metabolites on the kidneys as the role of cigarette smoking is complicated.

In summary, our data found that cigarette smoking was negatively associated with more severe decline eGFR in patients with T2DM and DN, especially for young DN patients with DR. although the significant results in renal outcomes were not obvious in our study.

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## Compliance with ethical standards

**Conflict of interest** The authors have no conflict of interest that is relevant to this article.

**Ethical approval** The ethics committee of West China Hospital approved this research. The study protocol was in compliance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

**Informed consent** Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

## References

1. Cho NH, Shaw JE, Karuranga S et al (2018) IDF diabetes atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract 138:271–281
2. Wang L, Gao P, Zhang M et al (2017) Prevalence and ethnic pattern of diabetes and prediabetes in China in 2013. JAMA 317:2515–2523
3. Menke A, Casagrande S, Geiss L, Cowie CC (2015) Prevalence of and trends in diabetes among adults in the United States, 1988–2012. JAMA 314:1021–1029
4. Alicic RZ, Rooney MT, Tuttle KR (2017) Diabetic kidney disease: challenges, progress, and possibilities. Clin J Am Soc Nephrol 12(12):2032–2045
5. Saran R, Li Y, Robinson B et al: US renal data system 2015 annual data report: epidemiology of kidney disease in the United States. Am J Kidney Dis 2016; 67(3 Suppl 1):Svii,1–305
6. O'Shaughnessy MM, Hogan SL, Poulton CJ et al (2017) Temporal and demographic trends in glomerular disease epidemiology in the Southeastern United States, 1986–2015. Clin J Am Soc Nephrol 12(4):614–623
7. Okada K, Osuga J, Kotani K et al (2012) Current smoking status may be associated with overt albuminuria in female patients with

type 1 diabetes mellitus: a cross-sectional study. Tob Induc Dis 10(1):12–12
8. Hsu CC, Hwang S, Tai TY et al (2009) Cigarette smoking and proteinuria in Taiwanese men with Type 2 diabetes mellitus. Diabet Med 27(3):295–302
9. Maeda I, Hayashi T, Sato KK et al (2011) Cigarette smoking and the association with glomerular hyperfiltration and proteinuria in healthy middle-aged men. Clin J Am Soc Nephrol 6(10):2462–2469
10. Halimi JM, Giraudeau B, Vol S et al (2000) Effects of current smoking and smoking discontinuation on renal function and proteinuria in the general population. Kidney Int 58(3):1285–1292
11. Baggio B, Budakovic A, Dalla Vestra M et al (2002) Effects of cigarette smoking on glomerular structure and function in type 2 diabetic patients. J Am Soc Nephrol 13(11):2730–2736
12. Li L, Zhang X, Li Z et al (2017) Renal pathological implications in type 2 diabetes mellitus patients with renal involvement. J Diabetes Compl 31(1):114–121
13. Anguiano Gómez L, Lei Y, Kumar Devarapu S, Anders HJ (2017) The diabetes pandemic suggests unmet needs for 'CKD with diabetes' in addition to 'diabetic nephropathy'-implications for preclinical research and drug testing. Nephrol Dial Transpl. https://doi.org/10.1093/ndt/gfx219
14. Mannam P, Rauniyar N, Lam TT et al (2016) MKK3 influences mitophagy and is involved in cigarette smoke-induced inflammation. Free Radic Biol Med 101:102–115
15. Furuichi K, Yuzawa Y, Shimizu M et al (2018) Nationwide multicentre kidney biopsy study of Japanese patients with type 2 diabetes. Nephrol Dial Transpl 33(1):138–148
16. Zhang J, Wang Y, Li L et al (2018) Diabetic retinopathy may predict the renal outcomes of patients with diabetic nephropathy. Ren Fail 40(1):243–251
17. Tervaert TWC, Mooyaart AL, Amann K et al (2010) Pathologic classification of diabetic nephropathy. J Am Soc Nephrol 21(4):556–563
18. Miyatake N, Moriyasu H, Sakano N et al (2010) Influence of cigarette smoking on estimated glomerular filtration rate (eGFR) in Japanese male workers. Acta Med Okayama 64(6):385–390
19. Coresh J, Astor BC, Greene T et al (2003) Prevalence of chronic kidney disease and decreased kidney function in the adult US population: third national health and nutrition examination survey. Am J Kidney Dis 41(1):1–12
20. Nosadini R, Tonolo G (2004) Relationship between blood glucose control, pathogenesis and progression of diabetic nephropathy. J Am Soc Nephrol 15(90010):1–5
21. Benowitz NL, Jacob (1984) P 3rd: daily intake of nicotine during cigarette smoking. Clin Pharmacol Ther 35(4):499–504
22. Kozlowski LT, Mehta NY, Sweeney CT et al (1998) Filter ventilation and nicotine content of tobacco in cigarettes from Canada, the United Kingdom, and the United States. Tob Control 7(4):369–375
23. Armitage A, Dollery C, Houseman T et al (1978) Absorption of nicotine from small cigars. Clin Pharmacol Ther 23(2):143–151
24. Pankow JF (2001) A consideration of the role of gas/particle partitioning in the deposition of nicotine and other tobacco smoke compounds in the respiratory tract. Chem Res Toxicol 14(11):1465–1481
25. Patterson F, Benowitz N, Shields P et al (2003) Individual differences in nicotine intake per cigarette. Cancer Epidemiol Biomark Prev 12(5):468–471
26. Benowitz NL, Jacob P 3rd (1985) Nicotine renal excretion rate influences nicotine intake during cigarette smoking. J Pharmacol Exp Ther 234(1):153–155
27. Willi C, Bodenmann P, Ghali WA et al (2007) Active smoking and the risk of type 2 diabetes—a systematic review and meta-analysis. JAMA 298:2654–2664
28. Tomaszewski M, Charchar FJ, Maric C et al (2007) Glomerular hyperfiltration: a new marker of metabolic risk. Kidney Int 71:816–821
29. Benjamin LE (2001) Glucose, VEGF-A, and diabetic complications. Am J Pathol 158:1199–1206
30. Ritz E, Benck U, Franek E et al (1998) Effects of smoking on renal hemodynamics in healthy volunteers and in patients with glomerular disease. J Am Soc Nephrol 9:1798–1804
31. Hansen HP, Rossing K, Jacobsen P et al (1996) The acute effect of smoking on systemic haemodynamics, kidney and endothelial functions in insulin-dependent diabetic patients with microalbuminuria. Scand J Clin Lab Invest 56:393–399
32. Halimi JM, Philippon C, Mimran A (1998) Contrasting renal effects of nicotine in smokers and non-smokers. Nephrol Dial Transpl 13:940–944
33. Pawlik WW, Jacobson ED, Banks RO (1985) Actions of nicotine on renal function in dogs. Proc Soc Exp Biol Med 178:585–590
34. Tamaoki L, Oshiro-Monreal FM, Helou CM (2009) Effects of nicotine exposure on renal function of normal and hypercholesterolemic rats. Am J Nephrol 30:377–382
35. Macklin KD, Maus AD, Pereira EF et al (1998) Human vascular endothelial cells express functional nicotinic acetylcholine receptors. J Pharmacol Exp Ther 287:435–439
36. Nasr SH, D'Agati VD (2007) Nodular glomerulosclerosis in the nondiabetic smoker. J Am Soc Nephrol 18:2032–2036
37. Heeschen C, Jang JJ, Weis M et al (2001) Nicotine stimulates angiogenesis and promotes tumor growth and atherosclerosis. Nat Med 7:833–839
38. Wang H, Yu M, Ochani M et al (2003) Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. Nature 421:384–388
39. Halimi JM, Giraudeau B, Vol S et al (2000) : Effects of current smoking and smoking discontinuation on renal function and proteinuria in the general population. Kidney Int 58:1285–1292
40. Rossing P, Hougaard P, Parving HH (2002) Risk factors for development of incipient and overt diabetic nephropathy in type 1 diabetic patients: a 10-year prospective observational study. Diabetes Care 25:859–864
41. Ejerblad E, Fored CM, Lindblad P et al (2004) Association between smoking and chronic renal failure in a nationwide population based case—control study. J Am Soc Nephrol 15:2178–2185 9
42. Hallan SI, Orth SR (2011) Smoking is a risk factor in the progression to kidney failure. Kidney Int 80:516–523
43. Jain G, Jaimes EA (2013) Nicotine signaling and progression of chronic kidney disease in smokers. Biochem Pharmacol 15(8):1215–1223 86(
44. Lhotta K, Rumpelt HJ, König P et al (2002) Cigarette smoking and vascular pathology in renal biopsies. Kidney Int 61(2):648–654
45. Leone A, Landini L (2013) Vascular pathology from smoking: look at the microcirculation! Curr Vasc Pharmacol 11(4):524–530
46. Orth SR, Hallan SI (2008) Smoking: a risk factor for progression of chronic kidney disease and for cardiovascular morbidity and mortality in renal patients—absence of evidence or evidence of absence? Clin J Am Soc Nephrol 3:226–236
47. Agarwal PK, van den Born J, van Goor H et al (2012) Renoprotective effects of long-term oral nicotine in a rat model of spontaneous proteinuria. Am J Physiol Renal Physiol 302:F895–F904