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Serum and urinary transforming growth factor beta 1 as biochemical markers in diabetic nephropathy patients

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ABSTRACT

Diabetic nephropathy is a common complication of diabetes mellitus. Transforming growth factor beta 1 (TGF-β1) is considered to be one of the major cytokines involved in the regulation of extracellular matrix (ECM) synthesis and degradation. Discrepant results were reported for urine TGF-β1 in diabetic patients. The aim of the present study is to investigate urine and serum TGF-β1 in patients with type II diabetes with and without nephropathy.

The study was performed on 72 patients with type II diabetes (26 macroalbuminuria, 27 microalbuminuria and 19 normoalbuminuria) together with 30 healthy subjects to serve as controls. Urinary and serum TGF-β1 and urine albumin were investigated by Elisa, plasma glucose, whole blood glycated hemoglobin, Urinary and serum total protein, creatinine, were determined by colorimetric methods. Urine and serum TGF-β1 were significantly increased in all diabetic groups being more pronounced in the macroalbuminuria group than micro and normoalbuminuria groups. Also urine total protein was increased, being more pronounced in macroalbuminuria group than other groups. Urinary and serum TGF-β1 showed high positive correlation with urinary total protein concentration in macro and microalbuminuria groups r = (0.9, 0.9, 0.8 and 0.89) respectively. The results revealed significant increase in Urinary and serum TGF-β1 and their concentrations in urine were parallel to urine proteins and albumin concentrations; urinary and serum TGF-β1 showed high positive correlation with urinary total protein, so it could be used as a marker with total protein and albumin to confirm the diabetic nephropathy in type II diabetic patients.

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1. Introduction

Diabetes mellitus is a metabolic disease, which is characterized by high glucose levels in blood (hyperglycemia) and urine (glucosuria). Diabetes affects more than 170 million people worldwide and the number will rise to 370 million people by 2030. About one third of those affected, will eventually have progressive deterioration of renal function (Chen et al., 2013). A long standing diabetic state of diabetes mellitus can result in several severe chronic complications: cardiovascular complications, diabetic retinopathy, diabetic neuropathy and diabetic nephropathy (Ramezani et al., 2007). Diabetic nephropathy (DN) is one of the most severe complications of diabetes mellitus (El Mesallamy et al., 2008). The classical definition of diabetic nephropathy is a progressive rise in urine albumin excretion, coupled with increasing blood pressure, leading to declining glomerular filtration and eventually end stage kidney failure (Obineche and Adem, 2005). Diabetic nephropathy is characterized structurally by renal hypertrophy and by progressive mesangial deposition of extracellular matrix (ECM) characterizing glomerulosclerosis, which is associated with progressive glomerular capillary occlusion, albuminuria and a progressive fall in glomerular filtration rate (GFR). Overt diabetic nephropathy is characterized by persistent proteinuria (>500 mg/24 h) or macroalbuminuria (>300 mg/24 h) (Rivarola et al., 1999). In the natural history of the disease, proteinuria is preceded by stages of excessive glomerular filtration and of microalbuminuria (30–300 mg/24 h), which signals an increased risk of progression to overt nephropathy (Williams, 2005). The degree of proteinuria correlates with the progression of glomerulosclerosis and tubulointerstitial fibrosis (Wolf and Ziyadeh, 2007).

Transforming growth factor beta (TGF-β1) is a multifunctional cytokine implicated in the pathogenesis of many forms of progressive renal disease, including diabetic nephropathy (McKnight et al., 2007). TGF-β1 directly stimulates the transcription of many extracellular matrix genes in renal cells including mesangial, endothelial and tubular cells. In addition, it decreases collagenase production and simultaneously stimulates expression of tissue inhibitors of metalloproteinases, resulting in inhibition of extracellular matrix turnover. Deposition of extracellular matrix components, including fibronectin and collagen types I, III and IV, is an important component of the scarring observed during the progression of glomerulosclerosis and tubulointerstitial fibrosis. An increase in the synthesis as well as a decrease in turnover of these proteins is responsible for the net accumulation of extracellular matrix (Fukuda et al., 2009).

A number of molecular mediators and intracellular signaling pathways that have been identified in diabetic kidney injury have been found to stimulate the renal TGF-β1 activity as an intermediary step. These mediators are: high glucose concentration, early and advanced products of nonenzymatic glycation of proteins, oxidative stress, glomerular hypertension, de *novo* synthesis of diacylglycerol and protein kinase C activation, glucosamine overproduction, and high levels of vasoactive substances such as intrarenal angiotensin II, endothelin, and thromboxane (Ziyadeh, 2004). Several clinical studies have established that TGF-β1 is increased in the kidneys of diabetic patients (Sharma and McGowan, 2000). Urinary TGF-β1 measurement has been suggested as a marker for diabetic nephropathy in some studies as reported by Sato et al. (1998) who found higher TGF-β1 excretion in diabetic patients well correlated to the state of nephropathy, other studies have not shown the association of urinary TGF-β1 with diabetic nephropathy as reported by Eija et al. (2000) who did not find difference in urinary TGF-β1 excretion between microalbuminuric and normoalbuminuric patients.

The aim of the present work is to study the role of TGF-β1 as biochemical marker in diabetic nephropathy which was monitored by glycemic status and kidney function measurements and to explore the correlation between serum TGF-β1 and urinary TGF-β1 in diabetic nephropathy although it has been suggested as a marker for diabetic nephropathy not all studies had shown the association of urinary TGF-β1 with nephropathy.

2. Patients and methods

2.1. Patients

This study included 102 subjects which classified into 2 main groups.

Group (I): thirty adult hypertensive healthy volunteers served as control. They were on antihypertensive therapy with captopril. The control group was selected as hypertensive healthy subjects since all the diabetic patients in group II were hypertensive. The systolic and diastolic BP values for all subjects were shown in Table 1.

Group (II): seventy two previously diagnosed type 2 diabetic patients from outpatients' clinic of the National Institute for Urology and Nephrology, Cairo, Egypt. All type 2 diabetic patients met the criteria of American Diabetes Association (ADA) for type 2 diabetes. All type 2 diabetic subjects were not receiving any medications other than hypoglycemic drugs and hypotensive drug (Captopril) and were not complaining of any chronic or acute illness.

This group was subdivided into the following subgroups according to albumin excretion rate (AER):

- **Group IIa** nineteen diabetic patients with normoalbuminuria having (AER<30 mg/g creatinine).
- **Group IIb** Twenty seven diabetic patients with microalbuminuria having (AER 30–300 mg/g creatinine).
- **Group IIc** Twenty six diabetic patients with macroalbuminuria having (AER > 300 mg/g creatinine).

Full history including age, sex, diabetic duration and treatment were recorded for all subjects. The studied groups were matched as regards age and sex. Written informed consent was obtained from the subjects and this study was approved by the Ethics Committee of the National Research Center.

2.2. Exclusion criteria

Chronic liver diseases, heart diseases, coronary artery diseases (CAD), malignancy, autoimmune diseases, chronic renal