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Studying Sex Effect on CTGF, TGF-B1 Levels and Some Relevant Parameters in Iraqi Diabetic Patients with Glomeruli and Renal Tubules Fibrosis

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Abstract

Due to high blood sugar over long periods, the incidence and prevalence of type 2 diabetes are increasing throughout the world. Diabetic complications include microvascular and macrovascular that target the kidneys, nerves, eyes, and heart. Hence, the current study aimed to investigate the levels of connective tissue growth factor (CTGF) and transforming growth factor-β1 (TGF-β1) for both men and women and to demonstrate the effect of sex on it. In addition, some related biochemical factors in patients with diabetes and diabetic nephropathy are compared with those in healthy controls. The study included 120 males and females with an age range of (30-65) years old. Ninety patients with type 2 diabetes were subdivided into three groups on the basis of ACR criteria. All the individuals in the groups visited Baghdad Teaching Hospital, Medical City, and Al-Yarmouk Teaching Hospital during the period between December 2021 and May 2022. The CTGF and TFG-β1 levels were determined using the ELISA technique. Urea results showed statistically significant differences between diabetic nephropathy in the patient group and the control group in female cases. Still, there were no statistically significant differences between male patients with diabetic nephropathy and the control group. The results also revealed that there were statistically significant differences in albumin/creatinine ratio, estimated glomerular filtration rate, urea, fasting blood sugar, and creatinine between the diabetic nephropathy group and the healthy group for both men and women. From these results, CTGF and TGF-β1 represent good early prognostic markers in diabetic nephropathy.

Keywords: Diabetes mellitus, diabetic nephropathy, connective tissue growth factor, transforming growth factor-β1, albumin to creatinine ratio.

1. Introduction

Diabetes mellitus (DM) is a type of metabolic disease that occurs when patients suffer from elevated blood glucose levels, and their bodies do not respond to or develop insulin. Typically, diabetes presents in three forms: type 1 DM, type 2 DM (T2DM), and gestational DM, with greater emphasis placed on the first two forms [1]. Type 2 DM arises when the body does not respond to the production of insulin.

Diabetes may cause long-term injury and damage to various organs in chronic conditions, particularly the eyes, kidneys, nerves, heart, and blood vessels. Diabetes has entered pandemic proportions, impacting more than 400 million citizens worldwide, and has become of great significance in developed countries, too [2].

Type 2 DM, also known as T2DM, is the most prevalent form among the large and heterogeneous number of diabetes patients. T2DM is the most pervasive form among the large and heterogeneous number of diabetes patients. It emerged from the Global Burden of Diseases report in 2016 that T2DM and its complications have been responsible for the 22% rise in damage over the last ten years, which has had a significant consequence on public health. Diabetes mellitus has several long-term problems connected with it, such as chronic kidney disease (CKD) [3]. A phenomenon known as diabetes kidney disease (DKD) is characterized by alterations in the glomerular filtration rate, leakage of proteins (mainly albumin), metabolites, and ions into the urine, and the estimation of the glomerular filtration rate (GFR). It is a microvascular complication of diabetes characterized by injury to the capillaries, mesangial cell expansion, extracellular matrix accumulation, thickening of glomerular basement membrane GBM, and podocyte and glomerular injury, leading to glomerular sclerosis and tubulointerstitial fibrosis [4,5]. Connective tissue growth factor (CTGF) is engaged in numerous diseases, including the growth of tumors and tissue fibrosis, as well as multiple biological processes, including cell proliferation, angiogenesis, and wound healing. It is 38 kDa and cysteine-rich (22 cytosines in the N-terminal and 16 cytosines in the C-terminal region). 10. extracellular matrix protein. The mechanism causing excessive extracellular matrix accumulation and transforming growth factor-β (TGF-β1) has been suggested as the main trigger for both the increased collagen production and decreased matrix degradation pathways in fibrosis, being recognized as a central mediator in the development of fibrosis. Renal fibrosis is a common pathological manifestation of CKD [6].

Numerous diseases and ailments can cause CKD. The two most frequent causes that affect the nephrons include congenital illnesses, urinary tract infections, polycystic kidney disease, blockages, glomerulonephritis, medicines, toxins, and diabetes. This damage may render the kidneys incapable of removing squanders. Other kidney issues include tumors, cysts, and stones, hypertension and DM, and obesity may be non-conventional hazard factors [7,8].

This study aims to investigate the effect of sex on levels of CTGF and TGF-β1 in diabetic patients with or without diabetic nephropathy in comparison with healthy subjects.

2. Materials and Methods

2.1 Study design and patients

A total of 120 subjects; aged 30-65, participated in the study; 90 of them were patients with DM who visited Baghdad Teaching Hospital, Medical City, and Al-Yarmouk Teaching Hospital between December 2021 and May 2022, and 40 healthy people were the control group. Groups of analysis included:

1) The control group included 15 males and 15 females who were healthy-looking subjects without any diseases.

2) Diabetic patients: included 90 patients who were divided into three groups according to the albumin-to-creatinine ratio (ACR) criterion:

- Normoalbuminuria group included 15 male and 15 female patients with an ACR < 30 mg/g.
- Microalbuminuria group included 15 male and 15 female patients with the range of ACR 30-300 mg/g
- Macroalbuminuria group included 15 male and 15 female patients with an ACR >300 mg/g.

2.2 Biochemical analysis

Seven mL of blood from the antecubital vein was withdrawn and divided into two parts. Part 1: 5 mL were placed in gel tubes and solidified at room temperature for 30 minutes. After centrifuging for 10 minutes, separated the serum and stored it in Eppendorf tubes. The first part was utilized to rapidly identify (fasting blood sugar, urea, and creatinine in serum) using an auto-spectrophotometer, which is a clinical chemistry analyzer that performs diagnostic tests. Also, it was utilized after being maintained at -20 °C to assess CTGF and TGF-B1, which were evaluated using a BioSource enzyme-linked immunosorbent test (ELISA) kit, USA. Part 2: 2 mL of the blood was kept in a test tube containing an anticoagulant for hemoglobin A1c (HbA1c) measurement by an I-chroma device.

2.3 Statistical analysis

The SPSS software version 26 was used to analyze the data statistically. The variables' means and standard deviations were reported. To find out whether there are statistically significant variations in the means of the four independent studied groups, one-way analysis of variance (ANOVA) is utilized (control, DM with normoalbuminuria, DM with microalbuminuria, and DM with macroalbuminuria) [9].

3. Results

As shown in **Table 1**, the mean ± standard deviation values parameters for the studied groups showed highly significant (p < 0.05) differences in blood sugar, HbA1C, creatinine, blood urea, eGFR levels, and albumin creatinine to ratio in urine.

Table 1 shows the mean ± SD for FBS and HbA1C in the three patient groups and the control group. The results revealed a significant difference (p = 0.0001) between the control group and the patients in the current study for both females and males. This means that sex does not affect the results obtained for the FBS and HbA1C groups.

The results of creatinine and urea for both females and males in patients and control groups are recorded in **Table 1**, and there were significant differences. Then, a comparison was made that included the patients' totals, each group separately, the control, and the results. The results of creatinine in both sex showed a significant difference (p = 0.0001) between macro groups (3.50 ± 2.85^{af}, 3.36 ± 2.99^{am}) and other studied groups that include micro-, normoalbuminuria, and control groups for both sex (1.50 ± 0.67^{b}, 0.91 ± 0.20^{b}, 0.70 ± 0.12^{b}) for female groups and (1.30 ± 0.74^{b}, 0.95 ± 0.27^{b}, 0.80 ± 0.06^{b}) for male groups, respectively. In contrast, results of urea in females revealed significant differences (p=0.0001) among macro- (88.83 ± 40.61^{a}), micro- (61.01 ± 22.12^{b}), and normoalbuminuria (35.93 ± 7.83^{c}) compared with control (27.50 ± 4.77^{c}) groups. At the same time, findings of urea for males indicated significant differences (p= 0.0001) between both macro- (81.82 ± 39.67^{a}) and micro- (59.24 ± 25.92^{a}) groups compared to both normoalbuminuria (32.93 ± 10.27^{b}) and control (34.15 ± 2.79^{b}) groups, as reported in **Table 1**.

Table 1. Glycemic profile and renal function test in the studied groups.

Parameters \ Groups	Mean± SD				*p*-value
	Control (n= 30)	Normo-albuminuria (n= 30)	Micro-albuminuria (n= 30)	Macro-albuminuria (n= 30)	
FBS (mg/dL)					
Female	87.75 ± 5.15 [b]	190.23 ± 34.20 [a]	199.66 ± 34.38 [a]	203.06 ± 50.05 [a]	0.0001**
Male	87.0 ± 6.60 [b]	215.0±47.74 [a]	222.66 ± 46.83 [a]	232.0 ± 84.21 [a]	
HbA1c (%)					
Female	5.13 ±0.32 [b]	9.08 ± 1.76 [a]	9.40 ± 1.18 [a]	10.79 ± 3.94 [a]	0.0001**
Male	5.23 ± 0.33 [b]	10.28 ± 1.97 [a]	10.76 ± 2.35 [a]	10.12 ± 1.63 [a]	
Creatinine (mg/dL)					
Female	0.70 ± 0.12 [b]	0.91 ±0.20 [b]	1.50 ± 0.67 [b]	3.50 ± 2.85 [a]	0.0001**
Male	0.80 ±0.06 [b]	0.95 ± 0.27 [b]	1.30 ± 0.74 [b]	3.36 ± 2.99 [a]	
Urea (mg/dL)					
Female	27.50 ± 4.77 [c]	35.93 ± 7.83 [c]	61.01 ± 22.12 [b]	88.83 ± 40.61[a]	0.0001**
Male	34.15 ± 2.79 [b]	32.93 ± 10.27 [b]	59.24 ± 25.92 [a]	81.82 ± 39.67 a	
eGFR (mL/min/1.73 m)					
Female	105.81 ± 13.30 [a]	80.26 ± 22.31 [b]	50.73 ± 30.22 [c]	29.46 ± 21.10 [d]	0.0001**
Male	113.30 ± 6.21 [a]	98.40 ± 21.10 [b]	81.93 ± 34.72 [bc]	44.17 ± 27.29 [c]	
ACR (mg/g)					
Female	-	15.86 ±5.37[c]	91.53 ± 59.70[b]	629.04 ± 248.6[a]	0.0001**
Male	-	16.06 ± 5.75[c]	130.80 ± 56.37[b]	504.64±212.92[a]	

- Significant difference between means using the ANOVA test at the 0.01 level.
- Significant variants are denoted by different small letters; non-significant variations are denoted by identical small letters.

The values of eGFR and ACR of the studied groups for both sex are recorded in **Table 1**. The results of eGFR in females revealed a significant (p= 0.0001) decrease in all patient groups (normoalbuminuria, micro-, and macro- (15.86 ± 5.37[a]), (91.53 ± 59.70[b]), and (629.04 ± 248.6[c]), respectively, compared with the control group (105.81 ± 13.30[a]). Similar results were found in males, where their eGFR values were found to decrease significantly (p= 0.0001). Patient groups include normoalbuminuria, microalbuminuria, and macroalbuminuria (98.40±21.10[b]), (81.93±34.72[bc]), and (44.17 ± 27.29[c]) compared with the control group (113.30 ± 6.21[a]). That means sex has no effect on eGFR.

Table 2 displays the serum biomarkers for all male and female participants. The result showed that there was a significant increase in the serum levels of TGF-β1 and CTGF in the female patient groups compared with the control group (p= 0.0001). When conducting a careful comparison between the patient groups, a significant increase was observed between the two groups (micro- and macroalbuminuria) and the control group in the level of TGF-β1. The results presented in the description of **Figure 1** indicate that there was a significant increase in TGF-β1 in albumin for males compared to the control group (p= 0.003). Also, when comparing the groups of patients, there were no significant differences between normal albuminuria and microalbuminuria in the control group. When comparing CTGF levels in the women's group, each group separately, with the control group, it is indicated that there were significant differences between the two groups (microalbumin and macroalbuminuria). For men, all patient groups showed statistically significant differences from the healthy group.

Table 2. The TGF-β1 and CTGF levels in the studied groups.

Groups / Parameters	Mean±SD				*p*-value
	Control (n= 30)	Normo-albuminuria (n= 30)	Micro-albuminuria (n= 30)	Macro-albuminuria (n= 30)	
TGF-B1 (mg/dL)					
Female	263.25 ± 86.22 [a]	515.48 ± 154.28 [ab]	953.43 ± 410.44 [b]	680.46 ± 321.25 [c]	0.0001**
Male	257.37± 100.76 [a]	673.88 ± 296.05 [ab]	712.47 ± 370.23 [ab]	1167.6 ± 1094.2 [b]	0.003**
CTGF (pg/mL)					
Female	14.45 ± 9.64 [a]	46.06 ± 41.90 [ab]	77.04 ± 50.12 [bc]	92.76 ± 46.92 [c]	0.0001**
Male	24.57 ± 15.45 [a]	77.03 ± 46.97 [b]	77.73 ± 40.44 [b]	83.55 ± 53.94 [b]	0.002**

- Significant difference between means using the ANOVA test at the 0.01 level.
- Significant variants are denoted by different small letters; non-significant variations are denoted by identical smal letters.

Similarly, ACR values for females revealed a significant increase in patient groups (normoalbuminuria, micro-, and macro-). The same results were obtained for males; thus, sex has no effect on ACR in the case of patient groups, as shown in **Figures 1** and **2**.

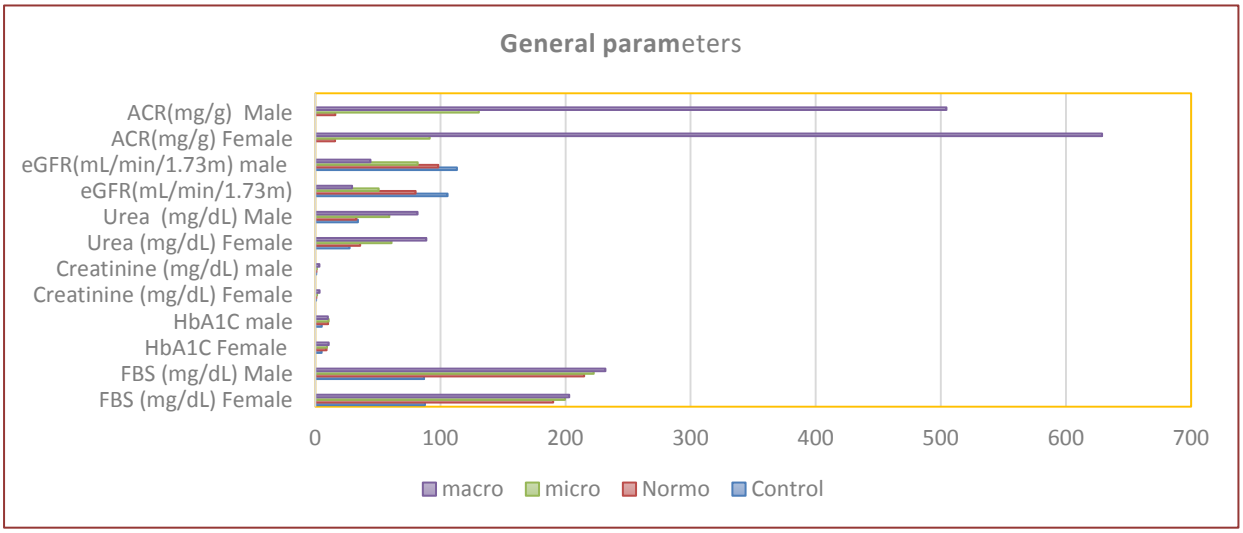

Figure 1. Box plot showing the effects of sex on CTGF and TGF-B1 levels between patients (normo-, micro-, macroalbuminuria) and control groups.

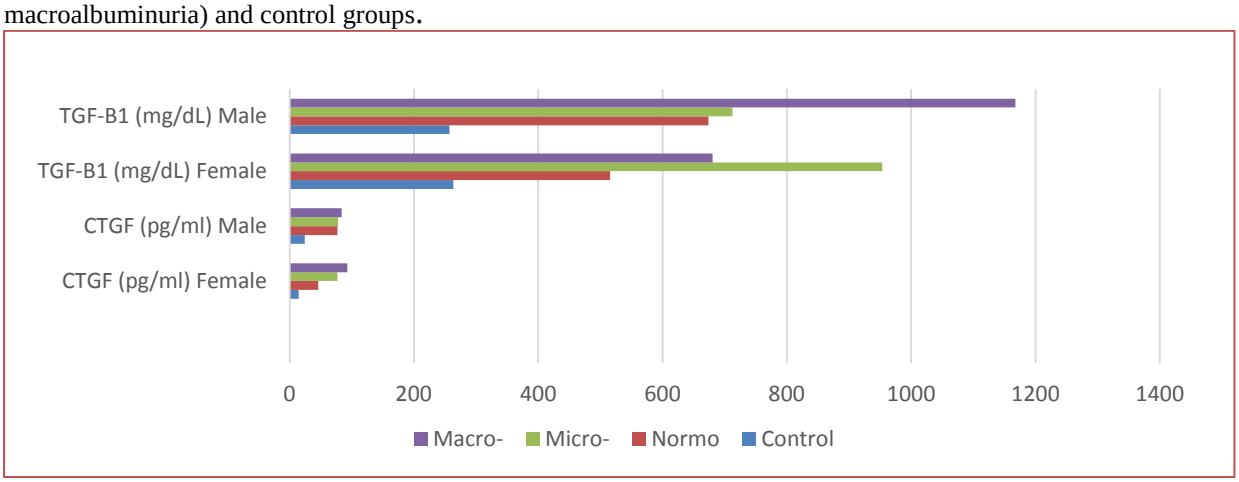

Figure 2. Box plot showing the effects of sex on the CTGF ,TGF- β1 levels between patients (normo-, micro-, macroalbuminuria) and control groups.

4. Discussion

Numerous investigations have been carried out to determine the causes of glomerulus damage and fibrosis, as well as how to detect these conditions early [10]. The current investigation was undertaken for diabetic patients with or without nephropathy (micro or macroalbuminuria), which was characterized by hyperglycemia (FBS), proteinuria (ACR), and eGFR values as dependent criteria for the classification of patient groups, in addition to other indicators such as urea and creatinine that support the criterion of DKD. Hyperglycemia, fibrosis, and inflammation are linked to risk factors for DKD stimulation and development. Chronic kidney disease is characterized by a progressive loss or impairment of kidney function as a result of glomerulus and tubular damage [11].

The current study did not find any effect of sex on the rate of hyperglycemia. The results of the study were not in agreement with many other studies. An increase in fasting blood sugar was found in patients with micro or total albumin urine compared to normal albuminuria in both men and women. The current study's findings demonstrated that sex has no impact on groups of men and women's [12] urea and creatinine levels. The present findings imply that female kidneys appear to be better protected than male kidneys [13]. The protection of the kidneys may be brought about by an increase in female NO (nitric oxide) levels. The NO is essential for tubular transport, autoregulation, and modulation in the kidney, NO production has been reported to decrease as CKD advances. As men age, their renal blood vessels become more dependent on nitric oxide. By controlling the expression and activity of NO synthase, testosterone deficiency may exacerbate endothelial dysfunction by reducing NO levels [14].

Albumin to creatinine ratio evaluation in urine was recommended for the estimation of albuminuria in the diagnosis of diabetic nephropathy in patients with T2DM. Due to the high incidence of microalbuminuria in diabetic patients, the development of glomerular and tubular fibrosis is attributed to endothelial damage [15]. So, the prevention of kidney disease progression depends on early diagnosis; hyperglycemia leads to severe kidney damage that causes a decline in eGFR. Therefore, eGFR can be helpful as a marker for early renal impairment diagnosis in patients with T2DM [16]. Both albuminuria and eGFR are significantly better indicators of end-stage renal disease, but urine ACR was found to have a more significant impact on clinical practice than eGFR. As a result, the presence of albuminuria indicates damage to both the glomeruli and the tubules, whereas an eGFR disorder only indicates glomerular damage. According to the findings of this study, assessment of ACR and GFR could serve as helpful markers in this setting for the early detection of diabetic nephropathy, the prevention of overt nephropathy, and the progression to end-stage renal disease. Sex had no impact on ACR or GFR in the current study [17].

In general, CTGF expression is modest in healthy adult kidneys but dramatically elevated in a number of renal disorders, where it plays a critical role in the progression of glomerular and tubule interstitial fibrosis [18]. Involved in cell migration, proliferation, and differentiation, CTGF either works directly to advance fibrosis or functions as a factor downstream of TGF-β1. In addition, CTGF controls the expression and activity of TGF- and bone morphogenetic protein (BMP), thereby playing an essential role in the process of kidney repair [19].

Elevated plasma CTGF in individuals with CKD is an independent risk factor for the development of end-stage renal disease and is closely correlated with glomerular filtration rate. As a result, CTGF may serve as a biological indicator of kidney fibrosis. The current study

showed an increase in connective tissue growth factor, indicating the occurrence of glomerular and tubular damage and fibrosis in diabetic patients [20]. This result is in agreement with Koszegi et al., who found DM is a significant health concern, impairing the quality of life and diminishing the life expectancy of millions of people. The DKD affects more than 20% of all diabetic patients, and due to limited therapeutic options, it remains the leading cause of CKD.

The CKD are defined by the buildup of extracellular matrix (ECM) components in the glomeruli (glomerular fibrosis, glomerulosclerosis), and the tubular interstitium, and TGF-β1 has been identified as a critical mediator in the genesis of CKD (tubulointerstitial fibrosis) [21]. One of the main reasons for decreased glomerular filtration rate in CKD is the formation of fibrosis, which is contributed to by the three main types of glomerular cells (visceral epithelial cells, mesangial cells, and endothelial cells) [22].

The TGF-B1 causes mesangial expansion due to mesangial cell hypertrophy, proliferation (and eventually apoptosis), and extracellular matrix ECM synthesis. It also causes endothelial to mesenchymal transition, which results in glomerular myofibroblasts, a significant source of ECM, and podocytopenia caused by podocyte apoptosis and detachment from the glomerular basement membrane [23].

The TGF-β1 has been shown to mediate several key tubular pathological events during CKD progression, namely fibroblast proliferation, epithelial to mesenchymal transition, tubular and fibroblast ECM production, and epithelial cell death, leading to tubular cell deletion and interstitial fibrosis. TGF-β1 has been shown to mediate several key tubular pathological events during CKD progression, namely fibroblast proliferation, epithelial to mesenchymal transition, tubular and fibroblast ECM production, and epithelial cell death, leading to tubular cell deletion and interstitial fibrosis [24, 25].

Through the results of our current study, there was a similar effect on the high level of TGF-β1 for both male and female groups, and our results agree with Al-Maiyaly, who found that high-level TGF-β1 plays a significant role in the extracellular accumulation of protein kinase PK in both men and women [26-28]. Activity is reported for its ability to increase the production of ECM. TGF-β1PK inhibitors can prevent hyperglycemia or diabetes-induced increases in ECM accumulation and production of TGF-β1 in mesangial cells or renal glomeruli [29, 30].

5. Conclusion

According to the results obtained, there were no differences in the levels of CTGF and TFG-β1 in male and female participants who were studied separately in DKD. So, this study conclude that sex has no effect.

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Conflict of Interest

There are no conflicts of interest.

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Ethical Clearance

The Scientific Committee at the University of Baghdad has approved this work for both the College of Science for Women and the College of Medicine.

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