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RESEARCH ARTICLE

The potential of N-glycosylation profiles as biomarkers for monitoring the progression of Type II diabetes mellitus towards diabetic kidney disease

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Abstract

Background On a global scale, type II diabetes mellitus (T2DM) remain a major health problem and it is the driver for chronic kidney disease (CKD). Despite this association, we still do not have sufficient biomarkers to anticipate better outcomes. N-glycosylation profiles are robust biomarkers and can be used for early monitoring of the progression of T2DM towards CKD.

Methods In this cross-sectional study, we recruited 241 T2DM patients from January to May 2016. Demographic and anthropometric data were collected, following which fasting blood samples were collected for clinical analyses. Renal function decline was determined by estimation of glomerular filtration rate (eGFR) and N-glycosylation profiles were analysed by Ultra-performance liquid chromatography (UPLC).

Results The prevalence of undiagnosed CKD was 31.53%. Compared to men, women had a statistically significantly higher HbA1c ($p = 0.031$), TG ($p = 0.015$), HDL-c ($p < 0.0001$), creatinine (<0.0001), urea ($p < 0.028$) and uric acid ($p < 0.0001$). T2DM patients with undiagnosed CKD had higher serum creatinine (145.75 ± 50.83 vs 88.59 ± 19.46, $p < 0.0001$), higher uric acid (361.10 ± 115.37 vs 294.54 ± 97.75; $p < 0.0001$) and higher urea (5.17 ± 2.35 vs 3.58 ± 1.19; $p < 0.0001$). After performing logistic regression and adjusting for age, sex and BMI, three N-glycan peaks [OR (95%CI): (GP12 (0.05(0.01–0.54), $p = 0.013$)); GP16 (0.61(0.43–0.87), $p = 0.006$)); GP22 (0.60(0.39–0.92), $p = 0.018$)) were associated with renal function.

Conclusion There was an increased prevalence of undiagnosed CKD among T2DM patients. This prevalence is the consequence of uncontrolled modifiable risk factors, which collectively may lead to end stage renal disease (ESRD). Although, the identified N-glycans could not adequately predict incident CKD, our investigation indicates the potential role of N-glycosylation in renal function and that their inclusion may improve risk stratification for CKD.

Keywords Chronic kidney disease · Diabetes mellitus · Hypertension · Metabolic risk factors

Introduction

Chronic kidney disease (CKD) is a life-threatening condition responsible for many morbidities and mortalities worldwide [1–4]. According to a systematic analysis on the global burden of diseases, it is the 18th cause of premature deaths [5]. However, apart from premature deaths being the worst outcome, those who survive it are prone to lifelong consequences including frequent hospitalisation [6, 7], cognitive impairment [8], poor quality of life [9] and overwhelming healthcare costs [7].

CKD is established based on kidney damage or decline in function over a 3-month period [10]. For many years, both the National Institute for Health Excellence (NICE) [11] and the Kidney Outcomes Quality Initiative (KDOQI) [12] have recognised the estimates of glomerular filtration rate (eGFR)

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as a proxy measure of kidney function. Usually, eGFR estimates are evaluated using different equations or formulae such as the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) [13], Modification of Diet in Renal Disease (MDRD) [14], Cockcroft-Gault [15] and cystatin based formula [16]. Based on the eGFR estimates derived from these equations, CKD is classified as follows: Stage 1 (> 90 mL/min/1.73 m^2); stage 2 (60–89 mL/min/1.73 m^2); stage 3 (30–59 mL/min/1.73 m^2); stage 4 (15–29 mL/min/1.73 m^2) and stage 5 (< 15 mL/min/1.73 m^2) [1, 10, 17]. These evaluations have thus far, enabled disease labelling, risk stratification, intervention, drug dosing and prognostication [18].

The main risk factors for CKD are proteinuria [19], glomerulonephritis [20], nephrolithiasis [21], hypertension [22] and type II diabetes (T2DM) but amongst them, T2DM has been suggested to be the main driver of CKD [18]. Indeed, the projected trajectory is nearly half of all patients with T2DM may suffer from kidney dysfunction at some stage in their life [23]. This is because T2DM leads to oxidative stress and chronic inflammation that in turn fuels many abnormalities including endothelial dysfunction, mesangial-cell contraction, glomerular fibrosis and mesangial expansion. When untreated, these complications then advance into end-stage renal disease (ESRD) [18, 24, 25]. At ESRD, patients can only survive under kidney replacement therapy (i.e. dialysis or kidney transplantation).

Ghana, like many other countries in sub-Saharan Africa (SSA) and worldwide, has large numbers of people with T2DM and if the current trend persists, it will not be surprising to realise an explosion of T2DM and its associated CKD complications in the future [26, 27]. Early recognition of risk factors will promote better treatment and improve survival [26, 27]. However, CKD awareness among T2DM sufferers is generally low in this region [28]. In part, this can be attributed to limited health care resources which in turn, has slowed the commitment to research, health screening and surveillance. In fact, only a few studies have reported the prevalence of CKD in Ghana [28, 29], and these studies were restricted to only some risk factors and failed to adequately explore other potential risk factors. Moreover, several studies have indicated the role of genetic and environmental factors to the pathophysiology of CKD but the contribution of epigenetic factors or posttranslational modifications is scarcely documented.

N-glycosylation is a widely recognised process where complex oligonucleotides (glycans) are pinned to asparagine residues of proteins [30]. When bound to proteins, glycans affect their trafficking, turnover, and other physiochemical properties including solubility and stability [31–34]. Glycans are stable in normal conditions but are aberrant in abnormal or environmental perturbations [35]. Thus, glyco-profiling is a unique approach to deciphering the complexities in both healthy and pathophysiological conditions. For example, N-glycans are defective in inflammatory diseases such as rheumatoid arthritis [36] andsystematic lupus erythematosus (SLE) [37]. However, the role of N-glycans in renal function remains scarce.

Therefore, in a hospital based clinical study, we have used the CDK-EPI equation in conjunction with multiple metabolic risk factors to determine CKD risk among T2DM patients in Ghana. In addition, this study profiles N-glycans in T2DM with or without CKD.

Study design and methods

This cross-sectional study was conducted from January 2016 to May 2016. Of the 260 T2DM participants recruited for the study, analyses were performed on 241 participants because of missing biochemical data. Recruitment for the study was based on a purposive sampling approach where T2DM patients who reported at the Diabetic Centre, Komfo Anokye Teaching Hospital (KATH) were invited to participate. KATH is a referral hospital with over 1200 beds with not less than 100 diabetic/hypertensive patients attending the hospital every week [38].

Inclusion and exclusion criteria

This study was conducted in consultation with clinicians and qualified health professionals. T2DM was diagnosed by clinicians at KATH and it was established based on the international classification of disease (ICD-10-CM Diagnosis Code E11.9). Each patient was carefully examined and their medical records thoroughly reviewed. As a result, we excluded all those individuals who were suffering from cancer, arthritis, infectious diseases, cardiovascular disease, thyroid disorders, pituitary disorders and adrenal disorders. The study did not include pregnant and lactating mothers. Since T2DM is largely a disease of ageing, the study recruited only individuals who were 30 years and above. Furthermore, to limit potential confounding and the likelihood of recruiting participants with type 1 diabetes, we excluded participants on insulin injections.

Demographic and anthropometric examination

Previous and current history of disease, family history of T2DM and hypertension were collected. In addition, information on health status and history of smoking and alcohol consumption as well as current physical activity were also collected using a structured questionnaire. Weight (kg) and height (cm) were measured with a standard stadiometer (SECA, Hamburg, Germany). These data were used to determine the body mass index (BMI); calculated as BMI = weight (kg)/ [height (m)]^2. Waist and hip circumference were measured in cm using a tape measure and waist-to-hip ratio (WHR) was calculated as WHR = waist (cm)/hip (cm).

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using a standard sphygmomanometer (Omron HEM711DLX, UK).

Clinical data

After an overnight fast, blood samples were collected from each participant. Samples were collected into tubes containing ethylenediaminetetraacetic acid (EDTA) anticoagulant, gel separator and fluoride oxalate. Samples were centrifuged (Mendelssohn, USA) at 3000 g at 4 °C for 10 mins (centrifuge Eppendorf 5702R, Germany) to separate the whole blood. Plasma glucose levels were measured using a glucose oxidase method (Roche Diagnostics, COBAS INTEGRA 400 Plus, USA). Serum levels of total cholesterol (TC), triglycerides (TG) and high density lipoprotein (HDL) cholesterol were determined enzymatically with commercially available reagents (Elitech Clinical Systems Elitech Group; Roche Diagnostics, COBAS INTEGRA 400 Plus, USA). Serum lipid levels were quantified based on the National Cholesterol Education Program, Adult Treatment Panel (NCEP-ATP) III guidelines. Low-density lipoprotein (LDL) cholesterol was calculated by the Friedewald formula using the equation LDL = TC−[HDL + TG/5] [38]. In addition, serum creatinine, uric acid and urea were measured using commercially available reagents on the automated chemistry analyser (Elitech Clinical Systems Elitech Group; Roche Diagnostics, COBAS INTEGRA 400 Plus, USA). Quality controls were applied throughout all these assays.

Non-HDL was calculated as Non-HDL = total cholesterol-HDL. We then calculated eGFR using the CKD-EPI equation [39];

$$\mathrm{GFR} = 141 \times \min\left(S_{cr}/\kappa, 1\right)^{\alpha} \times \max\left(S_{cr}/\kappa, 1\right)^{-1.209} \times 0.993^{\mathrm{Age}} \times 1.018\ [\text{if female}] \times 1.159\ [\text{if black}]$$

N-glycan release and labelling

The release of glycans from glycoproteins on a 96-well plate is a unique method for *N*-glycan analysis. Prior to the analyses, samples were randomised on multiple plates to avoid bias, experimental errors and make data comparable. Briefly, plasma samples (10 μl) were aliquoted in a 96-well plate and denatured with 20 μl 2% (*w*/*v*) sodium dodecyl sulphate (SDS; Invitrogen, USA), incubated at 65 °C for 10 mins and cooled to room temperature for 30 mins. Following this, 10 μl of 4% (*v*/v) Igepal CA-630 (Sigma-Aldrich, USA) was added and mixed. N-glycans were then detached from glycoproteins after the addition of 1.2 U of peptide *N*-glycosidase F (PNGase F; Promega, USA) in 10 μl 5x PBS and 18 h incubation at 37 °C. The released N-glycans were labelled with 2-amino benzamide (2-AB, Sigma-Aldrich) solution but prior to this, a labelling mixture of 2-AB (19.2 mg/ml) and 2-picoline borane (2-PB, 44.8 mg/ml; Sigma Aldrich) in dimethylsulfoxide (DMSO, Sigma Aldrich) and glacial acetic acid (Merck, Germany) mixture (70:30 *v*/v) was prepared. Subsequently, 25 μl of the labelling mixture was added to each glycan sample in the plate, sealed, shaken and incubated for 2 h at 65 °C. Shortly thereafter, excess label and reducing agents in samples were removed by hydrophilic interaction liquid chromatography solid phase extraction (HILIC-SPE) on a hydrophilic 0.2 μm AcroPrep GHP filter plate (Pall Corporation, USA) using vacuum manifold (Millipore Corporation, USA). Samples were first cooled for 30 mins after which 700 μl of cold (4 °C) acetonitrile (ACN) was added to each sample. Prior to loading samples in the wells, the wells of GHP filter plate were washed with 200 μl of 70% (*v*/v) ethanol, 200 μl of ultrapure water and equilibrated using 200 μl of cold (4 °C) 96% (v/v) ACN. Samples were then loaded into the equilibrated GHP filter plate, incubated briefly and washed with 5 × 200 μl of cold (4 °C) 96% ACN. Two times 90 μl of ultrapure water was added whilst shaking, followed by centrifugation at 164 g for 5 mins (centrifuge 5804, rotor A-2-DWP, Eppendorf, Germany) in each step, to elute N-glycans from the GHP filter plate. Eluted glycans in a total volume of 180 μl were stored at −20 °C until further analysis.

Ultra-performance liquid chromatography

Separation of fluorescently labelled N-glycans was achieved on a HILIC on an Acquity UPLC instrument (Waters, USA) which comprised of a sample manager, quaternary solvent manager and fluorescence (FLR) detector set with excitation wavelength of 250 nm and an emission wavelength of 428 nm. Using a Waters BEH Glycan chromatography column with dimensions 150 × 2.1 mm i.d. and 1.7 μm BEH particles, labelled N-glycans were then separated in the presence of 100 mM ammonium formate as solvent A (pH 4.4) and ACN as solvent B. A linear gradient of 30–47% solvent A at a flow rate of 0.56 ml/min was applied while separation temperature was maintained at 25 °C and sample temperature at 10 °C. Calibration of the system was done using external standards of hydrolysed and 2-AB labelled glucose oligomers following which the data was automatically processed and integrated. Automatically integrated chromatograms were manually corrected. With the same intervals of integration, the total plasma glycome was separated into 39 glycan peaks and the relative abundance of each N-glycan peak expressed as a percentage of the total integrated area. From these 39 directly measured N-glycan peaks, an additional 21 derived glycan traits were calculated. These are high branching (HB), low branching (LB), neutral or no sialylation (S0), monosialylated (S1), disialylated (S2), trisialylated (S3), tetrasialylated (S4), agalactosylated (G0), monogalactosylated

(G1), digalactosylated (G2), trigalactosylated (G3), tetragalactosylated (G4), antennary fucosylated (FUC_A), core fucosylated (FUC_C), biantennary (BA), biantennary agalactosylated (A2), biantennary galactosylated (A2G), monosialylated biantennary (BAMS), disialylated biantennary (BADS), triantennary (TRIA) and tetraantennary (TA) traits. **(**Supplementary Table 1**)** [40].

Statistical analysis

All continuous data was recorded as mean ± standard deviation and percentages for categorical variables. Association between CKD and metabolic risk factors were performed using linear regression and multiple logistic regression models. Odds ratios (ORs) at 95% confidence intervals (95% CI) were recorded for logistic regression analysis. Normalisation and batch correction on the UPLC data were performed in order to control for non-biological variability. Normality distribution of data was checked by the Kolmogov Smirnoff test as well as visualisation of QQ plots. However, because of the skewed nature of N-glycan data, interquartile ranges (IQRs) were used to describe the data. Depending on the normality distribution, between groups comparisons for continuous variables were performed using Mann-Whitney U-tests or Student-t tests and intergroup comparisons of categorical variables were performed using Chi-square tests. The Spearman correlation method was used to calculate the correlation coefficients (rho) between biochemical parameters and N-glycans. A $p < 0.05$ was considered statistically significant.

Results

All participants were physically examined, and their biochemical and clinical data collected. The mean ages of participants were 58.95 ± 10.98 years and 57.04 ± 10.77 years for men and women, respectively. There was female dominance in this study and the ratio of males to females was 99/142. This ratio was not surprising since the registered data from KATH shows that there are more women with T2DM than men. Also, compared to men, women were more willing and consented to participate in the study. Albeit, the age ranges for men and women were similar.

After stratifying by gender, women were generally obese compared to men when BMI (23.9% vs. 10.4%; $p = 0.001$) was used as an obesity index. A higher proportion of men compared to women had a history of smoking (28.3% vs. 3.5%; $p < 0.001$) and alcohol consumption (58.6% vs. 31.7%; $p < 0.0001$). There was a significantly higher HbA1c ($p = 0.031$), TC ($p = 0.015$), HDL-c ($p < 0.0001$), creatinine (<0.0001), urea ($p < 0.028$) and uric acid levels ($p < 0.0001$) among women compared to men. Levels of SBP, DBP, FPG, TG, VLDL-c, eGFR and coronary risk ratio among females were not significantly different between males and females ($p > 0.05$). Generally, men engaged in more moderate physical activity compared to women ($p < 0.035$) (Table 1).

CKD was more prevalent in females than males. Age ($p < 0.0001$), education ($p = 0.023$), occupation ($p < 0.035$) and physical activity ($p = 0.038$) were significantly associated with CKD. Meanwhile, being elderly [aOR = 28.86 (3.26–225.9); $p = 0.0002$], retired [aOR = 3.21 (1.48–6.94) $p = 0.0036$)], or primarily sedentary [aOR = 2.28 (1.29–4.01); $p = 0.005$], were significant independent risk factors for CKD after adjusting for age and gender. Surprisingly, T2DM patients who had significant CKD (eGFR <60 ml/min/1.73m^2) had lower plasma glycaemic levels (HbA1c 7.88% vs 8.45%; $p< 0.049$) but had higher serum creatinine (145.75 ± 50.83 vs 88.59 ± 19.46, $p < 0.0001$), high uric acid (361.10 ± 115.37 vs 294.54 ± 97.75, $p < 0.0001$) and high urea (5.17 ± 2.35 vs 3.58 ± 1.19; $p < 0.0001$). However, there were no significant differences between the mean lipid profile and FPG among participants with CKD compared to those without CKD ($p > 0.05$) (Table 2).

After adjusting for age and gender, high SBP [aOR = 1.81 (1.08–3.26); $p = 0.024$)], HbA1c [aOR = 0.5 (1.28–0.89) $p = 0.017$], and high TG [aOR = 2.38 (1.21–4.70); $p = 0.024$)] were significant independent risk factors for CKD (Table 3).

In the bivariate analysis, there was a significant negative relationship between urea ($\beta = -2.62$; $p < 0.0001$) and uric acid ($\beta = -0.02$; $p = 0.0161$) with CKD. The r^2 indicated that the cause of CKD was influenced by 30.6% of urea and 4.2% of uric acid. Inverse and non-significant relationships were observed between CKD and age, SBP, FPG, TC, TG, HDL-c, non-HDL-c, LDL-c, coronary risk ratio, and VLDL-c (Table 4).

In the bivariate analysis, it was shown that urea and uric acids were independently associated with CKD. After including uric acid, TC, SBP, DBP, Urea, FPG, BMI, TG, age, HDL-c, HbA1c, coronary risk, VLDL-c and LDL-c in the multivariate linear regression model, they influenced CKD by 55.7% ($r^2 = 0.557$). When the significant predictors, urea and uric acid were included in the model, CKD was influenced by 37.21% ($r^2 = 0.3721$). The predictive equation for this model was CKD =72.07–3.08*Urea-7.30*Uric acid (Table 5).

Differential plasma N-glycan patterns in T2DM with CKD and those without CKD

The IQRsof all measured N-glycans are shown in Table 6 and there were distinct levels of N-glycans between T2DM with CKD and those without it. Generally, GP10 (FA2G2), GP16 (FA2G2S[6]1) and GP22 (FA2G2S[3,6]2) were higher among in T2DM without CKD compared to T2DM with CKD. In contrast, GP14 (A2G2S[6]1, T2DM with CKD than those without it ($p < 0.05$). However, in

Table 1 Characteristics of study participants

Characteristics	Total	Men ($n = 99$)	Women ($n = 142$)	p value
Age (years)	57.99 ± 10.88	58.95 ± 10.98	57.04 ± 10.77	0.18
Body mass index (kg/m^2)				
Underweight	9(3.8)	5(5.1)	4(2.8)	**0.015**
Normal weight	107(44.6)	53(54.1)	54(38.0)	
Overweight	80(33.3)	30(30.6)	50(35.2)	
Obese	44(18.3)	10(10.4)	34(23.9)	
Education				**0.0001**
Tertiary	36(14.9)	21(21.2)	15(10.6)	
Senior high school	57(23.7)	33(33.3)	24(16.9)	
Junior high school	78(32.4)	30(30.3)	48(33.8)	
Lower primary	28(11.6)	8(8.1)	20(14.1)	
No formal education	42(17.4)	7(7.1)	35(24.6)	
Marital status				
Married	164(68.0)	91(91.9)	73(51.4)	**0.0001**
Never married	4(1.7)	1(1.0)	3(2.1)	
Divorced	25(10.4)	5(5.0)	20(14.1)	
Widowed	48(19.9)	2(2.0)	46(32.4)	
Occupation				
Employed	133(55.2)	67(67.7)	66(46.5)	**0.0001**
Retired	35(14.5)	18(18.2)	17(12.0)	
Unemployed	51(21.1)	8(8.1)	43(30.3)	
Informal employment	22(9.1)	6(6.1)	16(11.3)	
Systolic blood pressure (mmHg)	139.60 ± 24.32	140.73 ± 24.01	138.48 ± 24.64	0.483
Diastolic blood pressure (mmHg)	81.61 ± 13.75	81.74 ± 13.75	81.47 ± 13.74	0.878
Fasting plasma glucose (mmol/l)	9.11 ± 4.42	8.75 ± 4.51	9.48 ± 4.34	0.213
Glycated haemoglobin (%)	8.22 ± 2.06	7.93 ± 1.97	8.51 ± 2.15	**0.031**
TC (mmol/l)	4.69 ± 1.22	4.49 ± 1.09	4.89 ± 1.36	**0.015**
TG (mmol/l)	1.26 ± 0.56	1.21 ± 0.55	1.32 ± 0.57	0.13
HDL-c (mmol/l)	1.34 ± 0.31	1.24 ± 0.28	1.44 ± 0.33	**0.0001**
NonHDL-c (mmol/l)	3.35 ± 1.19	3.25 ± 1.03	3.45 ± 1.36	0.208
VLDL-c (mmol/l)	2.78 ± 1.13	2.70 ± 0.99	2.86 ± 1.27	0.145
LDL-c (mmol/l)	0.58 ± 0.26	0.55 ± 0.25	0.60 ± 0.26	0.301
Coronary risk	4.98 ± 1.48	5.09 ± 1.33	4.88 ± 1.64	0.286
Creatinine (mmol/l)	108.42 ± 40.2	119.81 ± 38.64	97.03 ± 41.76	**0.0001**
Urea (mmol/l)	4.13 ± 1.80	4.38 ± 1.95	3.87 ± 1.65	**0.028**
Uric acid (mmol/l)	324.24 ± 96.61	374.52 ± 100.73	273.95 ± 92.49	**0.0001**
eGFR (per mL/min/1.73m^2)	73.35 ± 25.15	73.04 ± 24.96	73.66 ± 25.33	0.852
Family history and activity				
Diabetes family history (yes)	184(76.3)	68(68.7)	116(81.7)	0.053
Smoking (yes)	33(13.7)	28(28.3)	5(3.5)	**0.0001**
Drinking (Yes)	103(42.7)	58(58.6)	45(31.7)	**0.0001**
Physical activity				
Primarily sedentary	80(32.8)	32(31.7)	48(33.8)	**0.035**
Moderate activity	161(66.8)	67(67.7)	94(66.2)	

Data is expressed as mean ± standard deviation or (n %). Statistically significant differences ($p < 0.05$) are bold

Supplementary Table 2, there were no statistical significance differences between N-glycan traits in T2DM + CKD and those without CKD.

After performing logistic regression, GP6 (FA2[6]BG1), GP 7 (M6D1-D2), GP10 (FA2G2), GP14 (A2G2S[6]1), GP16 (FA2G2S[6]1) and GP22

Table 2 Characteristics of study participants with or without CKD risk

Variables	Total	CKD	No CKD	$\times^2$	*p*	aOR (95%CI)	*p*
Gender				0.38	0.315		
Male	99(41.1)	33(44.0)	66(39.8)			1#	
Female	142(58.9)	42(56.0)	100(60.2)			0.84(0.48–1.45)	0.573
Age (years)				40.53	**0.0001**		
31–40	14 (5.8)	1(0.0)	13 (8.4)			1#	
41–50	49(20.3)	7(9.3)	42(25.3)			2.16 (0.24–19.29)	0.671
51–60	81(33.6)	18(24.0)	63(38.0)			3.71 (0.45–30.36)	0.288
61–70	68(28.2)	30(40.0)	38(22.9)			10.26(1.27–82.98)	**0.013**
71–80	29(12.0)	20(26.7)	9(5.4)			28.86(3.26–225.9)	**0.0002**
Education				11.32	**0.023**		
Tertiary	36(14.9)	11(14.7)	25(15.1)			1.0#	
Senior high school	57(23.7)	14(18.7)	43(25.9)			0.74(0.29–1.88)	0.632
Junior high school	78(32.4)	28(37.3)	50(30.1)			1.27(0.54–2.96)	0.673
Lower primary	28(11.6)	3(4.0)	25(15.1)			0.27(0.06–1.09)	0.072
No formal education	42(17.4)	19(25.3)	23(13.9)			1.88(0.74–4.77)	0.244
Marital Status				2.2	0.699		
Married	164(68.0)	47(62.7)	117(70.5)			1#	
Never married	4(1.7)	1(1.3)	3(1.8)			0.82(0.08–8.18)	0.998
Divorced/separated	25(10.4)	8(10.7)	17(10.2)			1.17(0.47–2.89)	0.814
Widowed	48(19.9)	19(25.3)	29(17.5)			1.63(0.83–3.19)	0.159
Occupation				10.35	**0.035**		
Employed	133(55.2)	33(44.0)	100(60.2)			1#	
Retired	35(14.5)	18(24.0)	17(10.2)			3.21(1.48–6.94)	**0.0036**
Unemployed	51(21.1)	17(22.7)	34(20.4)			1.51(0.75–3.06)	0.269
Informal employment	22(9.1)	7(9.3)	15(9.0)			1.41(0.53–3.76)	0.599
Physical activity				8.4	**0.038**		
Primarily sedentary	79(32.8)	35(45.9)	45(27.1)			2.28(1.29–4.01)	**0.0051**
Sedentary with activity	161(66.8)	41(54.7)	120(72.3)			1#	
Biochemical data							
FPG (mmol/l)	9.05 ± 4.49	8.71 ± 4.72	9.39 ± 4.27				0.27
HbA1c (%)	8.16 ± 2.08	7.88 ± 2.07	8.45 ± 2.08				0.049
TC (mmol/l)	4.72 ± 1.28	4.71 ± 1.29	4.73 ± 1.27				0.94
TG (mmol/l)	1.29 ± 0.55	1.37 ± 0.54	1.22 ± 0.57				0.71
HDL-c (mmol/l)	1.34 ± 0.33	1.33 ± 0.37	1.36 ± 0.30				0.612
NonHDL-c (mmol/l)	3.37 ± 1.25	3.37 ± 1.27	3.37 ± 1.23				0.979
LDL-c (mmol/l)	2.78 ± 1.17	2.75 ± 1.18	2.80 ± 1.16				0.74
Creatinine (μmol/l)	117.17 ± 35.14	145.75 ± 50.83	88.59 ± 19.46				**<0.0001**
Urea (mmol/l)	4.38 ± 1.77	5.17 ± 2.35	3.58 ± 1.19				**<0.0001**
Uric acid (μmol/l)	327.82 ± 106.56	361.10 ± 115.37	294.54 ± 97.75				**<0.0001**
eGFR (per mL/min/1.73 m2)	66.02 ± 15.39	46.48 ± 11.19	85.57 ± 19.60				**<0.0001**

aOR: adjusted odds ratio, CI confidence interval. Multivariate regression model was adjusted for age and gender; #: reference, Statistically significant differences ($p < 0.05$) are bold

(FA2G2S[3,6]2) were significant in the crude (unadjusted) models ($p < 0.05$) (Table 7). However, after adjusting for Age, gender and BMI, only GP 12, GP16 and GP 22 were significant. There were no significant associations in the derived traits after performing logistic regression (Supplementary Table 3).

Table 3 Association between CKD and metabolic risk factors

Variables	Total (n %)	CKD	No CKD	x^2	*p* value	aOR (95%CI)	*p* value
Body mass index							
Underweight	9(3.8)	6(8.0)	3(1.8)	8.47	**0.037**	4.48(1.06–19.04)	0.059
Normal weight	107(44.6)	33(44.0)	74(44.8)			1.0#	
Overweight	80(33.3)	19(25.3)	61(37.0)			0.69(0.36–1.34)	0.324
Obese	44(18.3)	17(22.7)	27(16.4)			1.41(0.67–2.93)	0.446
Systolic blood pressure				5.1	**0.017**		
Normal systolicBP	132(54.8)	33(44.0)	99(59.6)				1.0#
High systolicBP	109(45.2)	42(56.0)	67(40.4)			1.81(1.08–3.26)	**0.024**
Diastolic blood pressure							
Normal diastolicBP	177(74.1)	53(72.6)	124(74.7)	0.11	0.43	1.0#	0.74
High diastolicBP	62(25.9)	20(27.4)	42(25.3)			1.11(0.59–2.08)	
Fasting blood glucose				1.83	0.11		0.18
Normal	94(39.0)	34(45.3)	60(36.1)			1.0#	
High	147(61.0)	41(54.7)	106(63.9)			0.68 (0.39–1.18)	
Glycated Haemoglobin				5.6	**0.014**		**0.017**
Normal	74(30.8)	31(41.3)	43(26.1)			1.0#	
High	166(69.2)	44(58.7)	122(73.9)			0.5(0.28–0.89)	
Cholesterol				1.05	0.19		0.307
Normal	154(64.2)	51(68.9)	103(62.0)			1.0#	
High	86(35.8)	23(31.1)	63(38.0)			0.74(0.41–1.32)	
Triglycerides				6.46	**0.01**		**0.011**
Normal	199(82.6)	55(73.3)	144(86.7)			1.0#	
High	42(17.4)	20(26.7)	22(13.3)			2.38(1.21–4.70)	
HDL-c				2.66	0.074		0.104
Normal	189(78.4)	54(72.0)	135(81.3)			1.0#	
Low	52(21.6)	21(28.0)	31(18.7)			1.69(0.89–3.20)	
Non-HDL-c				0.29	0.345		0.592
Normal	117(48.8)	38(51.4)	79(47.6)			1.0#	
High	123(51.3)	36(48.6)	87(52.4)			0.86(0.49–1.49)	
LDL-c				0.43	0.304		
Normal	106(44.2)	35(47.3)	71(42.8)			1.0#	0.516
High	134(55.8)	39(52.7)	95(57.2)			0.83(0.48–1.44)	

aOR: adjusted odds ratio, CI confidence interval. Multivariate regression model was adjusted for age and gender; #: reference. Statistically significant differences ($p < 0.05$) are bold

Discussion

Prevalence and metabolic risk factors that characterise CKD among 241 T2DM patients were evaluated. Here, 31.53% of T2DM patients had CKD as defined by eGFR <60 ml/min/1.73 m^2. This prevalence rate is comparable to a previous study conducted among 280 T2DM patients in an urban community in Ghana [28] with the difference being 1.53%. As the determination of prevalence of CKD is based on the CKD-EPI Creatinine Equation in both studies, the slight increase in CKD in our study can be attributed to the proportion of the aged population in our study and the likelihood that these aged individuals may have been suffering from other clinical conditions (e.g. nephrosclerosis and undiagnosed ischaemic kidney disease) which we could not diagnose at the time of our investigation.

Surprisingly, compared with T2DM patients without CKD, those with CKD in our present study had lower HbA1c levels (Table 2), however, the clinical implications of this is negligible, given the difference was only 0.57% and a *p* value of 0.049. Besides, as shown in Table 2, the levels of FPG in T2DM with CKD and T2DM without CKD were not statistically significantly different. Other plausible explanation is the fewer number of T2DM participants with CKD compared to those without it.

Table 4 Bivariate relationship between predictors and CKD < 60 ml/min/1.73m^2

Factors	β(95%CI)	SE	r^2	*p* value
Age (years)	−0.20(−0.43 to 0.03)	0.11	0.041	0.0820
BMI (kg/m^2)	0.19(−0.04 to 0.42)	0.11	0.037	0.0968
SBP (mmHg)	−0.05(−0.28 to 0.19)	0.12	0.002	0.7004
DBP (mmHg)	0.14(−0.09 to 0.38)	0.12	0.021	0.2150
FPG (mmol/l)	−0.19(−0.75 to 0.36)	0.28	0.007	0.4831
HbA1c (%)	0.46(−0.80 to 1.71)	0.63	0.007	0.4720
Urea (mmol/l)	−2.62(−3.55 to −1.70)	0.46	0.306	**< 0.0001**
TC (mmol/l)	−0.64(−2.67 to 1.40)	1.02	0.005	0.5348
TG (mmol/l)	−3.81(−8.55 to 0.92)	2.38	0.034	0.1128
HDL-c (mmol/l)	−0.64(−7.61 to 6.33)	3.50	0.000	0.8551
Non-HDL-c (mmol/l)	−0.63(−2.69 to 1.43)	1.04	0.005	0.5452
LDL-c (mmol/l)	−0.43(−2.65 to 1.80)	1.12	0.002	0.7036
Coronary risk	−0.69(−2.24 to 0.85)	0.77	0.011	0.3752
VLDL-c (mmol/l)	−8.16(−18.68 to 2.36)	5.28	0.032	0.1263
Uric Acid (mmol/l)	−0.02(−0.04 to 0.00)	0.01	0.042	**0.0161**

The use of BMI as an obesity index has been criticised, hence the association between BMI and CKD has been conflicting in many studies. For example, whereas one study showed that the risk of developing advanced kidney malfunction is threefold higher in patients with BMI > 30, another study showed that the association between BMI and kidney dysfunction was insignificant [41, 42]. The present study agrees with the latter in that BMI was not an independent risk factor for CKD. Perhaps, complementing BMI with other fat indicators or measurements such as visceral fat and fat mass index (FMI) would have yielded a better association.

Consistent with other studies [43, 44], our findings show that T2DM patients with high systolic BP had increased odds for CKD (Table 3). Although it remains controversial whether elevated blood pressure is the cause or the consequence of kidney dysfunction, it has been suggested that elevated blood pressure promotes arterial stiffening and intimal thickening in the kidney parenchyma and subsequently results in glomerulosclerosis [45, 46]. Similarly, T2DM patients with high TG levels had increased odds for developing CKD. This confirms the findings by other studies [41, 47]. Although the exact mechanism is not fully understood, it has been suggested that the correlation between high TG and CKD is related to regulation of lipoprotein lipase activity during TG catabolism. This enzyme is regulated by two proteins: apolipoprotein C-II and apolipoprotein C-III that act antagonistically. That is, whilst apolipoprotein C-II activates lipoprotein lipase, apolipoprotein C-III inhibits it. During CKD, there is an increase in apolipoprotein C-III (i.e. decrease apolipoprotein C-II/C-III ratio) and this results in lipase inactivation and subsequent TG accumulation [48–50].

Table 5 Multivariate relationship between predictors and CKD < 60 ml/min/1.73m^2

Predictors	Standardized β	*p* value
Model 1		
Age (years)	−0.045	0.719
BMI (Kg/m^2)	0.148	0.167
SBP (mmHg)	−0.183	0.137
DBP (mmHg)	0.228	0.082
FPG (mmol/l)	−0.068	0.591
HbA1c (mmol/l)	0.113	0.383
Urea*(mmol/l)	−0.647	**<0.0001**
TC (mmol/l)	−0.678	0.869
TG (mmol/l)	5.061	0.139
HDL-c (mmol/l)	−0.038	0.973
Non-HDL-c (mmol/l)	0.598	0.877
LDL-c (mmol/l)	0.041	0.788
Coronary risk	0.14	0.685
VLDL-c (mmol/l)	−5.157	0.109
Uric Acid*(mmol/l)	−0.195	**0.014**
r^2	**0.557**	
Adjusted r^2	**0.441**	
(Constant)	**71.969**	
p value	**<0.0001**	
Model 2		
Urea (mmol/l)	−0.6536	**< 0.0001**
Uric Acid (mmol/l)	−0.2446	**0.0178**
r^2	0.372	
Adjusted r^2	**0.354**	
(Constant)	**72.069**	
p value	**<0.0001**	

Standardized β for predictors in the model 1: (Constant), Uric Acid, TC, DBP, SBP, urea, FPG, HbA1c, BMI, TG. Age, HDL-c, coronary risk, VLDL-c, LDL-c

Standardized β for best predictors in model 2: (Constant), urea, uric acid. Statistical significance ($p < 0.05$) are bold

*Significant predictors in model 1

Thus far, we have used routine biochemical markers to establish the presence of CKD amongst T2DM, however, it is worth investigating the potential role of post-translational modification in kidney function. From the results, it was evident that N-glycosylation was associated with age and gender as previously reported [51–59] and this is attributable to hormonal differences (Fig. 1). The results of this present study showed that core fucosylated N-glycans without bisection [GP 10 (FA2G2), GP 16 (FA2G2S[6]1) and GP22 (FA2G2S[3,6]2) was significantly higher in T2DM patients with normal renal function (Table 6). Our findings agree with that of Barrios et al. [59] who showed that core fucosylated glycans that lack bisecting GlcNAc was associated with decreased risk of CKD. Core fucosylated N-glycan is

Table 6 N-glycan traits in normal and CKD

		Normal		CKD			
N-glycan	Structure	Median(IQR)	Range	Median(IQR)	Range	W	*p*
GP1	FA2	6.36(2.79)	(5.02–7.81)	6.61(2.90)	(5.39–8.29)	18,846	0.260
GP2	FA2B	2.37(0.85)	(2.02–2.87)	2.47(0.72)	(2.08–2.81)	19,007	0.425
GP3	A2[6]BG1	0.08(0.05)	(0.07–0.12)	0.09(0.03)	(0.07–0.11)	8801	0.992
GP4	FA2[6]G1	4.39(1.20)	(3.98–5.19)	4.77(1.70)	(3.68–5.39)	8731	0.878
GP5	FA2[3]G1	1.83(0.64)	(1.57–2.21)	1.84(0.66)	(1.48–2.15)	8411	0.419
GP6	FA2[6]BG1	1.18(0.31)	(1.04–1.36)	1.31(0.57)	(1.00–1.58)	18,446	0.052
GP7	M6D1-D2	0.99(0.18)	(0.92–1.09)	0.95(0.23)	(0.83–1.07)	7897	0.063
GP8	A2G2	1.12(0.31)	(0.99–1.30)	1.10(0.37)	(0.97–1.34)	8650	0.750
GP9	A2BG2	0.09(0.04)	(0.09–0.12)	0.09(0.03)	(0.09–0.12)	8603	0.678
GP10	FA2G2	3.70(1.50)	(3.14–4.64)	3.46(1.39)	(2.89–4.28)	7688	**0.022***
GP11	FA2BG2	0.73(0.21)	(0.62–0.83)	0.71(0.19)	(0.62–0.81)	8677	0.792
GP12	A2[3]BG1S[3]1	1.00(0.16)	(0.93–1.09)	0.97(0.18)	(0.89–1.06)	8077	0.136
GP13	FA2[3]G1S[3]1	0.81(0.19)	(0.72–0.91)	0.81(0.32)	(0.66–0.99)	19,213	0.707
GP14	A2G2S[6]1	10.75(1.48)	(9.97–11.46)	11.19(1.64)	(10.46–12.11)	18,049	**0.006***
GP15	A2BG2S[6]1	0.38(0.11)	(0.34–0.45)	0.38(0.10)	(0.34–0.44)	8690	0.813
GP16	FA2G2S[6]1	5.82(1.35)	(5.16–6.52)	5.28(1.17)	(4.67–5.84)	7158	**0.001***
GP17	FA2BG2S[3]1	1.56(0.56)	(1.33–1.90)	1.58(0.48)	(1.32–1.81)	8780	0.958
GP18	A2G2S[3,6]2	3.40(0.76)	(3.01–3.77)	3.35(0.63)	(3.11–3.74)	19,338	0.904
GP19	M9	1.12(0.22)	(1.00–1.22)	1.08(0.22)	(0.97–1.20)	8136	0.171
GP20	A2G2S[3,6]2	25.47(3.68)	(23.55–27.23)	25.65(4.67)	(23.38–28.06)	19,094	0.536
GP21	A2BG2S[3,6]2	0.51(0.17)	(0.44–0.61)	0.51(0.17)	(0.44–0.61)	8780	0.958
GP22	FA2G2S[3,6]2	4.38(0.96)	(4.01–4.97)	4.12(1.17)	(3.76–4.93)	7531	**0.009***
GP23	FA2BG2S[3,6]2	1.89(0.68)	(1.61–2.29)	1.99(0.66)	(1.66–2.32)	19,036	0.460
GP24	A3G3S[3,6]2	1.73(0.62)	(1.41–2.03)	1.84(0.73)	(1.38–2.11)	19,217	0.713
GP25	A3BG3S[3,6]2	0.15(0.06)	(0.12–0.19)	0.16(0.06)	(0.14–0.19)	18,544	0.081
GP26	A3G3S[3,3]2	1.63(0.48)	(1.38–1.87)	1.68(0.59)	(1.36–1.95)	19,095	0.537
GP27	A3G3S[3,3,3]3	0.48(0.34)	(0.30–0.64)	0.50(0.37)	(0.34–0.72)	18,936	0.346
GP28	A3G3S[3,3,6]3	0.80(0.32)	(0.64–0.96)	0.82(0.29)	(0.63–0.92)	8612	0.692
GP29	FA3G3S[3,3,3]3	0.19(0.06)	(0.16–0.22)	0.18(0.05)	(0.16–0.21)	8498	0.529
GP30	A3G3S[3,3,6]3	5.89(2.01)	(4.86–6.87)	5.94(2.19)	(4.64–6.84)	8598	0.671
GP31	FA3G3S[3,3,6]3	0.52(0.29)	(0.42–0.72)	0.50(0.21)	(0.42–0.63)	8218	0.229
GP32	A3F1G3S[3,3,3]3	1.46(0.57)	(1.24–1.82)	1.56(0.55)	(1.25–1.80)	19,136	0.594
GP33	A4G4S[3,3,3]3	1.83(1.44)	(1.17–2.61)	2.03(1.51)	(1.34–2.85)	18,932	0.342
GP34	A4G4S[3,3,6]3	0.40(0.15)	(0.33–0.48)	0.41(0.10)	(0.34–0.45)	8706	0.838
GP35	A4F1G3S[3,3,3]3	0.26(0.15)	(0.19–0.35)	0.29(0.16)	(0.21–0.36)	18,959	0.371
GP36	A4G4S[3,3,3,3]4	0.44(0.11)	(0.40–0.51)	0.45(0.13)	(0.39–0.53)	19,149	0.612
GP37	A4G4S[3,3,3,3]4	0.49(0.21)	(0.39–0.60)	0.48(0.19)	(0.38–0.58)	8512	0.548
GP38	A4G4S[3,3,3,6]4	0.90(0.27)	(0.78–1.06)	0.91(0.24)	(0.77–1.01)	8632	0.722
GP39	A4F1G4S[3,3,3,6]4	0.51(0.25)	(0.41–0.67)	0.55(0.23)	(0.45–0.67)	18,916	0.325

Data presented as median interquartile range (IQR). W-Wilcoxon statistic**,** Tests of significance were two tailed (*$p < 0.05$) and are bold

an important molecule in notch signalling, growth factor receptor expression and adhesion molecule activity. Moreover, bisecting *N*-acetyl glucosamine on IgG facilitates antibody dependent cellular cytotoxicity (ADCC) or a pro-inflammatory state since the presence of bisecting *N*-acetyl glucosamine on N-glycans inhibits core fucosylation and indirectly promotes the binding of IgG molecules to Fcγ receptor III [54, 58]. Further, after performing a logistic regression and adjusting for covariates, CKD was associated with an increased levels of complex N-glycans (GP12, GP16 and GP22) (Table 7). These identified N-glycans have crucial role in kidney function. For example, GP12, a

Table 7 Logistic regression analysis of N-glycans crude in and adjusted models

	Crude				Age + Gender + BMI			
	B	S.E.	OR(95%CI)	*p*	B	S.E.	OR(95%CI)	*p*
GP1	0.064	0.047	1.07(0.97–1.17)	0.176	0.061	0.051	1.06(0.96–1.18)	0.232
GP2	0.241	0.213	1.27(0.84–1.93)	0.257	0.045	0.240	1.05(0.65–1.67)	0.851
GP3	−2.153	3.384	0.12(0.00–88.22)	0.525	−5.967	4.221	0.00(0.00–10.04)	0.157
GP4	−0.029	0.130	0.97(0.75–1.25)	0.821	0.021	0.147	1.02(0.77–1.36)	0.886
GP5	−0.167	0.287	0.85(0.48–1.48)	0.559	−0.204	0.323	0.82(0.43–1.54)	0.527
GP6	1.062	0.474	2.89(1.14–7.33)	**0.025***	0.751	0.518	2.12(0.77–5.85)	0.147
GP7	−1.851	0.908	0.16(0.03–0.93)	**0.041***	−1.826	0.960	0.16(0.03–1.06)	0.057
GP8	−0.217	0.428	0.81(0.35–1.86)	0.612	−0.501	0.533	0.61(0.21–1.72)	0.347
GP9	−3.519	3.145	0.03(0.00–14.07)	0.263	−4.957	3.629	0.01(0.00–8.64)	0.172
GP10	−0.375	0.150	0.69(0.51–0.92)	**0.012***	−0.291	0.183	0.75(0.52–1.07)	0.112
GP11	−0.203	0.785	0.82(0.18–3.80)	0.796	−0.249	0.845	0.78(0.15–4.09)	0.768
GP12	−2.043	1.028	0.13(0.02–0.97)	**0.047***	−2.915	1.175	0.05(0.01–0.54)	**0.013***
GP13	0.830	0.632	2.29(0.67–7.90)	0.189	0.336	0.728	1.40(0.34–5.82)	0.645
GP14	0.262	0.118	1.30(1.03–1.64)	**0.026***	0.132	0.130	1.14(0.88–1.47)	0.313
GP15	−0.811	0.795	0.44(0.09–2.11)	0.308	−1.039	0.880	0.35(0.06–1.99)	0.238
GP16	−0.491	0.154	0.61(0.45–0.83)	**0.001***	−0.494	0.180	0.61(0.43–0.87)	**0.006***
GP17	0.062	0.301	1.06(0.59–1.92)	0.836	−0.012	0.327	0.99(0.52–1.88)	0.971
GP18	0.071	0.260	1.07(0.65–1.79)	0.784	−0.100	0.296	0.91(0.51–1.62)	0.736
GP19	−1.445	0.894	0.24(0.04–1.36)	0.106	−1.410	1.014	0.24(0.03–1.78)	0.164
GP20	0.012	0.052	1.01(0.92–1.12)	0.816	0.011	0.058	1.01(0.90–1.13)	0.854
GP21	−0.612	1.062	0.54(0.07–4.35)	0.564	−1.227	1.145	0.29(0.03–2.77)	0.284
GP22	−0.493	0.196	0.61(0.42–0.90)	**0.012***	−0.514	0.218	0.60(0.39–0.92)	**0.018***
GP23	0.189	0.204	1.21(0.81–1.80)	0.353	−0.019	0.233	0.98(0.62–1.55)	0.935
GP24	0.099	0.290	1.10(0.63–1.95)	0.733	0.310	0.323	1.36(0.73–2.57)	0.336
GP25	5.190	3.072	179.53(0.44–746.43)	0.091	3.261	3.489	26.07(0.03–28.75)	0.350
GP26	0.209	0.368	1.23(0.60–2.53)	0.571	0.656	0.447	1.93(0.80–4.63)	0.142
GP27	0.531	0.521	1.70(0.61–4.73)	0.308	0.160	0.596	1.17(0.37–3.78)	0.788
GP28	−0.349	0.650	0.71(0.20–2.52)	0.591	0.110	0.721	1.12(0.27–4.58)	0.879
GP29	−1.254	3.278	0.29(0.00–176.23)	0.702	−2.296	3.580	0.10(0.00–112.27)	0.521
GP30	−0.051	0.090	0.95(0.80–1.13)	0.569	0.061	0.104	1.06(0.87–1.30)	0.560
GP31	−0.897	0.738	0.41(0.10–1.73)	0.224	−0.333	0.875	0.72(0.13–3.99)	0.704
GP32	0.155	0.336	1.17(0.61–2.25)	0.645	0.657	0.419	1.93(0.85–4.39)	0.118
GP33	0.135	0.130	1.14(0.89–1.48)	0.299	0.061	0.148	1.06(0.80–1.42)	0.681
GP34	−0.826	1.408	0.44(0.03–6.91)	0.557	0.314	1.790	1.37(0.04–45.73)	0.861
GP35	0.960	1.091	2.61(0.31–22.16)	0.379	0.686	1.231	1.99(0.18–22.18)	0.577
GP36	0.736	1.676	2.09(0.08–55.80)	0.660	1.495	1.971	4.46(0.09–212.20)	0.448
GP37	−0.796	0.868	0.45(0.08–2.47)	0.359	−0.198	0.966	0.82(0.12–5.45)	0.838
GP38	−0.396	0.671	0.67(0.18–2.51)	0.555	0.110	0.776	1.12(0.24–5.11)	0.888
GP39	0.241	0.632	1.27(0.37–4.39)	0.703	−0.113	0.715	0.89(0.22–3.63)	0.875

OR: odds ratio, logistic regression model was adjusted for age, gender and BMI. Two tailed *$p < 0.05$ is significant and are bold

monogalactosylated, monosialylated biantennary N-glycan with bisecting GlcNAc, has previously been associated with increased HbA1c levels among CKD patients with type I diabetes mellitus (T1DM). Also, GP16 has been associated with albumin-to-creatinine ratio and eGFR slope in T1DM [55]. Plausible explanation in the context of renal function are that increased plasma glycemia triggers the flux of glucose in the hexosamine pathway. In turn, this leads to the production of uridine-diphosphate N-acetylgucosamine, which acts as a substrate in N-glycosylation and increase the production of complex N-glycans. In parallel, this events indirectly upregulate epidermal growth receptor; a molecule which as

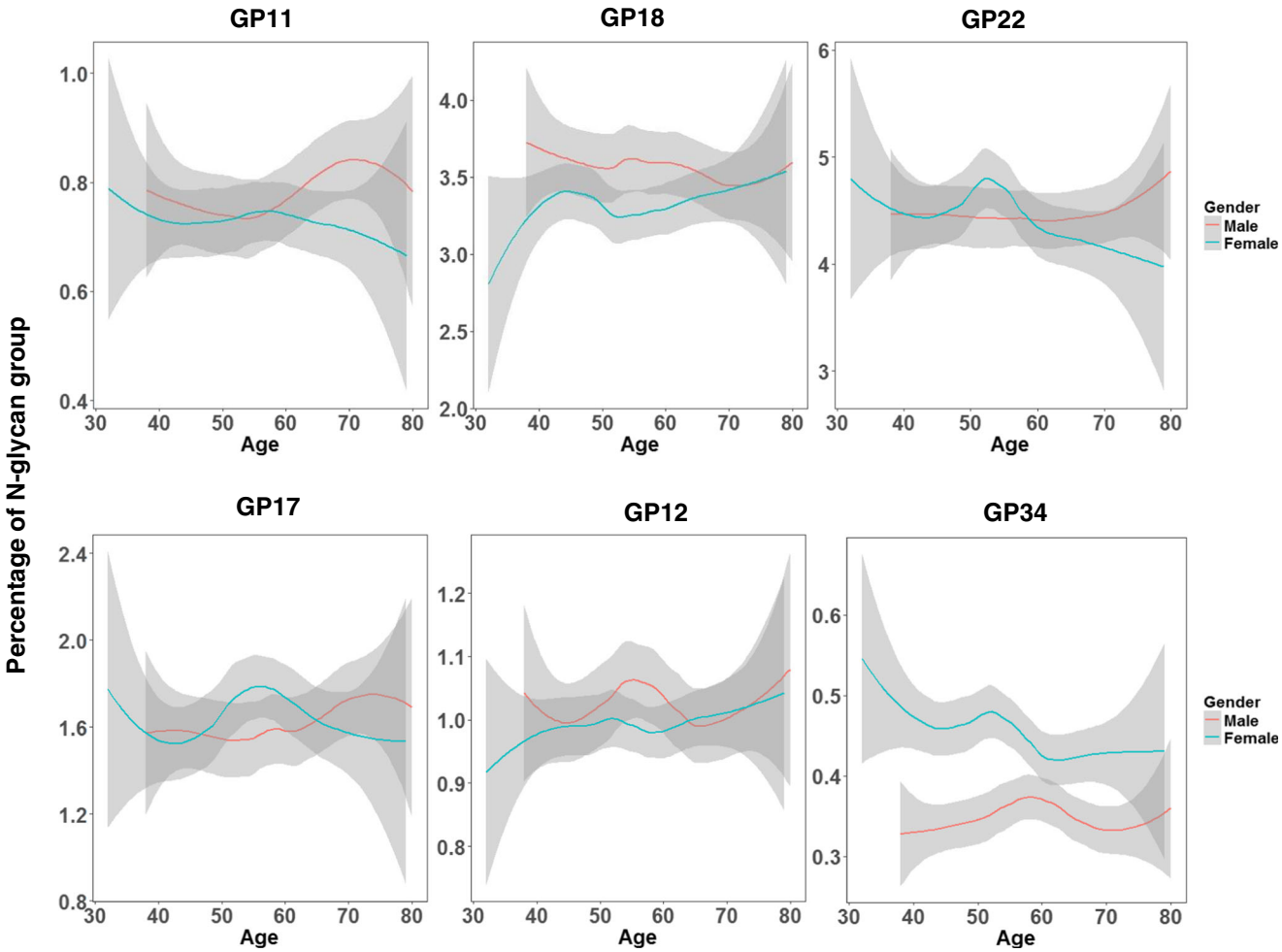

Fig. 1 Loiss plots of the relationship between N-glycans and Age among cases. Blue and red curves are fitted linear regression models. The shaded region is the 95% confidence intervals on the fitted values

been suggested to be central in renal function. Over activation of EGF receptor may underlie diabetic kidney disease [54, 55]. Further, since the identified N-glycans (GP12 and GP16) have been implicated in renal function in both T1DM and T2DM, this result suggests a possible mechanistic similarity between the two main types of diabetes. However, further investigation is warranted to gain additional insights.

A previous study has shown that IgG galactosylation is associated with complement activation and renal damage [56] and that decreased IgG galactosylation is linked with CKD. On the contrary, the present study showed that galactosylated N-glycan GP14 (A2G2S[6]1), was higher in T2DM patients with CKD compared to those without it. Also, after performing logistic regression in the crude model, agalactosylated N-glycans GP7 (M6D1-D2), core fucosylated monogalactosylated N-glycans GP6 (FA2[6]BG1) and digalactosylated N-glycans (GP14) were found to be associated with increased risk of CKD. These discrepancies may be attributed to the glycoprotein under investigation. In this study, total N-glycome was measured, exploring the whole plasma proteins whereas the previous study focused on IgG N-glycan. Exploring total plasma N-glycome is more beneficial as it reflects N-glycosylation and the relative abundance of proteins in circulation.

The present study has revealed the independent association between elevated uric acid, urea and creatinine and renal dysfunction as previously reported [60–65] (Tables 4 and 5). Subsequently, we sought to investigate how N-glycans correlate with these renal function markers. Some important highlights were that trigalactosylated [(GP26, GP30, GP31, GP32) and tetragalactosylated structures (GP 34 and GP36) were negatively correlated with creatinine whereas core fucosylated (GP4, GP5, GP20) and trigalactosylated N-glycans (GP30) significantly correlated with uric acid amongst T2DM patients with CKD (Fig. 2 and Supplementary Tables 4 & 5). However, these analyses does not adequately distinguish or adequately predict incident CKD. This can be blamed on the fewer number of T2DM patients with CKD and the impact of antidiabetic medications. A recent study has shown that the majority of the participants utilised different medications including

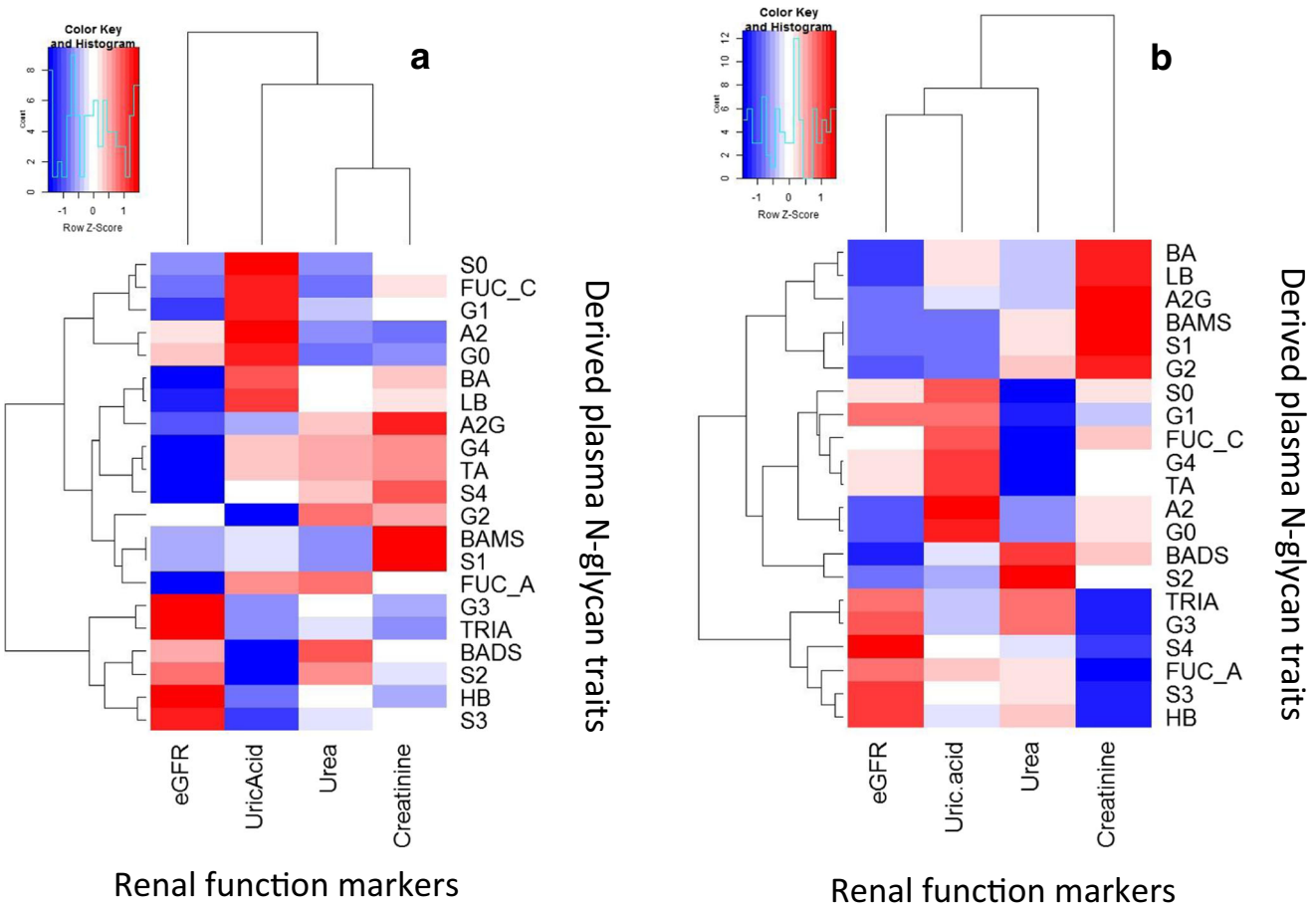

Fig. 2 Correlation between derived plasma N-glycan traits with or without CKD. (a) No CKD (b) CKD-T2DM. LB (rs = 0.21, $p = 0.008$), BA (rs = 0.22, $p = 0.005$), A2G (rs = 0.18, $p = 0.025$), S1 (rs = 0.19, $p = 0.017$), and BAMS (rs = 0.19, p = 0.017) were positively correlated with creatinine among T2DM without CKD. However, S3 (rs = −0.19, $p = 0.013$), G3 (rs = −0.23, $p = 0.004$), FUC_A (rs = −0.19, $p = 0.014$) and TRIA (rs = −0.23, $p = 0.003$) were negatively associated with creatinine. There were no statistically significant correlations between N-glycans and urea and uric acid in this group. On the other hand, TRIA was negatively associated with creatinine among T2DM with CKD. S0 (rs = 0.26, $p = 0.023$), G1 (rs = 0.27, $p = 0.021$) and FUC_C (rs = 0.30, $p = 0.035$) were positively associated with uric acid whereas S2 (rs = −0.30, $p = 0.011$), G3 (rs = −0.25, $p = 0.036$), BADS (rs = −0.26, $p = 0.028$), TRIA (rs = −0.025, $p = 0.029$) were negatively associated with uric acid in this group.

diuretics, statins, glucocorticoids and anti-malarial agents [27]. These medications may influence serum uric acid levels and hence, the observed results could be overestimated.

The present study has limitations that need to be mentioned. First, metabolic risk factors such as blood pressure, blood glucose, lipid profiles and kidney dysfunction markers were limited to only one measurement whereas CKD presence should be established following multiple estimates for over 3 months. Again, because the study was a cross-sectional one, we were unable to determine the direction or the causal relationship between risk factors and CKD.

Outlook and perspectives

This study provides a stimulus for future research. Here, we have established the presence of CKD by estimating GFR with CKD-EPI equation. Apart from the aforementioned biomarkers, imaging techniques should be employed to establish the presence or absence of CKD. For example, ultrasonographic techniques can be used to examine the size and texture of the kidneys and determine possible abnormalities. In addition, spectral doppler and color doppler with ultrasonography as well as computer tomography, elastography and radiography are powerful tools for detecting CKD. Further, these efforts can be complemented with histopathological methods (e.g. renal biopsy) to confirm diagnosis [66–69].

Conclusion

The present study showed that undiagnosed CKD is prevalent among the T2DM patients. This was established based on the CKD-EPI equation. However, reporting incident CKD was not sufficient with this equation alone and

therefore we explored the N-glycosylation profiles of the participants. The study revealed specific complex N-glycans that were associated with fucosylated N-glycans. Future investigations may better reveal the role of the identified complex N-glycans and renal function.

Compliance with ethical standards

Conflict of interest The authors declared that they have no conflict of interest

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