ASSESSMENT OF URINARY CYSTATIN C AND NGAL IN DIABETIC- AND NON-DIABETIC KIDNEY DISEASE OVER 40 YEARS FEMALE WITH TYPE 2 DIABETES

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Abstract: Background: Altered kidney structure and function is the most important evidence of diabetic nephropathy (DN), also known as diabetic kidney disease (DKD), and is an important cause of permanent renal impairment. Urinary albumin/creatinine proportion and glomerular filtration rate are utilised in ordinary work practise in the Republic of Iraq to detect DKD. These routine tests, on the other hand, do not always detect initial DN damage. In the current study, early kidney damage is detected by urine biomarker NGAL and Cystatin C, even if the patients do not show any symptoms. **Research design and methods :**This study used a cross-sectional design all 180 participant are female with type 2 diabetes mellitus the ages of 40 and 70. Were enrolled during the period between June 2021 to February in 2022. Those patients were attended to Diabetic center for admitted in the Teaching Hospitals of Wasit Governorate (Iraq). After taking informed consent from each. We chose n=67 NDKD and n=113 DKD (eGFR 60 mL/min/1.73 m^2 and uACR >30 mg/g) from the participants who were separated into two groups based on the ratio of eGFR to uACR. **Results:** The DKD group had significantly higher concentration of of NGAL and Cystatin C in urine than the NDKD group, according to the study's findings. In both groups with diabetes, receiver operating characteristic (ROC) curves where uNCR was known to be greater than uNCR for estimating eGFR/uACR were found to be better than uNCR for estimating eGFR/uACR, which gave the uNCR: AUC 0.973, (95%CI): (0.921, 1.000), Specificity 97%, Sensitivity 90 %, PPV 98%, NPV 93% with accuracy 93% and precision 98%, (log-rank test P=0.005). **Conclusions:** Urine markers such as NGAL and Cystatin C It may serve as a possible technique for detecting cases with a significant clinical suspicion of DKD, particularly if it is attributable to uACR.

Keywords: Type 2 Diabetic Mellitus, Diabetic Kidney Disease, NGAL Cystatin C

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INTRODUCTION

DKD is thought to be caused by diabetes and the high blood sugar that comes with it [1]. DKD, which is the leading cause of end-stage kidney disease (ESRD), affects half of people with type 2 diabetes [2]. With the fast-increasing frequency of DKD, biomarkers that can predict the development and severity of DKD are in high demand. An increment in systemic inflammation has been associated to T2DM [3]. Chronic inflammation has a role in the onset and progression of DKD in T2DM patients [4]. DKD has a direct association with other pathological conditions such as oxidative stress, glomerular damage, tubular injury, and inflammatory responses. [5]. DKD is diabetes-related CKD, defined as a glomerular filtration rate (GFR) of < 60 mL/min/1.73 m^2 and/or a urine albumin: creatinine ratio of ≥30 mg/g creatinine [6].

There is no requirement for a kidney sample if these conditions are followed. Proteinuria (major albuminuria) or perhaps microalbuminuria is not a reliable indicator of renal deterioration in individuals with type 2 diabetes, implying a non-proteinic form of diabetic kidney disease defined by an eGFR of <60 mL/min/1.73 m^2 and a UACR of < 300 mg/gm. [7]. In T2DM, Cystatin C and Neutrophil gelatinase associated lipocalin (NGAL) were shown to be two novel potential indicators in serum. Urine NGAL (uNGAL) and Urine Cystatin C (uCys C) were shown to be considerably higher in T2DM patients, suggesting that they might be used as early indicators of tubular and glomerular markers, respectively. Other recent evaluations on the potential of biomarkers in DKD early detection may be found here. [8]

Cystatin C is a tiny protein that the body filters via the glomeruli and has a strong relationship with glomerular filtration rate (GFR) [9]. The effect is almost non-existent for age (more than a year), sex, muscle mass and inflammatory diseases [10]. CysC is distinguished from other indications of renal failure by its power to remain unbound to proteins and flow freely through the glomeruli. In healthy persons, CysC, like other low-molecular-weight proteins with minimal or only limited tubular secretion, is filtered almost completely by the glomeruli and reabsorbed nearly totally in the proximal tubules. The readings of this biomarker rise with decreasing GFR in renal impairment. When there is early renal impairment, CysC rises when GFR and creatinine concentrations are still within acceptable ranges, according to several studies. [11,12]

The NGAL is used as a biomarker for renal injury since its release is similar to the reaction to tubular damage [13]. NGAL, a tiny protein (25 kDa) related to the lipocalin protein family that is detected in both urine and serum and generated in the renal tubules following inflammation and tissue injury, is one of the most significant tubular biomarkers in the detection of acute and chronic kidney disease. [14]. It is produced even before albumin is detected in the urine, as it needs two to four hours to appear [15]. In the early stages of DN, the uNGAL increases, then decreases as the disease progresses. In other words, the quantity of albuminuria is inversely proportional to uNGAL [16]. The therapy of metabolic and hemodynamic disturbances is critical for the prevention and delay of DKD development. DKD is a global problem with substantial social and economic implications; research should focus on coming up with novel ways to combat this dreadful disease [17]. Clinical and experimental research revealed that the progression in rabies and how it occurs can be determined by means of knowledge of renal tubular damage [18]. DKD can be detected and treated early, delaying the onset of ESRD. eGFR and urine albumin excretion appear to be the most predictive factors. Renal risk scores are still in their early stages of and 113 DKD (uACR ≥30 mg/g) participants from 2 categories (Diabetes group (DKD) "standard clinical renal function was abnormal") after they volunteered for a physical examination. New and traditional measurements for diabetic kidney patients were measured for two sets of urine and venous blood samples, and these biomarkers are: Serum NGAL (Neutrophil Gelatinase-Associated Lipocalin), Serum Cystatin C, Serum Creatinine, uACR. Normal and fasting blood glucose indices, as well as HbA1c, were used to confirm T2DM. Use venous blood in the morning to measure creatinine, uCysC, uNGAL, HbA1c, and blood sugar after a 12-hour fast. Special tubes were used to test uCysC and uNGAL, which were placed in a centrifuge and stored in deep freeze (-80°C). The concentrations of uCysC and uNGAL were measured by enzyme-linked immunosorbent assay (ELISA) and enzyme quantitative immunoassay (sandwich antibody duplex) by (Melsin medical co., limited, China). uCysC has a conventional biomarker value of 0.62-1.11 mg/L. At mean concentrations of 0.97 mg/L, the inter-assay coefficient of variation (CV) was 5.05 %, and at mean concentrations of 1.90 mg/L, it was 4.87 %. The measurement frequency for NGAL is 10.0-1500 ng/mL, whereas the calibration range is 0.0-1500 ng/mL. A concentration of up to 131.7 ng/mL is deemed normal. The UACR were measured from a urine sample, and the uACR were regarded abnormal when it values larger than 30 mg/g, which refers to patients with DKD in this study; and the analysis is planned to have a <10% error in total CV (coefficient of variation). The uACR was measured from a urine sample, and results more than 30 mg/g were considered abnormal. This refers to the patients in this research who have DKD. The results are reliable to by 10% of the total CV (coefficient of variation). The estimated glomerular filtration rate (eGFR) is CKD-EPI eGFR = 141 min (SCr /κ, 1)α max (SCr /κ, 1) $^{-1.209}$ x0.993^{age} , as computed by the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) creatinine equation. [x1.018 (if a woman)] or [x1.159 (if a black person)] SCr in mol/l is κ 61.9 for females and 79.6 for men, α is -0.329 for females and -0.411 for males, min shows the minimum of SCr/κ or 1, max represents the maximum of SCr/κ or 1.[20]

development, and most models contain flaws [19]. Here, we'll look for a sensitive biomarker that may be used to correctly classify diabetic individuals with CKD and predict disease progression. In this investigation, uNCR levels in T2DM patients with DKD were shown to be considerably greater than those in T2DM patients with non-diabetic kidney disease (NDKD).

METHODS

Study design and Sample Collection

This study employed a cross-sectional study design, with 180 patients with Type 2 diabetes, all of whom were women, ranging in age from 40 to 70 years. Were enrolled during the period between June 2021 to February in 2022. Those patients were attended to Diabetic center for admitted in the Teaching Hospitals of Wasit, Region (Iraq). After taking informed consent from each; The individuals were separated into two groups based on their urine albumin to creatinine ratio (uACR). We chose 67 NDKD (uACR <30 mg/g)

The study's major goal is to determine the percentage of T2DM patients who have CKD (eGFR 60 ml/min/1.73 m^2 or albumin creatinine ratio [uACR] ≥30 mg/g or ≥3 mg/mmol, or both). The evolution of albuminuria from normal albuminuria (urine albumin to creatinine ratio [uACR], 30 mg/g) to microalbuminuria (uACR 30-299 mg/g) to macroalbuminuria (uACR ≥300 mg/g) can be used to predict the clinical course of diabetic renal patients. An empirical method was used to calculate the rate of progression of CKD in the presence of non-proteinuria (uACR 30-299 mg/g) and proteinuria (uACR ≥300 mg/g). Then the study patients' renal dysfunction assessment (eGFR/uACR) was used to classify the patients. If urine is stored at room temperature, it can be used for tests for up to 24 hours, and the shelf life can be extended to 7 days by storing it at 2-8°C. The medical history must be taken from the patient because it has an important role in diagnosis and treatment. We exclude patients with active urinary tract infection, T1DM, pregnant patients, gestational diabetes, other renal disorders, patients on glucocorticoid medication, chronic conditions, active malignancy, or patients who demanded to be excluded from the trial.

STATISTICAL ANALYSIS

R was used to conduct statistical analysis (version 4.0.2). The demography and lab tests findings were analysed using standardised statistical techniques. The Pearson parametric correlation (Pearson's correlation coefficient r with P-value) was used to determine whether the values of two variables are associated; the connection of general and biochemical parameters with diabetic kidney disease was investigated using Pearson Chi-squared and ANOVA tests; descriptive statistics (Mean ± SD); and operating receiver curve (ROC) responsiveness and specificity were established. Multiple regression analysis was a method for examining the connection between one dependent factor and one or more independent factors; the null hypothesis was rejected using the p-value.

RESULTS

Participant Information

In Table 1, diabetic kidney disease (DKD) patients were classified as per their level of renal dysfunction based on the ratio of kidney function to the amount of protein in the urine (eGFR/uACR). DKD staging, which is useful for planning, follow-up, and administration, is created using this data. Individuals with an uACR of < 3 mg/mmol and an eGFR of more than > 60 mL/min/1.73 m2 were classified as having 'No DKD.' Persons with DKD had an uACR > 3 mg/mmol or an eGFR <60 mL/min/1.73 m2. Gender, age, hypertension, HBA1c percent, SCr, and eGFR-Cr were not statistically different in both diabetes cohorts (p≥0.05). On the other hand, the mean values of patients with DKD were insignificantly different from NDKD in the two groups; from where the mean values of other variables like Hypertensive, known duration of diabetes, showed significant difference higher in DKD group than DKD (p ≤0.05).

Table 1. Demographic and biochemical parameter of diabetes groups

Variables (nss=180)	**NDKD eGFR/UACR (n =67)**	**DKD eGFR/UACR (n = 113)**	**P-Value**
Age (years) (mean±SD)	45.5 ± 9.80	47.46 ± 8.72	0.068
Hypertensive (yes/ no) (n)	23/7	27/3	<0.08
Diabetes (mean±SD)			
Known duration (years)	5.7±3.0	13.0±5.2	<0.005
HBA1c %	6.43 ± 0.35	8.95 ± 2.53	0.787
DKD parameters (mean±SD)			
SCr (mg/dl)	0.65 ± 0.11	0.83 ± 0.38	0.297
eGFR-Cr (ml/min/1.73m2)	109.55 ± 11.33	96.21 ± 13.20	0.723
UACR (mg/g)	18.24 ± 4.83	112.17 ± 67.78	<0.001
Albuminuria status (n)			
Normoalbuminuria	30	0	---
Microalbuminuria	0	13	---
Macroalbuminuri	0	17	---
CKD stage (1/2/3a/3b/4/5) (n)	10/20/0/0/0/0	0/4/16/9/1/0	---

Data are presented as the mean ± SD, unless otherwise indicated. NDKD, non-diabetic kidneydisease; DKD, diabetic kidney disease. A P value of <0.05 indicates significance.

In Table 2, And on the same division of the first table of patients' groups, the mean values of patients with DKD were insignificantly different from NDKD in the two groups also; from where the mean values of other potential biomarkers analysis in study patients like uACR, uCysC, uCCR, uNGAL, uNCR showed significant difference higher in DKD group than DKD (p ≤0.001).

Table 2. Other potential biomarkers analysis in study patients (mean±SD)

Variables (nss=180)	NDKD eGFR/UACR (n =67)	DKD eGFR/UACR (n = 113)	P-Value
uCysc (mg/L)	125.03 ± 85.32	215.41 ± 92.35	<0.001
uCCR (lg/g)	158.03 ± 96.21	380.71 ± 227.08	<0.001
uNGAL (ng/ml)	18.12 ± 12.34	70.93 ± 34.35	<0.001
uNCR (lg/g)	23.11 ± 11.93	118.71 ± 55.05	<0.001

Data are presented as the mean ± SD, A P value of <0.001 indicates highly significance.

Table 3 shows the results of ROC analysis of uACR with uNGAL and uCCR, as well as the area under the curve, as indicated in Table 2 and Figures 1 and 2. When comparing diabetic kidney patients to non-diabetic kidney patients, the uNCR: AUC 0.973 (95 % CI): (0.921, 1.000), Specificity 97 %, Sensitivity 90 %, PPV 98 %, NPV 93 % with accuracy 93 percent and precision 98 % was found to be better than the uCCR: AUC 0.872 (95 % CI) (0.784, 0.959), Specificity For predicting diabetic kidney patients as well as non-diabetic kidney patients of type 2 diabetes patients, PPV 98 %, NPV 93 %, accuracy 81, and precision 93 % were used.

Table 3. ROC curve (Area under curve (AUC) for Urine NGAL and Urine Cystatin C)

Parameters		**AUC (95 % CI)**	**Sensitivity%**	**Specificity%**	**PPV%**	**NPV%**	**Accuracy%**	**Precision%**
NDKD	**uNCR**	0.956 (0.813-0.963)	83	77	71	87	79	71

	uCCR	0.803 (0.643-0.970)	71	79	72	77	75	72
DKD	**uNCR**	0.973 (0.921-1.000)	90	97	98	88	93	98
	uCCR	0.872 (0.784-0.959)	66	93	93	67	81	93
eGFR/Uacr		1.000 (0.995-1.000)	100	100	100	100	100	100

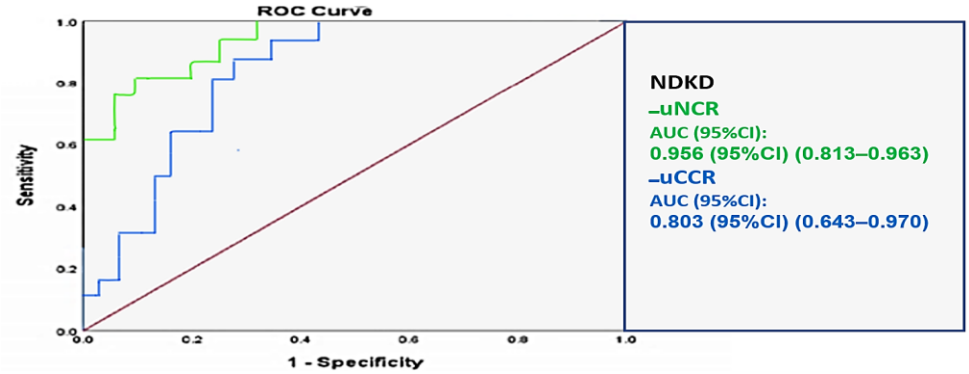

Figure 1. ROC analysis of uNCR and uCCR in non-diabetic kidney patients with uACR as status variable

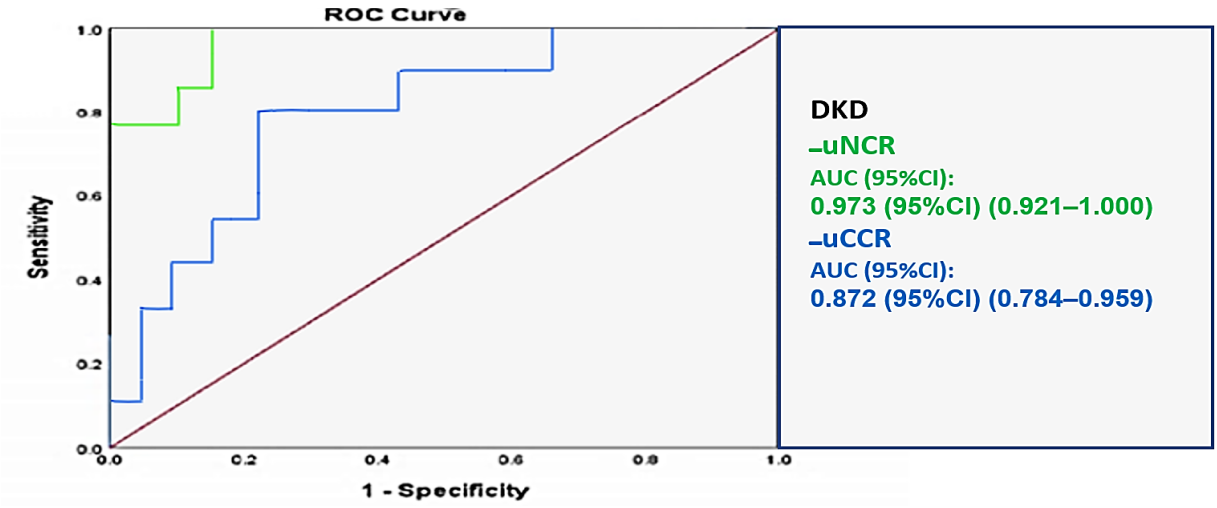

Figure 2. ROC analysis of uNCR and uCCR diabetic kidney patients with uACR as status variable

DISCUSSION

Increasing data from experimental and clinical investigations shows that only a small percentage of individuals with CKD/DKD achieve end-stage renal disease (ESRD), with the most dying from other reasons before reaching ESRD [21]. Here, we'll look for a sensitive biomarker that can be used to correctly classify diabetic individuals with DKD and predict disease progression. In this study, uNCR and uCCR levels were shown to be significantly greater in DKD patients than in Therefore, it is important to stratify diabetic patients at high risk of progressing from DKD to ESRD by adopting a precise and accurate biomarker to distinguish between NDKD and DKD. The kidney damage indicators albumin, NGAL, and cystatin C are measured in urine to detect kidney impairment in people NDKD patients. In both basic and multivariate logistic regression models, the level of uNCR was demonstrated to be a risk factor for DKD in diabetic patients with CKD.DKD is a large and rising expensive health-care burden, which is projected to continue to climb as the population ages and T2D rates rise. Despite current best practise standards of therapy, such as lifestyle change, blood pressure control, and the utilization of renin-angiotensin-aldosterone system blockers, the rate of progression to ESKD in patients with CKD is still high [22].

with pre-diabetes. Makris et al., Conti et al., Makris et al., Makris et al., Makris et al., Makris et al., Makris et al., Makris et al., Makris et al., Makris et al., Makris et al., Makris et al., Makris et al., Makris et [23, 24]. The results of Laquonetti et al. [25, 26] Wu et al. [25, 26] to calculate the mean value of NGAL (12.65

ng/ml). Diabetes and normal albuminuria linked with diabetes were shown to have increasing amounts of NGAL and Cystatin C than healthy controls [26, 27]. The results of the current research were similar to those found by Jeon et al. [29] According to the aforementioned source, Cystatin C levels in diabetic persons with normal albuminuria and microalbuminuria are 0.06 ± 0.06 and 0.32 ± 1.14 mg/L, respectively. In the study by Wu et al., the UNCR values in diabetic patients with normoalbuminuria and microalbuminuria were 12.54 (8.08–27.61) and 14.99 (8.7–25.88) lg/g, respectively; the values in the research by [33, 34] were 69.2 (29.3–120.4) and 123.6 (59.4–184.60) lg/g, respectively. The uNCR levels in the present research were comparable to those obtained in the prior two studies. More study is needed to corroborate the aforementioned results since conventional normality values have yet to be established. The levels of uACR, NGAL, cystatin C, uCCR, and uNCR in normoalbuminuria patients are less than those in microalbuminuria patients, as shown in Table 1. Individuals with micro and macroalbuminuria, and also glomerular hyperfiltration, show an increase in these symptoms. According to Nauta, Wu, and Assal et al. [32, 33, 35] In healthy controls, urinary NGAL levels were increased, normal albuminuria, microalbuminuria, and macroalbuminuria. Cystatin C levels increased with albuminuria [29, 30]. Pre-diabetes individuals with normal albuminuria showed greater levels of NGAL and cystatin C than diabetic patients with normal albuminuria. The prior group's eGFR was greater, demonstrating this. According to Fu WJ et al., people with type 2 diabetes and glomerular hyperfiltration showed greater amounts of NGAL in their urine than those with normal filtration. [36] The pre-diabetes stage had not been researched for the aforementioned example till the time of authoring the research. In the case of excessive glomerular filtration causing tube injury, increasing pressure and flow might cause functional and structural alterations. Renal tubular injury can be determined by measuring the level of the glycoprotein NGAL, which results from damaged renal epithelial cells. In diabetic nephropathy, renal insufficiency can be known by the level of NGAL production [36, 37]. As for cystatin C, it is not tubular secreted and is freely filtered by the glomeruli and is mostly absorbed by the proximal tubules. If it is secreted, it is one of the signs of tubular and glomerular dysfunction [38, 36]. Diabetic patients with early renal impairment have a DN disorder and the cause is believed to be a tubulo-interstitial infection. People with diabetes who had normal albuminuria owing to glomerular hyperfiltration had a greater amount. Premature tubular failure is implicated of diabetic kidney disease, as evidenced by the preceding. In pre-diabetes and diabetes, there was a favorable correlation between UACR and uNCR. Albuminuria is a symptom of glomerular injury. One-third of diabetes individuals with microalbuminuria had glomerulopathy. Lower urinary tubular indices at baseline were strongly related with regression of microalbuminuria in two-year research by Vaidya et al. [39]. Endocytosis of progressive glycation end products (AGEs) in proximal tubular epithelial cells by megalin in diabetics can induce cellular damage. Megalin concentrations in urine were shown to be linked with the severity of diabetic kidney disease in studies evaluating megalin as a tubular biomarker [38]. Kim et al. [30] discovered that uACR was positively associated with NGAL in normoalbuminuria (r = 0.603, p0.05) and microalbuminuria (r = 0.204, p0.05) groups.Nauta et al. [35] obtained similar results (b = 0.45, p <0.001). We explored the relationship between diabetes and pre-diabetes and biomarkers in people with diabetes and pre-diabetes. A little increase in urine NGAL and Cystatin C with of albuminuria in the normoalbuminuria range might be due to decreased tubular absorption. The addition of new biomarkers (NGAL and Cystatin C) to existing tests (uACR and eGFR) for early DN diagnosis aids in the creation of suitable management and treatment strategies, which are critical for reducing DKD morbidity and mortality in T2D patients. Despite the fact that multiple studies on Cystatin C and NGAL have been done, cut-off values for normality have yet to be thoroughly defined in the literature by bigger observational research before being included in T2D patients' DKD risk assessments.

CONCLUSION

For primary care physicians, there is a clear possibility for more risk-reduction techniques in individuals with T2D and early stages of renal failure. Urine eGFR/uACR is not sensitive or specific enough to detect these alterations early on. NGAL and Cystatin C are two urine indicators. Early identification of nephropathy in those with pre-diabetes and diabetes has shown promising results, particularly when connected to uACR.

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Consent of Ethics: Administrative approval was taken from all places where samples were collected, as well as written and oral consent was taken for all participants in the research

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REFERENCE

i. Falkevall A, Mehlem A, Palombo I, Heller Sahlgren B, Ebarasi L, He L, Ytterberg AJ, Olauson H, Axelsson J, Sundelin B, Patrakka J, Scotney P, Nash A, Eriksson U Cell Metab. 2017 Mar 7; 25(3):713-726.

ii. Tuttle KR, Bakris GL, Bilous RW, Chiang JL, de Boer IH, Goldstein-Fuchs J, Hirsch IB, Kalantar-Zadeh K, Narva AS, Navaneethan SD, et al. Diabetic kidney disease: a report from an ADA consensus conference. Diabetes Care. 2014;37(10):2864–83.

iii. Calder PC, Albers R, Antoine JM, Blum S, Bourdet-Sicard R, Ferns GA, Folkerts G, Friedmann PS, Frost GS, Guarner F, et al. Inflammatory disease processes and interactions with nutrition. Br J Nutr. 2009;101(Suppl 1): S1–45.

iv. Park DJ, Choi SE, Xu H, Kang JH, Lee KE, Lee JS, Choi YD, Lee SS. Chronicity index, especially glomerular sclerosis, is the most powerful predictor of renal response following immunosuppressive treatment in patients with lupus nephritis. Int J Rheum Dis. 2018.

v. Zhang D, Ye S & Pan T.: The role of serum and urinary biomarkers in the diagnosis of early diabetic

nephropathy in patients with type 2 diabetes., PeerJ., 2019;7: e7079

vi. Guideline Development Group. Clinical Practice Guideline on management of patients with diabetes and chronic kidney disease stage 3b or higher (eGFR <45 mL/min). Nephrol Dial Transplant 2015; 30(Suppl 2): ii1–ii142

vii. Yamanouchi M, Furuichi K, Hoshino J, Toyama T, Hara A, Shimizu M, Kinowaki K, Fujii T, Ohashi K, Yuzawa Y, Kitamura H. Nonproteinuric versus proteinuric phenotypes in diabetic kidney disease: a propensity score–matched analysis of a nationwide, biopsy-based cohort study. Diabetes care. 2019 May 1;42(5):891-902.

viii. Papadopoulou-Marketou N, Skevaki C, Kosteria I, et al. NGAL and cystatin C: two possible early markers of diabetic nephropathy in young patients with type 1 diabetes mellitus: one year follow up. Hormones (Athens). 2015;14(2):232-240.

ix. Dharnidharka VR, Kwon C, Stevens G. Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis. Am J Kidney Dis 2002; 40: 221-226.

x. Finney H, Newman DJ, Thakkar H, et al. Reference ran-ges for plasma cystatin C and creatinine measurements in premature infants, neonates, and older children. Arch Dis Child 2000; 82: 71-75.

xi. Husain SA, Willey JZ, Moon YP, et al. Creatinine-versus cystatin C-based renal function assessment in the Northern Manhattan. Study 2018; 13: e0206839.

xii. Mussap M, Vestra MD, Fioretto P, et al. Cystatin C is a more sensitive marker than creatinine for the estimation of GFR in type 2 diabetic patients. Kidney Int 2002; 61: 1453-1461.

xiii. Buonafine M, Martinez-Martinez E, Jaisser F. More than a simple biomarker: the role of NGAL in cardiovascular and renal diseases. Clin Sci 2018; 132:909-923.

xiv. Kapoula GV, Kontou PI, Bagos PG. Diagnostic accuracy of neutrophil gelatinase-associated lipocalin for predicting early diabetic nephropathy in patients with type 1 and type 2 diabetes mellitus: A systematic review and metaanalysis. J Appl Lab Med 2019; 4:78-94.

xv. Assal HS, Tawfeek S, Rasheed EA, et al. Serum cystatin C and tubular urinary enzymes as biomarkers of renal dysfunction in type 2 diabetes mellitus. Clin Med Insights Endocrinol Diabetes 2013; 6:7-13.

xvi. Sagoo MK & Gnudi L. Diabetic nephropathy: an overview. Diabetic Nephropathy. 2020

xvii. Sharif E, Alwakeel M. Neutrophil gelatinase associated lipocalin: Is not an early marker inductor for diabetic nephropathy in qatari population. Biomed J Scientific Tech Res 2019; 22:16345-16355

xviii. Kim SS, Song SH, Kim IJ, et al. Urinary cystatin C and tubular proteinuria predict progression of diabetic nephropathy. Diabetes Care 2013; 36:656-61.

xix. Duru OK, Middleton T, Tewari MK, Norris K. The Landscape of Diabetic Kidney Disease in the United States. Curr Diab Rep. 2018;18(3):14.

xx. Inker LA, Schmid CH, Tighiouart H, et al.; CKD-EPI Investigators. Estimating glomerular filtration rate from serum creatinine and cystatin C. N Engl J Med 2012; 367:20–29

xxi. Willis M, Nilsson A, Kellerborg K, Ball P, Roe R, Traina S, Beale R, Newell I. Cost-Effectiveness of Canagliflozin Added to Standard of Care for Treating Diabetic Kidney Disease (DKD) in Patients with Type 2 Diabetes Mellitus (T2DM) in England: Estimates Using the CREDEM-DKD Model. Diabetes Therapy. 2021 Jan;12(1):313-28.

xxii. Seidu S, Barrat J, Khunti K. Clinical update: the important role of dual kidney function testing (ACR and eGFR) in primary care: identification of risk and management in type 2 diabetes. Primary care diabetes. 2020 Aug 1;14(4):370-5.

xxiii. Makris K, Nikolaki E, Nanopoulos K, Pirqakis KM, et al. Measurement of Cystatin C in human urine by particle enhanced turbidimetric immunoassay on automated biochemistry analyzer. Clin Biochem. 2013;46(12):1128–30.

xxiv. Conti M, Moutereau S, Zater M, Lallali K, et al. Urinary Cystatin C as a specific marker of tubular dysfunction. Clin Chem Lab Med. 2006;44(3):288–91.

xxv. Woo KS, Choi JL, Kim BR, Kim JE, et al. Urinary neutrophil gelatinase-associated lipocalin levels in comparison with glomerular filtration rate for evaluation of renal function in patients with diabetic chronic kidney disease. Diabetes Metab J. 2012;36: 307–13.

xxvi. Lacquaniti A, Donato V, Pintaudi B, Vieste GD, et al. ‘‘Normoalbuminuric’’ diabetic nephropathy: tubular damage and NGAL. Acta Diabetol. 2013; 50:935–42

xxvii. Makris K, Nikolaki E, Nanopoulos K, Pirqakis KM, et al. Measurement of Cystatin C in human urine by particle enhanced turbidimetric immunoassay on automated biochemistry analyzer. Clin Biochem. 2013;46(12):1128–30.

xxviii. Woo KS, Choi JL, Kim BR, Kim JE, et al. Urinary neutrophil gelatinase-associated lipocalin levels in comparison with glomerular filtration rate for evaluation of renal function in patients with diabetic chronic kidney disease. Diabetes Metab J. 2012;36: 307–13.

xxix. Jeon YK, Kim MR, Huh JE, Mok JY, et al. Cystatin C as an early biomarker of nephropathy in patients with type 2 diabetes. J Korean Med Sci. 2011; 26:258–63.

xxx. Kim SS, Song SH, Kim IJ, Jeon YK, et al. Urinary Cystatin C and Tubular proteinuria predict progression of diabetic nephropathy. Diabetes Care. 2013; 36:656–61.

xxxi. Garg V, Kumar M, Mahapatra HS, Chitkara A, Gadpayle AK, Sekhar V. Novel urinary biomarkers in pre-diabetic nephropathy. Clinical and experimental nephrology. 2015 Oct;19(5):895-900.

xxxii. Assal HS, Tawfeek S, Rasheed EA, Lebedy DE, et al. Serum cystatin C and tubular urinary enzymes as biomarkers of renal dysfunction in type 2 diabetes mellitus. Clin Med Insights Endocrinol Diab. 2013; 6:7–13.

xxxiv. Wu J, Ding Y, Zhu C, Shao X, et al. Urinary TNF-a and NGAL are correlated with the progression of nephropathy in patients with type 2 diabetes. Exp Ther Med. 2013; 6:1482–8.

xxxv. Fu WJ, Xiong SL, Fang YG, Wen S, et al. Urinary tubular biomarkers in short-term type 2 diabetes mellitus patients: a crosssectional study. Endocrine. 2012;41(1):82–8.

xxxvi. Nauta FL, Boertien WE, Bakker SJL, Goor HV, et al. Glomerular markers are elevated in patients with diabetes. Diabetes Care. 2011; 34:975–81.

xxxvii. Fu WJ, Li BL, Wang SB, Chen ML, et al. Changes of the tubular markers in type 2 diabetes mellitus with glomerular hyperfiltration. Diabetes Res Clin Pract. 2012;95(1):105–9.

xxxviii. Mori K, Lee HT, Rapoport D, Drexler IR, et al. Endocytic delivery of lipocalin-siderophore-iron complex rescues the kidney from ischemia-reperfusion injury. J Clin Invest. 2005;115(3): 610–21.

xxxix. Sauriasari R, Safitri DD, Azmi NU. Current updates on protein as biomarkers for diabetic kidney disease: a systematic review. Therapeutic Advances in Endocrinology and Metabolism. 2021 Oct; 12:20420188211049612.

xl. Vaidya VS, Waikar SS, Ferguson MA, et al. Urinary biomarkers for sensitive and specific detection of acute kidney injury in humans. Clin Transl Sci. 2008;1(3):200–8.