Estimation of transforming growth factor-beta 1 as a marker of renal injury in type II diabetes mellitus

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ABSTRACT

Objectives: To evaluate the serum transforming growth factor-beta 1 level in type II diabetic patients with diabetic nephropathy (DN) and to assess its use as a marker of renal injury in type II diabetes.

Methods: Sixty patients with type II diabetes mellitus (DM), who attended the outpatient internal medicine clinic in Cairo University Hospital, Egypt from January 2003 to March 2003, were the subjects of the present study and compared to 10 healthy age- and gender-matched control subjects They were divided into 6 major groups according to degree of metabolic control, as determined by glycosylated hemoglobin (HbA1c), the rate of urinary albumin excretion (UAE) and serum creatinine level. Serum transforming growth factor-beta 1 level was assessed by enzyme linked immunosorbent assay (ELISA).

Results: Serum transforming growth factor-beta 1 level was significantly increased in micro albuminuric (UAE 20-200 ug/minute), macro albuminuric (UAE >200 ug/minute) and overtly nephropathic diabetic patients with renal impairment compared to healthy controls (p<0.05). In addition, serum transforming growth factor-beta 1 level was significantly increased in type II diabetic patients with poor glycemic control (HbA1c >7.6%) compared to patients with good glycemic control (HbA1c 5.5-7.6%). Serum transforming growth factor-beta 1 level was significantly increased in hypertensive DM patients compared to normotensive DM patients (p<0.05). There was a strong correlation between serum transforming growth factor-beta 1 level and HbA1c, blood urea, serum creatinine and 24-hour urinary protein excretion (p<0.01).

Conclusions: Our data strongly support the hypothesis that hyperglycemia may trigger the activation of transforming growth factor-beta 1 which in turn mediates progressive renal damage in type II DM. Increased serum transforming growth factor-beta 1 may be useful as a marker of diabetic renal disease as it shows a close association with the parameters of renal injury in type II diabetes.

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Diabetic nephropathy (DN) is the most common cause of end stage renal failure (ESRD) in western world. Diabetic nephropathy follows a well outline clinical course, starting with microalbuminuria through proteinuria, azotemia and culminating in ESRD.[1] Before the onset of overt proteinuria, there are various renal functional changes including renal hyperfiltration, and increasing capillary permeability to macromolecules.[1] Morphological features such as accumulation of extracellular matrix proteins and thickening of glomerular basement membrane have been recognized as the pathological hallmark of diabetic nephropathy and appear to occur prior to microalbuminuria.[1,2] Transforming growth factor beta 1 (TGF-ß1) is a multifunctional cytokine with potent fibrogenic properties.[3] It plays a key role in the deposition of extracellular matrix via stimulation of synthesis of extracellular matrix protein and inhibition of extracellular matrix degradation proteinases.[4,5] A recent study has demonstrated increased glomerular and circulating TGF-ß1 in patients with type II diabetes mellitus (DM) with mild and advanced nephropathy implicating its role in the development of DN.[6] The aim of the present study was to evaluate serum TGF-ß1 in type II diabetic patients, both controlled and uncontrolled patients, in variable stages of DN in order to clarify its association with diabetic kidney disease and to assess its use as a marker of renal injury in diabetic patients.

Methods. Sixty outpatients with type II DM in various stages of DN, who attended the outpatient internal medicine clinic from January 2003 to

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March 2003, were the subjects of the present study besides 10 healthy age and gender matched controls. They were divided into 2 main groups according to the level of glycosylated hemoglobin (HbA1c) detected by colorimetric method (Stanbio laboratory, Texas, USA): Group I consisted of 31 patients with type II DM with good metabolic control (HbA1c 5.5-7.6%). Group II consisted of 29 patients with type II DM with poor metabolic control (HbA1c above 7.6%). Each group was further subdivided into 3 subgroups according the degree of nephropathy determined by blood urea, serum creatinine, 24-hour urinary protein excretion and urinary albumin excretion rate (UAE) estimated by Micral Test® kit, supplied by Roche. The 6 subgroups were: Group A was consisted of 11 DM patients with good glycemic control and microalbuminuria (UAE 20-200 ug/minute). They were 7 males and 4 females with mean age of 54.90±5.97 years. The mean duration of DM was 11.27±4.47 years. Three (27.27%) patients were smokers and 4 (36.36%) were hypertensive. Group B was consisted of 8 DM patients with good glycemic control and macroalbuminuria (UAE >200 ug/minute). They were 5 males and 3 females with mean age of 55.25±8.41 years. The mean duration of DM was 12.75±5.09 years. Five (62.5%) patients were smokers and 4 (50%) were hypertensive. Group C was 12 DM patients with good glycemic control, overt nephropathy and renal impairment (serum creatinine >5 mg/dl). They were 7 males and 5 females with mean age of 51.25±2.67 years. The mean duration of DM was 14.58±3.70 years. Seven (58.3%) patients were smokers and 9 (75%) were hypertensive. Group D was consisted of 9 DM patients with poor glycemic control and microalbuminuria (UAE 20 - 200 ug/minute). They were 6 males and 3 females with mean age of 57.15±7.26 years. The mean duration of DM was 7.39±5.23 years. Five (55.6%) patients were smokers and 4 (44.4%) were hypertensive. Group E was consisted of 12 DM patients with poor glycemic control and macroalbuminuria (UAE >200 µg/minute). They were 5 males and 7 females with mean age of 57.50±7.54 years. The mean duration of DM was 10.75±3.14 years. Seven (58.33%) patients were smokers and 7 (58.33%) were hypertensive. Group F was consisted of 8 DM patients with poor glycemic control, overt nephropathy and renal impairment (serum creatinine >5 mg/dl). They were 5 males and 3 females with mean age of 56.37±6.83 years. The mean duration of DM was 14.5±4.21 years. Four (50%) patients were smokers and 7 (87.5%) were hypertensive. In addition, 10 age and gender matched healthy volunteers served as controls. They were 5 males and 5 females with mean age of 57.4±4.65 years. All study subjects gave informed consent and the study protocol was approved by the Cairo University Hospital Ethics committee. Serum TGF-ß1 level was assessed by enzyme linked immunosorbent assay (ELISA) (BioSource Europe S.A).

Data were expressed as mean±standard deviation. The Student t test was used for group comparisons of the quantitative parameters. Linear regression analysis was performed to evaluate the presence of a statistically significant relationship between serum TGF-ß1 level and other baseline variables. *P* value <0.05 was considered statistically significant.

Results. Serum TGF-ß1 level was significantly increased in all diabetic groups compared to control ($p<0.05$) (**Table 1**). In addition, serum TGF-ß1 level was significantly increased in type II DM patients with poor metabolic control (group II, HbA1c >7.6%) compared to type II DM patients with good glycemic control (group I, HbA1c 5.5-7.6%), both with variable

Table 1 - Clinical and biochemical data of the study groups.

Parameter	Group A	Group B	Group C	Group D	Group E	Group F	Control
Age (year)	54.90±5.97	55.25±8.41	51.25±2.67	57.15±7.26	57.50±7.54	56.37±6.83	57.4±4.65
Gender (M:F)	7:4	5:3	7:5	6:3	5:7	5:3	5:5
FBS (mg/dl)	167.82±32.61*	198.90±42.53*	145.41±8.96*	241.11±57.59*	241.08±55.68*	202.34±43.2*	82.11±6.67
HBA_{1C} %	7.07±6.65*	7.15±0.29*	6.95±0.51*	8.62±1.15*	10.3±1.54*	8.12±1.23*	5.61±0.72
UAE (ug/min)	35.45±18.64*	>200*	>200*	73.33±21.79*	>200*	>200*	-
24/hour urinary protein (g/day)	0.10±0.03	1.31±0.43*	2.15±0.94*	0.16±0.05	1.67±0.83*	2.87±0.54*	0.04±0.01
Serum creatinine (mg/dl)	0.96±0.29	1.88±1.03*	5.98±1.65*	1.12±0.28	1.98±0.97*	6.22±1.3*	0.7±0.12
Blood urea (mg/dl)	39.27±12.19	63.5±22.4*	125.23±8.97*	44.11±4.04	69.91±16.35*	180.45±23*	22.1±4.45
Serum TGF-$ß_1$ (ng/dl)	137.8±69.50*	329.25±41.46*	406.83±109.81*	256.67±51.54*	514.82±159.23*	508.75±101.62*	29.6±10.58

Data are expressd as mean±SD. *$p<0.05$, FBS - fasting blood sugar, HbA1c - glycosylated hemoglobin, TGF-$ß_1$ - transforming growth factor-beta 1 UAE - urinary albumin excretion

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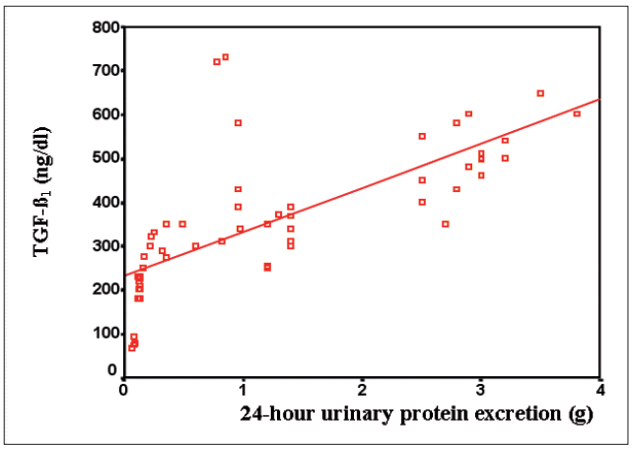

Figure 1 - Relationship between serum transforming growth factor-beta 1 (TGF-ß1) and 24-hour urinary protein excretion in the study groups. (r=0.693, p<0.01).

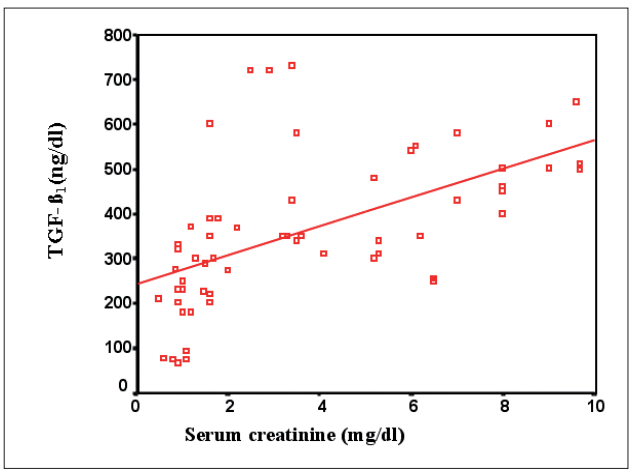

Figure 2 - Relationship between serum transforming growth factor-beta 1 (TGF-ß1) and serum creatinine in the study groups (r=0.693, p<0.01).

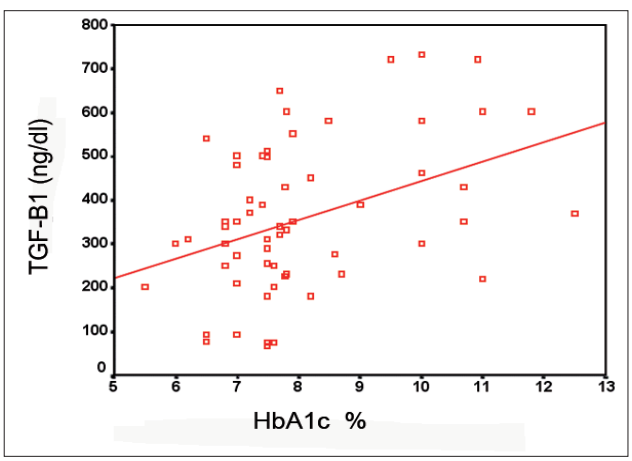

Figure 3 - Relationship between serum transforming growth factor-beta 1 (TGF-ß1) and HbA1c in the study groups (r=0.693, p<0.01).

degree of DN (433.03±166.75 versus 291.36±144.20, p<0.05). Serum TGF-ß1 level was also significantly increased in hypertensive type II DM patients compared to normotensive type II DM patients (437.23±158.39 versus 251.48±113.47 respectively, p<0.05). There were significant positive correlations between serum TGF-ß1 level and 24-hour urinary protein excretion (r=0.69, p<0.01) (**Figure 1**), serum creatinine (r=0.54, p<0.01) (**Figure 2**), blood urea (r=0.522, p<0.01), and HbA1c level (r=0.40, p<0.01) (**Figure 3**). No significant correlation was found between serum TGF-ß1 level and age, gender, duration of DM or fasting blood sugar (p>0.05).

Discussion. The present study revealed significant increase in serum TGF-ß1 level in diabetic groups with variable degrees of diabetic nephropathy compared to healthy controls (p<0.05). This finding is consistent with the previous observations indicating a pathological role of TGF-ß1 in the development of pathological manifestations of DN.[7] Elevation of circulating active TGF-ß1 in a transgenic mouse model was associated with progressive glomerular disease characterized by mesangial expansion, accumulation of extracellular matrix proteins and interstitial fibrosis.[8] Glomerular TGF-ß1 mRNA over-expression was demonstrated in experimental diabetic models as well as in human biopsy samples of DN.[9,10] Several studies suggested that diabetic kidneys act as a factory for production of TGF-ß1 releasing it into the systemic circulation. In contrast to normal kidneys that extract TGF-ß1 from the blood, diabetic kidneys produce and release TGF-ß1 into the renal veins leading to elevated serum and urinary level in patients with type II DM.[7] Bordin et al[6] demonstrated increased glomerular and circulating levels of TGF-ß1 in type II DM patients with mild and advanced nephropathy. In addition, one study showed marked increase in circulating TGF-ß1 level in patients having type II DM with DN compared to those without renal involvement.[11] Serum TGF-ß1 level was demonstrated to be increased at the onset of type II diabetes and remained elevated throughout the disease even in normoalbuminuric patients, thus highlighting the impact of hyperglycemia on the production of this profibrotic cytokine.[12] Serum TGF-ß1 level was significantly increased in patients with poor glycemic control with variable degree of renal affection compared with those with good glycemic control and comparable degree of renal affection (p<0.05). In addition, serum TGF-ß1 level showed significant positive correlation with HbA1c (p<0.01). These findings confirm the direct link between hyperglycemia and activation of TGF-ß1. It was suggested that glucose may be a direct activator of latent TGF-ß1 level.[11] Glucose stimulates de novo

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synthesis of diacylglycerol (DAG), generated from glycolytic intermediates through the polyol pathway, elevated DAG then leads to activation of protein kinase C (PKC) which increases TGF-ß1 synthesis and matrix protein synthesis in measngial and tubular cells.[13] Glomerular TGF-ß1 mRNA levels were found to closely correlate with HbA1c levels in diabetic patients.[14,15] The present study demonstrated significant positive correlation between serum TGF-ß1 level and blood urea, serum creatinine and 24 hour urinary protein excretion ($p<0.01$). This close association between circulating TGF-ß1 and the development and progression of renal disease has been previously confirmed in type II diabetic patients.[2] Urinary TGF-ß1 excretion was also elevated in type II diabetic patients with severe mesangial expansion.[16] One recent study showed significant positive correlations between serum TGF-ß1 level and urinary albumin excretion rate and serum creatinine level, the 2 strong parameters of disease progression, in type II diabetic patients.[11] These data strongly support the hypothesis that TGF-ß1 is the key mediator of diabetic renal hypertrophy and extracellular matrix expansion in experimental and human DN. The present work showed significant increase in serum TGF-ß1 level in hypertensive diabetic patients compared to normotensive diabetics. This finding was demonstrated previously by Li et al,[17] who showed circulating levels of TGF-ß1 to be significantly correlated with blood pressure levels. TGF-ß1 was shown to be an important regulator of blood pressure and vascular remodeling via stimulation of endothelin-1 and /or rennin secretion.[17] A positive correlation has been demonstrated recently between serum TGF-ß1 level and diastolic blood pressure readings in type II diabetic patients with DN suggesting its close association with the severity of vascular and renal disease in diabetes mellitus.[18] TGF-ß1 was demonstrated to be a useful marker for evaluating the severity and progression of hypertensive renal disease as determined by urinary albumin excretion.[19] There was no significant correlation between serum TGF-ß1 level and age and the duration of diabetes. These results are in agreement with Sharma et al,[7] who found renal production of TGF-ß1 neither correlate with age of patients nor the duration of diabetes. No significant difference was found in serum TGF-ß1 level between male and female subjects of the present study and this was previously reported in a recent study.[20] In addition, no significant difference was found in serum TGF-ß1 between smokers and non-smokers in the present study. This finding is contradictory to that of Esmatjes et al,[21] who reported higher serum TGF-ß1 level in smoker patients with type I diabetes suggesting that tobacco consumption may amplify the effect of hyperglycemia on the production of TGF-ß1.

To conclude, type II diabetic patients with diabetic nephropathy exhibited increased circulating TGF-ß1 that was significantly correlated with the degree of renal involvement, suggesting an association between serum TGF-ß1 and the development of diabetic renal disease. Serum TGF-ß1 may, therefore, represent one parameter that can be used to evaluate the progression of diabetic nephropathy.

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