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ORIGINAL RESEARCH

Prognostic Value of Hemoglobin Concentration on Renal Outcomes with Diabetic Kidney Disease: A Retrospective Cohort Study

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Objective: Diabetic kidney disease (DKD) patients with anemia face an elevated risk of glomerular filtration rate decline. However, the association between hemoglobin and estimated Glomerular Filtration Rate (eGFR) progression remains to be elucidated.

Methods: A retrospective cohort of 815 subjects with DKD was followed from January 2010 to January 2023. A Cox proportional hazard regression model was utilized to explore the predictive role of hemoglobin in renal outcomes. Renal outcomes were defined as a composite endpoint, including a 50% decline in eGFR from baseline or progression to End-Stage Renal Disease (ESRD). To unveil any nonlinear relationship between hemoglobin and renal outcomes, Cox proportional hazard regression with cubic spline functions and smooth curve fitting was conducted. Additionally, subgroup analyses were performed to identify specific patient populations that might derive greater benefits from higher hemoglobin.

Results: Among the 815 DKD subjects, the mean age was 56.482 ± 9.924 years old, and 533 (65.4%) were male. The mean hemoglobin was 121.521±22.960 g/L. The median follow-up time was 21.103±18.335 months. A total of 182 (22.33%) individuals reached the renal composite endpoint during the study period. After adjusting for covariates, hemoglobin was found to exert a negative impact on the renal composite endpoint in patients with DKD (HR 0.975, 95% CI [0.966, 0.984]). A nonlinear relationship between hemoglobin and the renal composite endpoint was identified with an inflection point at 109 g/L. Subgroup analysis unveiled a more pronounced association between hemoglobin and renal prognosis in males.

Conclusion: Hemoglobin emerges as a predictive indicator for the renal prognosis of diabetic kidney disease in China. This study reveals a negative and non-linear relationship between hemoglobin levels and the renal composite endpoint. A substantial association is noted when hemoglobin surpasses 109 g/L in relation to the renal composite endpoint.

Keywords: hemoglobin, eGFR decline, ESRD, diabetic kidney disease, non-linear, Cox proportional-hazards regression

Background

Diabetic kidney disease (DKD) is the most common cause of end-stage renal disease (ESRD).[1] Early detection and improved management may slow down the progression of renal function and reduce its complications. However, prediction factors of DKD progression are not fully understood.

Anemia is a major complication resulting from chronic kidney disease (CKD) and also a risk factor for cardiovascular events and eGFR decline. In a Chinese cross-sectional study, low eGFR (<60mL/min/1.73m^2) were associated with SBP, retinopathy, TC, TG, and anemia.[2] A study with 526 patients with type 2 diabetes during the follow-up period showed that anemia was an independent risk factor for rapid eGFR decline.[3] In another study, which included a total of 160,031 patients, anemia was a risk factor associated with adverse chronic renal outcomes.[4] According to a predicted model,

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when adding the risk factor of anemia to the conventional factors, it revealed the predictive performance of severe interstitial fibrosis and tubular atrophy (IF/TA) in DKD was improved,[5] also in severe IFTA, lower Hb level was associated with a higher risk of death.[6] Early identification and treatment of anemia have been shown to slow renal progression and delay renal replacement therapy in non-dialysis CKD patients. It also improves cardiac function, reducing hospitalization and mortality rates.

In a multi-center study conducted in China, anemia was present in 51.5% of patients with CKD who were not undergoing dialysis, and the prevalence was higher in patients with diabetic nephropathy (DN) (68.0%) than in patients with hypertensive renal damage (56.6%) or chronic glomerulonephritis (46.1%).[7] A Chinese cross-sectional study showed that the prevalence of anemia was 40–60% in patients with DKD.[8] The risk of anemia in patients with diabetes is estimated to be 2–3 times higher than that in patients without diabetes.[9]

DKD patients are more likely to suffer severe and earlier anemia compared to non-DKD patients. The mechanisms of anemia among DKD patients are complicated, including greater bleeding tendency associated with antiplatelet effect, less O_2 sensing due to autonomic neuropathy or renin-angiotensin-aldosterone system inhibitor use, inhibitory effect of inflammatory cytokines, urinary loss of erythropoietin (EPO), and poor response to EPO.[10]

The relationship between anemia and renal prognosis of DKD remains unclear and controversial. Since studies about anemia in DKD patients are rare and the factor of anemia in predicting DKD progression needs further investigation, hence we conducted a cohort study to investigate whether hemoglobin is independently associated with renal composite endpoint in Chinese DKD people.

Materials and Methods

Study Design

This was a retrospective cohort study using records from Guangdong Provincial People's Hospital. The target-independent variable was hemoglobin at baseline. The outcome variable was a renal composite endpoint (dichotomous variable: 0 = did not reach renal endpoint events, 1 = reached renal endpoint events, renal composite endpoint includes 50% eGFR declined from baseline and/or DKD proceeded to ESRD).

Study Population

Participants diagnosed with DKD with T2DM from inpatient or outpatient department of nephrology in Guangdong Provincial People's Hospital from Jan 2010 to Jan 2023 (n=1212) were included. The diagnosis of T2DM was made based on criteria established by the American Diabetes Association.[11] DKD was diagnosed based on the 2020 guideline of KDIGO,[12] which includes urinary albuminuria level (urinary albumin creatinine ratio≥30mg/g and/or eGFR<60 mL/min/1.73 m^2), while excluding CKD induced by other causes, or proved by renal biopsy. The exclusion criteria were patients without at least one follow-up (n=311); Patients without baseline eGFR (n=23); Patients with eGFR<15mL/min/1.73 m^2 (n=23) at baseline; Patients without baseline Hb (n=31) (see flowchart for details in Figure 1). All patients provided written informed consent, and the study was approved by the institutional review board of the Guangdong Provincial People's Hospital.

Outcomes

The outcomes consisted of two renal endpoints, which included more than 50% decline of baseline eGFR, or eGFR < 15 mL/min/1.73m^2. The remaining patients were followed up with until January 1, 2023. Dichotomous variable: 0 = did not reach renal endpoint events, 1 = reached renal endpoint events.

Variates

Hemoglobin (Hb)

The diagnostic criteria for anemia were Hb < 120 g/L in females and Hb < 130 g/L in males according to World Health Organization.[13]

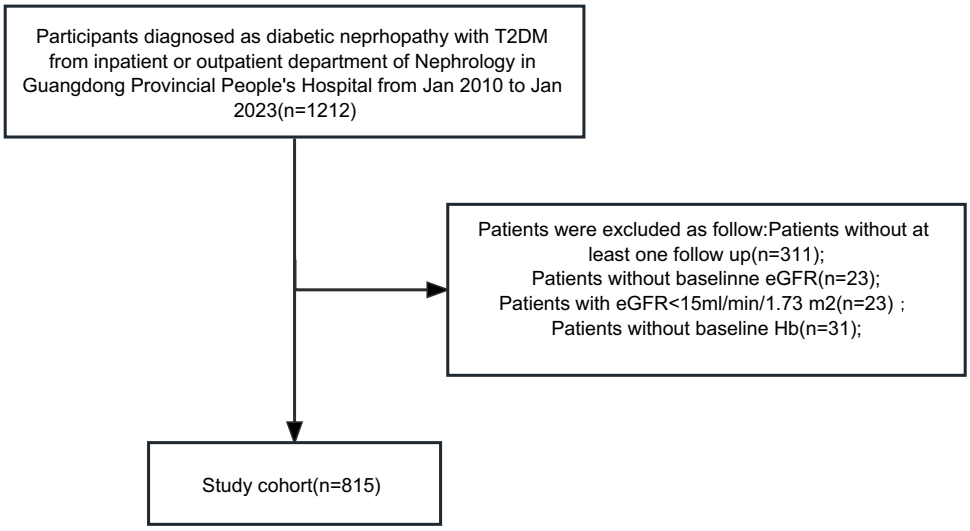

Figure 1 Study design and flowchart of study participants.

Covariates

We select the covariates according to our clinical experience and the previous literature. The covariates were as follows: (1) continuous variables: age, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipid cholesterol (LDL-C), triglycerides (TG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (Bun), serum creatinine (Scr), uric acid (UA), Cystatin C (Cys C), white blood cell (WBC), total protein (TP), albumin (ALB), serum potassium (K), serum magnesium (MG), serum calcium (Ca), serum phosphorus (P), glycosylated hemoglobin (HbA1c), 24-hour urine albumin (24h UP), 24-hour urine protein (24h UAlb); (2) categorical variables: gender.

Information on demographic characteristics (age, gender) was collected through each visit to hospital. Height, weight, and blood pressure were measured by trained staff. BMI was calculated as weight in kilograms divided by height in meters square (kg/m^2). eGFR was calculated by CKD-EPI equation.[14] Standard mercury sphygmomanometers were applied to measure blood pressure.

Statistical Analysis

Participants were stratified by hemoglobin quartiles. Quantitative variables with normal distribution were expressed as the mean ± standard deviation, and variables with skewed distribution were expressed as the median (quartile), and categorical variables were expressed as the frequency (percentage). We used the One-Way ANOVA test (normal distribution), the χ^2 (categorical variables), or the Kruskal–Wallis *H*-test (skewed distribution) to test for differences among different hemoglobin quartile groups.

To assess the impact of modifiable risk factors in hemoglobin changes, we used univariate and multivariate Cox proportional risk regression models to construct three models, including unadjusted model (crude model: unadjusted covariate), minimum adjusted model (Model I: only adjusted sociodemographic variables, including gender, age, BMI, systolic blood pressure, diastolic blood pressure), and fully adjusted models (Model II: adjusted covariates including age, gender, BMI, systolic blood pressure, diastolic blood pressure, ALT, AST, BUN, TC, TG, LDL-C, HDL-c, Scr, UA, CysC, TP, ALB, K, CA, P, HbA1c, 24h-UP, 24h-UAlb).

In order to investigate the nonlinear relationship between hemoglobin and renal prognosis, a Cox proportional hazards regression model with cubic spline functions and smooth curve fitting was used to state the non-linear relationship. In addition, the two-piecewise Cox proportional-hazards regression model was used to further elaborate the non-linearity between hemoglobin and renal prognosis.

The subgroup analyses were conducted using a stratified Cox proportional-hazards regression model across various subgroups (gender, age, UA, ALB, BMI, SBP, and DBP). Continuous variables such as age (<60 and ≥60 years old), UA (<420μmol/L and ≥420μmol/L), ALB (<30g/L and ≥30g/L), BMI (<25 and ≥25kg/m^2), SBP (<140 and ≥140 mmHg), DBP (<90 and ≥90 mmHg)[15–17] were transformed to a categorical variable based on the clinical cut point. Then, we adjusted each stratification for all factors (Models were adjusted for age, gender, BMI, SBP, DBP, ALT, AST, BUN, TC, TG, LDL-C, HDL-c, Scr, UA, CysC, TP, ALB, K, CA, P, HbA1c, 24h UP, and 24h UAlb, but not adjusted for stratification variables in each model). Finally, tests for interaction were conducted with and without interaction terms.

The number of participants with missing data of SBP (n=216), DBP (n=216), BMI (n=251), WBC (n=403), ALT (n=448), AST (n=555), BUN (n=6), TC (n=54), TG (n=53), LDL-C (n=71), HDL-c (n=71), UA (n=70), CYSC (n=571), TP (n=71), ALB (n=50), K (n=82), Ca (n=81), P (n=176), HbA1c (n=81), 24hUP (n=96), and 24hUAlb (n=84) were 26.50%, 26.50%, 30.80%, 49.45%, 54.97%, 68.10%, 0.74%, 6.63%, 6.50%, 8.71%, 8.71%, 8.59%, 70%, 8.71%, 6.13%, 10.06%, 9.94%, 21.60%, 9.94%, 11.78%, and 10.31%, respectively. Missing data of covariants was handled by multiple imputations method. The imputation model included age, sex, SBP, DBP, BMI, WBC, ALT, AST, BUN, TC, TG, LDL-C, HDL-c, UA, CysC, TP, ALB, K, Ca, P, HbA1c, 24hUP, and 24hUAlb. Missing data analysis procedures use missing-at-random (MAR) assumptions.

All results were written according to the STROBE statement.[18] Both R statistical software packages (http://www.r-project.org, The R Foundation), SPSS 27.0 (SPSS Inc., Chicago, IL, USA), and Empower Stats (X&Y Solutions, Inc., Boston, MA, http://www.empowerstats.com) were used to conduct all analyses. Statistical significance was defined as P-values less than 0.05 (two-sided).

Results

Baseline Characteristics of Participants

The baseline characteristics of study participants are listed in Table 1. The mean age was 56.48 ± 9.92 years old, and 533 (65.40%) were male. The mean hemoglobin was 121.52±22.96 g/L. During a median follow-up time of 21.103±18.335 months, 182 (22.33%) individuals had experienced a final diagnosis of renal composite endpoint. Participants were divided into subgroups according to hemoglobin quartiles (<104g/L, ≥105g/L to <120g/L, ≥121g/L to <137g/L, ≥138g/L). Compared to the Q1 (<104g/L) quartile group, SBP, BUN, UA, CHOL, LDL-C, HDL-C, AST, K, P, Scr, 24hUP, 24hUalb decreased significantly in the Q4 (≥138g/L) quartile group, while DBP, BMI, TG, ALT, TP, ALB, Ca, HbA1c,

Table 1 Baseline Characteristics of All the Patients at Enrollment (n=815)

Hb Quartiles	Q1 (56–104g/L)	Q2 (105–120g/L)	Q3 (121–137g/L)	Q4 (138–187g/L)	P-value
Participants	204	195	203	213	
SBP (mmHg)	149.734 ± 22.865	148.030 ± 21.677	142.886 ± 22.706	143.364 ± 22.497	0.003
DBP (mmHg)	80.241 ± 12.718	83.365 ± 12.244	81.821 ± 12.546	86.704 ± 14.199	<0.001
BMI (kg/m^2)	23.962 ± 3.630	25.185 ± 3.553	25.758 ± 3.640	25.986 ± 3.943	<0.001
Age (years)	55.711 ± 9.945	56.682 ± 9.800	58.463 ± 8.834	55.150 ± 10.728	0.004
WBC (*10^9/L)	7.633 ± 2.136	7.859 ± 2.152	7.896 ± 2.161	7.470 ± 1.773	0.119
BUN (mmol/L)	12.677 ± 5.706	10.056 ± 4.467	7.790 ± 3.078	6.503 ± 2.368	<0.001
UA (μmol/L)	438.414 ± 119.760	424.422 ± 118.138	412.553 ± 124.423	406.382 ± 108.543	0.031
Cys C (mg/L)	2.256 ± 0.949	1.845 ± 0.764	1.575 ± 0.715	1.284 ± 0.619	<0.001
CHOL (mmol/L)	5.433 ± 2.073	5.700 ± 2.067	5.641 ± 1.957	5.176 ± 1.734	0.029
LDL-C (mmol/L)	3.325 ± 1.429	3.594 ± 1.469	3.391 ± 1.310	3.184 ± 1.047	0.018
HDL-C (mmol/L)	1.164 ± 0.402	1.124 ± 0.333	1.167 ± 0.333	1.054 ± 0.302	0.002
TG (mmol/L)	1.745 (1.110–2.662)	1.940 (1.315–2.865)	1.800 (1.200–3.035)	1.990 (1.360–3.100)	0.044
ALT (U/L)	19.823 (13.000–29.459)	22.843 (16.996) 18.000 (12.149–27.718)	18.281 (14.000–28.000)	23.000 (16.229–34.000)	<0.001
AST (U/L)	20.974 (14.369–26.763)	19.000 (14.000–26.131)	19.000 (14.980–25.000)	20.468 (16.681–30.609)	0.006
TP (g/L)	61.192 ± 10.050	66.383 ± 8.967	67.487 ± 9.132	69.409 ± 7.145	<0.001
ALB (g/L)	31.073 ± 6.926	35.423 ± 6.536	36.711 ± 6.701	39.792 ± 5.101	<0.001

(*Continued*)

Table 1 (Continued).

Hb Quartiles	Q1 (56–104g/L)	Q2 (105–120g/L)	Q3 (121–137g/L)	Q4 (138–187g/L)	P-value
K (mmol/L)	4.186 ± 0.621	4.143 ± 0.600	3.991 ± 0.524	3.927 ± 0.490	<0.001
MG (mmol/L)	0.861 ± 0.112	0.843 ± 0.115	0.844 ± 0.122	0.837 ± 0.124	0.202
Ca (mmol/L)	2.180 ± 0.195	2.274 ± 0.153	2.291 ± 0.146	2.330 ± 0.117	<0.001
P (mmol/L)	1.420 ± 0.297	1.303 ± 0.224	1.206 ± 0.201	1.184 ± 0.206	<0.001
HbA1c (%)	7.684 ± 1.804	8.183 ± 2.223	8.650 ± 2.450	8.723 ± 2.154	<0.001
Hb (g/L)	91.747 ± 9.660	113.412 ± 4.642	129.148 ± 5.135	150.192 ± 9.735	<0.001
SCr (μmol/L)	181.691 ± 76.955	148.128 ± 72.286	116.516 ± 59.612	100.538 ± 44.273	<0.001
eGFR (mL/min per 1.73 m^2)	40.494 ± 23.267	51.679 ± 27.822	64.926 ± 28.116	78.554 ± 26.907	<0.001
Gender					<0.001
Male	121 (59.314%)	107 (54.872%)	119 (58.621%)	186 (87.324%)	
Female	83 (40.686%)	88 (45.128%)	84 (41.379%)	27 (12.676%)	
24hU-pro	3109.665 (1199.253–5435.745)	1487.041 (449.555–4744.710)	618.860 (182.663–2847.709)	273.773 (124.044–651.340)	<0.001
24hU-alb	1862.170 (657.950–3344.025)	890.900 (163.355–2549.350)	289.800 (61.368–1772.710)	88.400 (23.200–358.800)	<0.001
Renal biopsy					<0.001
No	134 (65.686%)	158 (81.026%)	173 (85.222%)	198 (92.958%)	
Yes	70 (34.314%)	37 (18.974%)	30 (14.778%)	15 (7.042%)	

Notes: Continuous variables were summarized as mean (SD) or medians (quartile interval); categorical variables were displayed as percentage (%).
Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipid cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Scr, serum creatinine; UA, uric acid; Cys C, Cystatin C; WBC, white blood cell; TP, total protein; ALB, albumin; K, serum potassium; MG, serum magnesium; Ca, serum calcium; P, serum phosphorus; HbA1c, glycosylated hemoglobin; 24hU-pro, 24-hour urine albumin; 24hU-alb, 24 -hour urine protein.

eGFR increased in the Q4 (≥138g/L) group. Besides, Q4 (≥138g/L) group had a lower proportion of female, older people, and renal biopsy when compared to Q1 (<104g/L) group.

Figure 2 shows the distribution of Hb levels. The results indicated that the distribution level of Hb was normal in the range from 56 to 187 g/L, with an average of 122g/L.

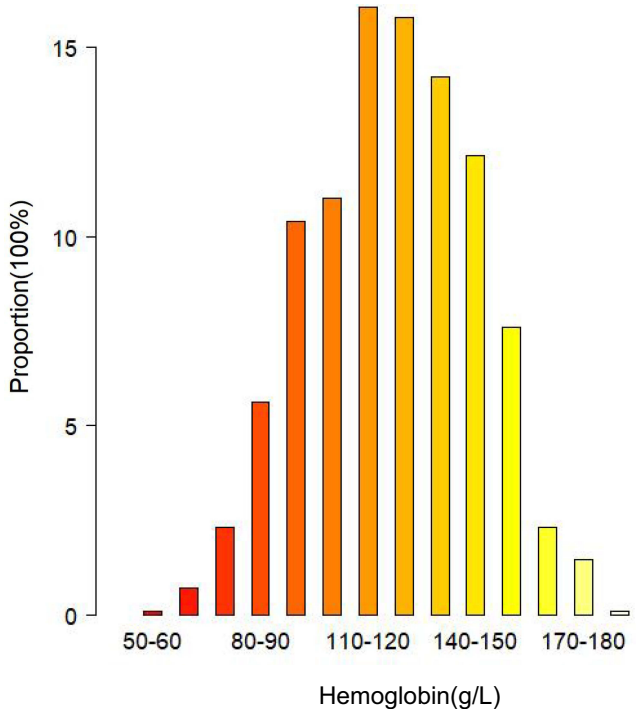

Figure 2 Showed the distribution of Hb levels. It showed a normal distribution within a range from 56g/L to 187g/L (with an average of 121.5g/L).

Table 2 Incident Rate of Renal Composite Endpoint

Hb	Participants (n)	Renal Composite Endpoint Events(n)	Incidence Rate (95% CI)(%)	Cumulative Incidence (Per 100 Person-Year)
Total	815	182	22.331 (19.466–25.197)	12.875
Q1 (<104g/L)	204	82	40.196 (33.411–46.981)	32.875
Q2 (105–120g/L)	195	54	27.692 (21.356–34.029)	18.840
Q3 (121–137g/L)	203	35	17.241 (12.001–22.482)	7.978
Q4 (≥138g/L)	213	11	5.164 (2.168–8.160)	2.506
P for trend			<0.0001	

The Incidence Rate of Renal Composite Endpoint with DKD

Table 2 reveals that 182 (22.331%) participants had experienced renal composite endpoint during a median follow-up time of 21.103±18.335 months. The total cumulative incidence rate of all participants was 12.875 per 100 person-years. The cumulative incidence of the four Hb quartile groups was 32.875, 18.840, 7.978, and 2.506 per 100 person-years, respectively.

The distribution of different CKD stages and albuminuria severity levels with different hemoglobin levels. Figure 3A shows the relationship between eGFR and hemoglobin in male DKD patients. Figure 3B shows the relationship between

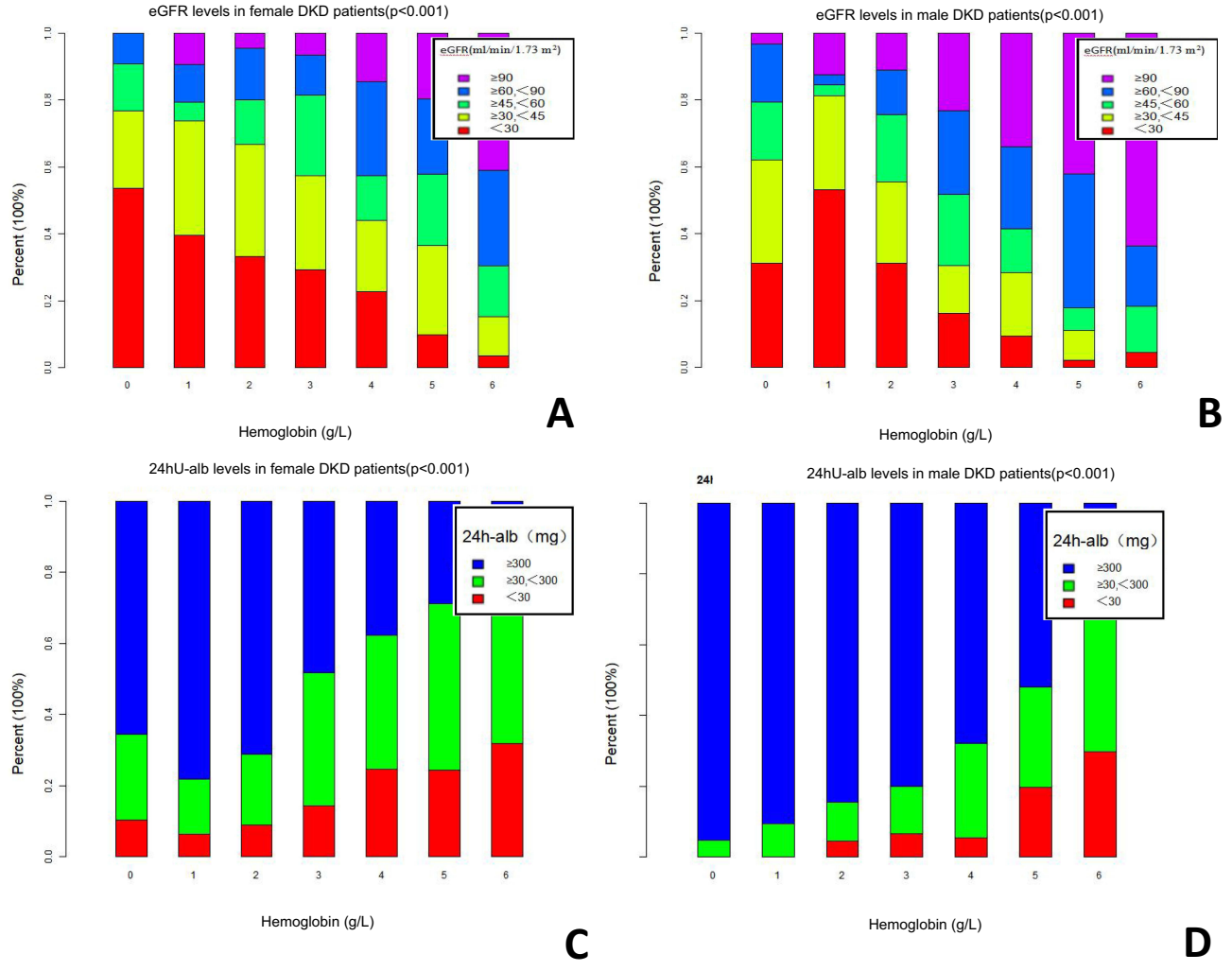

Figure 3 The distribution of different eGFR levels and 24hU-alb severity levels with different hemoglobin levels. (**A**) The relationship between eGFR and Hb in male DKD patients. (**B**) The relationship between eGFR and Hb in female DKD patients. (**C**) The relationship between 24hU-alb and Hb in male DKD patients. (**D**) The relationship between 24hU-alb and Hb in female DKD patients.

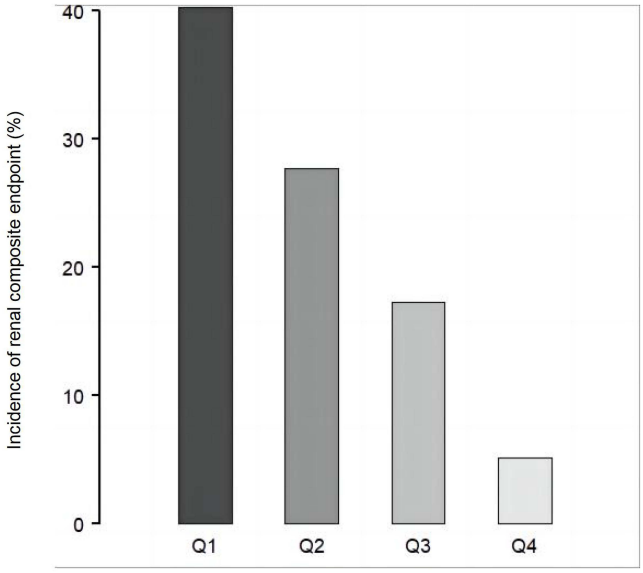

Figure 4 Incidence of renal composite endpoint according to Hb quartiles. This figure showed that participants within the highest Hb group had lower incidence rate of renal composite endpoint compared to the group with the lowest Hb ($p < 0.0001$ for trend).

eGFR and hemoglobin in women with DKD. Figure 3C shows the relationship between albuminuria and hemoglobin in men with DKD. Figure 3D shows the relationship between albuminuria and hemoglobin in women with DKD(Figure 3).

The incidence rate (%) of each Hb group was 40.196 (95% CI: 33.411–46.981), 27.692 (95% CI: 21.356–34.029), 17.241 (95% CI: 12.001–22.482), and 5.164 (95% CI: 2.168–8.160), respectively. Participants within the highest Hb group had lower incidence rates of renal composite endpoint compared to the group with the lowest Hb ($p < 0.0001$ for trend) (Figure 4).

The Results of Univariate Analyses Using Cox Proportional-Hazards Regression Model

The univariate analyses showed that incidence of normoglycemia was positively related to SBP (HR=1.015, 95% CI: 1.008, 1.022), 24hUP (HR=1.000, 95% CI: 1.000, 1.000), 24hUAlb (HR=1.000, 95% CI: 1.000, 1.000), Scr (HR=1.013, 95% CI: 1.012, 1.015), BUN (HR=1.141, 95% CI: 1.114, 1.168), UA (HR=1.001, 95% CI: 1.000, 1.003), K (HR=1.615, 95% CI: 1.246, 2.093), P (HR=3.856, 95% CI: 2.355, 6.314), MG (HR=5.867, 95% CI: 1.882, 18.292), CysC (HR=2.898, 95% CI: 2.421, 3.468), CHOL (HR=1.119, 95% CI: 1.061, 1.181), LDL-C (HR=1.281, 95% CI: 1.165, 1.408), HDL-C (HR=1.587, 95% CI: 1.063, 2.368); and negatively related to BMI (HR=0.900, 95% CI: 0.859, 0.943), age (HR=0.973, 95% CI: 0.959, 0.987), Hb (HR=0.961, 95% CI: 0.955, 0.968), eGFR (HR=0.963, 95% CI: 0.956, 0.970), Ca (HR=0.014, 95% CI: 0.007, 0.028), HbA1C (HR=0.894, 95% CI: 0.834, 0.959), and ALB (HR=0.883, 95% CI: 0.866, 0.900) (Table 3).

Kaplan–Meier survival curves for renal survival rate stratified by the Hb groups are shown in Figure 5. There were significant differences in the renal survival between the Hb quartile groups (Log rank test, $p < 0.0001$). The probability of

Table 3 The Results of Univariate Analysis of Renal Composite Endpoint

Variable	Statistics	HR (95% CI)	*P* value
SBP (mmHg)	146.668 ± 22.742	1.015 (1.008, 1.022)	<0.00001
DBP (mmHg)	83.405 ± 13.307	1.004 (0.993, 1.015)	0.46333
BMI (kg/m^2)	25.310 ± 3.357	0.900 (0.859, 0.943)	0.00001
SEX			
Male	534 (65.441%)	1.0	
Female	282 (34.559%)	0.747 (0.547, 1.021)	0.06722
Age (years)	56.461 ± 9.937	0.973 (0.959, 0.987)	0.00015
Hb (g/L)	121.527 ± 22.947	0.961 (0.955, 0.968)	<0.00001
24hUP	2461.425 ± 3241.219	1.000 (1.000, 1.000)	<0.00001
24hUAlb	1390.625 ± 1833.980	1.000 (1.000, 1.000)	<0.00001
SCr (μmol/L)	136.146 ± 71.376	1.013 (1.012, 1.015)	<0.00001
BUN (mmol/L)	9.215 ± 4.725	1.141 (1.114, 1.168)	<0.00001

(*Continued*)

Table 3 (Continued).

Variable	Statistics	HR (95% CI)	*P* value
UA (μmol/L)	425.199 ± 116.725	1.001 (1.000, 1.003)	0.02042
eGFR (mL/min per 1.73 m^2)	59.255 ± 30.212	0.963 (0.956, 0.970)	<0.00001
K (mmol/L)	4.040 ± 0.561	1.615 (1.246, 2.093)	0.00029
Ca (mmol/L)	2.269 ± 0.163	0.014 (0.007, 0.028)	<0.00001
P (mmol/L)	1.280 ± 0.245	3.856 (2.355, 6.314)	<0.00001
MG (mmol/L)	0.847 ± 0.131	5.867 (1.882, 18.292)	0.00229
HbA1c (%)	8.359 ± 2.224	0.894 (0.834, 0.959)	0.00162
Cys C (mg/L)	1.583 ± 0.772	2.898 (2.421, 3.468)	<0.00001
WBC (*10^9/L)	7.526 ± 2.078	1.018 (0.950, 1.091)	0.61731
TC (mmol/L)	5.446 ± 1.923	1.119 (1.061, 1.181)	0.00004
TG (mmol/L)	2.370 ± 1.908	1.002 (0.940, 1.067)	0.95493
LDL-C (mmol/L)	3.368 ± 1.310	1.281 (1.165, 1.408)	<0.00001
AST (U/L)	23.766 ± 14.275	0.997 (0.987, 1.007)	0.57404
ALT (U/L)	25.721 ± 24.524	0.998 (0.992, 1.004)	0.48887
ALB (g/L)	35.971 ± 6.886	0.883 (0.866, 0.900)	<0.00001
HDL-C (mmol/L)	1.130 ± 0.347	1.587 (1.063, 2.368)	0.02378

Notes: Continuous variables were summarized as mean (SD) or medians (quartile interval); categorical variables were displayed as percentage (%).

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipid cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Scr, serum creatinine; UA, uric acid; Cys C, Cystatin C; WBC, white blood cell; ALB, albumin; K, serum potassium; MG, serum magnesium; Ca, serum calcium; P, serum phosphorus; HbA1c, glycosylated hemoglobin; 24hU-pro, 24-hour urine albumin; 24hU-alb, 24 -hour urine protein.

renal survival gradually decreased with decreasing Hb, indicating that the group with the highest Hb had the highest rate of renal survival with DKD.

The Results of Multivariate Analyses Using Cox Proportional-Hazards Regression Model

Three Cox proportional-hazards regression models were built to explore the relationship between Hb and incident renal composite endpoint (Table 4). In the unadjusted model (Crude model), an increase of 1g/L of Hb was connected with a 4% decrease in renal composite endpoint with DKD (HR=0.960, 95% CI: 0.954, 0.967, *P*<0.00001). In Model I, we adjusted for demographic variables (SBP, DBP, BMI, Gender, and Age), each additional 10g/L of Hb increased the risk of renal composite endpoint by 41% with DKD (HR=0.959, 95% CI: 0.952, 0.966, *P*<0.00001). While in fully adjusted model (Model II), each additional 1g/L of Hb increased the risk of renal composite endpoint by 2.5% with DKD (HR=0.975, 95% CI: 0.966, 0.984, *P*<0.00001).

When we divided patients into non-anemia group and anemia group and set the non-anemia group as a reference. It revealed that the anemia group was related to the increased risk of renal composite endpoint (Crude model: HR=6.698, 95% CI: 4.537, 9.887, *P*<0.001; Model I: HR=6.527, 95% CI: 4.336, 9.8254, *P*<0.001; Model II: HR=2.592, 95% CI: 1.622, 4.140, *P*<0.001; Model III: HR=2.781, 95% CI: 1.626, 4.756, *P*<0.001) (Table 4). In addition, we set the lowest quartile as a reference, the higher quartiles of Hb were distinctly associated with decreased risk of renal composite endpoint (Crude model, Q2:HR=0.562, 95% CI: 0.398, 0.794, *P*<0.001; Q3:HR=0.208, 95% CI: 0.139, 0.312, *P*<0.001; Q4:HR=0.064, 95% CI: 0.034, 0.121, *P*<0.001) (Model I, Q2:HR=0.580, 95% CI: 0.408, 0.824, *P*<0.001; Q3:HR=0.237, 95% CI: 0.156, 0.361, *P*<0.001; Q4: HR=0.043, 95% CI: 0.022, 0.087, *P*<0.001) (Model II, Q2:HR=0.875, 95% CI: 0.587, 1.304, *P*=0.51189; Q3:HR=0.505, 95% CI: 0.315, 0.810, *P*<0.001; Q4: HR=0.141, 95% CI: 0.064, 0.312, *P*<0.001). The results also showed that the trends consistent with the result when Hb categorical values were continuous variables (Table 4).

We also used a GAM to insert the continuity covariate into the equation as a curve. The result remained consistent with the fully adjusted model (Model III). We still got similar results. In the fully adjusted model (Model III, GAM model), an increase of 1g/L of Hb was connected with a 2.7% decrease in renal composite endpoint with DKD (HR=0.973, 95% CI:

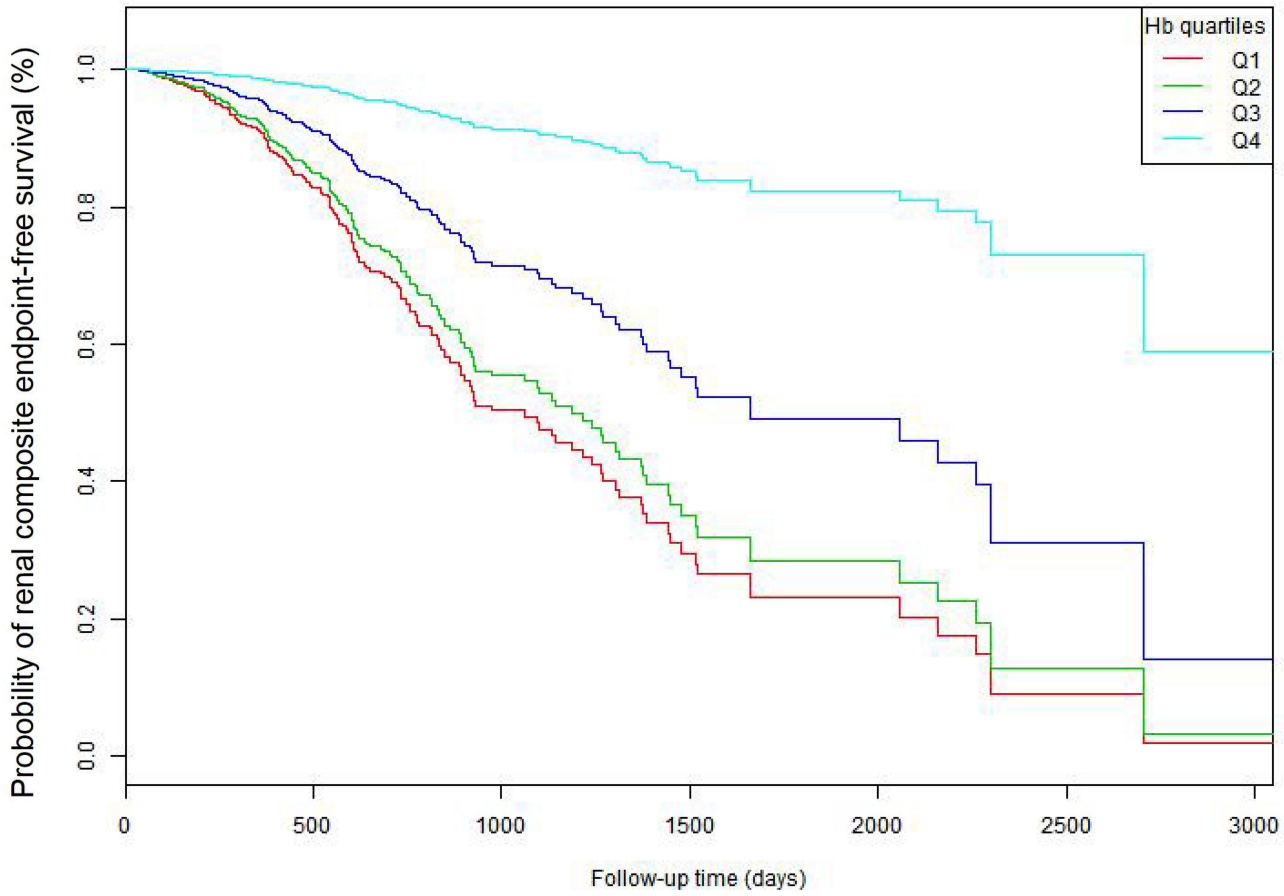

Figure 5 Kaplan–Meier survival curve. The probability of renal composite endpoint-free survival differed significantly between the Hb quartiles (Log rank test, p < 0.001). The probability of renal composite endpoint-free survival gradually decreased with decreasing Hb, suggesting that the group with the highest Hb had the highest probability of renal composite endpoint-free survival.

0.962, 0.984, *P*<0.00001). And the anemia group was related to the increased risk of renal composite endpoint (Model III, GAM model, HR=2.781, 95% CI: 1.626, 4.756, *P*<0.001). Besides, the Q3 and Q4 quartiles of Hb were associated with decreased risk of renal composite endpoints (Model III, GAM model, Q3: HR=0.467, 95% CI: 0.265, 0.821, *P*=0.00820)

Table 4 Relationship Between Hb and Renal Composite Events in Different Models

Exposure	Crude Model (HR, 95% CI, *P*)	Model I (HR, 95% CI, *P*)	Model II (HR, 95% CI, *P*)	Model III (HR, 95% CI, *P*)
Hb	0.960 (0.954, 0.967) <0.00001	0.959 (0.952, 0.966) <0.00001	0.975 (0.966, 0.984) <0.00001	0.973 (0.962, 0.984) <0.00001
Anemia				
No	1.0	1.0	1.0	1.0
Yes	6.698 (4.537, 9.887) <0.00001	6.527 (4.336, 9.825) <0.00001	2.592 (1.622, 4.140) 0.00007	2.781 (1.626, 4.756) 0.00019
Hb Quartile				
Q1	1.0	1.0	1.0	1.0
Q2	0.562 (0.398, 0.794) 0.00106	0.580 (0.408, 0.824) 0.00234	0.875 (0.587, 1.304) 0.51189	0.856 (0.523, 1.400) 0.53472
Q3	0.208 (0.139, 0.312) <0.00001	0.237 (0.156, 0.361) <0.00001	0.505 (0.315, 0.810) 0.00458	0.467 (0.265, 0.821) 0.00820
Q4	0.064 (0.034, 0.121)	0.043 (0.022, 0.087)	0.141 (0.064, 0.312)	0.118 (0.045, 0.307)
	<0.00001	<0.00001	<0.00001	0.00001
P for trend	<0.00001	<0.00001	<0.00001	0.00001

Notes: Crude model adjust for: None. Model I adjust for: SBP; DBP; BMI; Gender; Age. Model II adjust for: SBP; DBP; BMI; Gender; Age; BUN; UA; Cys C; TC; LDL-C; HDL-C; TP; ALB; ALT; K; CA; P; HbA1C; 24hUP; 24hUalb. Model III adjust for: SBP (Smooth); DBP (Smooth); BMI (Smooth); Gender; Age (Smooth); BUN (Smooth); UA (Smooth); Cys C (Smooth); TC (Smooth); LDL-C (Smooth); HDL-C (Smooth); TP (Smooth); ALB (Smooth); ALT (Smooth); K (Smooth); Ca (Smooth); P (Smooth); HbA1C (Smooth); 24hU-pro (Smooth); 24hU-alb (Smooth). Restricted cubic spline were applied.

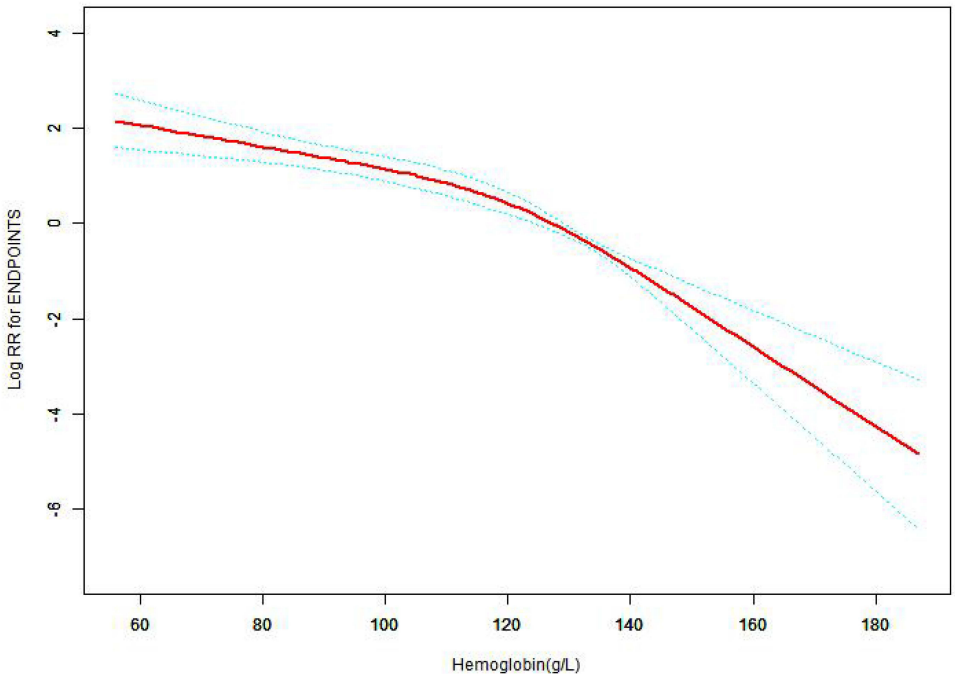

Figure 6 The non-linear relationship between Hb and the renal composite endpoint among DKD patients. We used a Cox proportional hazards regression model with cubic spline functions to evaluate the relationship between Hb and renal composite endpoint. The result showed that the relationship between Hb and renal composite endpoint was non-linear, with the inflection point of Hb being 109g/L.

(Model III, GAM model, Q4: HR=0.118, 95% CI: 0.045, 0.307, *P*<0.001) (Table 4). The results obtained from all of the GAM analyses indicated the well-robustness of the relationship between Hb and renal prognosis.

The Non-linearity Addressed by Cox Proportional Hazards Regression Model With Cubic Spline Functions

A non-linear relationship was detected between Hb and renal composite endpoint by the Cox proportional hazards regression model with cubic spline functions analyses (Figure 6). To determine the best fit model, we used a log-likelihood ratio test, it showed that the P for the log-likelihood ratio test was <0.001. By recursive algorithm, we got the inflection point of 109g/L (Table 5).

On the right side of the inflection point by two piecewise Cox proportional-hazards regression model, the HR and 95% CI were 0.946 (0.929, 0.962). That indicates when Hb level is greater than 109g/L, the risk of renal endpoint became smaller as Hb level increased.

While on the left side of the inflection point by two piecewise Cox proportional-hazards regression model, the relationship between Hb and renal endpoint events was not statistically significant (HR=1.008, 95% CI: 0.990, 1.026, *P*=0.378) (Table 5).

The Results of Subgroup Analyses

Subgroup analyses showed that there was no significant interaction between Hb and incident renal composite endpoint according to strata of age, SBP, DBP, BMI, UA, and ALB (Table 6). The results showed that Gender could modify the

Table 5 The Results of Two-Piecewise Regression Linear Model

Incidence of Normalization of Blood Glucose	HR, 95% CI	*P*
Fitting model by standard Cox regression	0.976 (0.967, 0.985)	<0.0001
Fitting model by two-piecewise Cox regression		
Inflection points of Hb		
< 109	1.008 (0.990, 1.026)	0.3782
> 109	0.946 (0.929, 0.962)	<0.0001
P for log-likelihood ratio test		<0.0001

Notes: We adjusted SBP; DBP; BMI; Gender; Age; BUN; UA; Cys C; TC; LDL-C; HDL-C; ALB; ALT; AST; K; Ca; P; HbA1C; 24hU-pro; 24hU-alb.

Table 6 Results of Subgroup Analysis and Interaction Analysis

Subgroup	Endpoints-Free	Endpoints		HR (95% CI)	P	P for Interact
All patients	633	182				
Gender						0.0267
Male	409	124	0.975 (0.965, 0.984)	0.975 (0.965, 0.984)	<0.0001	
Female	224	58	1.000 (0.979, 1.022)	1.000 (0.979, 1.022)	0.9876	
Age						0.2204
<60	358	118	0.976 (0.966, 0.987)	0.976 (0.966, 0.987)	<0.0001	
≥60	275	64	0.987 (0.972, 1.003)	0.987 (0.972, 1.003)	0.1059	
SBP						0.1721
<140	275	54	0.971 (0.956, 0.987)	0.971 (0.956, 0.987)	0.0003	
≥140	358	128	0.984 (0.973, 0.995)	0.984 (0.973, 0.995)	0.0041	
DBP						0.4057
<90	427	124	0.984 (0.974, 0.994)	0.984 (0.974, 0.994)	0.0024	
≥90	206	58	0.976 (0.960, 0.993)	0.976 (0.960, 0.993)	0.0049	
BMI						0.1258
<25	302	103	0.987 (0.975, 0.999)	0.983 (0.971, 0.996)	0.03140	
≥25	331	79	0.974 (0.961, 0.987)	0.980 (0.968, 0.992)	<0.0001	
UA						0.9824
<420	339	85	0.981 (0.969, 0.993)	0.981 (0.969, 0.993)	0.0021	
≥420	294	97	0.981 (0.969, 0.993)	0.981 (0.969, 0.993)	0.0022	
ALB						0.0842
<30	83	79	0.988 (0.975, 1.002)	0.988 (0.975, 1.002)	0.0993	
>30	550	103	0.973 (0.962, 0.985)	0.973 (0.962, 0.985)	<0.0001	

Notes: The model above is adjusted for SBP, DBP, Gender, Age, BUN, UA, Cys C, TC, LDL-C, HDL-C, ALB, ALT, AST, K, Ca, P, HbA1C, 24hU-pro, 24hU-alb, eGFR, but not adjusted for stratification variables.

relationship between Hb and incident renal composite endpoint (P for interaction < 0.05). And a stronger association was observed in males (OR=0.975, 95% CI: 0.965, 0.984). In contrast, weaker association was observed in females (HR=1.000, 95% CI: 0.979, 1.022) (Table 6).

Discussion

The purpose of this study was to examine the association between hemoglobin (Hb) levels and renal prognosis in patients with diabetic kidney disease (DKD). Our findings reveal a negative correlation between Hb levels and the renal composite endpoint in DKD patients. Elevated Hb levels are linked to a reduced risk of renal composite endpoint during follow-up, while lower Hb levels are associated with an increased risk of renal endpoints. Furthermore, a non-linear relationship between Hb and renal endpoints is observed, with an inflection point at 109 g/L. When Hb exceeds 109 g/L, the incidence of renal composite endpoint decreases as Hb levels increase.

Moreover, subgroup analysis indicates that gender serves as a modifying factor. Specifically, in male patients, Hb demonstrates a more pronounced negative correlation with renal prognosis. In other words, higher Hb levels are associated with a lower risk of estimated Glomerular Filtration Rate (eGFR) decline in males. Higher Hb proves to be a more robust protective factor for male DKD patients compared to their female counterparts.

Anemia is common among patients with CKD and is associated with risk of progression of kidney disease, cardiovascular disease, and mortality.[19] A United Kingdom cross-sectional study had found that 59% older adult with diabetes suffered from anemia.[20] A multicenter study performed in Italy showed that 61.7% prevalence of anemia happened in person with diabetes and CKD.[21] Similarly, another cross-sectional study including 101 DM patients with CKD stages III–IV had found the anemia prevalence of 60%.[22] In our study, the prevalence of anemia in DKD patients was 55.58% (n=453), which is similar to the prevalence in other studies. A multicenter, cross-sectional survey in China showed that the prevalence of anemia was significantly higher in patients with DKD (68.0%) than in patients with hypertensive renal impairment (56.6%) or chronic glomerulonephritis (46.1%) (P<0.001). Besides, the prevalence of

anemia in DKD patients with CKD II stage reached 51.2%, and reached 91.4% in DKD patients with CKD V stage.[7] It revealed that DKD patients had a higher prevalence of anemia and that anemia happened earlier compared to other CKD by another pathogeny. However, some studies have found wide variation in the prevalence of anemia among people with DKD. It was reported that in an Australian study,[23] 11.50% of adults with diabetes had anemia, similar to the study conducted in India (12.13%).[24] But those studies had included those diabetic patients with and without CKD, so the prevalence of anemia was much lower than in our study. The high prevalence in our study indicates that DKD patients who are at risk of developing anemia should be screened regularly and managed more proactively.

Anemia has been associated with an increased risk of eGFR decline. Prior study had revealed that diabetic patients who underwent a kidney biopsy and biopsy-proven DN cases were found to have a higher prevalence of anemia than the non-DKD group and anemia was associated with eGFR decline.[5] The Japanese study[6] also enrolled patients with biopsy-proven diabetic nephropathy and improved that the risks of renal events were higher for lower tertiles of Hb. But in a 3-year prospective, multicenter cohort study, a total of 1138 pre-dialysis CKD patients were recruited and categorized into two groups (DKD and non-DKD groups). Propensity score matching was performed to adjust for confounding factors, resulting in 197 patients being assigned to DKD and non-DKD groups, respectively. Cox regression analyses showed that decreased hemoglobin (Hb) was a risk factor for all-cause death endpoint, but not a risk factor for 50% eGFR decline and initiation of kidney replacement therapy.[25] Therefore, whether hemoglobin is independently associated with renal prognosis is still controversial.

In our study, it was found that Hb was negatively correlated with 50% eGFR decline and progress to ESRD. And there was a nonlinear correlation between Hb and renal prognosis in diabetic kidney disease people, with an infection point of 109g/L. It is important to highlight that this study primarily investigated the impact of Hb on renal prognosis in individuals with diabetic kidney disease. The findings of this study have clinical significance as they provide insights into early interventions for individuals with DKD and might potentially prevent rapid progression to ESRD.

The mechanisms underlying anemia in patients with Diabetic Kidney Disease (DKD) and its association with poorer renal outcomes, compared to those without anemia, remain not fully understood. Anemia in these patients may result from erythropoietin (EPO) deficiency and an insensitive response to EPO. Additionally, increased levels of circulating inflammatory cytokines, such as TNF-α, IL-6, and TGF-β, accelerate the apoptosis of erythroid progenitor cells. Chronic hyperglycemia and advanced glycation end products may contribute to oxidative stress, leading to decreased erythrocyte deformability and a shortened life expectancy.[26] Patients with diabetic nephropathy experiencing anemia might be affected by hypoglycemic drugs. Thiazolidinediones (TZD) can increase plasma volume, leading to blood dilution and a subsequent decrease in hemoglobin. Metformin might cause intestinal malabsorption, preventing the absorption of Vitamin B12.[27] Inflammation and renal tubular injury may also play a role in the pathogenesis of anemia. A prior study reported that interstitial lesions, rather than glomerular lesions, were a significant predictor of renal prognosis in patients with diabetic nephropathy, type 2 diabetes, and massive proteinuria. Interstitial lesions might lead to severe anemia, reflecting interstitial impairment.[28] Moreover, anemia may exacerbate renal fibrosis by inducing renal tissue hypoxia through the stimulation of cytokine production, including hypoxia-inducing factor (HIF)-1. These fibroblast growth factors could contribute to the activation of renal interstitial fibroblasts.

Our research has revealed that in subgroup analysis, the relationship between Hb and renal prognosis is stronger among the male population. Previous studies have found that females have a higher prevalence of anemia compared to males. Apart from erythropoietin deficiency, there are numerous other factors contributing to anemia, such as blood loss, iron deficiency due to menstrual bleeding, hormonal variations, and the potential presence of nutritional deficiencies.

Observational studies have indicated that females undergoing ESA treatment exhibit lower hemoglobin levels than males. Females with CKD require higher doses of ESA therapy.[29] Despite higher incidence of CKD in females, the deterioration of renal function progresses more rapidly in males.[30] In a cross-sectional study of 12,055 ambulatory patients, the relationship between renal function and hemoglobin (or hematocrit) differed in men and women. Both men and women developed a statistically significant decrease in eGFR when hemoglobin (or hematocrit) decreased. But the change in eGFR was greater in men than in women.[31] Sex hormones are thought to be one of the factors affecting the progression of CKD. Estrogen can be anti-fiber and anti-cell oxidation, while testosterone has been shown in animal tests to have pro-inflammatory effects. By activating the RAAS system, testosterone increases oxidative stress and fibrosis,

resulting in pathogenic effects, while estrogen can inhibit the above processes.[32] Gender differences in nitric oxide metabolism and oxidative stress also play an important role in the progression of CKD. Low nitric oxide levels are associated with endothelial cell dysfunction in patients with CKD.[33] The mechanism for a stronger link between anemia and deterioration of renal function among men was still unclear. But the proinflammatory effect of testosterone and anemia causing renal hypoxia and aggravated renal fibrosis by stimulating the production of cytokines might cause a stronger effect on decreased renal function. Further studies were needed to find out the mechanism. In our study, it appeared that serum hemoglobin was much more distinguished between males than females (P for trend; <0.001 for male vs 0.015 for females). Decreased hemoglobin had a greater effect on eGFR among males. Clinically, more attention should be paid to DKD anemic males to prevent the serious impact of decreased hemoglobin on renal function.

The nonlinear relationship between Hb level and renal composite endpoints in DKD patients had great clinical significance. It promotes clinical consultation and optimization of decision-making in the prevention of deterioration of renal function, and its inflection point provides clinicians with the prognosis of patients with DKD. From the perspective of treatment, controlling Hb levels through medications or lifestyle interventions to maintain Hb around 109g/L may effectively reduce the risk of patients progressing to 50% eGFR decline and ESRD.

The advantages of our study are as follows: 1) Our study is a relatively large sample size study of diabetic kidney patients. 2) For the first time, our study described the relationship between Hb and the renal prognosis in Chinese DKD patients, and found the inflection point of Hb (Hb inflection point 109g/L) through non-linear correlation statistical analysis, which provided prognostic prediction for screening DKD groups as well as provided strong data support for reducing the occurrence of renal composite endpoint. 3) We used subgroup analysis and found a stronger association between Hb and renal outcomes in specific populations (males). 4) In order to avoid the reduction of statistical test efficiency and bias caused by direct exclusion of missing values, we use multivariate multiple interpolation to estimate missing values.

However, there are some shortcomings in our study: 1) As all the other observational studies although known potential confounders such as BMI, SBP, and age were controlled, there were still uncontrolled or unmeasured confounders. 2) The patient's medication regimen (anti-anemia drugs, hypoglycemic drugs, lipid-lowering drugs, uric-lowering drugs, antihypertensive drugs, etc.) was not included in the follow-up, these drugs might influence the outcome. In short, we still could not judge the effects on the renal outcomes with drugs and lifestyle. 3) Further studies are needed to improve the renal outcomes in other countries and areas.

Conclusion

DKD patients are more likely to suffer severe and earlier anemia compared to non-DKD patients. DKD patients with anemia have a higher risk of eGFR decline. Thus, early identification and intervention of anemia may delay the risk of renal function worsening. Our study showed that Hb was a good and simple index to predict the renal outcome of patients with DKD, when Hb > 109, patients with DKD are less likely to proceed to renal composite endpoints. Therefore, people with DKD might benefit from limiting Hb value to around 109g/L, instead of progressing to renal composite endpoints.

Abbreviations

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipid cholesterol; ALT, alanine amino-transferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Scr, serum creatinine; UA, uric acid; Cys C, Cystatin C; WBC, white blood cell; TP, total protein; ALB, albumin; K, serum potassium; MG, serum magnesium; Ca, serum calcium; P, serum phosphorus; HbA1c, glycosylated hemoglobin; 24hU-pro, 24-hour urine albumin; 24hU-alb, 24-hour urine protein; DKD, diabetic kidney disease; ESRD, end-stage renal disease; eGFR, estimated glomerular filtration rate; EPO, erythropoietin; DN, diabetic nephropathy; CKD, chronic kidney disease; IFTA, interstitial fibrosis and tubular atrophy; T2DM, type 2 diabetes mellitus; CI, confidence interval; HR, Hazard ratio; GAM, Generalized additive model; SD, Standard deviation; KDIGO, Kidney Disease: Improving Global Outcomes.

Data Sharing Statement

The original contributions presented in the study are included in the article/Figures Material. Further inquiries can be directed to the corresponding author.

Ethics Statement

All individuals provided written informed consent after being fully briefed and advised about the study procedures. This study was approved by the Ethics Committee of Guangdong Provincial People's Hospital. It was conducted in compliance with the Declaration of Helsinki.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests in this work.

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