BioBacta

Journal of Bioscience and Applied Research
www.jbaar.org

Diagnostic performances of tumor necrosis factor-alpha and type IV collagen for diabetic nephropathy in type 2 diabetic patients

Mohamed M. Omran[1,*] Anhar F. Elmetwaly[2], Tarek M. Emran[3], Ahmed A. Eldeeb[4], Arafa A. Belal[2], Faten Z. Mohamed [5]

[1] Chemistry Department, Faculty of Science, Helwan University, Cairo, Egypt

[2] Chemistry Department, Faculty of Science, Port Said University, Port Said, Egypt

[3] Clinical Pathology Department, Faculty of Medicine, Al-Azhar University, Damietta, Egypt

[4]Nephrology Unit, Faculty of Medicine, Mansoura University, Mansoura, Egypt

[5] Chemistry Department, Faculty of Science, Zagazig University, Egypt

Short Title: Predicting diabetic nephropathy

***Correspondence:**
***Mohamed Mostafa Omran, PhD**
Chemistry Department, Faculty of Science,
Helwan University, Ain Helwan, 11795, Cairo, Egypt
E-mail: drmmomran@science.helwan.edu.eg; drmmomran@yahoo.com

DOI : 10.21608/jbaar.2022.258696

Abstract

Background: Diabetic nephropathy is a serious complication of both type 1 and type 2 diabetes. It's also called diabetic kidney disease (DKD). In the United States, about 1 in 3 people with diabetes have diabetic nephropathy. This necessitates identifying better biomarkers that diagnose diabetic nephropathy. The study aimed to evaluate tumor necrosis factor α (TNFα) and type IV collagen as potential biomarkers for the detection of diabetic nephropathy and its progression in patients with type 2 diabetes. **Materials and Methods:** A total of 88 subjects were included in this cross-sectional study. Patients were classified into three major groups; diabetics with DKD group (n=50), diabetics without DKD group (n=28), and healthy control group (n=10). TNFα and type IV collagen levels were measured in all subjects. The diagnostic value of single and combined TNFα and type IV collagen was assessed by the area under the receiver operating characteristic (AUC). **Results:** For discrimination between diabetics with DKD from healthy individuals, the most efficient marker was TNF-α (AUC= 0.81, 70% sensitivity, and 70% specificity. For discriminant between DM patients with DKD from DM patients without DKD, the most efficient marker was type IV collagen (AUC= 0.77, 69% sensitivity, and 73% specificity. Interestingly, we developed a new index for differentiating between DM and DM-DKD based on two blood markers (TNF-α and type IV collagen). The AUC of the developed index was 0.93; 0.79 for discriminated DM with DKD from DM

Received: July 1, 2022. Accepted : August 14, 2022. Published: September 9, 2022

without DKD. The AUC of the developed index was 0.90 for discriminated early DKD from healthy individuals. Also, The AUC of the developed index was 0.83 for discrimination early from late DKD among DM patients.

Conclusions: TNFα and type IV collagen may be potentially useful for early detection and to discriminate diabetics with DKD from DM without DKD.

Abbreviations: DKD; diabetic kidney disease- TNFα; Tumor necrosis factor-alpha.

Keywords: Type 2 diabetes mellitus; diabetic nephropathy; diagnosis; Tumor necrosis factor-alpha, type IV collagen.

INTRODUCTION:

The epidemiology of DM around the world has reached epidemic proportions of about 350 million people and is predictable to grow to about 550 million people by 2035. In Egypt, the prevalence of DM is about 20% and about 42% of diabetic patients had nephropathy (Gheith et al., 2015). Diabetic nephropathy (DN) is a chronic complication of both types of DM. More than 40% of diabetics will develop chronic kidney disease (CKD), with a significant number who will develop end-stage kidney disease (ESKD) requiring renal replacement therapies (dialysis and/or transplantation). DN is a frequent and severe complication of diabetes mellitus (DM). Its diagnosis in incipient stages may allow prompt interventions and an improved prognosis. Towards this aim, biomarkers for detecting early DN can be used (Alicic et al., 2017; Hung et al., 2021; Pugliese et al., 2020). Tumor necrosis factor-α (TNF-α) is a proinflammatory cytokine that was originally described as antitumorigenic and produced by immune cells like macrophages and lymphocytes; however, further studies revealed it is also produced by endothelial and epithelial cells (Ramseyer et al., 2013). TNF-α is elevated in chronic inflammatory states such as diabetes. There now are convincing data regarding the role of inflammation in the pathogenesis of renal damage in DM and the implication of TNF-α in the initiation of inflammatory cascade. Indeed, inhibition of inflammatory cell recruitment into the kidney is protective in experimental diabetic nephropathy (Navarro et al., 2006; Kong et al., 2022). Many clinical studies in patients with DN have reported that the urinary concentration of TNF-α is elevated in diabetic subjects as compared with non-diabetic individuals also with diabetic subjects with DN and these concentrations increase concomitantly with the progression of DN. These findings indicate a potential relationship between the elevated levels of this inflammatory cytokine and the development and progression of renal injury in DM (Chen et al., 2017; Donate-Correa et al., 2015). In the kidney, type IV collagen is the main constituent of both glomerular and tubular basement membranes as well as the mesangial matrix**.** It is critical for establishing and maintaining the glomerular filtration barrier to albumin**.** Urinary type IV collagen was significantly increased in both normoalbuminuric and microalbuminuric patients of type 2 DM compared with healthy controls, and urinary type IV collagen significantly correlated with the amount of albuminuria (Cosgrove et al., 2017; Miner et al., 2020; Mahendran et al., 2016). We aimed to determine the potential of TNF-α and type IV collagen biomarkers for the detection of diabetic nephropathy and its progression in patients with type 2 diabetes.

PATIENTS AND METHODS

Patients: Overall, 88 coherent Egyptian diabetic patients who were operated on between January 2018

to May 2020 from Al-Azhar teaching Hospital, Damietta, Egypt were enrolled in this study prospectively. Patients were classified into three major groups; the DM with DKD group (n=50), DM without DKD group (n=28), and the healthy control group (n=10). The diagnosis of DM was done based on American Association criteria. We calculated the eGFR value by using MDRD GFR Equation. Our excluded criteria involved type 1 diabetes mellitus, non-diabetic kidney disease, and other causes of proteinuria (urinary tract infection, severe hypertension, and hematuria). The study was approved by the Ethics Committee of the Faculty of Medicine, Al- Azhar University, New Damietta (Code IRB 00012367). Informed written consent was signed by all patients in compliance with the ethical guidelines of the 1975 Helsinki Declaration.

Blood samples:

Blood was obtained from each subject and collected in the morning after fasting overnight and another sample was collected two hours after breakfast. Part of the samples was collected on K-EDTA as an anti-coagulant and analyzed directly for complete blood count (CBC) and glycated hemoglobin (HbA1c) test. The other part of the samples is allowed to clot at room temperature before centrifugation at 3000 rpm for 10 minutes and aliquoted into two micro-centrifuge tubes. The first aliquot was used for routine investigations but the second one was stored at −20 °C and thawed only at the time of the assay. The severity of DKD has been categorized into five stages according to eGFR where G1, GFR ≥90 mL/min/1.37m2; G2, 60 to 89 mL/min/1.37m2; G3, 30 to 59 mL/min/1.37m2; G4, 15 to 29 mL/min/1.37m2; and G5 < 15 mL/min/1.37m2. Diabetic patients have DM- DKD (group III) were divided into two subgroups: early (G1-G2) and late (G3-G4) stages.

Biochemical analysis:

Fasting blood sugar, postprandial blood sugar, liver function tests, kidney function tests, and albumin to creatinine ratio were measured on an automated biochemistry analyzer (Hitachi 917; Roche Diagnostics). HbA1c was measured by (MISPA-i2, Agappe, India) automated analyzer.). Based on the Sandwich-ELISA technique; Serum Tumor necrosis factor-α (TNF-α) and Human type IV collagen (H Col IV) levels were analyzed according to the manufacturers' instructions of (SinoGeneClon Biotech Co., Ltd; Catalog No: SG-00425; Hongkong, China) and the resulted color was measured using a spectrophotometric plate reader at a wavelength of 450 nm where it is proportionally related to the concentrations by an absorbance reader (Tecan Austria GmbH, Sunrise – Basic TECAN).

Data analysis:

Statistical analyses were carried out via a statistical software package SPSS 22.0 for Microsoft Windows, SPSS Inc.). Frequency and percent distribution were used to describe categorical variables whereas quantitative/continuous variables were described as mean ± SD or median and inter-quarter range. Comparison of categorical factors (across outcomes groups; i.e., DM- DKD group vs. DM) was set by chi-square tests while continuous quantitative variables were analyzed by t-tests or analysis of variance (ANOVA). The correlation was evaluated by Pearson's or spearman correlation coefficient. Statistical significance was set at a $P<0.05$ with a 95% confidence interval. For the multivariate analysis, ROC curve analysis was established for all combinations to decide which one was the most accurate in detecting DM-CKD. The area under the ROC curves (AUC) was plotted, and then the best cut-off values were determined for both markers. The diagnostic accuracy of both type IV collagen and tumor necrosis factor-α was calculated by sensitivity, specificity, positive

predictive value (PPV), and negative predictive value (NPV).

RESULTS:

As illustrated in table 1, DM- DKD showed significantly increased kidney profile tests (creatinine, uric acid, urea) and diabetic profile tests (fasting blood sugar, HbA1C) levels than DM (p-value ranging from 0.024 - <0.0001). There was a significant decrease ($p < 0.0001$) in albumin levels. The levels of candidate markers (TNF and type IV collagen) increased by $P < 0.001$ in DM- DKD than in DM patients. Simple linear regression analysis was performed to examine the correlation between tumor necrosis factor-α and type IV collagen and kidney profile tests. The values of correlation of candidate markers and corresponding p values were listed in table 2. The TNF and type IV collagen negatively correlated with EGFR levels with significant values of $p = 0.001$ and $p = 0.006$; respectively.

3.7 Diagnostic power of TNF-α and type IV collagen:

Using the ROC curve, we assessed the diagnostic powers of TNF-α and type IV collagen to discriminate among different groups as presented in table 3. For discriminating between DM- DKD from healthy individuals, the most efficient marker was TNF-α (AUC= 0.81, 70% sensitivity, and 70% specificity. For discriminating between DM- DKD from patients with DM, the most efficient marker was type IV collagen (AUC= 0.77, 69% sensitivity, and 73% specificity. For discriminating between diabetics with late DKD from diabetics with early DKD, the most efficient marker was type IV collagen (AUC= 0.76, 69 % sensitivity, and 73% specificity; table 3. The AUC of the TNF and type IV collagen were 0.70 and 0.97 for discriminated late DM- DKD from healthy.

Development of a novel index for DM-DKD

We developed a new index for differentiating between DM and DM- DKD based on two blood markers (TNF-α and type IV collagen). It can be calculated as = 1.22 + 0.54 X TNF + type IV collagen x 0.148. The mean ±SD of the TPN index was 2.1±0.05; 2.3±0.19; 2.6±0.34 in healthy, DM, and DM-DKD; respectively. Its level was 2.5±0.40 in males while 2.4±0.28 in females without significant differences ($p= 0.29$). The values of correlation of developed index with other's markers and corresponding p values were listed in table 2. The diagnostic performances of the developed index were higher than corresponding single markers; table 3. The AUC of the developed index was 0.93; 0.79 for discriminated DM- DKD from healthy and DM. The AUC of the developed index was 0.83 for discriminated late DM- DKD from early DM-CKD. The AUC of the developed index was 0.90 for discriminated early DM-DKD from healthy.

Table1. Laboratory data of the studied groups

Variable	Healthy control group	DM group	DM-DKD group	P value	Post-hoc test		
					Healthy vs. DM	Healthy vs. DM- DKD	DM vs. DM- DKD
Age (Year)	58.0 ± 7.4	61.3 ± 8.5	62.2 ± 10.2	0.43	0.28	0.24	0.81
FBS (mg/dl)	88.2 ± 12.7	211.3 ± 78	232.3 ± 71.8	<0.001	< 0.0001	< 0.0001	< 0.0001
HbA1C (%)	5.5 ± 1.5	7.2 ± 2.5	7.3 ± 2.6	0.024	< 0.0001	< 0.0001	0.02
Creatinine (mg/dl)	0.88 ± 0.11	0.95 ± 0.26	4.05 ± 1.7	<0.001	0.95	< 0.0001	< 0.0001
G. F. R. (ml/min)	94.0±28.9	90.7±20.8	20.1±12.8	<0.0001	0..96	< 0.0001	< 0.0001
Urea (mg/dl)	28.5±10.4	36.6±10.9	130.8±57.4	<0.0001	0.91	< 0.0001	< 0.0001
Uric acid (mg/dl)	4.6 ± 1.3	4.8 ± 1.6	8.22 ± 1.8	<0.001	0.93	< 0.0001	< 0.0001
Albumin (g/dl)	4.3 ± 0.26	4.2 ± 0.42	3.2 ± 0.74	<0.0001	0.96	< 0.0001	< 0.0001
ACR	-	17.8 (8-28)	70 (35-635)	<0.0001	-	-	<0.0001
TNF-α (pg/ml)	31 (27 – 42)	96 (29 – 118)	96 (39–302)	0.001	0.006	0.02	0.05
Type IV collagen	615(400 – 890)	400 (300 – 700)	1704(502-4710)	0.001	0.06	0.02	< 0.0001

DKD:diabetic kidney disease **FBS:** fasting blood sugar**, TNFα**; Tumer necrosis factor alpha. **HbA1C:** Hemoglobin A1c**, GFR:** Glomerular filtration rate, **ACR:** Albumin creatinine ratio

Table 2. Correlations between TNF-α, type IV collagen with other kidney profile tests

Parameters	TNF-α		Type IV collagen	
	r	P	r	P
Creatinine	0.33	0.02	0.28	0.08
Uric acid	0.44	0.005	0.24	0.33
Urea	0.48	0.001	0.32	0.04
Urea	0.46	0.001	0.41	0.007
TNF	-	-	0.1	0.35
Type IV collagen	0.1	0.35	-	-

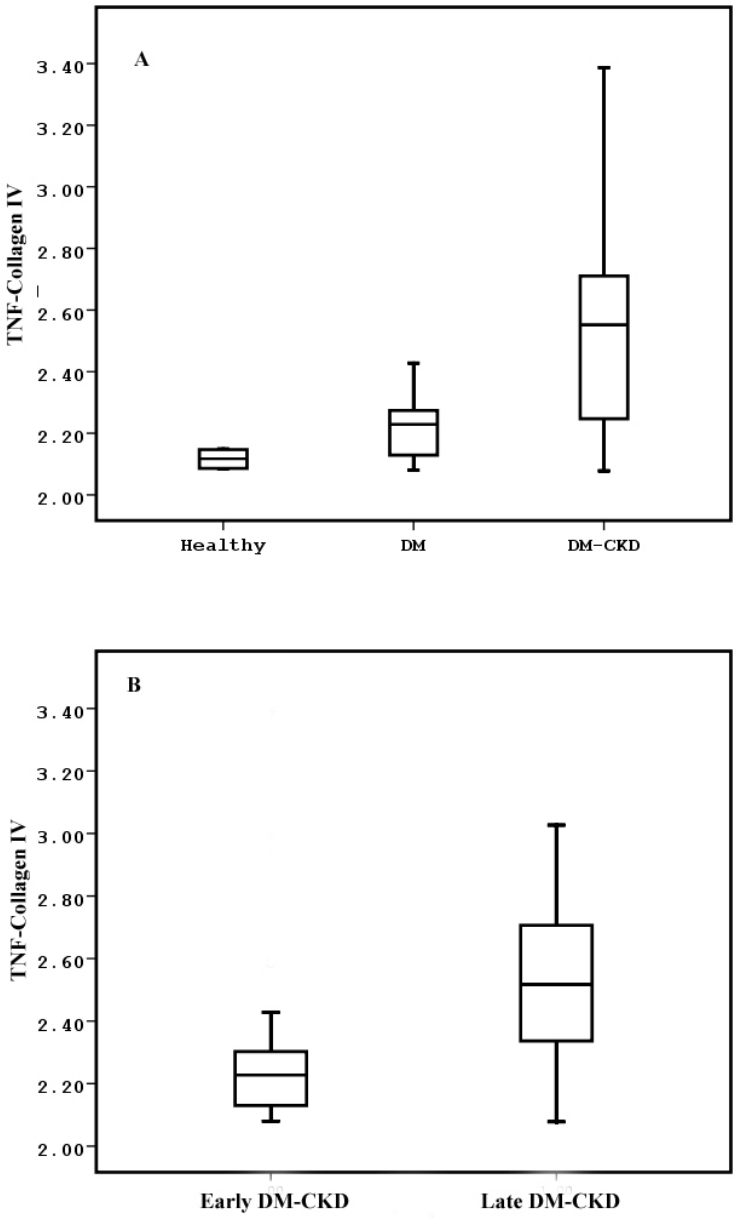

Figure 1. Level of TNF-Type IV collagen in study groups. A. level of TNF-Type IV collagen in healthy, DM, DM- DKD. B. A. level of TNF- Type IV collagen in early and late DM-DKD.

Table 3. Diagnostic power of single and combined TNF-α and Type IV collagen.

Markers	AUC (95% CI)	P value	Cut off	Sen	Spec	PPV	NPV
Healthy vs DM							
TNF-α	0.79 (0.64-0.93)	0.007	40	60	90	94	45
Type IV collagen	0.70 (0.53 – 0.83)	0.06	630	77	30	77	30
TNF + Type IV collagen	0.74 (0.59 – 0.89)	0.02	2.2	70	60	84	40
Healthy vs DM-DKD							
TNF-α	0.81 (0.70-0.90)	< 0.0001	40	70	70	92	30
Type IV collagen	0.73 (0.60-0.83)	0.002	630	67	70	92	29
TNF + Type IV collagen	0.93 (0.86-0.99)	< 0.0001	2.2	83	90	92	50
Diabetes vs DM-DKD							
TNF-α	0.60 (0.62-0.54)	0.11	40	67	40	65	39
Type IV collagen	0.77(0.66-0.88)	< 0.0001	630	69	73	82	58
TNF+ Type IV collagen	0.79 (0.48-0.71)	< 0.0001	2.2	86	40	71	63
Early DM-DKD vs late DM-DKD							
TNF-α	0.65 (0.46-0.74)	0.24	40	70	42	80	29
Type IV collagen	0.67 (0.53-0.80)	< 0.0001	630	38	92	93	31
TNF+ Type IV collagen	0.83 (0.74-0.89)	< 0.0001	2.2	86	52	70	75

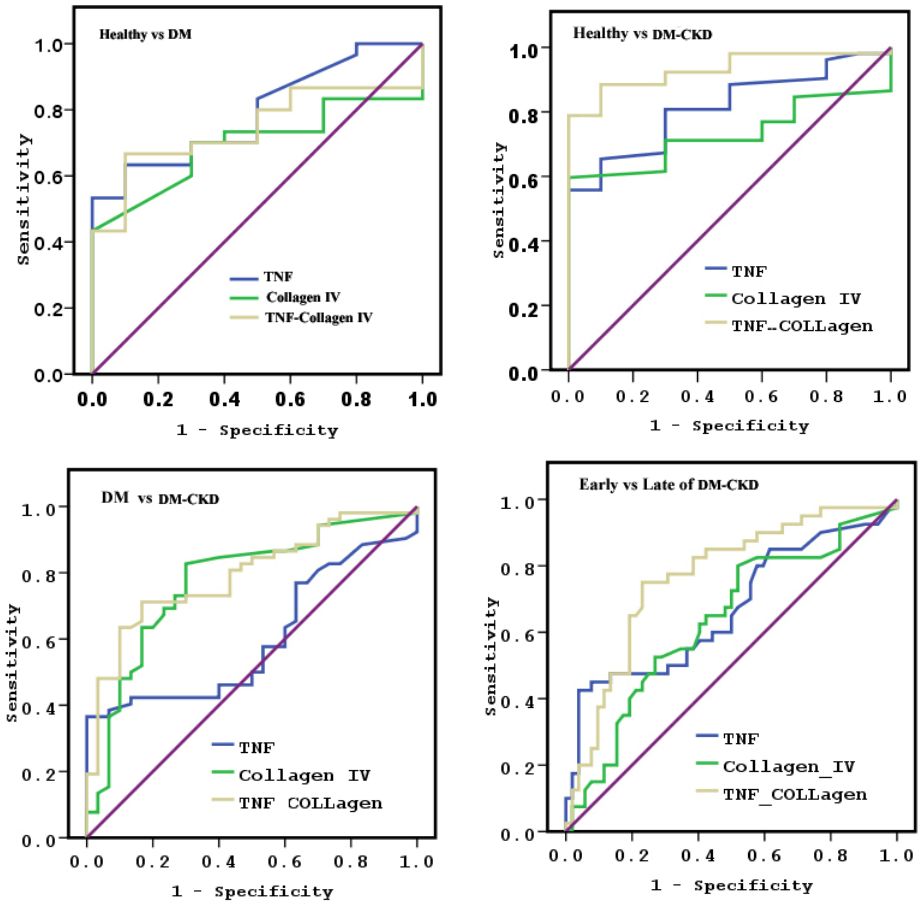

Figure 2. AUC of single and combined TNF and collagen-IV. **A**. For discriminated healthy vs DM. **B**. For discriminated healthy vs DM-DKD. **C**. For discriminated DM vs DM-DKD. **D.** For discriminated early vs late chronic kidney diseases

DISCUSSION:

Diabetic nephropathy is the main cause of chronic kidney disease and represents the most common and serious complication of diabetes (Lim, 2014). DN is characterized by the progressive accumulation of ECM and ECM turnover regulation depends on the balance between its synthesis and degradation (Garcia-Fernandez et al., 2020). There has been an important achievement in finding associated biomarkers in all aspects of diabetic nephropathy. Thus, there is now a focus on novel biomarkers that have higher sensitivity and specificity for earlier detection of DN and a more precise prediction of its progression to end stages (Gluhovschi et al., 2016; Uwaezuoke et al., 2017; Thipsawat, 2021; Yan et al., 2021). In the present study, the levels of candidate markers (TNF and type IV collagen) increased by $P < 0.001$ in DM- DKD than in DM patients. The inflammatory response could be activated by biochemical, metabolic, or hemodynamic disorders when a large number of white blood cells gather in the kidney. Pro-inflammatory cytokine such as TNF-α was significantly associated with DM. These results revealed that TNF-α might be an early biomarker of kidney damage in diabetic patients (Aghadavod et al., 2016). type IV collagen is the main component of the glomerular basement membrane and

extracellular matrix and does not pass through the glomerular filtration barrier under normal conditions. Therefore, type IV collagen could be used as a biomarker of basement membrane injury. type IV collagen could reflect morphological renal alterations in patients with type 2 DM (Bülow et al., 2019). High blood sugar enhances endogenous TGF-b1 production, which then works in an autocrine fashion on the cell to induce type IV collagen production. Hyperglycemia stimulates multiple inflammatory mechanisms directly and by gene transcription factors, resulting in protein accumulation in the nephron extracellular matrix with albumin leakage (Nikolov et al., 2016; Kostov et al., 2021; Cohen et al., 2001; Ban and Twigg, 2008; Gu et al., 2020; Cohen-Bucay et al., 2012; Mahendrane et al., 2016). In the present study, the TNF and type IV collagen negatively correlated with eGFR levels with significant p values = 0.001 and p = 0.006; respectively. The correlation between TNF and eGFR may reflect reduced renal filtration by the kidney. The levels of TNF measured in the present study increased overall with reduced eGFR (Kamei et al., 2018). In the present study, for discriminant between DM- DKD from DM patients without DKD, the most efficient marker was type IV collagen (AUC= 0.77) flowed by TNF-α (AUC=0.62).

ROC analysis revealed that TNF had better discrimina tion between patients with nephropathy and healthy controls than the

estimated glomerular filtration rate [0.87 vs 0.76) (Kamei et al., 2018). Using AUC analysis, type IV collagen had 0.91 (with 92% sensitivity and 89% specificity) for the normoalbumin group, 0.96 (with 92% sensitivity and 93% specificity) for microalbumin patients, and 0.99 (with 96% sensitivity and 94% specificity) for macroalbumin patients (Hamid et al., 2021). In the present study, the AUC of the developed index (TNF and type IV collagen) was 0.93; 0.79; 0.83 for discriminating DM with DKD from healthy, DM without DKD, and control. The urinary transferrin (Tf), immunoglobulin G (IgG), neutrophil gelatinase-associated lipocalin (NGAL), and TNF-α were significantly related to the albumin to creatinine ratios (UACR). The AUC were calculated; urinary IgG (0.89), NGAL (0.88), Tf (0.86), TNF-α (0.76), and the combination of urinary Tf + IgG + TNF-α + NGAL (0.92) showed good diagnostic value for early-stage DN (Zhang et al., 2019). In conclusion: a two-marker index (TNF-α and type IV collagen) could improve the diagnosis of diabetics with DKD with high sensitivity and specificity.

REFERENCES:

Aghadavod E, Khodadadi S, Baradaran A, Nasri P, Bahmani M, Rafieian-Kopaei M. Role of Oxidative Stress and Inflammatory Factors in Diabetic Kidney Disease. Iran J Kidney Dis. 2016; 10(6):337-343.

Alicic RZ, Rooney MT, Tuttle KR. Diabetic Kidney Disease: Challenges, Progress, and Possibilities. Clin J Am Soc Nephrol. 2017;12(12):2032-2045.

Ban CR, Twigg SM. Fibrosis in diabetes complications: pathogenic mechanisms and circulating and urinary markers. Vasc Health Risk Manag. 2008;4(3):575-96.

Bülow RD, Boor P. Extracellular Matrix in Kidney Fibrosis: More Than Just a Scaffold. J Histochem Cytochem. 2019; 67(9):643-661.

Chen YL, Qiao YC, Xu Y, Ling W, Pan YH, Huang YC, Geng LJ, Zhao HL, Zhang XX. Serum TNF-α concentrations in type 2 diabetes mellitus patients and diabetic nephropathy patients: A systematic review and meta-analysis. Immunol Lett. 2017;186:52-58.

Cohen MP, Shearman CW, Lautenslager GT. Serum type IV collagen in diabetic patients at risk for nephropathy. Diabetes Care. 2001;24(8):1324-7.

Cohen-Bucay A, Viswanathan G. Urinary markers of glomerular injury in diabetic nephropathy. Int J Nephrol. 2012;2012:146987.

Cosgrove D, Liu S. Collagen IV diseases: A focus on the glomerular basement membrane in Alport syndrome. Matrix Biol. 2017;57-58:45-54.

Donate-Correa J, Martín-Núñez E, Muros-de-Fuentes M, Mora-Fernández C, Navarro-González JF. Inflammatory cytokines in diabetic nephropathy. J Diabetes Res. 2015;2015:948417.

Garcia-Fernandez N, Jacobs-Cachá C, Mora-Gutiérrez JM, Vergara A, Orbe J, Soler MJ. Matrix Metalloproteinases in Diabetic Kidney Disease. J Clin Med. 2020;9(2):472.

Gheith O, Farouk N, Nampoory N, Halim MA, Al-Otaibi T. Diabetic kidney disease: world wide difference of prevalence and risk factors. J Nephropharmacol. 2015;5(1):49-56.

Gluhovschi C, Gluhovschi G, Petrica L, Timar R, Velciov S, Ionita I, Kaycsa A, Timar B. Urinary Biomarkers in the Assessment of Early Diabetic Nephropathy. J Diabetes Res. 2016;2016:4626125.

Gu YY, Liu XS, Huang XR, Yu XQ, Lan HY. Diverse Role of TGF-β in Kidney Disease. Front Cell Dev Biol. 2020;8:123.

Hamid GS, Ghudhaib KK, Allawi AA. Association of type IV collagen with progression of diabetic nephropathy disease in Iraqi patients under typical criteria. association of type iv collagen with progression of diabetic nephropathy disease in Iraqi patients under typical criteria. Biochem. Cell. Arch 2021; 21: 1471-1475.

Hung PH, Hsu YC, Chen TH, Lin CL. Recent Advances in Diabetic Kidney Diseases: From Kidney Injury to Kidney Fibrosis. Int J Mol Sci. 2021;22(21):11857.

Kamei N, Yamashita M, Nishizaki Y, Yanagisawa N, Nojiri S, Tanaka K, Yamashita Y, Shibata T, Murakoshi M, Suzuki Y, Gohda T. Association between circulating tumor necrosis factor-related biomarkers and estimated glomerular filtration rate in type 2 diabetes. Sci Rep. 2018;8(1):15302.

Kong L, Andrikopoulos S, MacIsaac RJ, Mackay LK, Nikolic-Paterson DJ, Torkamani N, Zafari N, Marin ECS, Ekinci EI. Role of the adaptive immune system in diabetic kidney disease. J Diabetes Investig. 2022;13(2):213-226.

Kostov K, Blazhev A. Serum Anti-Collagen IV IgM and IgG Antibodies as Indicators of Low Vascular Turnover of Collagen IV in Patients with Long-Term Complications of Type 2 Diabetes. Diagnostics (Basel). 2021;11(5):900.

Lim AKh. Diabetic nephropathy - complications and treatment. Int J Nephrol Renovasc Dis. 2014;7:361-81.

Mahendran KB, Bhaskar MV, Santha K, Inmozhi R, Perumal KK. Plasma and Urinary Type IV Collagen Levels for Early Detection of Nephropathy in Type 2 Diabetes Mellitus Patients. Int J Health Sci (Qassim). 2016;10(4):492-498.

Mahendran KB, Bhaskar MV, Santha K, Inmozhi R, Perumal KK. Plasma and Urinary Type IV Collagen Levels for Early Detection of Nephropathy in Type 2 Diabetes Mellitus Patients. Int J Health Sci (Qassim). 2016;10(4):492-498.

Miner JH. Type IV collagen and diabetic kidney disease. Nat Rev Nephrol. 2020;16(1):3-4.

Navarro JF, Mora-Fernández C. The role of TNF-alpha in diabetic nephropathy: pathogenic and therapeutic implications. Cytokine Growth Factor Rev. 2006;17(6):441-50.

Nikolov A, Tsinlikov I, Tsinlikova I, Nicoloff G, Blazhev A, Garev A. Serum anti-collagen type IV IgM antibodies and development of diabetic nephropathy in diabetics with essential hypertension. Cent Eur J Immunol. 2016;41(1):86-92.

Pugliese G, Penno G, Natali A, Barutta F, Di Paolo S, Reboldi G, Gesualdo L, De Nicola L; Italian Diabetes Society and the Italian Society of Nephrology. Diabetic kidney disease: new clinical and therapeutic issues. Joint position statement of the Italian Diabetes Society and the Italian Society of Nephrology on "The natural history of diabetic kidney disease and treatment of hyperglycemia in patients with type 2 diabetes and impaired renal function". J Nephrol. 2020;33(1):9-35.

Ramseyer VD, Garvin JL. Tumor necrosis factor-α: regulation of renal function and blood pressure. Am J Physiol Renal Physiol. 2013 304(10):F1231-42.

Thipsawat S. Early detection of diabetic nephropathy in patient with type 2 diabetes mellitus: A review of the literature. Diab Vasc Dis Res. 2021;18(6):14791641211058856.

Uwaezuoke SN. The role of novel biomarkers in predicting diabetic nephropathy: a review. Int J Nephrol Renovasc Dis. 2017;10:221-231.

Yan Z, Wang G, Shi X. Advances in the Progression and Prognosis Biomarkers of Chronic Kidney Disease. Front Pharmacol. 2021;12:785375.

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.