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Connective tissue growth factor among elderly patients with type 2 diabetes: Correlation with diabetic nephropathy

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Abstract

Background: Diabetic nephropathy remains the most common cause of end stage renal disease. Connective tissue growth factor is an extracellular protein involved in the development of diabetic nephropathy.

Objective: To evaluate serum connective tissue growth factor level in elderly patients with type 2 diabetes and to assess the correlation with markers of diabetic nephropathy.

Subjects and methods: Fifty elderly type 2 diabetic patients with diabetic nephropathy and fifty age and sex-matched control were enrolled. All subjects underwent detailed history taking, clinical examination and anthropometric measurements assessment. Laboratory investigations included serum connective tissue growth factor, fasting blood glucose, post prandial blood glucose, glycated haemoglobin, lipid profile, C-reactive protein, serum creatinine, blood urea, urine analysis, urinary albumin to creatinine ratio and estimated glomerular filtration rate.

Results: The 50 patients were 35(70 %) females and 15 (30%) males with a mean age of 67.76± 3.04 years and disease duration were 11.3 ± 4.7 years. The mean serum CTGF in patients was 53.72 ± 21.22 ng/dl and in control was 22.28 ± 1.96 ng/dl ($p < 0.001$). Serum connective tissue growth factor significantly correlated with glycated haemoglobin, serum creatinine, urinary albumin to creatinine ratio and estimated glomerular filtration rate.

Conclusion: Connective tissue growth factor expression in the serum of patients with diabetic nephropathy was significantly higher than controls and was significantly correlated with markers of diabetic nephropathy.

Keywords: Diabetes, Diabetic nephropathy, connective tissue growth factor, albumin creatinine ratio, glomerular filtration rate.

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INTRODUCTION

Diabetic nephropathy (DN) is considered the commonest cause of end-stage renal disease (ESRD), accounting for up to 50% of the cases in Western societies. Generally, about 25% of type 1 and type 2 diabetic patients develop evidence of nephropathy and type 2 patients less progress to end-stage renal disease, this could be

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explained by competing mortality from cardiovascular diseases, with fewer patients reaching ESRD. However, because of greater prevalence of type 2 diabetes, the majority of diabetes patients presenting for treatment of ESRD (dialysis or transplantation) have type 2 diabetes.[1]

Diabetic nephropathy or diabetic kidney disease (DKD) is defined by the presence of albuminuria (increased urinary albumin excretion is defined as ≥ 30 mg/g) and reduction in estimated glomerular filtration rate (e GFR) in patients with long history of diabetes (> 10 years' duration of type 1 diabetes; may be present at diagnosis in type 2 diabetes) and is usually associated with retinopathy.[2] As regard histopathological changes in diabetic nephropathy, it is usually associated with glomerular changes in the form of diffuse and nodular mesangial proliferation, and thickening of the glomerular basement membrane, due to excessive accumulation of extracellular matrix. These changes lead to the occlusion of glomerular capillaries and progressive glomerular dysfunction.[3]

Additionally, podocytopathy can occur which is characterized by shortening, thinning and detachment of the podocyte foot processes from the glomerular basement membrane.[4] Moreover, the endothelial cells fenestrated area is reduced and the attachment between the glomerular endothelial cells and the adjacent glomerular cells is distorted.[5]

Connective Tissue Growth Factor (CTFG) plays an important role in the development of diabetic glomerulosclerosis by promoting transient cytoskeletal actin breakdown in mesangial cells, high fibronectin production, type I and IV collagen and mesangial cell hypertrophy.[6] CTGF have four domains, each of which can bind several ligands capable of modifying their function. CTGF can interact with a wide range of cellular receptors including integrins, tyrosine receptor kinase, heparin 28 sulfate proteoglycans, extracellular matrix proteins, fibronectin, vascular endothelial growth factor (VEGF), transforming growth factor-beta (TGF-β), etc.[7] With so many potential interactions, it is predictable that CTGF affects a multitude of different biological events. Mason et al. have demonstrated that CTGF stimulates the profibrotic effects of transforming growth factor-beta (TGF-β), by amplifying the TGF-β-Smad2/3 signaling pathway.[8]

The aim of the current work was to explore the association between serum CTGF with markers of renal disease in elderly type 2 diabetic patients with diabetic nephropathy.

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SUBJECTS AND METHODS

This cross-sectional study included fifty elderly type 2 diabetic patients with diabetic nephropathy. Patients were enrolled from Internal Medicine Department, Alexandria University Hospitals, Egypt. 50 healthy control subjects matched for age and sex with the patients were enrolled as control group. Patients with type 1 diabetes mellitus, chronic liver disease, autoimmune disorders that might affect the kidney and those with age less than 65 years were excluded.

The study was conducted after approval by our institutional ethics committee no 0305983, in accordance with the ethical guidelines of the Declaration of Helsinki. All subjects were informed about the study and informed written consent was obtained from all subjects.

All patients underwent detailed history taking including duration of diabetes, type of anti- diabetic medications, family history of diabetes and presence of co-morbidities. Clinical examination as well as screening for diabetic complications were performed.

Fasting blood samples were collected for the following investigations:

Complete blood picture, fasting plasma glucose, 2 hour post prandial blood glucose, glycated haemoglobin (HbA1c), total cholesterol, triglycerides, high-density lipoprotein (HDL), low density lipoprotein (LDL), serum creatinine, blood urea, urine analysis urinary albumin to creatinine ratio (ACR), serum albumin, alanine transaminase, C-reactive protein (CRP), estimated glomerular filtration rate. Serum levels of CTGF were assessed with a sandwich enzyme-linked immunosorbent assay (ELISA).

Statistical analysis of the data

Data was analysed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Data were represented as numbers and percentages. For continuous data, they were tested for normality by the Shapiro-Wilk test. Distributed data were represented as range mean and standard deviation. Mann Whitney test was used for comparison between two groups for not normally distributed quantitative variables and the Spearman coefficient was used to correlate between not normally distributed quantitative variables. The significance of the obtained results was judged at the 5% level.

RESULTS

The fifty patients were 15 (30%) males and 35 (70%) females with mean age of 67.76 year and disease duration was 11.3 year. The fifty controls were 20 (40%)

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males and 30(60%) females with mean age of 68.5 year. The demographic and clinical characteristics of the patients are shown in table (1).

The laboratory findings of the patients and control subjects are presented in table (2).

The mean CRP in patients was 5.45 mg/L, and in controls the mean CRP was 9.72 mg/L with significant statistical difference ($p<0.001$) as shown in figure (1).

The mean S. CTGF in patients was 53.72 ng/ml, and in controls the mean S. CTGF was 22.28 ng/ml with significant statistical difference ($p<0.001$) as shown in figure (2).

Among patients with diabetic nephropathy, there was significant correlation between S. CTGF and HbA1c, Urinary albumin creatinine ratio, GFR as shown in table (3), figure (3,4,5).

Values of S.CTGF were used to constuct the ROC curve as shown in the area under the curve (AUC). The confidence intervals were estimated to assess the accuracy of CTGF level in discriminating patients with diabetic nephropathy as shown in figure (6). The AUC of S.CTGF level showed a high level of accuracy (AUC = 0.988, $P < 0.001$) and the estimated cutoff value > 30 ng/ml can discriminate patients with diabetic nephropthy with 92% senstivity and 94% specificity as shown in table (4).

Table (1): Demographic and clinical characteristics of the studied cases (n = 50)

	Mean ± SD
Female: male	35:15(7:3)
Age (years)	67.76 ± 3.04
BMI (kg/m^2)	29.82 ± 3.68
Disease duration (years)	11.30 ± 4.70
Mean arterial blood pressure (mm Hg)	107.42 ± 7.11
Diabetic complications:	
Retinopathy	44 (88%)
Neuropathy	47(94%)
Cerebrovascular disease	11 (22%)
Ischemic heart disease	16 (32%)
Peripheral arterial disease	21(42%)
Medications:	
Insulin	11 (22%)
Sulphonyl urea	41(82%)
Metformin	43(86%)
DDP4	33(66%)
SGLT-2	14(28%)

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Table (2): Laboratory findings of the patients and control

	Cases (n = 50)	Control (n = 50)	Test of Sig.	p
FBG (mg/dl)	144.90 ± 27.02	86.64 ± 8.34	t=14.571*	<0.001*
PPBG (mg/dl)	232.44 ± 39.72	116.14 ± 11.07	t=19.943*	<0.001*
HbA1c %				
Mean ± SD	8.18 ± 0.65	5.16 ± 0.72	U= 48.0*	<0.001*
Median (Min. – Max.)	8.0 (7.30 – 10.10)	5.0 (4.30 – 9.50)		
Serum creatinine (mg/dl)				
Mean ± SD	1.65 ± 0.54	0.85 ± 0.14	U= 96.0*	<0.001*
Median (Min. – Max.)	1.55 (0.90 – 2.80)	0.80 (0.60 – 1.15)		
BUN (mg/dl)				
Mean ± SD	26.58 ± 6.38	12.56 ± 4.10	U= 71.0*	<0.001*
Median (Min. – Max.)	25.0 (18.0 – 39.0)	11.50 (7.0 – 23.0)		
CRP (mg/L)				
Mean ± SD	9.72 ± 5.39	5.45 ± 3.33	U= 690.50*	<0.001*
Median (Min. – Max.)	9.0 (2.80 – 20.0)	5.0 (2.80 – 20.0)		
Urinary albumin creatinine ratio (mg/gm)				
Mean ± SD	678.32 ± 409.92	21.24 ± 6.22	U= 0.000*	<0.001*
Median (Min. – Max.)	563.5 (175 – 1550)	22 (11 – 35)		
GFR (ml/min)				
Mean ± SD	61.26 ± 18.94	85.96 ± 6.67	U= 200.50*	<0.001*
Median (Min. – Max.)	65.0 (23.0 – 82.0)	86.0 (73.0 – 99.0)		
S. CTGF (ng/ml)				
Mean ± SD	53.72 ± 21.22	22.28 ± 3.51	U= 30.50*	<0.001*
Median (Min. – Max.)	47.0 (29.0 – 95.0)	21.0 (19.0 – 33.0)		

U: Mann Whitney test SD: **Standard deviation**
p: p-value for comparing between **Cases** and **Control**
*: Statistically significant at $p \leq 0.05$

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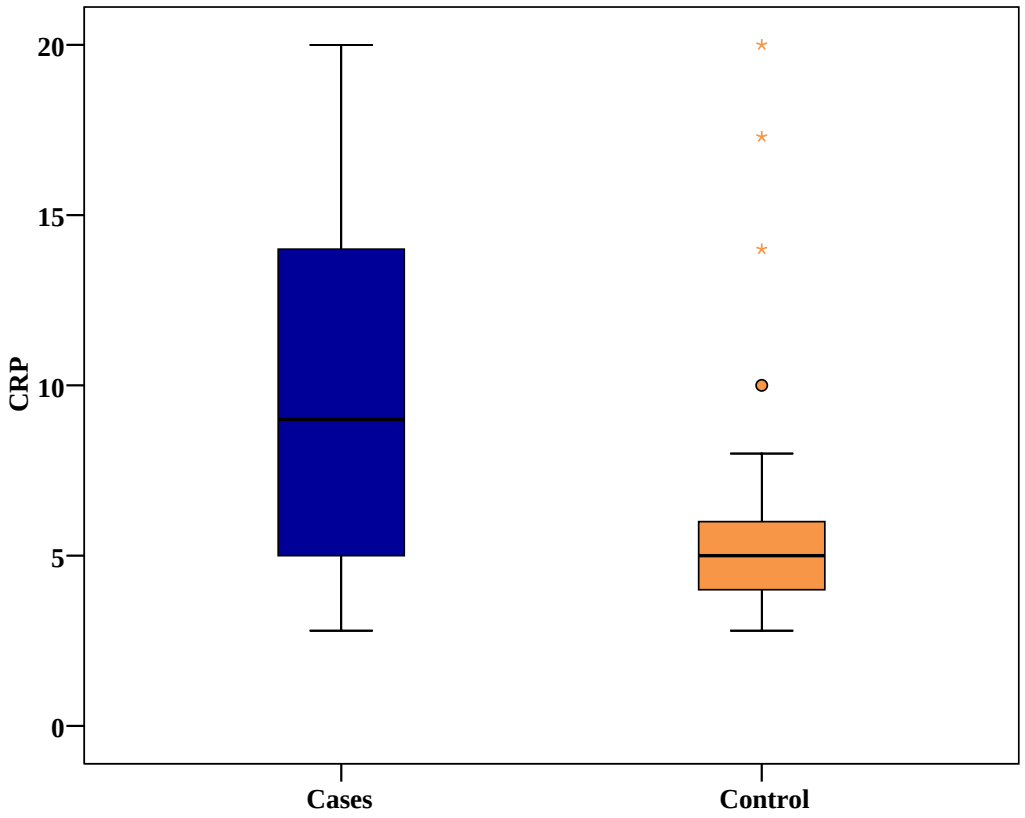

Figure (1): Comparison between the two studied groups according to CRP.

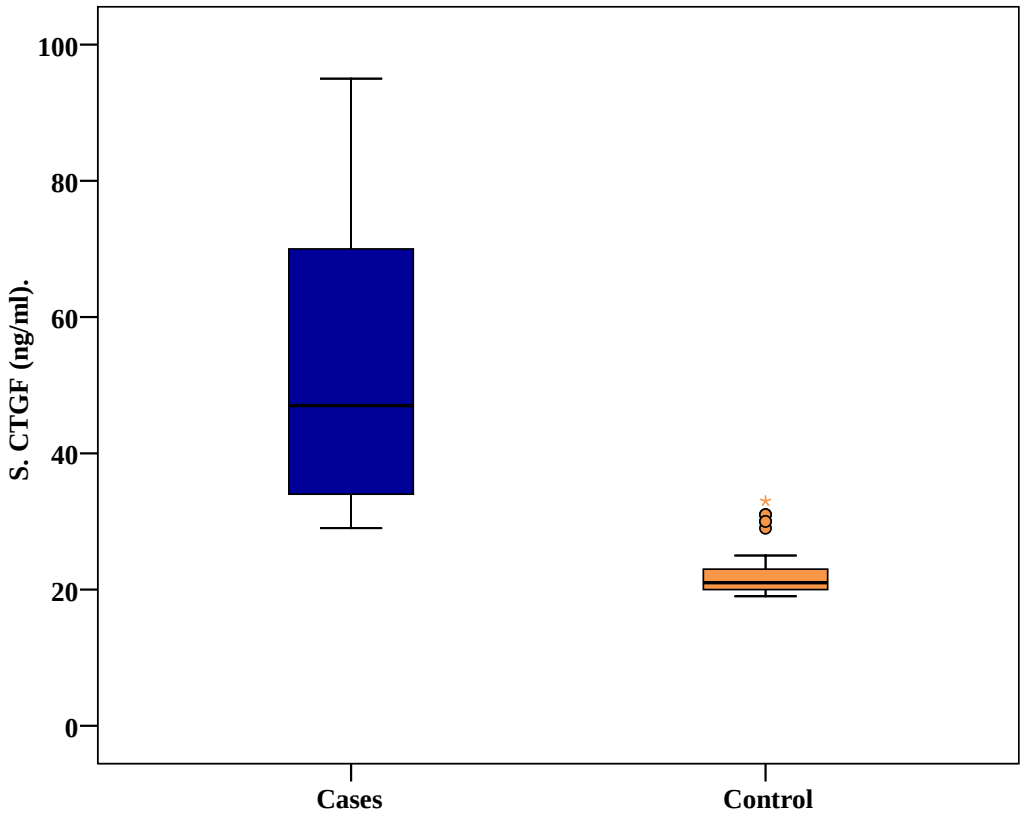

Figure (2): Comparison between the two studied groups according to S. CTGF (ng/ml).

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Table (3): Correlation between S-CTGF and different variables in the patients. (n = 50)

	S. CTGF (ng/ml)	
	r_s	p
Age (years)	0.090	0.533
BMI (kg/m^2)	-0.111	0.442
FBG (mg/dl)	0.526*	<0.001*
PPBG (mg/dl)	0.664*	<0.001*
HbA1c %	0.798*	<0.001*
Serum creatinine (mg/dl)	0.866*	<0.001*
BUN (mg/dl)	0.769*	<0.001*
CRP (mg/L)	0.903*	<0.001*
Urinary albumin creatinine ratio mg/gm)	0.929*	<0.001*
e-GFR (ml/min)	-0.843*	<0.001*

r_s: Spearman coefficient
*: Statistically significant at $p \leq 0.05$

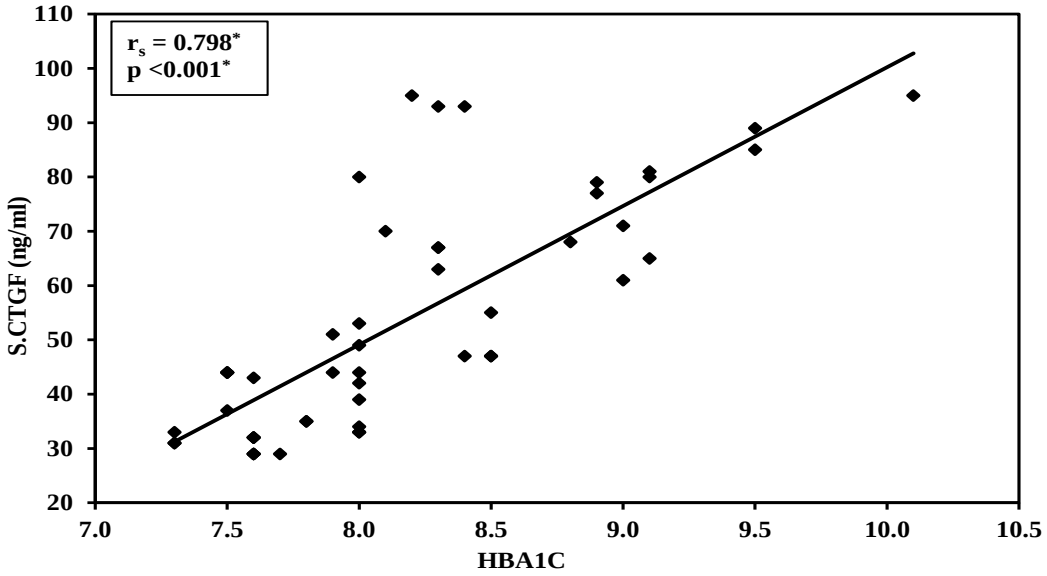

Figure (3): Correlation between S-CTGF and HbA1c for cases (n = 50).

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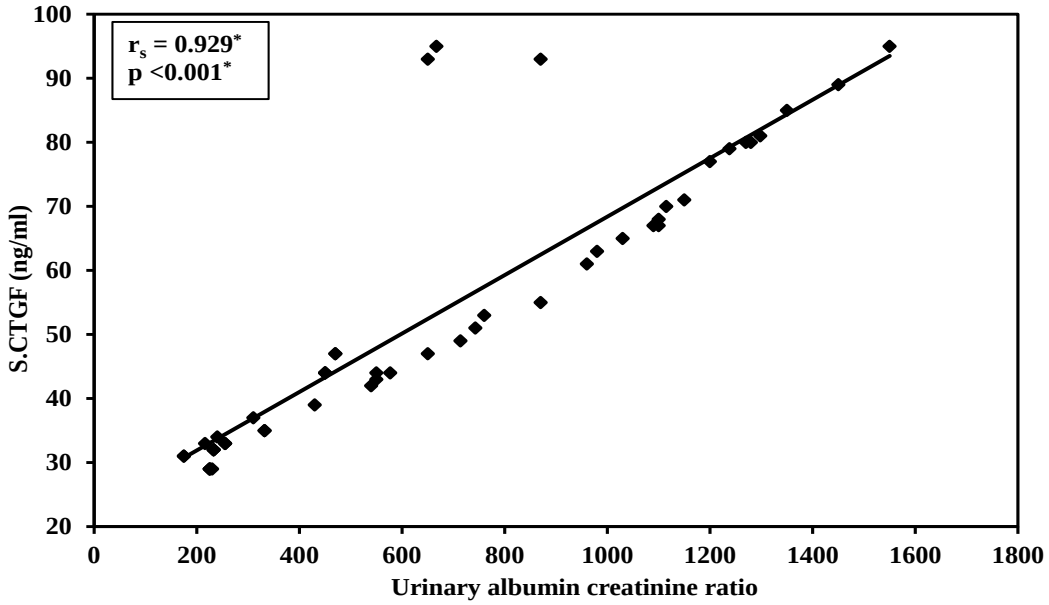

Figure (4): Correlation between S-CTGF and urinary albumin creatinine ratio for cases (n = 50)

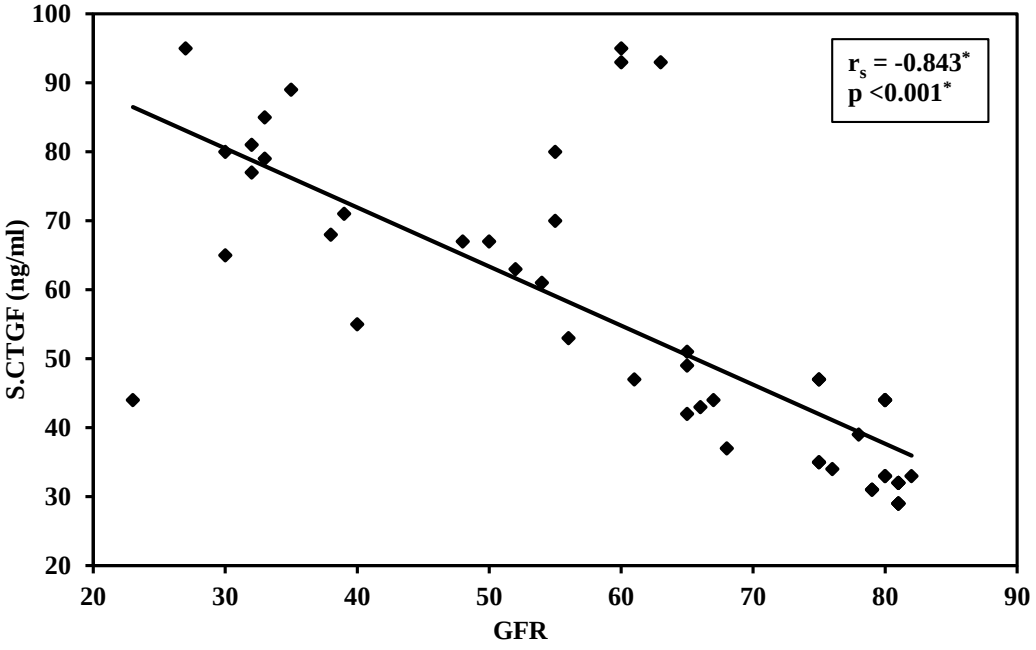

Figure (5): Correlation between S-CTGF and GFR for cases (n = 50)

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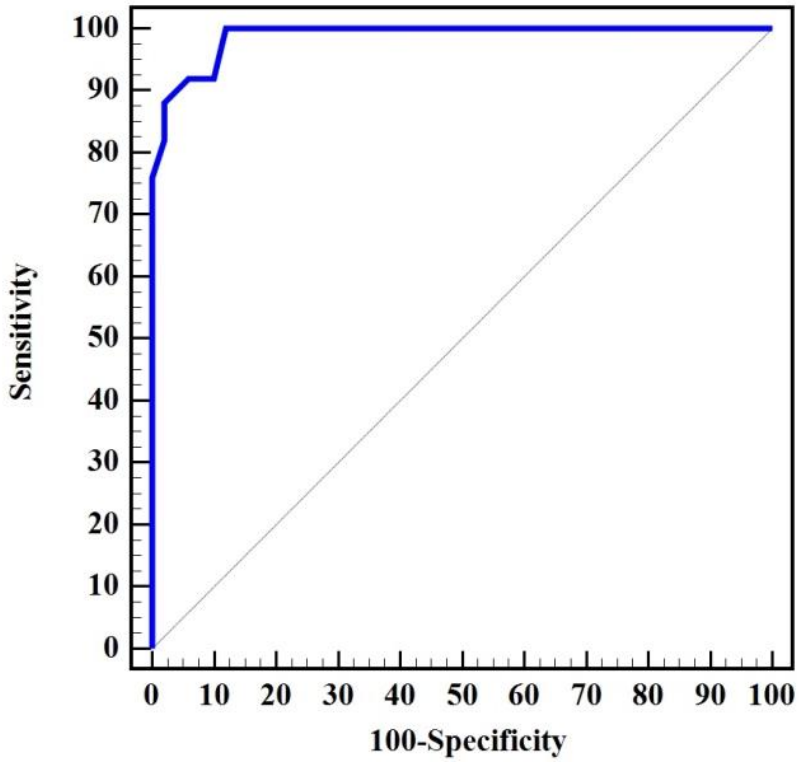

Figure (6): **ROC curve for S. CTGF (ng/ml) to discriminate patients (n = 50) from control (n = 50)**

Table (4): **Diagnostic performance for S. CTGF (ng/ml) to discriminate patients (n = 50) from control (n = 50)**

	AUC	p	95% C.I	Cut off	Sensitivity	Specificity	PPV	NPV
S. CTGF (ng/ml)	0.988	<0.001*	0.974 – 1.000	>30	92.0	94.0	93.9	92.2

AUC: Area Under a Curve
p value: Probability value
CI: Confidence Intervals
NPV: Negative predictive value
PPV: Positive predictive value
*: Statistically significant at $p \leq 0.05$

Discussion

All diabetic patients should be screened for diabetic nephropathy by looking for microalbuminuria. Although the cut-off point for microalbuminuria detection is an albumin/creatinine ratio of more than 2.5 mg/g, many other disorders such as urinary tract infections, fever, and hypertension may lead to microalbuminuria in diabetic patients.[9] So, the test should be repeated after 6 months, so there is a need to search for a novel biomarker for DN such as CTGF and some authors found that CTGF was

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correlated for the presence of microalbuminuria among patients with DN.[10] So, this study was aiming to explore the association between CTGF and microalbuminuria.

The mean age of our patients was 67.76, this increase in the age of the patients was in agreement with Tziomalos et al. Who found that the old age is a risk factor for DN.[11] The association between advanced age and DN may suggest that the long duration of exposure to hyperglycemia lead to increased risk of developing nephropathy in old age.

In the present study, a positive correlation between the serum CTGF level and s. creatinine, BUN, FBG, 2h PPBG, HbA1c and UACR (p-value < 0.001) were detected.

Also in our study, there is a negative correlation between the serum crap level and the estimated GFR (p-value < 0.001) detected. Such results might suggest that any rise in the CTGF level may be correlated with decrease in renal functions and could be used as early marker for nephropathy in diabetic patients.

CTGF overexpression has been found in many renal fibrotic disorders including chronic allograft nephropathy, IgA nephropathy and DN.[12]

Riser et al. reported that CTGF was elevated in the urine samples of diabetic rats and persons with diabetes[13] Subsequently, several groups have found higher urinary levels of CTGF in persons with diabetes than in healthy individuals,[14, 15] which indicate that it could be considered as a marker for diabetic nephropathy. In persons with diabetes, plasma CTGF levels were found to be higher in patients with macroalbuminuria than in those with a normal albumin level in urine. CTGF was an independent predictor of ESRD and correlated with the rate of decline in the GFR.[16]

Urinary CTGF was studied for its correlation with renal allograft fibrosis in a cohort of 315 transplant recipients. Following this cohort for 2 years, Metalidis et. al. compared CTGF concentrations with protocol biopsies at 3-, 12-, and 24 months post-transplantation. Urinary CTGF levels were correlated with the degree of interstitial fibrosis. In a subset of patients, CTGF levels at 3 months were associated with long-term moderate and severe fibrosis at the 24-month time point.[17]

Limitation of the study include the following: First, being a cross sectional study with single measurement of serum CTGF, so causality cannot be determined, and multiple measurement are needed over time. Second, relatively small sample size and lastly being conducted at single center and single ethnic group.

CONCLUSIONS

Serum connective tissue growth factor among elderly patients with type 2 diabetes correlates with markers of diabetic nephropathy.

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