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Original Article

Plasma endoglin in Type2 diabetic patients with nephropathy

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ABSTRACT

Background: Diabetic nephropathy may be a common complication of diabetes mellitus. Endoglin is glycoprotein located on cell surfaces of endothelial cells and is part of the transforming growth factor beta (TGF- β) receptor. Endoglin expression is enhanced in endothelial cells during injury and inflammation. The aim of this study was to estimate the plasma level of soluble endoglin (sEng) in type 2 diabetic patients (with and without nephropathy). Also to explore its availability as marker for disease progression.

Methods: In this study, sixty eight patients with type 2 diabetes mellitus (T2DM) were included; the patients were sub-grouped to normoalbuminuria without nephropathy and moderately increased albuminuria (microalbuminuria) with nephropathy groups with 13 individuals as control group. Plasma soluble endoglin level was determined using ELISA technique. Fasting plasma glucose (FPG), glycated haemoglobin (HbA1c), lipid profile, and creatinine were determined using colorimetric assay, whereas glomerular filtration rate (GFR) was calculated.

Results: The plasma level of sEng of both normoalbuminuria group 1 and microalbuminuria group 2 were significantly higher when compared to control group. While, the plasma level of sEng in microalbuminuria group 2 was nonsignificant lower when compared to normoalbuminuria group 1. Also, there was a significant positive association between plasma level of sEng and HbA1c, HDL-C and urinary albumin concentration in normoalbuminuria group.

Conclusion: Plasma level of soluble Endoglin is markedly increase prior to alteration in endothelial function, and increases to lesser extent with the developing of diabetic nephropathy which indicated disease progression and development of vascular abnormalities.

1. Introduction

Approximately 40% of diabetics will evolve diabetic nephropathy (DN) [1]. Diabetic nephropathy is a metabolic disorder with high morbidity and mortality and is now the driving cause of end-stage kidney disease [2]. Diabetic patients with nephropathy have high rates of mortality due to cardiovascular complication [3]. Mortality in diabetic patients with nephropathy is almost 20–40 times higher than in patients without nephropathy [4]. People who have diabetic nephropathy have low glomerular filtration rate (GFR) and persistent albuminuria [5]. Kidney Disease Improving Global Outcomes (KDIGO) incorporates the albuminuria level (given as albumin creatinine ratio (ACR) (in mg per g) and divided into three albuminuria categories: Normal-to-mildly increased albuminuria (A1) known as normoalbuminuria (ACR < 30 mg/g), moderately increased albuminuria (A2) previously known as microalbuminuria (ACR 30–300 mg/g) and severely increased albuminuria, formerly known as macroalbuminuria, (ACR >300) [6,7]. Progress to end-stage kidney disease, is one of the common significant cause in the development of organ damage and indirectly increase mortality in diabetic patients [8]. Diabetic nephropathy pathogenesis is not clear but several mechanisms are believed to participate in its development as hyperglycemia, advanced glycation end products, protein kinase C, oxidative stress, inflammation, and poly (ADP-ribose) polymerase activation [8]. At present, there is no effective clinical treatment and kidney damage

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is reduced mainly by lowering blood glucose [9]. Endoglin (CD 105), also called TGF-β receptor III, is a transmembrane receptor glycoprotein for TGF-β1 and TGF-β3 which regulates TGF- β signalling [10]. It is mostly expressed on proliferating endothelial cells, activated macrophages, smooth muscle cells and fibroblast. Expression of endoglin is enhanced in vessels during various pathological situations like hypoxia or injury [11].

Thus, the aim of this study is to explore the possibility of plasma endoglin as early biomarker of kidney impairment in normoalbuminuric patients with diabetes, also the disease progression in patient with diabetic nephropathy.

2. Materials and methods

2.1. Study subjects

This study was conducted on 68 patients with type 2 diabetes mellitus were recruited from National Institute of Diabetes and Endocrinology, Cairo, Egypt. They were divided into two groups according to urinary albumin concentration (UAC); First group was 43 patients with normoalbuminuria (<30 mg/day). They were 17 males and 26 females, of less than 5 years of disease development; their mean age was 56.63 ± 5.47 years. Second group was 25 patients with microalbuminuria (30–300 mg/day). They were 11 males and 14 females. The onset of disease was between 5 and 15 years; their mean age was 57.16 ± 5.93 years. In addition, 13 apparently healthy individuals (7 males and 6 females) were included in this study as control group; their mean age was 52.92 ± 6.97 years. Exclusion criteria were acute illness at time of the study, systemic chronic inflammation, and history of deep or superficial venous thrombosis, thrombocytosis, liver disorders, malignancy, hypertension and cardiac disease. This study was approved by the research ethics committee of general Organization of Teaching Hospitals and Institutes. Written informed consent was obtained from control subjects and all patients.

2.2. Methods

Ten ml fasting venous blood samples were taken to determine fasting plasma glucose (FPG), glycated haemoglobin (HbA1c), total cholesterol, triglycerides (TG), low density lipoprotein (LDL-C) and high density lipoprotein (HDL-C), creatinine and plasma sEng concentrations. Available biochemical commercial kits were utilized for determination of plasma glucose concentrations by glucose oxidase reaction [12], glycated haemoglobin measured according to the method of [13], using ion exchange high-performance liquid chromatography (BioRad D-10)®. Total cholesterol, triglycerides and HDL-C were estimated spectrophotometrically while LDL-C was calculated mathematically; LDL-C = TC-HDL-C-(TG/5) [14]. Serum creatinine level was measured using colorimetric method [15]. GFR was estimated using Modification of Diet in Renal Disease equation [16]. Urine samples were collected for the determination of urinary albumin concentration by turbidimetric immunoassay [17] (biosystem SA, Barcelona, Spain). Plasma soluble endoglin (sEng) concentration was measured by using an enzyme linked Immunosorbent assay method (Human Endoglin; R&D Systems, Minneapolis, MN, USA), according to the manufacturer instructions [18].

2.3. Statistical analysis

All statistical data were presented as means ± standard deviation (M ± SD) and compared by unpaired Student's t-test. All analysis and graphics were performed using GraphPad prism 6.0 (GraphPad software 2010). Correlations were tested by Pearson's correlation analysis. Differences were considered statistically significant at P value > 0.05.

3. Results

Sixty eight uncontrolled T2DM participants were enrolled in the study to determine the role of soluble endoglin in diabetic nephropathy pathogenesis. Among them, 43 T2DM patients were normoalbuminuria their age mean was (56.63 ± 5.47), 25 of them are microalbuminuria; their age mean was (57.16 ± 5.93) and 13 subjects were apparently healthy as control; their age mean was (52.92 ± 6.97) as shown in Table 1.

There was a significant difference between body mass index (BMI) of both patients groups when compared to control group ($p < 0.05$, Table 1) with no significance difference between patients groups.

Regarding fasting plasma glucose (FPG) was significantly increased in both patients groups when compared to control group ($p < 0.001$, Table 1), glycated haemoglobin (HbA1c) show the same pattern as FPG ($p < 0.001$, Table 1) without significance difference between patients groups for both parameters.

Serum creatinine did not reach significant level between group 1 and group 2 when compared to control group, while there was a high significant difference between patient groups, there was increased in group 2 than in group 1 ($p < 0.01$, Table 2). Also, glomerular filtration rate in group 1 and group 2 showed no statistically significant difference when compared to control group, while it was significantly decreased in group 2 when compared to group 1 ($p < 0.01$, Table 2).

Urinary albumin concentration did not show any significant difference between group 1 and control, while in group 2, there was a high significance difference when compared to control and group 1 ($p < 0.001$, Table 2).

In both patient groups, we found that, total cholesterol, Triglycerides (TG), and LDL cholesterol (LDL-C) levels were significantly increased in both patient groups compared with control group, whereas HDL cholesterol levels were significantly lower in both patients group when compared to control ($P < 0.001$, Table 3).

Regarding the plasma level of sEng, both patients groups normoalbuminuria and microalbuminuria had significantly higher sEng concentrations when compared to the control group ($p < 0.001$). While there was no significant difference in sEng levels between the normoalbuminuria group and the microalbuminuria group (Table 4).

Plasma level of soluble endoglin showed a significant positive correlation with HbA1c (%), HDL (mg/dL) and UAC (mg/L) in normoalbuminuria group but there is no significant correlation was observed in microalbuminuria group (Table 5).

The overall performance of sEng in both patients groups was assessed by ROC curve analysis. The best cut off point for plasma sEng was 3.6 in both patient groups with 97.7% sensitivity and 100% specificity producing area under the curve (AUC) 0.997 in normoalbuminuria group ($p < 0.001$, Fig. 1a), and 100% sensitivity and 100% specificity producing AUC = 1.00 for microalbuminuria group ($p < 0.001$, Fig. 1b).

4. Discussion

Despite the fact that microalbuminuria is regarding the standard test for diagnosis of an early and reversible DN, the sensitivity to precisely detect disease progression remains unsatisfied [19]. Therefore, recognise more sensitive diagnostic markers for better monitoring of disease pathogenesis could largely facilitate earlier diagnosis and prevent disease progression. In the present study, both diabetic groups showed significantly higher body mass index when

Table 1
The baseline characteristics of the studied groups.

Parameters	Groups		
	Control (n = 13)	Normoalbuminuria group 1 (n = 43)	Microalbuminuria group 2 (n = 25)
Male (%)	7 (53.9%)	17 (39.5%)	11 (44%)
Female (%)	6 (46.2%)	26 (60.5%)	14 (56%)
Age (years)	52.92 ± 6.97	56.63 ± 5.47	57.16 ± 5.93
BMI (Kg/m^2)	27.33 ± 4.75	33.91 ± 3.39[a]	33.52 ± 4.19[a]
FPG (mg/dl)	91.92 ± 6.71	223.1 ± 87.23[a]	241.7 ± 67.57[a]
HbA1c (%)	4.54 ± 0.93	6.93 ± 1.70[a]	7.40 ± 1.56[a]

BMI: body mass index, FPG: fasting plasma glucose, HbA1c: glycated hemoglobin, M ± SD: mean ± standard deviation, a: significant from control group at P < 0.05.

Table 2
Kidney function tests in all the studied groups.

Parameters	Groups		
	Control (n = 13)	Normoalbuminuria group1 (n = 43)	Microalbuminuria group 2 (n = 25)
Creatinine (mg/dL)	0.75 ± 0.11	0.77 ± 0.16	0.96 ± 0.40[b]
GFR (ml/min per 1.73 m^2)	105.6 ± 29.14	115.9 ± 23.89	96.99 ± 28.65[b]
UAC (mg/L)	10.39 ± 2.97	14.06 ± 8.03	72.18 ± 33.31[ab]

GFR: glomerular filtration rate, UAC: urinary albumin concentration, a: significant from control group at P value < 0.01, b: significant from Normoalbuminuria patients group at P value < 0.01, values are represented as M ± SD: mean ± standard deviation.

Table 3
Lipid profile in all studied groups.

Parameters	Groups		
	Control (n = 13)	Normoalbuminuria group1 (n = 43)	Microalbuminuria Group 2 (n = 25)
TG (mg/dL)	113.31 ± 22.99	183.23 ± 11.42[a]	190.96 ± 27.41[a]
T-Cholesterol (mg/dL)	144.08 ± 19.04	224.98 ± 11.40[a]	220.33 ± 9.55[a]
HDL-C (mg/dL)	52.23 ± 4.38	35.86 ± 3.04[a]	35.40 ± 2.80[a]
LDL-C (mg/dL)	69.18 ± 19.44	152.46 ± 11.62[a]	149 ± 8.85[a]

TG: triglyceride, T-Cholesterol: total cholesterol, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, a: significant from control group at P value < 0.001, values are represented as mean ± SD: mean ± standard deviation.

Table 4
Plasma level of sEng (ng/ml) in all the studied groups.

Parameter	Groups		
	Control (n = 13)	Normoalbuminuria group1 (n = 43)	Microalbuminuria Group 2 (n = 25)
sEng (ng/mL)	1.747 ± 0.93	9.595 ± 3.49[a]	8.827 ± 2.67[a]

[a] Significant from control group at P value < 0.001, values are represented as M ± SD: mean ± standard deviation.

Table 5
Pearson's correlation between sEng and HbA1c, HDL and UAC in normoalbuminuria and microalbuminuria groups.

Parameters	Groups			
	Normoalbuminuria group		Microalbuminuria group	
	sEng		sEng	
	r	P	r	P
HbA1c (%)	0.4	0.02*	0.2	0.30
HDL-C (mg/dL)	0.3	0.04*	−0.05	0.82
UAC (mg/L)	0.3	0.04*	0.1	0.75

HbA1C: glycated hemoglobin, HDL-C: high density lipoprotein, UAC: urinary albumin concentration, r: correlation coefficient,*: significant at P value < 0.05.

compared to control subjects (Table 1, p < 0.001), consequently, it is important to consider the influence of obesity on development of microvascular complications in diabetic patients. These results agreed with Rossi et al. [20], who showed that the increase in BMI over time is associated with a parallel increase in ACR.

We found also that total cholesterol, Triglycerides, and LDL cholesterol levels were significantly high in both patient groups compare to control (Table 3), whereas HDL cholesterol levels were significantly lower in both patient groups (P < 0.001) compare with control. This agreed with Kachhawa et al. [21], who reported an increase in lipid profile except for HDL cholesterol in T2DM patients group compared to control group.

Dyslipidemia is noted in diabetic patients with early stage of kidney injury. However, the involvement of lipid abnormalities in the pathogenesis of kidney injury is still a point of research. Generally, diabetic dyslipidemia is due to impaired function of lipoprotein lipase which is localized in the endothelial cells, leading to increase serum levels of triglyceride and decrease high-density lipoprotein cholesterol.

Glycated and oxidized LDL which are smaller size play vital roles to induce vascular and renal cellular dysfunction [22]. Previous studies elucidated that dyslipidemia increase macrophage activation and exaggerated extracellular matrix expression in the glomeruli under diabetic conditions, leading to DN [22].

Our results showed that microalbuminuria developed in group 2

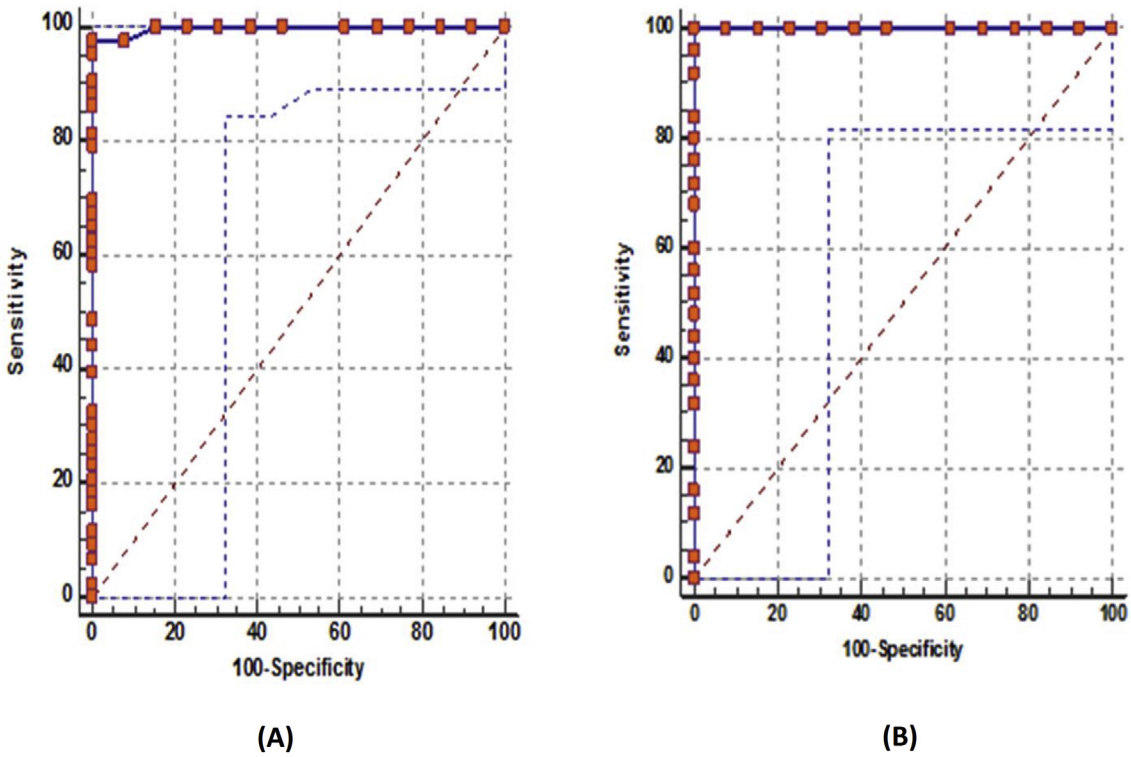

Fig. 1. (A) ROC curve of plasma sEng for T2DM normoalbuminuria group. (B): ROC curve of plasma sEng for T2DM microalbuminuria group.

diabetic patients. This result consistent with Wu et al. [23], who reported in their prospective study that microalbuminuria developed after 9-year duration of diabetes and 3 (1–6) years of follow-up. Moreover, obesity and weight gain in patients with T2DM due to insulin resistance may be involved in the pathology of microalbuminuria [24].

Microalbuminuria is an earlier sign of endothelial dysfunction and vascular damage [25]. Endothelial dysfunction is an important risk factor of many kidney and metabolic diseases, including, primary hypertension, cardiovascular and chronic kidney diseases and diabetic nephropathy [26].

Endoglin belongs to endothelial microparticles (EMPs) which are novel biomarkers facilitating the evaluation of endothelial function in DM-related vascular diseases. EMPs are membrane vesicles derived from apoptotic or activated endothelial [27].

The level of plasma EMPs is considerably higher in patients with type 2 DM than in normal individuals [28]. Moreover, its plasma level in patients with type 2 DM suffering from complications is significantly higher than in diabetic patients without complications [29].

Apoptotic endothelial cells express and release high level of endoglin when compared with activated endothelial cells [30]. Although more studies have shown that increased sEng level by itself is not capable of developing endothelial dysfunction in an animal model, but their finding does not abolish the possibility that sEng might lead to variation of endothelial function in the presence of other risk factors related to chronic vascular diseases [31].

Our result revealed a significant increment of plasma endoglin concentrations in both normoalbuminuria and microalbuminuria groups compared with control group. Furthermore, considering the inverse association between circulating sEng and GFR and the presence of slightly lower sEng concentration in the microalbuminuria group compared to the normoalbuminuria group and this in agreement with Emeksiz et al. [32], and a week significant positive correlation occurred between sEng and UAC in normoalbuminuria patients only and this was in agreement with Emeksiz et al. [32], who reported an increase of serum level of sEng in diabetic patients without nephropathy (normoalbuminuria) then with disease progression to microalbuminuria the level decreased.

We speculate that exposure to T2DM at first may give rise to an increase in serum sEng concentrations; but, with the development of microalbuminuria, a relative decline in sEng concentrations might be found and all these observations suggest that increased level of sEng may be an early indicator of functional vascular alterations due to diabetes and even might arise before the development of microalbuminuria and the manifestation of subclinical structural vascular alterations in T2DM.

Also, we found that, although did not reach significance level, endoglin plasma levels were inversely related to fasting plasma glucose in both patients groups and positive association with glycated haemoglobin in diabetic patients with normoalbuminuria group, this in agreement with Blázquez-Medela et al. [33], who reported a negative but non significance association between sEng and fasting plasma glucose in diabetic patients with normal blood pressure, and positive correlation with glycated haemoglobin in all diabetic patients groups with normal or high blood pressure. Therefore, sEng level may serve as a prognostic indicator of vascular pathologies associated with diabetes such as endothelial dysfunction, hypertension and cardiovascular risk [33].

SEng has been involved as a potential marker for endothelial dysfunction [34]. Hypoxia and oxidative stress are considered triggers of sEng release, which, in turn, inhibits the antiatherogenic effects induced by TGF-β [35].

SEng may be important in certain stages of vascular disease due to diabetes. The concentration of sEng may decline over time and this decrease may be due to decreased production or enhanced complex formation with other unidentified substances in the circulation [32].

5. Conclusion

Plasma soluble endoglin is markedly increased in the early stage of T2DM with normoalbuminuria while increase to lesser extent with the developing of DN with microalbuminuria. sEng might be used as a marker in addition to albuminuria in early stages of diabetic nephropathy. sEng levels may serve as a biomarker of vascular pathologies related to endothelial dysfunction.

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Conflicts of interest

The authors have no conflict of interest to declare.

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