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ORIGINAL RESEARCH

Prevalence of Diabetic Kidney Disease with Different Subtypes in Hospitalized Patients with Diabetes and Correlation Between eGFR and LncRNA XIST Expression in PBMCs

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ABSTRACT

Introduction: Diabetic kidney disease (DKD) has become the leading cause of end-stage kidney disease (ESKD) in most countries. Recently, long noncoding RNA XIST has been found involved in the development of DKD.

Methods: A total of 1184 hospitalized patients with diabetes were included and divided into four groups based on their estimated glomerular filtration rate (eGFR) and urinary albumin to creatinine ratio (UACR): normal control group (nDKD), DKD with normoalbuminuric and reduced eGFR (NA-DKD), DKD with albuminuria but without reduced eGFR (A-DKD), and DKD with albuminuria and reduced eGFR (Mixed), and then their clinical characteristics were analyzed. Peripheral blood mononuclear cells (PBMCs) of patients with DKD were isolated, and lncRNA XIST expression was detected by real-time quantitative PCR.

Results: The prevalence of DKD in hospitalized patients with diabetes mellutus (DM) was 39.9%, and the prevalence of albuminuria and decreased eGFR was 36.6% and 16.2%, respectively. NA-DKD, A-DKD, and Mixed groups accounted for 3.3%,23.7%, and 12.9%, respectively. Women with DKD had considerably lower levels of lncRNA XIST expression in their PBMCs compared to nDKD. There was a significant correlation between eGFR level and lncRNA XIST expression ($R = 0.390$, $P = 0.036$) as well as a negative correlation between HbA1c and lncRNA XIST expression ($R = -0.425$, $P = 0.027$) in female patients with DKD.

Conclusions: Our study revealed that 39.9% of DM inpatients who were admitted to the hospital had DKD. Importantly, lncRNA XIST expression in PBMCs of female patients with DKD was significantly correlated with eGFR and HbA1c.

Keywords: Diabetic kidney disease; Long noncoding RNA; Normoalbuminuric diabetic kidney disease; Xist

This article was revised due to update in abstract and key summary point.

Yingbei Lin, Peili Wu, and Lei Guo have contributed equally to this work and share the first authorship.

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Key Summary Points

Why carry out this study?

Diabetic kidney disease has become the leading cause of end-stage kidney disease in most countries

The prevalence of normoalbuminuric diabetic kidney disease has substantially increased over the past few decades, but the clinical characteristics remains under-characterized

What was learned from the study?

The prevalence of DKD in hospitalized patients with DM was 39.9%, normoalbuminuric and reduced eGFR, DKD with albuminuria but without reduced eGFR, and DKD with albuminuria and reduced eGFR groups accounted for 3.3%, 23.7%, and 12.9%, respectively

The clinical signs and symptoms of normoalbuminuria is different from other phenotypes of diabetic kidney disease

lncRNA XIST has potential as a biomarker to predict the progression of diabetic kidney disease

INTRODUCTION

Diabetic kidney disease (DKD) is one of the main complications of diabetes mellitus (DM). In recent years, the incidence of end-stage renal disease (ESRD) caused by diabetes has increased dramatically [1]. Traditionally, DKD begins with a progressive increase in albuminuria followed by a steady decrease in estimated glomerular filtration rate (eGFR) [2]. Recent research, however, suggested that a portion of patients with DM with normoalbuminuria have progressive renal insufficiency, referred to as normoalbuminuric diabetic kidney disease (NADKD) [3, 4]. The prevalence of DKD with this feature has substantially increased over the past few decades, according to several sizable, serial cross-sectional studies [5, 6]. Based on these, NADKD differs from albuminuric renal dysfunction in terms of its clinical features. The majority of patients with NADKD were women, and their conditions were frequently characterized by obesity, lipidemia, higher plasma glucose, and a reduced frequency of polyneuropathy [2, 7]. However, there is an apparent dearth of research on Asians, specifically Chinese individuals, regarding the clinical characteristics of normoalbuminuric DKD. Therefore, it is imperative to investigate the clinical traits associated with normoalbuminuric DKD, discover new molecular markers linked to the progression of DKD, and elucidate the role of these markers in the development of DKD.

Numerous studies have confirmed that long non-coding RNAs (lncRNAs) could regulate cell functions through a variety of pathways [8]. Among them, lncRNA XIST, a major regulator of X chromosome inactivation, has also been extensively found to play an important role in cell differentiation, proliferation, and metabolism [9–11]. In recent years, the study of lncRNA XIST in the kidney has revealed that it can regulate renal interstitial fibrosis in DKD and play a role as a biomarker in AKI treatment strategy [12, 13]. It has also been shown that lncRNA XIST is associated with the macrophage phenotype and thus may be involved in the DKD process [14–16], but its significance in patients with DKD remains to be clarified.

Therefore, in this study, 1184 hospitalized patients with DM were retrospectively analyzed to determine the prevalence and clinical characteristics of DKD, especially NADKD. In addition, we explored the relationship between lncRNA XIST expression of PBMCs in patients with DKD and the progression of DKD to provide novel biomarkers with the potential to indicate the progression of DKD.

METHODS

Study Participants

This was a retrospective cross-sectional study enrolling the patients who were diagnosed with T1DM or T2DM between January 2021 and December 2021 in Nanfang Hospital, Guangdong Province, China. We have developed reasonable exclusion criteria (Table S1). In the end, data from 1184 participants were included in the study. The algorithm for the selected participants is shown in Fig. S1. The cases were further divided into four groups based on eGFR and UACR as follows: nDKD: normal control group (UACR $<$ 3 mg/mmol and eGFR $\geq$ 60 ml/min/1.73 m^2), NA-DKD: DKD with normoalbuminuric and reduced eGFR (UACR $<$ 3 mg/mmol and eGFR $<$ 60 ml/min/1.73 m^2), A-DKD: DKD with albuminuria but without reduced eGFR (UACR $\geq$ 3 mg/mmol and eGFR $\geq$ 60 ml/min/1.73 m^2), and Mixed: DKD with albuminuria and reduced eGFR (UACR $\geq$ 3 mg/mmol and eGFR $<$ 60 ml/min/1.73 m^2). Peripheral blood was collected from outpatients who met the inclusion criteria.

Approval of the research protocol: The protocol for this research project was approved by a suitably constituted Ethics Committee of the institution and conforms to the provisions of the Declaration of Helsinki and the Committee of Medical Ethics of Nanfang Hospital, approval no. NFEC-2021–049. Informed consent was obtained from all subjects to further follow-up.

Diagnostic Criteria

DKD was defined as previously described [17], as the presence of albuminuria and/or reduced eGFR in the absence of signs or symptoms of other primary causes of kidney damage; eGFR using the CKD-EPI-creatinine formulae. Reduced eGFR was defined as eGFR $<$ 60 ml/min/1.73 m^2. Albuminuria was defined as urinary albumin to creatinine ratio (UACR) $\geq$ 3 mg/mmol. Coronary heart disease (CHD) was defined as myocardial infarction, ischemic heart disease, or angina pectoris. Cardiovascular diseases (CVD) were defined as previously described [18], including myocardial infarction, atrial fibrillation, heart failure, left ventricular hypertrophy, and stroke. Metabolic syndrome (MetS) was defined as the presence of three or more of the following: (1) BMI $>$ 30 kg/m^2; (2) blood pressure $>$ 130/85 mmHg and/or diagnosed hypertension; (3) fasting blood glucose $\geq$ 6.1 mmol/l or 2 h oral glucose tolerance test (OGTT) $\geq$ 7.8 mmol/l and/or have been diagnosed with diabetes and treated; (4) triglyceride $>$ 1.7 mmol/l; (5) high-density lipoprotein cholesterol $<$ 1.04 mmol/l.

Data Collection

General information and clinical manifestation were obtained through case history inquiry. Biochemical and physical examinations were conducted in related auxiliary departments of Nanfang Hospital. All the clinical characteristics and biochemical indexes were electronically collected through the electronic medical record system.

Isolation and Purification of Peripheral Blood Mononuclear Cells (PBMCs)

Anticoagulated 3–5-ml whole-blood samples from participants were centrifuged at 3500 rpm for 10 min, and then PBMCs were isolated using a commercially available Lymphoprep™ kit purchased from Haoyang Biological Manufacture Co. (Tianjin, China).

Quantitative Real-Time PCR

The total RNA of PBMCs was extracted using Trizol reagent (TAKARA), which was then reverse transcribed according to the PrimeScript RT Reagent Kit (TAKARA) manual. Quantitative real-time PCR (qRT-PCR) was performed in a Roche LightCycler 480II system (Roche, Basle, Switzerland) using a SYBR Green qPCR Kit (TAKARA). The $2^{-\Delta\Delta Ct}$ method with β-actin as an internal control was used to carry out relative quantification. The levels of XIST mRNA were normalized by the method of $2^{-\Delta CT}$; the normalized RNA-Seq data were subjected to

Table 1 General condition data of the study groups

	Overall (n = 1184)	nDKD (n = 711)	NADKD (n = 39)	ADKD (n = 281)	Mixed (n = 153)	P
T2DM (%)	1101 (93%)	648 (91.1%)	37 (94.9%)	270 (96.1%)*	146 (95.4%)	0.024
eGFR (ml/min/1.73m^2)	96.0 (74.7, 107.3)	100.8 (91.6, 110.8)	46.5 (36.1, 53.6)***	94.5 (79.3, 105.4)***,###	35.7 (23.0, 47.9)***	< 0.001
UACR (mg/mmol)	1.7 (0.8, 8.9)	1.0 (0.7, 1.5)	1.1 (0.8, 1.5)	10.8 (5.4, 26.4)***,###	36.4 (16.1, 119.0)***,###	< 0.001
HbA1c (%)	9.36 ± 2.47	9.41 ± 2.5	8.56 ± 2.67*	9.72 ± 2.37##	8.65 ± 2.28***	< 0.001
Male (%)	736 (62.2%)	454 (63.9%)	26 (66.7%)	164 (58.4%)	92 (60.1%)	0.363
Age (years)	54.4 ± 13.46	51.73 ± 13.21	62.36 ± 9.67***	56.15 ± 13.36***,#	61.5 ± 11.69***	< 0.001
Body mass index (kg/m^2)	24.02 ± 3.56	24.02 ± 3.63	23.72 ± 3.61	23.99 ± 3.44	24.14 ± 3.47	0.931
Duration of DM (m)	84 (12.8, 144)	60 (6, 120)	108 (36, 168)	120 (46, 156)***	120 (72, 240)***	< 0.001
Family history of DM (m)	409 (34.6%)	260 (36.6%)	8 (20.5%)	94 (33.5%)	47 (30.7%)	0.117
Age at DM diagnosis (years)	45.92 ± 12.1	44.92 ± 12.06	51.72 ± 8.9**	45.86 ± 11.5##	50.33 ± 13.14***	< 0.001
Family history of DM (%)	409 (34.6%)	260 (36.6%)	8 (20.5%)	94 (33.5%)	47 (30.7%)	0.117
Duration of hypertension (m)	36 (0, 120)	36 (0, 120)	90 (25, 120)	24 (0, 120)	60 (12, 120)**	< 0.001
Family history of hypertension (%)	173 (14.6%)	115 (16.2%)	5 (12.8%)	34 (12.1%)	19 (12.4%)	0.316
Smoking (%)	466 (39.4%)	280 (39.4%)	20 (51.3%)	104 (37.1%)	62 (40.5%)	0.394

eGFR estimated glomerular filtration rate, *UACR* urinary albumin/creatinine ratio, *DM* diabetes mellitus
vs. nDKD: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; vs. NA-DKD: $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$

correlation analysis. The primers used are listed in Table S2.

Statistical Analysis

Continuous variables are expressed as mean ± SD or median (interquartile range), and categorical variables are reported for frequency (%). For continuous variables, ANOVA and least significant difference test (LSD) were performed for normally distributed variables and the Kruskal-Wallis test for non-normally distributed variables, while the chi-square test was used for categorical variables and the rank sum test for grade data.

Table 2 Biochemical indicators and complications of the study groups

	Overall (n = 1184)	nDKD (n = 711)	NA-DKD (n = 39)	A-DKD (n = 281)	Mixed (n = 153)	P
WBC ($\times 10^9$/l)	6.96 ± 1.96	6.74 ± 1.82	7.55 ± 2.29*	7.05 ± 1.98*	7.65 ± 2.23***	< 0.001
HGB (g/l)	135.41 ± 19.92	141.02 ± 15.62	123.51 ± 24.51***	134.96 ± 19.33***,#	113.25 ± 20.76***	< 0.001
Fibrinogen (g/l)	3.2 (2.8, 3.9)	3 (2.6, 3.5)	3.6 (3.2, 4.8)***	3.4 (2.9, 4.1)***	4.35 (3.65, 5.4)***,#	< 0.001
K + (mmol/l)	3.95 ± 0.44	3.88 ± 0.39	4.03 ± 0.48	3.94 ± 0.43	4.29 ± 0.54***,#	< 0.001
Na + (mmol/l)	139.36 ± 2.87	139.51 ± 2.63	138.54 ± 4.89	139.22 ± 2.75	139.14 ± 3.43	0.463
Urea (mmol/l)	5.8 (4.7, 7.48)	5.3 (4.4, 6.3)	8.5 (6.8, 12.1)***	6 (4.7, 7.3)***,###	11.2 (8.4, 15.2)***	< 0.001
CR (μmol/l)	71 (58, 90)	67 (55, 77)	132 (113, 176)***	70 (56, 83.5)###	162 (126.5, 231.5)***	< 0.001
UA (μmol/l)	361.5 ± 104.8	343.8 ± 96.4	443.2 ± 133.6***	356.5 ± 97.5###	432.3 ± 109.0***	< 0.001
CysC (mg/l)	0.91 (0.78, 1.17)	0.84 (0.75, 0.96)	1.53 (1.37, 1.97)***	0.96 (0.82, 1.17)***,###	2.01 (1.61, 2.71)***	< 0.001
TG (mmol/l)	1.51 (1.09,2.37)	1.41 (1.02, 2.11)	1.81 (1.32, 2.52)	1.55 (1.16, 2.52)*	1.81 (1.31, 2.97)***	< 0.001
Total cholesterol (mmol/l)	4.98 ± 1.51	4.86 ± 1.27	4.62 ± 1.16	5.23 ± 1.81	5.19 ± 1.92	0.029
HDL-C (mmol/l)	1.12 ± 0.32	1.12 ± 0.29	0.99 ± 0.25*	1.16 ± 0.39##	1.11 ± 0.3	0.014
LDL-C (mmol/l)	3.13 ± 0.98	3.12 ± 0.93	2.9 ± 0.92	3.18 ± 1.01	3.15 ± 1.14	0.378
Homocysteine (μmol/l)	11 (9,13)	10 (8, 12)	16.5 (12, 22)***	11 (9, 13)***,###	17.5 (14, 22)***	< 0.001
PTH (pg/ml)	31.64 (24.08, 40.7)	31.45 (24.5, 38.51)	33.71 (24.59, 44.53)	28.34 (21.72, 36.72)	44.42 (26.78, 69.47)***	< 0.001
VitD (ng/ml)	21.55 ± 7.1	22.11 ± 6.65	23.47 ± 8.02	21.34 ± 6.91	19.14 ± 8.46***	< 0.001
FT4 (ng/dl)	1.03 ± 0.18	1.03 ± 0.18	1.01 ± 0.16	1.05 ± 0.2	0.97 ± 0.16***	< 0.001
FT3 (ng/dl)	2.61 ± 0.55	2.69 ± 0.52	2.35 ± 0.47***	2.6 ± 0.54*,##	2.31 ± 0.57***	< 0.001
TSH(mIU/l)	1.43 (0.94, 2.07)	1.4 (0.95, 1.97)	1.64 (0.91, 2.95)	1.37 (0.89, 2.08)	1.59(1, 2.19)	0.184
SBP (mmHg)	131.8 ± 19.5	128.3 ± 17.0	122.0 ± 19.4	135.6 ± 20.2***,##	143.1 ± 23.2***,###	< 0.001
DBP (mmHg)	80.3 ± 11.4	80.1 ± 11.1	77.2 ± 14.0	81.1 ± 11.8#	80.7 ± 11.7	0.203
IVS (mm)	10.5 (10, 12)	10 (9.7, 11)	11 (10, 12)	11 (10, 12)***	12 (11, 13)***	< 0.001
LVPW (mm)	10 (9.4, 11)	10 (9, 11)	10 (10, 11.1)	10 (9.3, 11.5)**	11 (10, 12)***,#	< 0.001
LVEF (%)	65.2 ± 5.1	65.4 ± 5.0	65.5 ± 6.1	65.0 ± 5.3	64.6 ± 4.7	0.254
Prevalence of comorbidities (%)						
CHD	95 (8%)	48 (6.8%)	5 (12.8%)	24 (8.5%)	18 (11.8%)	0.123
Stroke	134 (11.3%)	50 (7%)	2 (5.1%)	46 (16.4%)*	36 (23.5%)*	< 0.001
CVD	209 (17.7%)	89 (12.5%)	6 (15.4%)	66 (23.5%)*	48 (31.4%)*	< 0.001
MetS	581 (49.1%)	355 (49.9%)	22 (56.4%)	133 (47.3%)	71 (46.4%)	0.609
DR	490 (52.1%)	236 (41.5%)	17 (56.7%)	147 (64.5%)*	90 (78.9%)*	< 0.001
Diminished protective sensation	803 (81.6%)	462 (75.7%)	23 (88.5%)	199 (88.1%)*	119 (97.5%)*	< 0.001
Fatty liver disease	644 (58.5%)	391 (58.8%)	24 (64.9%)	156 (60.2%)	73 (52.5%)	0.390
Urolithiasis	201 (18.9%)	100 (15.7%)	13 (38.2%)*	51 (20.2%)	37 (27.2%)*	< 0.001

Table 2 continued

	Overall (n = 1184)	nDKD (n = 711)	NA-DKD (n = 39)	A-DKD (n = 281)	Mixed (n = 153)	P
Aorticsclerosis	794 (69.5%)	428 (62.8%)	35 (92.1%)*	201 (73.6%)*	130 (87.2%)*	< 0.001
Carotid plaque	636 (57.5%)	329 (49.1%)	27 (73%)*	170 (64.4%)*	110 (81.5%)*	< 0.001

WBC white blood cell count, *RBC* red blood cell count, *HGB* hemoglobin, *K* + serum potassium, *Na* + serum sodium, *UA* uric acid, *CysC* cystatin C, *CR* creatinine, *TG* triglycerides, *HDL-C* High-density lipoprotein cholesterol, *LDL-C* Low-density lipoprotein cholesterol, *PTH* parathyroid hormone, *VitD* vitamin D, *FT3* free triiodothyronine, *FT4* free thyroxine, *TSH* thyroid stimulating hormone, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *IVS* interventricular septum, *LVPW* left ventricular posterior wall thickness, *LVEF* left ventricular ejection fraction, *CHD* coronary heart disease, *CVD* cardiovascular diseases, *MetS* metabolic syndrome, *DR* diabetic retinopathy

vs. nDKD: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; vs NA-DKD: $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$

RESULTS

Baseline Characteristics of Patients

This study included a total of 1184 cases, the vast majority being diagnosed with T2DM, n = 1101 (93%). The average age was 54.4 years, and the number of male patients was 736 (62.2%). Of the 1184 patients, 473 (39.9%) were confirmed as having DKD, 434 (36.6%) as having albuminuria, and 192 (16.2%) as having renal impairment. NA-DKD prevalence was 39 (3.3%), A-DKD prevalence was 281 (23.7%), and Mixed prevalence was 153 (12.9%).

Patients with DKD had longer duration of diabetes than those in the nDKD group, and their ages as well as ages at DM diagnosis tended to be older, notably in the NA-DKD and Mixed groups. Although the course of hypertension did not differ between the NA-DKD and nDKD groups, there was a tendency for a longer duration of hypertension in the Mixed and NA-DKD groups compared to the nDKD group. More baseline characteristics of all participants in the study are provided in Table 1.

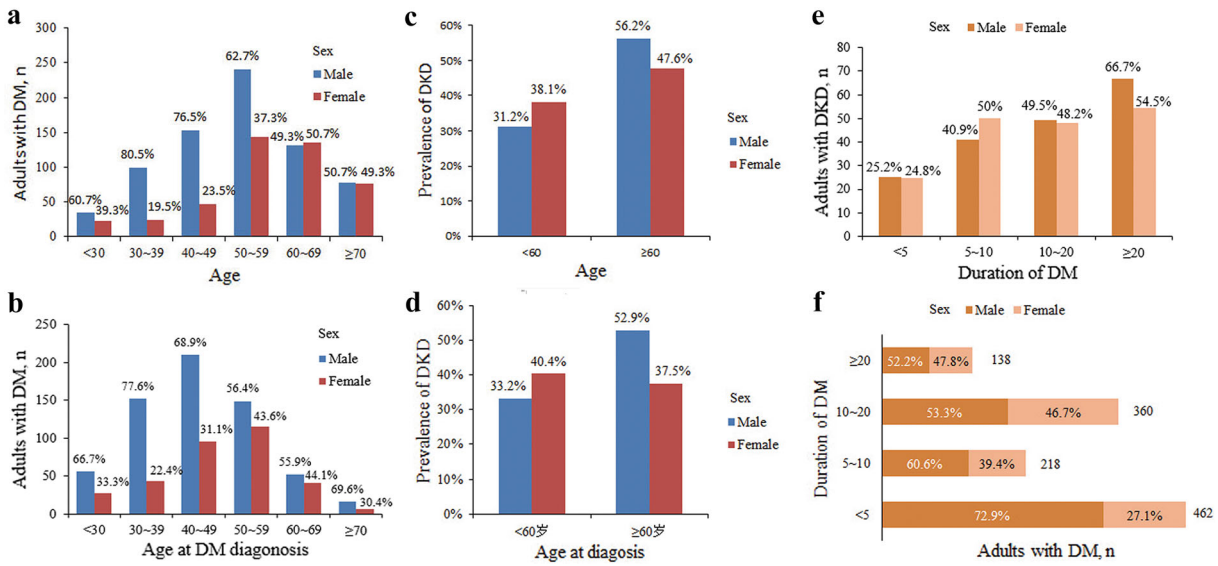

Fig. 1 Gender differences among participants

Sodium-dependent glucose transporters 2 inhibitors (SGLT2i) and glucagon-like peptide 1 receptor agonist (GLP-1RA) were recommended as kidney beneficial glucose-lowering medications, so the participants' use of these two drugs was further collected. The results show that 325 patients (27.3%) used GLP-1RA while 595 patients (50.3%) utilized SGLT2i. Of these, 186 patients (15.7%) took both types of medication while 450 patients (38.0%) took none. Notably, the Mixed group had the lowest percentage of SGLT2i ($p < 0.001$) (Table S3). Then, we looked into the connection between eGFR and SGLT2i use to further explore this. With declining eGFR level, there were noticeable drops in the utilization of SGLT2i or GLP-1RA (Fig. S2).

Comparison of Biochemical Indicators and Complications

An overview of the biochemical parameters observed in the study population is shown in Table 2. Those with DKD reported higher levels of white blood cell count (WBC), urea, cystatin C (CysC), homocysteine, and blood hypercoagulability but lower levels of hemoglobin (HGB) and free triiodothyronine (FT3) than control participants. Those with NA-DKD also had higher levels of WBC, and uric acid (UA), homocysteine but lower levels of high-density lipoprotein cholesterol (HDL-C) than those with the other DKD phenotypes. Compared to the other groups, the Mixed group's urine had much higher levels of 2-microglobulin, indicating serious tubular damage (Table S4).

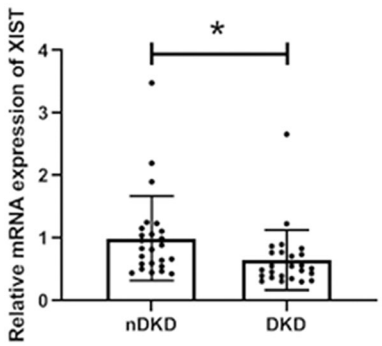

Fig. 2 Comparison of lncRNA XIST expression in PBMCs between DKD and nDKD in female patients

Since cardiovascular disease (CVD) is another important complication of diabetes [19], we further explored the differences in CVD-related indicators among the four groups. As shown in Table S3, patients with DKD have worse blood pressure than patients with nDKD, while the amount of hypertension in patients with NA-DKD is equivalent to that in patients with A-DKD but lower than that in Mixed group. Mixed and A-DKD groups had higher systolic blood pressure (SBP) compared to nDKD, and the heart restructure was more common. Interestingly, NA-DKD groups had lower SBP even though there was no statistically significant difference between nDKD and NA-DKD (Table S5).

Gender Differences

The results of the study show that the mean age and the age of DM diagnosis of men were significantly younger than that of women in DM population (52.46 ± 13.23 vs. 57.57 ± 13.26 years, 45.08 ± 11.78 vs. 47.53 ± 12.54 years) ($P < 0.001$). Before the age of 60 years, significantly more men than women were hospitalized with DM, while the number of male and female patients was about equal after the age of 60 years (Fig. 1a). Next, focusing on the age at DM diagnosis, more men than women were diagnosed with DM at either age. Similarly, there were some differences in the peak age of DM diagnosis in men and women, occurring at 40–49 and 50–59 years of age, respectively (Fig. 1b).

As demonstrated in Fig. 1c, the prevalence of DKD increased more rapidly with age in men than in women, with a prevalence of only 31.2% in men and 38.1% in women among those < 60 years of age, whereas in those > 60 years of age, the prevalence of DKD increased by 25% in men and only 9.5% in women. The prevalence of DKD increased with age at DM diagnosis in men but did not differ significantly in women (Fig. 1d). Furthermore, we focused on the duration of DM, and, as we widely perceived, the prevalence of DKD gradually

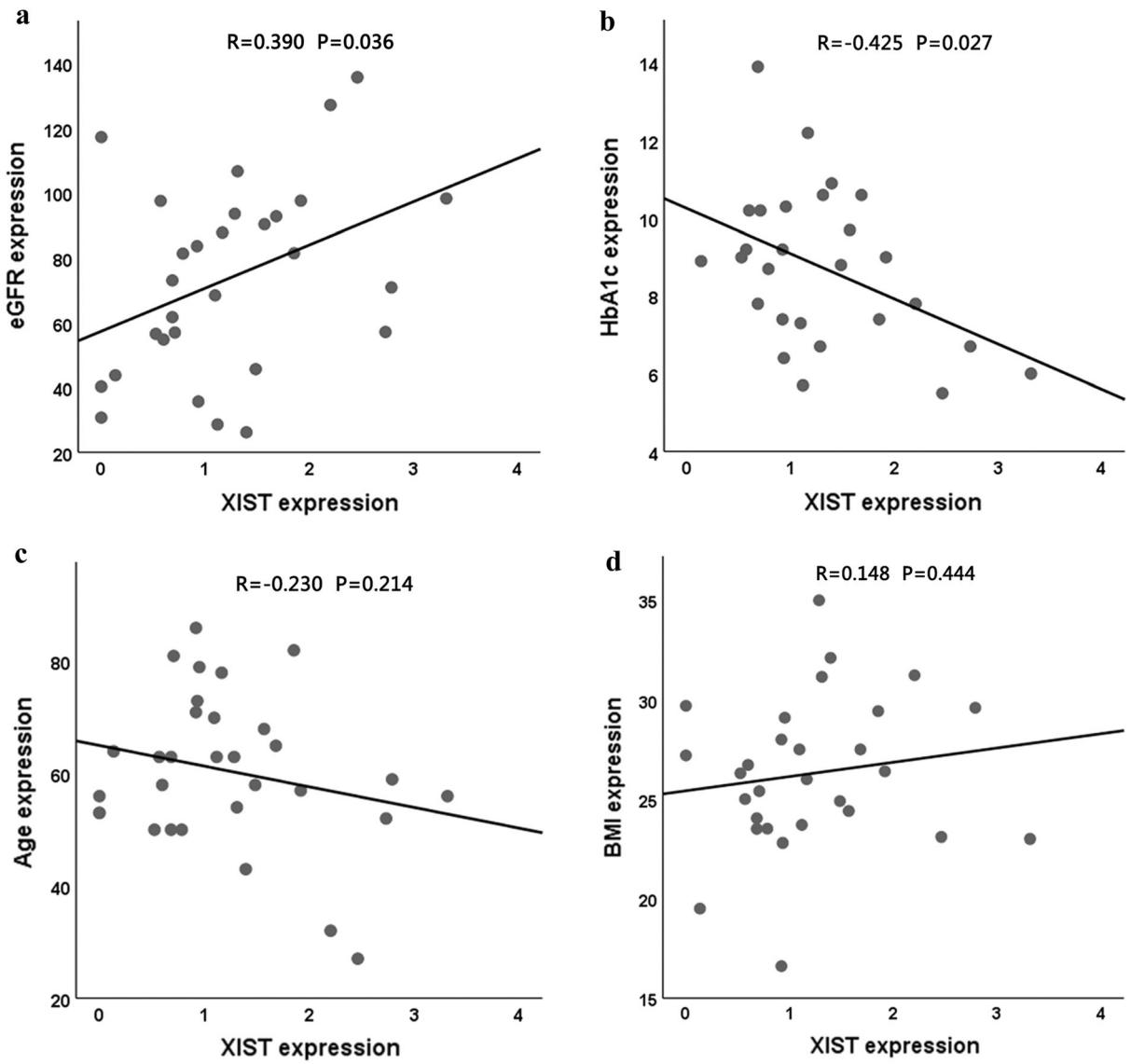

Fig. 3 Correlation analysis of lncRNA XIST expression levels with clinical indicators of DKD progression

increased with the duration of DM (Fig. 1e). Interestingly, however, the shorter the duration of DM, the greater the proportion of male patients among hospitalized patients (Fig. 1f).

Correlation of lncRNA XIST Expression Levels in PBMCs with Clinical Indicators of DKD

Based on the widely recognized important role of lncRNA XIST in sex dimorphism and previous related studies in our team [9, 20], we sought to further explore the relationship between lncRNA XIST and the gender differences in DKD obtained from the above analysis. We collected 50 female PBMC samples (patients with nDKD $n = 25$, patients with DKD $n = 25$), since lncRNA XIST expression in men was usually very low. As shown in Fig. 2, the expression of lncRNA XIST in patients with DKD was lower than in patients with nDKD. To further study the correlation between the expression of lncRNA XIST and clinical indicators in patients with DKD, we collected the clinical data and

PBMC samples from 31 female patients with DKD (Table S6). Scatter plots shown in Fig. 3 demonstrate significant positive correlations between the eGFR level and the expression of lncRNA XIST ($R = 0.390$, $P = 0.036$) and negative correlations between the HbA1c and the expression of lncRNA XIST ($R = -0.425$, $P = 0.027$) in female patients with DKD. Meanwhile, no correlation was suggested between age, BMI, and lncRNA XIST expression.

DISCUSSION

The clinical manifestations of DKD among patients with DM changed over time. We found that the prevalence of DKD among hospitalized patients with DM reached 39.9%, of which 16.2% exhibited decreased eGFR, and 36.6% exhibited albuminuria, and the prevalence of three DKD phenotypes, NADKD, ADKD, and Mixed, was 3.3%, 23.7%, and 12.9%, respectively. In the NHANES study, slightly different from our results, the prevalence of DKD in adults with DM was 26.2% in 2009–2014, while the prevalence of albuminuria and reduced eGFR was 15.9% and 14.1%, respectively [5]. We found that the prevalence of reduced eGFR is less than half of the prevalence of albuminuria, and the prevalence of reduced eGFR (20%) is also less than that of albuminuria (34%) in the people from Hong Kong [21], which is more than the prevalence of albuminuria in people from US and Japan [5, 22]. This shows the geographical variation of characteristics of DKD. We considered the variation and change in the prevalence of DKD mainly correlated with the enrollment population, detection rates, lifestyles, diet, and genes.

We next summarize the clinical characteristics of each DKD phenotype in an attempt to provide a basis for clinical diagnosis and treatment. Among patients with diabetes, compared with patients with nDKD, patients with DKD were older, were more likely to be complicated with aortic atherosclerosis and carotid plaque, and had higher levels of urea, CysC, and homocysteine but lower levels of HGB. Patients with NADKD were more likely to have urolithiasis and higher UA but lower HDL-C and HbA1c attainment rates. Patients with ADKD had a longer duration of diabetes and were more likely to be complicated with diminished protective sensation, diabetic retinopathy (DR), CVD, and cardiac structural changes. At the same time, the levels of SBP and triglycerides (TG) were higher, while the levels of albumin (ALB) and HbA1c attainment rate were lower. Patients in the Mixed group had a longer duration of diabetes and hypertension, had an older age at DM diagnosis, and were more likely to be complicated with CVD, DR, diminished protective sensation, and urolithiasis and higher levels of SBP, UA, and TG.

The patients with DKD were recommended an SGLT2i and GLP1-RA based on ADA guidelines; however, we found that > 30% of patients with DKD did not use kidney beneficial glucose-lowering medication. Special attention needs to be paid that Mixed groups use the least kidney beneficial glucose-lowering medication among patients with DKD, and since they had the worst kidney function, this indicated that their eGFR had significantly declined and they were experiencing especially severe medication distress. This suggests that additional medications tailored to these individuals are required and that the therapeutic quality, safety, and efficacy of currently available medications also need to be enhanced to continue treating patients with seriously reduced eGFR.

Improved understanding of sex and gender-specific differences in the etiology, mechanisms, and epidemiology of kidney disease could help nephrologists better address the needs of their patients [23]. A number of studies in recent years have focused on the role of gender differences in DKD. A single-center cohort study in 2012 showed that lower HDL-C levels appear to be associated with the progression of diabetic nephropathy in men but not in women [24]. In our study, we found that the shorter the duration of diabetes, the more patients with diabetes, especially male patients. This is similar to some findings among Chinese populations where the prevalence of diabetes is increasing fastest in younger patients [25], which may be related to the rapid changes in the economy and the increase in household income, which has led to an increase in diet and

changes to high-fat and high-sugar foods [26]. Our results also suggested that in the patients < 60 years old or diagnosed with diabetes, the prevalence of DKD in women was higher than in men; the opposite was true in the patients aged > 60 years old or diagnosed with diabetes after 60 years old. These results prompted us to see the sex differences in the clinical characteristics of the patients with DM.

LncRNA XIST is involved in diverse physiological and pathological processes; the expression of lncRNA XIST in PBMCs isolated from patients with T2DM has been proven higher than in that isolated from normoglycemic patients [25]. Studies have also shown that lncRNA XIST affects the immune function of B cells [26], and various immune pathways are involved in the occurrence and development of DM and its related complications [27], so lncRNA XIST may play a significant role in DM and DKD. In recent years, some studies have found that lncRNA XIST can play a role in DKD through the underlying ceRNA network. However, the correlation of lncRNA XIST with clinical indicators of DKD remains unknown [12, 28, 29]. As reported previously [30], the expression of lncRNA XIST in men was largely absent. In women, the expression of lncRNA XIST in patients with DKD was lower than in patients with nDKD. Otherwise, the expression of lncRNA XIST was positively correlated with eGFR level and negatively correlated with HbA1c. eGFR is known to be critical to the diagnosis and progression of DKD. Higher HbA1c was associated with an increased risk of CKD progression in patients with diabetes and remained an independent risk factor for adverse renal outcomes in patients with diabetes and CKD in patients with low carbamylation levels and no anemia [31]. According to a systematic review and meta-analysis of 20 cohorts, when HbA1c increased by 1%, the risk for DKD increased by 17% [32]. It is suggested that lncRNA XIST has great potential to participate in the development of DKD, but its mechanism needs to be further explored. Our investigation showed no correlations between age and lncRNA XIST expression, consistent with the results of a meta-analysis in cancer [33]. Thus, the variation in DKD prevalence with age, age of diabetes diagnosis, and duration of diabetes in women may not be attributed to differences in lncRNA XIST expression overages. Taking these into consideration, it is reasonable to assume that lncRNA XIST expression might be a slow and lasting protective factor for women, which is one of the reasons leading to a smaller increase in the prevalence of DKD with age, age at diabetes diagnosis, and duration of diabetes compared to men.

Limitations of our study cannot be shied away from, including that it is a small size, cross-sectional, single-center study and has possible enrollment bias. Meanwhile, further prospective and mechanistic studies are needed to confirm the causal relationship between the expression of lncRNA XIST and the changes in eGFR and HbA1c.

Overall, in this study we found that the prevalence of DKD in hospitalized patients with DM was 39.9% in economically advanced regions of southern China. LncRNA XIST expression in PBMCs of female patients with DKD is closely correlated with eGFR and HbA1c and therefore has potential as a biomarker of DKD progression.

CONCLUSIONS

Our study revealed that 39.9% of DM inpatients who were admitted to the hospital had DKD. Importantly, lncRNA XIST expression in PBMCs of female patients with DKD was significantly correlated with eGFR and HbA1c.

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Disclosures. Yingbei in, Peili Wu, Lei Guo, Qijian Feng, Ling Wang, Xiaochun Lin, Chuyi Yang, Nannan Liu, Churan Wen, Xuelin Lin, Xiaoqin Ma, Yaoming Xue, and Meiping Guan declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Compliance with Ethics Guidelines. Approval of the research protocol: The protocol for this research project has been approved by a suitably constituted Ethics Committee of the institution and it conforms to the provisions of the Declaration of Helsinki. Committee of Medical Ethics Committee of Nanfang Hospital, approval no. NFEC-2021–049. Informed consent was obtained from the subjects for further follow-up.

Data availability. The datasets supporting the conclusions of this article are included within the article.

REFERENCES

1. Cheng H-T, Xu X, Lim PS, Hung K-Y. Worldwide epidemiology of diabetes-related end-stage renal disease, 2000–2015. Diabetes Care. 2021;44:89–97. https://doi.org/10.2337/dc20-1913.

2. Fioretto P, Caramori ML, Mauer M. The kidney in diabetes: dynamic pathways of injury and repair. The Camillo Golgi Lecture 2007. Diabetologia. 2008;51:1347–55. https://doi.org/10.1007/s00125-008-1051-7.

3. Costacou T, Ellis D, Fried L, Orchard TJ. Sequence of progression of albuminuria and decreased GFR in persons with type 1 diabetes: a cohort study. Am J Kidney Dis. 2007;50:721–32. https://doi.org/10.1053/j.ajkd.2007.08.005.

4. In patients with type 1 diabetes and new-onset microalbuminuria the development of advanced chronic kidney disease may not require progression to proteinuria - PubMed n.d. https://pubmed.ncbi.nlm.nih.gov/19847154/. Accessed 29 Mar 2023.

5. Afkarian M, Zelnick LR, Hall YN, Heagerty PJ, Tuttle K, Weiss NS, et al. Clinical manifestations of kidney disease among US adults with diabetes, 1988–2014. JAMA. 2016;316:602–10. https://doi.org/10.1001/jama.2016.10924.

6. de Boer IH, Rue TC, Hall YN, Heagerty PJ, Weiss NS, Himmelfarb J. Temporal trends in the prevalence of diabetic kidney disease in the United States. JAMA. 2011;305:2532–9. https://doi.org/10.1001/jama.2011.861.

7. Boronat M, García-Cantón C, Quevedo V, Lorenzo DL, López-Ríos L, Batista F, et al. Non-albuminuric renal disease among subjects with advanced stages of chronic kidney failure related to type 2 diabetes mellitus. Ren Fail. 2014;36:166–70. https://doi.org/10.3109/0886022X.2013.835266.

8. Chen Y, Li Z, Chen X, Zhang S. Long non-coding RNAs: from disease code to drug role. Acta Pharm Sin B. 2021;11:340–54. https://doi.org/10.1016/j.apsb.2020.10.001.

9. Wu C, Fang S, Zhang H, Li X, Du Y, Zhang Y, et al. Long noncoding RNA XIST regulates brown preadipocytes differentiation and combats high-fat diet induced obesity by targeting C/EBPα. Mol Med.

2022;28:6. https://doi.org/10.1186/s10020-022-00434-3.

10. Lyon MF. Gene action in the X-chromosome of the mouse (*Mus musculus* L.). Nature. 1961;190:372–3. https://doi.org/10.1038/190372a0.

11. Richart L, Picod-Chedotel M-L, Wassef M, Macario M, Aflaki S, Salvador MA, et al. XIST loss impairs mammary stem cell differentiation and increases tumorigenicity through Mediator hyperactivation. Cell. 2022;185:2164-2183.e25. https://doi.org/10.1016/j.cell.2022.04.034.

12. Yang J, Shen Y, Yang X, Long Y, Chen S, Lin X, et al. Silencing of long noncoding RNA XIST protects against renal interstitial fibrosis in diabetic nephropathy via microRNA-93-5p-mediated inhibition of CDKN1A. Am J Physiol Renal Physiol. 2019;317:F1350–8. https://doi.org/10.1152/ajprenal.00254.2019.

13. Tang B, Li W, Ji T, Li X, Qu X, Feng L, et al. Downregulation of XIST ameliorates acute kidney injury by sponging miR-142-5p and targeting PDCD4. J Cell Physiol. 2020;235:8852–63. https://doi.org/10.1002/jcp.29729.

14. Pi L, Fang B, Meng X, Qian L. LncRNA XIST accelerates burn wound healing by promoting M2 macrophage polarization through targeting IL-33 via miR-19b. Cell Death Discov. 2022;8:220. https://doi.org/10.1038/s41420-022-00990-x.

15. Zhao Y, Yu Z, Ma R, Zhang Y, Zhao L, Yan Y, et al. lncRNA-Xist/miR-101-3p/KLF6/C/EBPα axis promotes TAM polarization to regulate cancer cell proliferation and migration. Mol Ther Nucleic Acids. 2021;23:536–51. https://doi.org/10.1016/j.omtn.2020.12.005.

16. Chen H, Guo Y, Cheng X. Long non-cording RNA XIST promoted cell proliferation and suppressed apoptosis by miR-423-5p/HMGA2 axis in diabetic nephropathy. Mol Cell Biochem. 2021;476:4517–28. https://doi.org/10.1007/s11010-021-04250-x.

17. American Diabetes Association Professional Practice Committee. 11. Chronic Kidney Disease and Risk Management: Standards of Medical Care in Diabetes-2022. Diabetes Care. 2022;45:S175–84. https://doi.org/10.2337/dc22-S011.

18. Ohno Y, Sone M, Inagaki N, Yamasaki T, Ogawa O, Takeda Y, et al. Prevalence of cardiovascular disease and its risk factors in primary aldosteronism: a multicenter study in Japan. Hypertension. 2018;71:530–7. https://doi.org/10.1161/HYPERTENSIONAHA.117.10263.

19. Strain WD, Paldánius PM. Diabetes, cardiovascular disease and the microcirculation. Cardiovasc Diabetol. 2018;17:57. https://doi.org/10.1186/s12933-018-0703-2.

20. Li J, Ming Z, Yang L, Wang T, Liu G, Ma Q. Long noncoding RNA XIST: mechanisms for X chromosome inactivation, roles in sex-biased diseases, and therapeutic opportunities. Genes Dis. 2022;9:1478–92. https://doi.org/10.1016/j.gendis.2022.04.007.

21. Jin Q, Luk AO, Lau ESH, Tam CHT, Ozaki R, Lim CKP, et al. Nonalbuminuric diabetic kidney disease and risk of all-cause mortality and cardiovascular and kidney outcomes in type 2 diabetes: findings from the Hong Kong Diabetes Biobank. Am J Kidney Dis. 2022;80:196-206.e1. https://doi.org/10.1053/j.ajkd.2021.11.011.

22. Kume S, Araki S-I, Ugi S, Morino K, Koya D, Nishio Y, et al. Secular changes in clinical manifestations of kidney disease among Japanese adults with type 2 diabetes from 1996 to 2014. J Diabetes Investig. 2019;10:1032–40. https://doi.org/10.1111/jdi.12977.

23. Carrero JJ, Hecking M, Chesnaye NC, Jager KJ. Sex and gender disparities in the epidemiology and outcomes of chronic kidney disease. Nat Rev Nephrol. 2018;14:151–64. https://doi.org/10.1038/nrneph.2017.181.

24. Hanai K, Babazono T, Yoshida N, Nyumura I, Toya K, Hayashi T, et al. Gender differences in the association between HDL cholesterol and the progression of diabetic kidney disease in type 2 diabetic patients. Nephrol Dial Transplant. 2012;27:1070–5. https://doi.org/10.1093/ndt/gfr417.

25. Sathishkumar C, Prabu P, Mohan V, Balasubramanyam M. Linking a role of lncRNAs (long noncoding RNAs) with insulin resistance, accelerated senescence, and inflammation in patients with type 2 diabetes. Hum Genomics. 2018;12:41. https://doi.org/10.1186/s40246-018-0173-3.

26. Yu B, Qi Y, Li R, Shi Q, Satpathy AT, Chang HY. B cell-specific XIST complex enforces X-inactivation and restrains atypical B cells. Cell. 2021;184:1790-1803.e17. https://doi.org/10.1016/j.cell.2021.02.015.

27. Tang SCW, Yiu WH. Innate immunity in diabetic kidney disease. Nat Rev Nephrol. 2020;16:206–22. https://doi.org/10.1038/s41581-019-0234-4.

28. Long B, Wan Y, Zhang S, Lv L. LncRNA XIST protects podocyte from high glucose-induced cell injury in diabetic nephropathy by sponging miR-30 and regulating AVEN expression. Arch Physiol

Biochem. 2023;129:610–7. https://doi.org/10.1080/13813455.2020.1854307.

29. Li G, Zhang J, Liu D, Wei Q, Wang H, Lv Y, et al. Identification of hub genes and potential ceRNA networks of diabetic nephropathy by weighted gene co-expression network analysis. Front Genet. 2021;12:767654. https://doi.org/10.3389/fgene.2021.767654.

30. Shenoda B, Tian Y, Alexander G, Aradillas-Lopez E, Schwartzman R, Ajit S. miR-34a-mediated regulation of XIST in female cells under inflammation. J Pain Res. 2018;11:935–45. https://doi.org/10.2147/JPR.S159458.

31. Tang M, Berg A, Rhee EP, Allegretti AS, Nigwekar S, Karumanchi SA, et al. The impact of carbamylation and anemia on HbA1c's association with renal outcomes in patients with diabetes and chronic kidney disease. Diabetes Care. 2023;46:130–7. https://doi.org/10.2337/dc22-1399.

32. Jiang W, Wang J, Shen X, Lu W, Wang Y, Li W, et al. Establishment and validation of a risk prediction model for early diabetic kidney disease based on a systematic review and meta-analysis of 20 cohorts. Diabetes Care. 2020;43:925–33. https://doi.org/10.2337/dc19-1897.

33. Zhu J, Kong F, Xing L, Jin Z, Li Z. Prognostic and clinicopathological value of long noncoding RNA XIST in cancer. Clin Chim Acta. 2018;479:43–7. https://doi.org/10.1016/j.cca.2018.01.005.