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Gene expression of connective tissue growth factor in relation to nephropathy in patients with type 2 diabetes

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Abstract: Diabetic Nephropathy (DN) is a chronic and progressive kidney disease that affects 45% of type 2 diabetes mellitus (T2DM) and type 1 diabetes mellitus (T1DM). DN is considered a major cause of renal failure globally. Connective tissue growth factor (CTGF) is an extracellular protein involved in the development of the DN. This study aims to prove the correlation between the CTGF and microalbuminuria and to determine the CTGF cut-off value at which we can diagnose microalbuminuria in DN patients. Our study is a cross-sectional study that included 75 patients with T2DM and 25 controls. We classified the patients into three groups, each group includes 25 patients; group1 (normoalbuminuria), group2 (microalbuminuria), and group3 (macroalbuminuria). Urine samples were collected from patients and control, centrifuged at ~1000 × g for 20 minute, then kept at 80 °C until real time PCR analysis. We found a significant difference between all groups regarding all variables (p-value < 0.001) except for age and uric acid (p-value 0.08 and 0.09) respectively. Spearman's correlation analysis of CTGF levels with different parameters in T2DM patients showed a significant positive correlation with creatinine, urea, fasting blood glucose (FBG),2 hours post prandial blood glucose (2h PPBG), glycosylated hemoglobin (HbA1c), and urine albumin to creatinine ratio (UACR) (p-value < 0.001). We did the multivariate regression analysis to detect the predictors of CTGF expression in diabetic patients, and we found that HbA1c and UACR were only the independent significant predictor of CTGF expression in diabetic patients (P<0.03 and 0.0001) respectively. Also, we found that the optimum cut-off value of CTGF for the identification of microalbuminuria is 1.085 pg/ml with 100% sensitivity and specificity. In conclusion, we confirm a significant elevation of CTGF in patients with DN. Also, we confirm that CTGF is considered a sensitive marker for the presence of microalbuminuria in patients with T2DM.

Keywords: Diabetic Nephropathy (DN), Connective Tissue Growth Factor (CTGF), Diabetes Mellitus (DM).

1. INTRODUCTION

Diabetes mellitus (DM), one of the metabolic disorders, which is characterized by hyperglycemia [1]. The most common forms of diabetes are; type 1 DM, in which absolute insulin deficiency is due to pancreatic beta cells destruction, and type 2 DM, in which hyperglycemia is due to insulin resistance [2]. The cause of type 1 DM is still unknown; however, it may be due to the interaction between environmental and genetic factors. Unhealthy food, obesity, sedentary life, and history of diabetes in the family are the main risk factors for type 2 DM [3]. In Egypt, the main risk factors include obesity, physical inactivity, pesticide exposure, chronic hepatitis C infection, smoking, and sedentary lifestyle [4].(DM prevalence is increasing worldwide, it increased in men from 4.3 to 9.0% in 2014, and in women from 5.0% to 7.9% [5].

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Egypt is considered one of the top ten diabetic countries worldwide, as listed by the International Diabetes Federation (IDF). Number of cases in adult Egyptians, which was reported by the IDF, is 10.9 million, with a prevalence of 20.9 [6]. Criteria for diagnosis of DM as described by the American Diabetes Association 2022; fasting blood glucose (FBG) ≥ 126 mg/dL, 2 hour post prandial blood glucose ≥ 200 mg/dL, Hemoglobin A1C ≥ 6.5%, a random plasma glucose ≥ 200 mg/dL in a symptomatic patients [7]. Diabetic patients are clinically presented with polydipsia, polyuria, and polyphagia and may be presented with complications such as hyperglycaemic hyperosmolar state, diabetic ketoacidosis (DKA), and hypoglycemia [8]. Vascular complications of the DM are considered the leading cause for death in those patients. These complications include macrovascular complications, which affect the cardiovascular system, and microvascular complications, such as diabetic retinopathy, neuropathy, and nephropathy [9].

Diabetic Nephropathy (DN) is a chronic and progressive kidney disease that affects 45% of type 1 and type 2 DM and develops about after 10–20 years of diabetes [10]. DN is considered a major cause of renal failure globally. Glomerular changes are the first to appear in DN. These glomerular changes include diffuse mesangial cell expansion and glomerular basement membrane thickening. This diffuse expansion produces a nodular collection of the mesangial matrix. these nodules are called Kimmelstiel-Wilson nodules which are present in the advanced stages. Glomerular changes may be associated with extra glomerular lesions such as interstitial inflammation, tubular atrophy, and tubulointerstitial fibrosis [11]. There are multiple pathways for the progression of DN. These pathways include pathway connective tissue growth factor (CTGF), pathway renin-angiotensin-aldosterone system, pathway formation of the advanced glycation end-product, pathway protein kinase C, pathway transforming growth factor-β1 activation, and pathway mitogen-activated protein kinase activation. Each of these pathways damages through multiple mediators or combined with another pathway [12].

Management of DN is a combination of multiple factors, including lifestyle modification and good control of blood glucose level, blood pressure, and lipid profile. However, the main (drugs) for the treatment of DN are angiotensin converting enzyme (ACE) inhibitors or the angiotensin II receptor blockers which decrease the level of proteinuria and will control the blood pressure [10]. Screening and early diagnosis of DN are crucial for all diabetic patients. Any diabetic patient should be screened for microalbuminuria. Although the albumin/creatinine ratio of 2.5 is the cut-off for microalbuminuria, albumin may be increased in the urine due to fever, urinary tract infection, and hypertension [13]. So, the patient should repeat the test over the following 3-6 months or measure another marker that will be elevated with DN, such as connective tissue growth factor (CTGF). CTGF is an extracellular protein involved in the development and progression of the DN. Some authors found that CTGF correlates with microalbuminuria which may be used as a biomarker for diagnosis of DN [14]. So, this study aims to furtherly investigate the correlation between the CTGF and microalbuminuria and to determine the CTGF cut-off value at which we can diagnose microalbuminuria in DN patients.

2. METHODS

2.1. Study populations

Our study design is a cross sectional. This study was done in faculty of pharmacy (girls), Al-Azhar University. Our research complied with the Helsinki Declaration's principles. Our data was protected confidentially. We included 75 patients with type 2 DM and 25 controls. We classified the patients according to the albuminuria into three groups, each group includes 25 patients; group1 (normoalbuminuria), group2 (microalbuminuria), and group3 (macroalbuminuria). Microalbuminuria was diagnosed if the albumin excretion rate (AER) was 20 - 200 µg/min. Macroalbuminuria was diagnosed by an AER 200 µg/min. We recruited the patient according to the following criteria:

2.1.1. The inclusion criteria

We included patients with type 2 DM with normo or micro or macroalbuminuria.

2.1.2. The exclusion criteria

Type 1 DM patients, Patients with upper or lower respiratory tract infection, Patients with a gastrointestinal infection, fever of unknown origin, patients with chronic liver disease, patients with cardiovascular disease, Patients with autoimmune disorders, patients with diseases that may increase the urinary excretion of protein as nephritic syndrome, dehydration conditions, patient with uncontrolled blood pressure, patients were having disturbed glomerular filtration rate (GFR) without albuminuria and patients on some drugs such as anti-inflammatory drugs, corticosteroids, ACE inhibitors, angiotensin II receptor blockers, and alcohol.

2.2. Data collection

Complete medical history was recorded and physical examination was performed to each patient during enrollment. Diagnosed Type 2DM patients were screened for diabetes duration, risk factors for heart disease, treatment history, family history, alcohol intake history, age, and other chronic illness.

Investigations including serum creatinine, serum uric acid, FBG, 2h PPBG ,HbA1c, eGFR using Cockroft and goult equation, UACR, CTGF measurement, and Abdominal Ultrasound were done to determine the kidney size and echogenicity.

2.3. Connective tissue growth factor assay

Urine samples collected from patients and healthy people were centrifuged at ∼1000 × g for 20 minute, then stored at − 80°C. Tissue homogenate was processed for RNA extraction followed by Reverse Transcriptase (for cDNA synthesis) and quantitative real-time PCR to quantify CTGF level in ng/ml.

Statistical analysis

All statistical analysis were done using the SPSS version 25 (IBM Corp., Armonk., NY., USA). Continuous variables were firstly examined for normality using the Shapiro–Wilk test. For parametric data, we presented with the mean and standard deviations. For non-parametric data, we presented them as median and interquartile range. For none parametric data we used the Kruskal–Wallis test to compare all four groups and followed by Mann–Whitney U-test to compare each two groups. for parametric variables; One Way ANOVA test was used to compare all four groups. Spearman's correlation was done to test for linear relations between variables. We did the multivariate regression analysis to investigate the predictors of CTGF expression in diabetic patients. ROC curve was plotted to determine the sensitivity, specificity, and cut-off value of CTGF as a marker of albuminuria.

3. RESULTS

Data of patients and controls are presented in (**Table 1)** We found a significant difference between all groups regarding all variables (p-value < 0.001) except for age and uric acid (p-value 0.08 and 0.09).

Table 1. Characteristics of the patients and control and comparison between them as regarding different variables**.**

	Group 1 (n=25) Type 2 DM patients with normoalbuminuria	**Group 2 (n=25) Type 2 DM patients with microalbuminuria**	**Group 3 (n=25) Type 2 DM patients with macroalbuminuria**	**Control (n=25)**	**P value within groups**	**P value between 2 groups**
Age (years) *	55.6 ± 10.33	53.92 ± 7.96	57.08 ± 9.42	50.16 ± 11.46	0.08	**P 1 = 0.5** **P 2 = 0.5** **P 3 = 0.08** **P 4 = 0.2** **P 5 = 0.1** **P 6 = 0.02 ****
Creatinine (mg/dl) ŧ	1.27 [1.04 – 1.5]	1.5 [1.3 – 1.7]	1.5 [1.31– 1.85]	0.9 [0.7 – 1]	0.0001	**P 1 < 0.009**** **P 2 < 0.03**** **P 3 < 0.001**** **P 4 = 0.5** **P 5 < 0.001**** **P 6 < 0.001****
Urea (mg/dl) *	50.3 ± 20.42	60.92 ± 13.85	68.56 ± 23.45	12.52 ± 4.12	0.0001	**P 1 < 0.03**** **P 2 < 0.005**** **P 3 < 0.001**** **P 4 = 0.1** **P 5 < 0.001**** **P 6 < 0.001****
Uric Acid (mg/dl) *	5.08 ± 1.49	5.73 ± 1.37	5.43 ± 1.56	4.74 ± 1.39	0.097	**P 1= 0.1** **P 2 = 0.4** **P 3 = 0.4** **P 4 = 0.4** **P 5 < 0.01**** **P 6 = 0.1**

Continue Table 1:

	Group 1 (n=25) Type 2 DM patients with normoalbuminuria	Group 2 (n=25) Type 2 DM patients with microalbuminuria	Group 3 (n=25) Type 2 DM patients with macroalbuminuria	Control (n=25)	P value within groups	P value between 2 groups
FBG (mg/dl) ŧ	150 [122.5 – 165]	175 [152 – 198.5]	225 [163.5 – 272.5]	86 [79 – 92.5]	0.0001	**P 1 < 0.01**** **P 2 < 0.001**** **P 3 < 0.001**** **P 4 < 0.003**** **P 5 < 0.001**** **P 6 < 0.001****
2h PPBG (mg/dl) ŧ	265.3 [231.5 – 301.5]	280 [240 – 375]	365 [274.5 – 404]	137 [128.5 – 146]	0.0001	**P 1 < 0.2** **P 2 < 0.001**** **P 3 < 0.001**** **P 4 = 0.07** **P 5 < 0.001**** **P 6 < 0.001****
HbA1c (glycosylated hemoglobin %) *	8.36 ± 1.58	9.93 ± 1.24	10.78 ± 1.74	4.89 ± 0.74	0.0001	**P 1 < 0.001**** **P 2 < 0.001**** **P 3 < 0.001**** **P 4 = 0.053** **P 5 < 0.001**** **P 6 < 0.001****
UACR (mg Alb / g creat) ŧ	10.2 [6.6 – 14.75]	116 [92 – 187]	549 [400 – 1036]	13.7 [8.1 – 17.55]	0.0001	**P 1 < 0.001**** **P 2 < 0.001**** **P 3 = 0.1** **P 4 < 0.001**** **P 5 < 0.001**** **P 6 < 0.001****
CTGF(ng/ml) ŧ	1.7 [1.12 – 2.1]	2.4 [1.4–4.25]	4.8 [3.45 – 7.45]	1.01 [1 – 1.02]	0.0001	**P 1 < 0.01**** **P 2 < 0.001**** **P 3 < 0.001**** **P 4 < 0.001**** **P 5 < 0.001**** **P 6 < 0.001****

Variables represented as (mean±SD) and compared with One Way ANOVA test and independent t-test, ŧ Variables represented as (median and IQR) and compared with Kruskal Wallis test between all groups and Mann–Whitney U-test to compare between each 2 groups. P1:** comparison between group1 and group2**, P2:** comparison between group1 and group3, **P3:** comparison between group1 and control group, **P4:** comparison between group2 and group3, **P5**: comparison between group2 and control group, **P6**: comparison between group3 and control group. **HbA1c**, glycated hemoglobin**; UACR,** urinary albumin–creatinine ratio**; CTGF,** connective tissue growth factor**; FBG,** fasting blood glucose**; 2hPPBG,** 2 hour post prandial blood glucose. *** statistically significant p value**.**

Group 3 including "25 patients with Type 2 DM with macroalbuminuriahad" had the highest CTGF level in comparison to groups 1 "25 Type 2 DM patients with normoalbuminuria", 2 "25 Type 2 DM patients with microalbuminuriaand", and control group (p-value < 0.001). However, CTGF level was lower in groups 1 than 2 and control group have the lowest CTGF level (p-value < 0.001). we compared each group with the control group and we found a statistically significant difference between the patients and control.

UACR was significantly different between patients in in groups 2 and 3, relative to control. However, there was no statistically significant difference between group 1 and control group in regard to UACR (p-value = 0.1). Spearman's correlation analysis of CTGF levels with different parameters in T2DM patients showed a significant positive correlation with creatinine, urea, FBG, 2h PPBG, HbA1c, and UACR (p-value < 0.001) **(Figure 1)**. However no significant correlation between age and uric acid level (p-value 0.1 and 0.5) was detected **(Table 2).**

Multivariate regression analysis was performed to investigate predictors of CTGF expression in diabetic patients **(Table 3)**. HbA1c and UACR were only the independent significant predictors of

CTGF expression in diabetic patients (P<0.03 and 0.0001) respectively. By drawing the ROC curve, we found the optimum cut of value of CTGF for the identification of microalbuminuria = 1.085 pg/ml with 100% sensitivity and specificity **(Figure 2).**

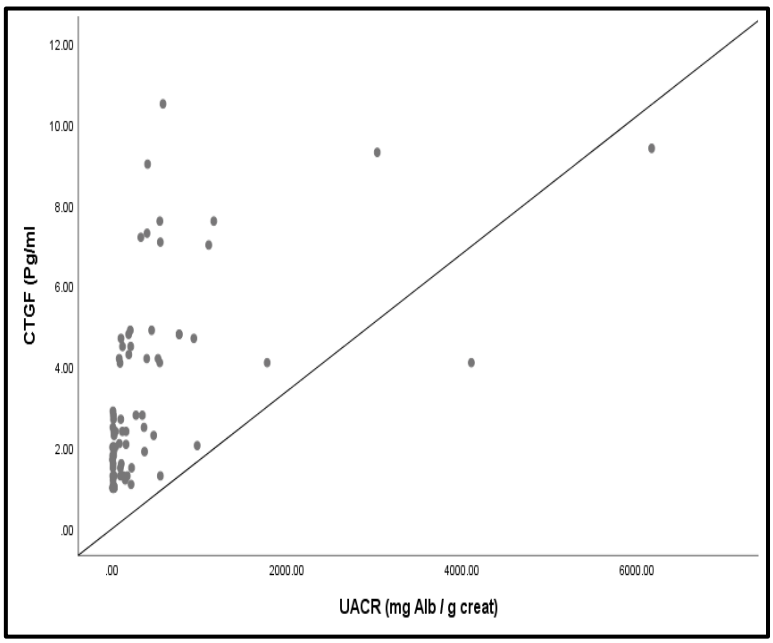

Figure 1. Spearman's correlation correlation between CTGF and UACR showing positive correlation.

Table 2. Correlation analysis of CTGF level with different parameters in T2DM patient

Parameters	CTGF (ng/ml)	
	r	P-value
Age	0.13	0.18
Creatinine (mg/dl)	0.47	0.001
Urea (mg/dl)	0.59	0.001
Uric Acid (mg/dl)	0.06	0.5
FBG (mg/dl)	0.6	0.001
2h PPBG (mg/dl)	0.56	0.001
HbA1c (glycosylated hemoglobin %)	0.63	0.001
UACR (mg Alb / g creat)	0.66	0.001

Table 3. Multivariate regression analysis to investigate predictors of CTGF expression in diabetic patients.

	CTGF (ng/ml)	
	Beta coefficient	**P value**
Creatinine (mg/dl)	-0.046	0.4
Urea (mg/dl)	0.002	0.8
FBG (mg/dl)	0.005	0.2
2h PPBG (mg/dl)	0.001	0.9
HbA1c (glycosylated hemoglobin %)	0.29	0.03
UACR (mg Alb / g creat)	0.001	0.0001

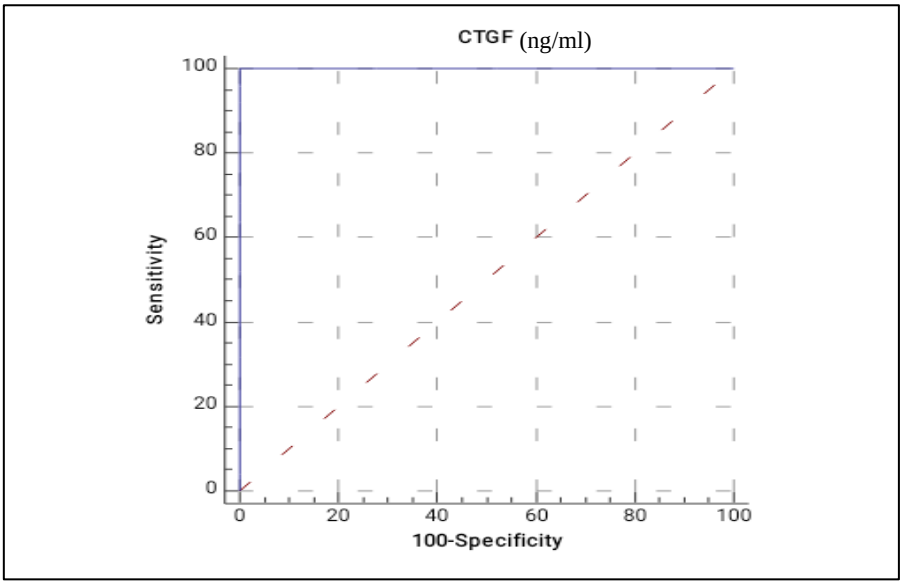

Figure 2. ROC curve of CTGF for prediction of microalbuminuria. Receiver operator characteristic (ROC) curve of CTGF for prediction of microalbuminuria at cut-off value ≥1.085 (mg/dl) had 100% sensitivity and specificity. Area under the curve was 1 and P<0.0001. CTGF; connective tissue growth factor (ng/ml)

4. DISCUSSION

Our study included 75 patients, divided into 3 groups according to the degree of albuminuria. The mean age of patients in each group was above 50 years old. This increase in the age of the patients agrees with Tziomalos *et a*l. 2015 who suggested that the old age is a risk factor for DN [15]. The association between advanced age and DN may be due to that prolonged exposure of those patients to hyperglycemia puts their kidney at the risk of developing nephropathy at old age.

In the present study, positive correlations between the CTGF level and creatinine, urea, FBG, 2h PPBG, HbA1c, and UACR (p-value < 0.001) were detected. Such results suggested that any elevation in the level of CTGF level may be associated with a decrease in the renal function, represented by elevation of the indicated parameter. HbA1c and UACR are the only independent significant predictor of CTGF expression in diabetic patients (P< 0.03 and 0.0001) respectively. That indicates if the level of HbA1c and UACR were elevated, we could predict upregulated expression of the CTGF.

CTGF level may be used as a screening tool for early detection of microalbuminuria with a cut-off value = of 1.085 pg/ml with 100% sensitivity and specificity. Elevation of the CTGF with kidney disease is explained by that CTGF is a cysteine-rich growth regulator upregulated by hyperglycemia. This factor promotes the mesangial cell hypertrophy, enhances the fibroblast proliferation in the kidney, and increases the extracellular matrix (ECM) accumulation [16], [17]. Therefore, plasma level of this factor in the diabetic patient may give information about the development of DN and other diabetic complications. (Karin G. F. Gerritsen et al.) suggesting that CTGF is elevated in DN due to increased local production of CTGF and decreased reabsorption as a result of tubular damage [14].

In agreement with our findings, CTGF was previously measured in the urine of DN patients to reveal the significant elevation of urinary CTGF in those patients and prove its correlation with UACR. Furthermore it has been reported that urinary CTGF is a sensitive marker for microalbuminuria (Mervat et al). These results agrees with our findings. Another study done by (Peggy Roestenberg, et al. 2004) measured the plasma N-terminal fragments of CTGF (CTGF-N), and they found that CTGF-N elevates in the diabetic patient; however, they found no significant difference between the total diabetic patients and controls, which disagree with our finding. This disagreement may be due to their smaller sample size, the type of their population was only type 1 DM, and they measured the CTGF-N. However, they found a significant difference between DN patients and the control group, which agrees with our findings. They also agree with us in the correlation between CTGF and HbA1c level (R=0.355, P = 0.005) and albuminuria (R=0.572, P < 0.001) [18]. An experimental study done by (Karin G. F. Gerritsen et al. 2015), found that CTGF Is Increased in the Kidney of diabetic patients, mainly in Medullary Tubules and Glomeruli, in consistence with our result [14].

Our study provides evidence of elevation of CTGF in diabetic patients secondary to hyperglycemia. Our study provides a certain cut-off value for CTGF level at which we can detect

microalbuminuria. A great strength point of our study is that this study recommends a screening tool for the early detection of microalbuminuria in diabetic patients. However, we have some limitations, such as the small sample size in each group. Also, our study is cross-sectional that cannot suggest the causal associations. Therefore., the effect of hyperglycemia on CTGF expression and the effect of CTGF on kidney functions needs more studies in the future. We recommend the CTGF as a marker for DN screening.

5. CONCLUSIONS

This study confirms the significant elevation of CTGF in patients with DN and its correlation with UACR as markers of disease severity. Furthermore, CTGF is suggested to be a sensitive marker for the presence of microalbuminuria in patients with T2DM.

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Conflicts of Interest: The authors declare that they have no competing interests.

Ethical Statement: The study was performed in line with the ethical rules and policies set out by the Ethics Committee of Faculty of Pharmacy, (Girls), Al-Azhar University (No. 194).

Author Contribution: All authors contributed in all steps of the work.

List of Abbreviations: CTGF: Connective tissue growth factor, T2DM: Type 2 diabetes mellitus, DN: Diabetic Nephropathy, T1DM: Type 1 diabetes mellitus, FBG: Fasting blood glucose, 2h PPBG: 2 hours post prandial blood glucose, HbA1c: Glycosylated hemoglobin, UACR: Urine albumin to creatinine ratio, DM: Diabetes mellitus, IDF: International Diabetes Federation, DKA: Diabetic ketoacidosis, ACE: Angiotensin converting enzyme inhibitors, AER: Albumin excretion rate .

REFERENCES

1. Petersmann A, Müller-Wieland D, Müller UA, Landgraf R, Nauck M, Freckmann G, et al. Definition, Classification and Diagnosis of Diabetes Mellitus. Exp Clin Endocrinol Diabetes [Internet]. 2019 Dec 20;127(S 01):S1–7.

2. Schmidt AM. Highlighting Diabetes Mellitus. Arterioscler Thromb Vasc Biol [Internet]. 2018 Jan;38(1).

3. Lovic D, Piperidou A, Zografou I, Grassos H, Pittaras A, Manolis A. The Growing Epidemic of Diabetes Mellitus. Curr Vasc Pharmacol [Internet]. 2020 Jan 27;18(2):104–9.

4. Hegazi R, El-Gamal M, Abdel-Hady N, Hamdy O. Epidemiology of and Risk Factors for Type 2 Diabetes in Egypt. Ann Glob Heal [Internet]. 2016 Apr 22;81(6):814.

5. Ding L, Xu Y, Liu S, Bi Y, Xu Y. Hemoglobin A1c and diagnosis of diabetes. J Diabetes [Internet]. 2018 May;10(5):365–72.

6. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, et al. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes Res Clin Pract [Internet]. 2022 Jan;183:109119.

7. Professional Practice Committee: Standards of Medical Care in Diabetes—2022. Diabetes Care [Internet]. 2022 Jan 1;45(Supplement_1):S3–S3.

8. Bettencourt-Silva R, Aguiar B, Sá-Araújo V, Barreira R, Guedes V, Marques Ribeiro MJ, et al. Diabetes-related symptoms, acute complications and management of diabetes mellitus of patients who are receiving palliative care: a protocol for a systematic review. BMJ Open [Internet]. 2019 Jun 14;9(6):e028604.

9. Cole JB, Florez JC. Genetics of diabetes mellitus and diabetes complications. Nat Rev Nephrol [Internet]. 2020 Jul 12;16(7):377–90.

10. Sagoo MK, Gnudi L. Diabetic Nephropathy: An Overview. In 2020. p. 3–7.

11. Qi C, Mao X, Zhang Z, Wu H. Classification and Differential Diagnosis of Diabetic Nephropathy. J Diabetes Res [Internet]. 2017;2017:1–7.

12. Samsu N. Diabetic Nephropathy:

Challenges in Pathogenesis, Diagnosis, and Treatment. Bellini MI, editor. Biomed Res Int [Internet]. 2021 Jul 8;2021:1–17.

13. Nazar CMJ. Diabetic nephropathy; principles of diagnosis and treatment of diabetic kidney disease. J nephropharmacology [Internet]. 2014;3(1):15–20.

14. Gerritsen KGF, Leeuwis JW, Koeners MP, Bakker SJL, van Oeveren W, Aten J, et al. Elevated Urinary Connective Tissue Growth Factor in Diabetic Nephropathy Is Caused by Local Production and Tubular Dysfunction. J Diabetes Res [Internet]. 2015;2015:1–11.

15. Tziomalos K, Athyros VG. Diabetic Nephropathy: New Risk Factors and Improvements in Diagnosis. Rev Diabet Stud [Internet]. 2015;12(1–2):110–8.

16. Expression of connective tissue growth factor in human renal fibrosis. 1998;53:853–61.

17. Wahab ANA. Glomerular expression of thrombospondin-1 , transforming growth factor-beta and connective tissue growth factor at different stages of diabetic nephropathy and their interdependent roles in mesangial response to diabetic stimuli. 2005;2650–60.

18. Roestenberg P, Van Nieuwenhoven FA, Wieten L, Boer P, Diekman T, Tiller AM, et al. Connective Tissue Growth Factor is Increased in Plasma of Type 1 Diabetic Patients with Nephropathy. Diabetes Care. 2004;27(5):1164–70.