

The Role of Urinary Connective Tissue Growth Factor in Early Detection of Diabetic Nephropathy in Elderly Type 2 Diabetics.

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Abstract:

Levels of connective tissue growth factor (CTGF; CCN-2) in plasma are increased in various fibrotic disorders, including diabetic nephropathy. Recently, several articles have reported a strong increase of urinary CTGF excretion (U.CTGF) in patients with diabetic nephropathy. **Objective:** This study evaluated U.CTGF in elderly patients with type 2 diabetics and its correlation with kidney functions tests, and degree of albuminuria. **Subjects:** This study included 45 elderly type 2 diabetic patients (group I) and compared with 15 elderly healthy controls (group II). **Method:** All subjects in this study were subjected to thorough history taking and clinical examination. Laboratory investigations done included: CBC, blood urea, serum creatinine, eGFR using MDRD method, ACR, complete urine analysis, fasting FBG, 2hppg,

HbA1c, U.CTGF was measured by sandwich ELISA.

Results: U.CTGF in patients with microalbuminuria (mean 5.05 ± 1.14 pg/mg creatinine [range 3.70 – 7.50]) was significantly higher than those with microalbuminuria (mean 1.71 ± 0.22 pg/mg creatinine [range 1.50– 2.20]), norm albuminuria (mean 0.25 ± 0.05 pg/mg creatinine [range 0.20 – 0.30]), and control subjects (mean 0.17 ± 0.06 pg/mg creatinine [range 0.10 – 0.30]).

Conclusions: U.CTGF level increased in elderly type 2 diabetes. The observed association of U.CTGF with albumin creatinine ratio (ACR) and glomerular filtration rate (GFR) might reflect a role of CTGF as a progression promoter in diabetic nephropathy.

Keywords: Connective Tissue Growth Factor, Diabetic Nephropathy, Elderly, T2DM.

Introduction:

Connective tissue growth factor (CTGF), a cysteine-rich, heparin-binding 38 KD growth factor protein⁽¹⁾ is expressed in mesenchymal cells such as fibroblasts and smooth muscle cells.⁽²⁾ In these latter cell types CTGF is up-regulated by TGF- β , which acts through a distinct element in the promoter region of the CTGF gene.⁽³⁾

Connective tissue growth factor (CTGF) has been associated with numerous fibrotic disorders, but it is of particular interest to diabetic nephropathy. Soon after its identification, the expression of CTGF mRNA was shown to be strongly up-regulated in human mesangial cells cultured under high glucose and in renal biopsies of patients with diabetic nephropathy.⁽⁴⁾

Abbreviations:

CBC: complete blood count, **e GFR:** estimated glomerular filtration rate, **ACR:** urinary albumin creatinine ratio, **FBG:** fasting blood glucose, **2hppg:** 2hours postprandial blood glucose

Connective tissue growth factor (CTGF) over expression in podocytes was critically involved in diabetes-related GBM thickening. Once induced, CTGF is able to initiate changes in ECM composition: it increased expression of fibronectin and enhanced fibronectin assembly into an insoluble matrix, increased type IV, type III and type I collagen production and up-regulated integrins on the cell surface, facilitating the deposition and assembly of ECM proteins.⁽⁵⁾

Understanding the different determinants of elevated U.CTGF in diabetes is essential for its proper interpretation as a biomarker. In the healthy kidney, filtered CTGF is almost completely reabsorbed in the proximal tubules by megalin-mediated endocytosis and that impairment of tubular reabsorption results in increased urinary excretion of CTGF. Reduced proximal reabsorption of U.CTGF, increased release of CTGF from damaged distal tubules also play a role with the majority of U.CTGF originating from an intrarenal source.⁽⁶⁾

CTGF levels were markedly increased in both plasma and urine of patients with diabetic nephropathy, but not in diabetic patients without nephropathy.⁽⁷⁾ Therefore, we set out to analyze U.CTGF in elderly patients with type 2 diabetes and how U.CTGF levels relate to clinical parameters associated with severity of diabetic nephropathy.

Research Design and Methods:

For the present study, CTGF levels were determined in early morning voided spot urine samples from 45 elderly type 2 diabetic patients and 15 healthy control subjects.

Patients were selected from the Renal and /or Diabetes Outpatient Clinics of Alexandria Main University Hospital for a study of diabetic nephropathy. Diabetic patients were categorized as having normoalbuminuria when ACR was persistently < 30 mg/g creatinine; microalbuminuria when ACR was between 30 and 300 mg/g creatinine of at least two of three consecutive visits to the outpatient clinic; macroalbuminuria if they had persistent albuminuria >300 mg/g creatinine and no other kidney or renal tract disease. Demographic and clinical data were recorded, including age, sex, and duration of diabetes, weight, height, blood pressure and medication. ACR was determined in early morning voided spot urine samples by turbidimetry.⁽⁸⁾ In venous blood samples, serum creatinine, blood urea was determined,⁽⁹⁾ and HbA1c was measured by affinity chromatography.⁽¹⁰⁾ Estimated glomerular filtration rate (eGFR) was calculated by the Modification of Diet in Renal Disease study equation (MDRD).⁽¹¹⁾ The study was performed after approval of the ethical committee of Alexandria University. All patients and control subjects gave their informed consent.

Enzyme-linked immunosorbent assay for U.CTGF

Urine content of CTGF was determined by a sandwich ELISA using 2 distinct monoclonal antibodies against the CTGF protein. The catching and detecting monoclonal antibodies bind distinct epitopes on the N-terminal half of the protein. This assay detects both CTGF N-terminal half fragments and the full length CTGF protein with detection limit of approximately

31.2-2,000 pg/ml. The microplate reader was read at 450 nm immediately. ⁽¹²⁾

Statistical analysis:

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. Qualitative data were described using number and percent. Quantitative data were described using range (minimum and maximum) mean, standard deviation and median. Comparison between different groups regarding categorical variables was tested using Chi-square test. For normally distributed data, comparison between the different groups were done using F-test (ANOVA) and Post Hoc test (Tukey's test) for pair wise comparison while for abnormally distributed data, comparison were done using Kruskal Wallis test and pair wise comparison was assessed using Mann-Whitney test.

Results:

U.CTGF excretion is increased in patients with diabetic nephropathy

General characteristics and clinical parameters of healthy subjects and diabetic patients are summarized in Table I. The U.CTGF/Creatinine ratio in macroalbuminuric group (mean 5.05 ± 1.14 pg/mg creatinine) was significantly higher than in microalbuminuric group (mean 1.71 ± 0.22 pg/mg creatinine) and normoalbuminuric group (mean 0.25 ± 0.05 pg/mg creatinine) and control subjects (mean 0.17 ± 0.06 pg/mg creatinine). A significant difference in U.CTGF/Creatinine ratio was observed between all studied groups and control ($p \leq 0.001$). There was statistically significant difference between normoalbuminuric group, microalbuminuric group

and macroalbuminuric. There was statistically significant difference between microalbuminuric group and macroalbuminuric ($p < 0.001$).

U.CTGF/Creatinine correlates with albuminuria (ACR) and with declined renal function as regard eGFR (tables II,III)

U.CTGF correlated with ACR in all patients ($R=0.979$, $P < 0.001$) and also in the subgroups of microalbuminuria ($R= 0.981$, $P < 0.001$) and macroalbuminuria ($R= 0.999$, $P < 0.001$).

We estimated GFR using Modification of Diet in Renal Disease study method described by Levey et al. ⁽¹¹⁾ U.CTGF inversely correlated with estimated GFR in all patients ($R= 0.796$, $p < 0.001$). When subgroups of patients were examined separately, this correlation was strongest in macroalbuminuric group ($R=-0.966$, $p < 0.001$) and also present in microalbuminuric group ($R= - 0.741$, $p = 0.002$).

Correlations between U.CTGF/Creatinine and different studied clinical Parameters (tables II,III)

In this study there was significant correlation between U.CTGF/Creatinine ratio, duration, HbA1c, Creatinine, systolic, and diastolic blood pressure in all patients ($p < 0.001$).

By analysis of these data in each group of this study we found that: the Correlation between U.CTGF/Creatinine ratio with duration, HbA1c, S. Creatinine, systolic and diastolic blood pressure was statistically significant only in microalbuminuric group (duration $p = 0.001$), (HbA1c $p = 0.008$), (S. Creatinine $p = 0.015$), (systolic $p = 0.001$), (diastolic $p < 0.001$).

Table (I): Comparison between the different studied groups according to different parameters

	Normal (Control) (n = 15)	Albuminuria			P
		Normo (n = 15)	Micro (n = 15)	Macro (n = 15)	
Sex					
Male	7 (46.7%)	7 (46.7%)	6 (40.0%)	8 (53.3%)	0.911
Female	8 (53.3%)	8 (53.3%)	9 (60.0%)	7 (46.7%)	
Age (in years)	62.93 ± 2.15	63.80 ± 3.63	62.67 ± 2.55	62.0 ± 1.81	0.316
Duration (in years)	-	1.0 (0.42 – 3.0)	5.0 (3.0 – 7.0)	12(10.0 – 15.0)	<0.001*
Sig.bet. Grps	-	p ₁ <0.001*, p ₂ <0.001*, p ₃ <0.001*			
FBG (mg/dl)	96.60 ± 8.69	190.47 ± 39.40	189.13 ± 33.12	250.33 ± 44.87	
p_{Con.}	-	<0.001*	<0.001*	<0.001*	<0.001*
Sig.bet. Grps	-	p ₁ =1.000, p ₂ <0.001*, p ₃ <0.001*			
Hb A1C %	5.0 – 5.80	8.25 ± 0.45	8.60 ± 0.76	9.78 ± 1.05	
p_{Con.}	-	<0.001*	<0.001*	<0.001*	<0.001*
Sig.bet. Grps	-	p ₁ = 0.517, p ₂ <0.001*, p ₃ <0.001*			
S. Creat. (mg/dl)	0.70 (0.60 – 0.90)	0.70 (0.60 – 0.90)	0.80 (0.80 – 1.0)	1.10 (0.70 – 3.10)	
p_{Con.}	-	0.744	0.003*	<0.001*	<0.001*
Sig.bet. Grps	-	p ₁ = 0.001*, p ₂ <0.001*, p ₃ = 0.003*			
eGFR (ml/min/m²)	98.11 ± 8.10	100.55 ± 14.64	82.72 ± 13.25	61.48 ± 25.0	
p_{Con.}	-	0.977	0.061	<0.001*	<0.001*
Sig.bet. Grps	-	p ₁ = 0.022*, p ₂ <0.001*, p ₃ = 0.004*			
ACR (mg/g creat)	2.60(1.80 – 6.50)	25.6(14.5 – 29.6)	68.4(45.9– 136.0)	2239(509.8-15425)	
p_{Con.}	-	<0.001*	<0.001*	<0.001*	<0.001*
Sig.bet. Grps	-	p ₁ <0.001*, p ₂ <0.001*, p ₃ <0.001*			
U. CTGF (pg/ml)	0.32 (0.25 – 0.38)	0.30 (0.20 – 0.65)	2.40 (1.50 – 6.20)	3.80 (1.90 – 8.50)	
p_{Con.}	-	0.414	<0.001*	<0.001*	<0.001*
Sig.bet. Grps	-	p ₁ <0.001*, p ₂ <0.001*, p ₃ = 0.048*			
U. CTGF/Creat. (picog/mg creat)	0.20 (0.10 – 0.30)	0.30 (0.20 – 0.30)	1.60 (1.50 – 2.20)	4.80 (3.70 – 7.50)	
p_{Con.}	-	0.001*	<0.001*	<0.001*	<0.001*
Sig.bet. Grps	-	p ₁ <0.001*, p ₂ <0.001*, p ₃ <0.001*			
Systolic (mmHg)	116.0 ± 9.49	128.67 ± 5.81	141.0 ± 8.49	146.67 ± 12.49	
p_{Con.}	-	0.003*	<0.001*	<0.001*	<0.001*
Sig.bet. Grps	-	p ₁ = 0.004*, p ₂ <0.001*, p ₃ = 0.357			
Distolic (mmHg)	77.67 ± 6.23	85.0 ± 4.23	92.0 ± 5.61	91.33 ± 7.43	
p_{Con.}	-	0.008*	<0.001*	<0.001*	<0.001*
Sig.bet. Grps	-	p ₁ = 0.012*, p ₂ = 0.027*, p ₃ =0.990			

Qualitative data was expressed using number and percent and was compared using Chi square or Monte Carlo test. Normally quantitative data was expressed in (Mean. ± SD) and was compared using F test (ANOVA) while for abnormally quantitative data expressed in Median (Min. – Max.) and was compared using Kruskal Wallis test.

p_{con.}: value for comparing between Normal (Control) and each other groups

p₁: value for comparing between Normo albumnuria with Micro albumnuria

p₂: value for comparing between Normo albumnuria with Macro albumnuria

p₃: value for comparing between Micro albumnuria with Macro albumnuria

Table (II): Correlation between U. CTGF/Creatinine with different studied parameters in total patients.

	U. CTGF/Creatinine	
	r_s	p
Age	-0.209	0.167
Sex (male = 1, female = 2)	-0.071	0.644
Duration of diabetes	0.928*	<0.001
FBG	0.236	0.118
HbA1c	0.691*	<0.001
S. Urea	-0.019	0.901
S. Creatinine	0.781*	<0.001
eGFR	-0.796*	<0.001
ACR	0.979*	<0.001
Systolic blood pressure	0.780*	<0.001
Diastolic blood pressure	0.575*	<0.001
BMI	0.178	0.241

Table (III): Correlation between U. CTGF/Creatinine with different studied parameters in each group.

		U. CTGF/Creatinine			
		Normal (Control) (n = 15)	Albuminuria		
			Normo (n = 15)	Micro (n = 15)	Macro (n = 15)
Duration of diabetes	r_s	-	0.456	0.778*	-0.083
	p	-	0.088	0.001	0.769
HbA1c	r_s	0.031	-0.102	0.654*	-0.100
	p	0.914	0.719	0.008	0.724
S. Creatinine	r_s	-0.241	0.360	0.614*	0.267
	p	0.387	0.188	0.015	0.336
eGFR	r_s	-0.357	-0.356	-0.741*	-0.966*
	p	0.191	0.192	0.002	<0.001
ACR	r_s	0.236	0.371	0.981*	0.999*
	p	0.397	0.173	<0.001	<0.001
Systolic blood pressure	r_s	0.257	0.168	0.746*	0.163
	p	0.356	0.550	0.001	0.561
Diastolic blood pressure	r_s	0.316	0.266	0.861*	0.231
	p	0.251	0.337	<0.001	0.407

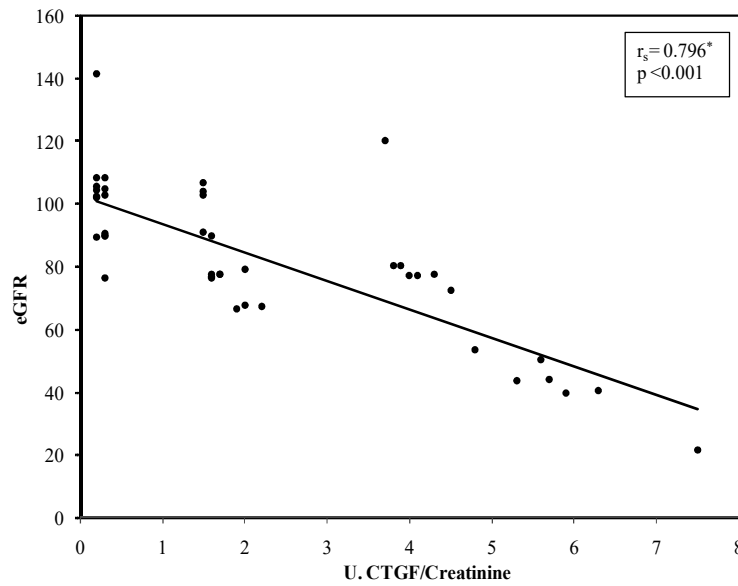


Figure (1): Correlation between U. CTGF/Creatinine with eGFR in total patients.

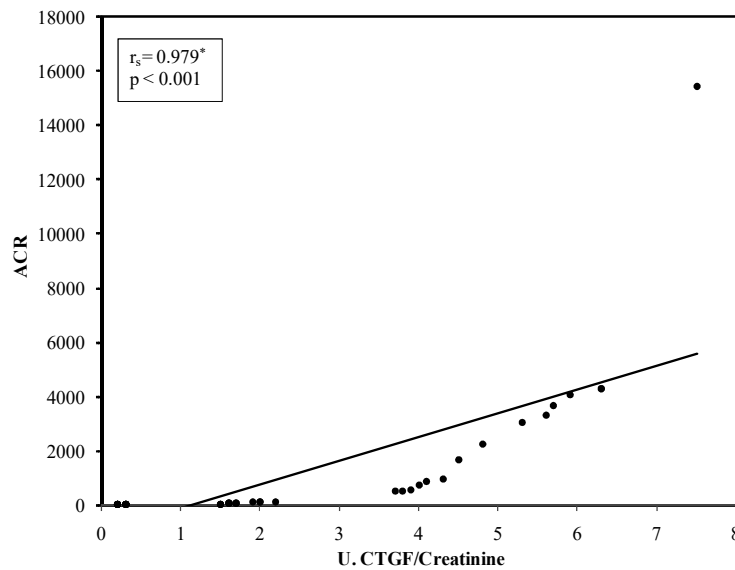


Figure (2): Correlation between U. CTGF/Creatinine with ACR in total patients.

Discussion:

In the present study, we analyzed U. CTGF excretion in 45 elderly type 2 diabetic patients and 15 healthy control subjects. Our results, confirmed that U. CTGF was significantly increased in diabetic nephropathy. To this, We added that in patients with diabetic nephropathy, U. CTGF was correlated with ACR ($R=0.979$, $P < 0.001$) and GFR ($R= 0.796$, $p < 0.001$), both are important clinical markers for severity of renal disease.

Elevated CTGF levels in the urine of patients with diabetic nephropathy was shown in multiple studies supporting our results.

In Yasuhiko Ito et al study,⁽¹³⁾ which included 333 patients, classified into 175 type 2 diabetic patients, 130 patients with non-diabetic renal disease and 28 healthy controls at the Faculty of Medicine Nagoya University, Nagoya Kyoritsu Hospital and

Chubu Rosai hospital. They noted that U.CTGF excretion was significantly higher in patients with DM renal insufficiency and chronic renal failure of DM nephropathy than in healthy controls. Urinary excretion levels of CTGF (U.CTGF/Creatinine) were significantly higher in diabetic nephropathy patients with chronic kidney disease (CKD) stages 4 and stage 5 compared to those with CKD stages 1, stage 2 and stage 3.

Nguyen TQ et al study⁽¹⁴⁾ the excretion of U.CTGF in 24 hours predicted an increase in 24 hours UAE in 318 type 1 diabetic patients and 29 normoglycemic controls. Patients were selected from the outpatient clinic at Steno Diabetes Center (Copenhagen, Denmark). They reported that U.CTGF in patients with diabetic nephropathy was significantly higher than in microalbuminuric patients and normoalbuminuric patients and control subjects.

This observation was supported also by Riser BL et al study,⁽¹⁵⁾ which quantified urinary CCN2 (CTGF) excretion in patients making routine visits to either the nephrology or the endocrinology clinics of Henry Ford Hospital. Patients were divided into two groups (renal disease group included 7 patients and the group without renal disease included 6 patients) and healthy controls. CCN2 levels were determined to be consistently low among the healthy volunteers. The values from the volunteer group did not differ from the diabetic patient group without renal disease as a whole. However, there was a significant difference between the control group and the diabetic patients with renal disease.

Tam F.W.K et al study⁽¹⁶⁾ included 43 adult diabetic patients (9 patients with type 1 DM, 34 patients with type 2 DM), whose attended to the Diabetic Clinic and Diabetic Nephropathy Clinic at Hammersmith Hospital, London, United Kingdom. They reported that low or undetectable levels of urinary CCN2 were observed in patients with normoalbuminuria, urinary CCN2 were

significantly higher level in microalbuminuria than those with normoalbuminuria. Urinary CCN2 were significantly higher level in those with macroalbuminuria ($p < 0.05$) than those with normoalbuminuria, but had a tendency to be lower than those with microalbuminuria.

Conclusion:

The main finding of this study was that, in patients with diabetic nephropathy U.CTGF was elevated and correlated with the severity of renal disease in terms of both ACR and decreased GFR. This suggests that CTGF, probably in conjunction with other factors, might act as a progression promoter in diabetic nephropathy.

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