Plasma long pentraxin 3 as a marker of endothelial dysfunction in early diabetic nephropathy

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Received 12 June 2013
Accepted 29 June 2013

The Egyptian Society of Internal Medicine
2013, 25:117–126

Objective
This study investigated the utility of measuring plasma long pentraxin 3 (PTX3) levels in the early detection of endothelial dysfunction compared with Von Willebrand factor (vWF) activity and flow-dependent arterial dilatation (well-known markers of endothelial dysfunction) in early diabetic nephropathy.

Materials and methods
A total of 50 Egyptian patients with type 2 diabetes and 20 healthy controls were recruited from the Diabetes, Endocrinology and Metabolism center, Faculty of Medicine, Cairo University. The diabetic patients were divided into two equal groups of comparable age and sex: group I consisted of patients with normal urinary albumin excretion and group II consisted of patients with microalbuminuria.

Results
In group II, the plasma PTX3 level was significantly higher (median value 2.3 ng/ml) and the mean flow-mediated dilatation (FMD; 0.433 ± 0.059) was significantly lower when compared with the control group (PTX3 1.15 ng/ml, FMD 0.901 ± 0.04; $P<00001$) and with group I (PTX3 1.2 ng/ml, FMD 0.627 ± 0.05; $P<0.0001$). The vWF activity (median value) was significantly higher in the two diabetic groups compared with controls (20.2, 16.3 and 4% in group I, group II, and controls, respectively; $P<0.0001$), with no significant difference between the two diabetic groups. There was a significant positive correlation between PTX3 levels and vWF activity ($P<0.001$), diabetes duration, and concentration of fasting blood sugar, HbA1c, cholesterol, and triglyceride and a significant negative correlation between PTX3 levels and FMD ($P<0.001$) in all diabetic patients. In group II, there was a significant positive correlation between PTX3 levels and vWF activity ($r=0.603$, $P=0.001$).

Conclusion
PTX3 may represent a useful endothelial dysfunction marker in early diabetes nephropathy.

Keywords:
endothelial dysfunction, long pentraxin 3, microalbuminuria, type 2 diabetes, vWF

Egypt J Intern Med 25:117–126

1110-7782

Introduction

Endothelial dysfunction has been demonstrated in type 1 and type 2 diabetes. It is incriminated in the early pathogenesis of atherosclerosis and is considered an early indicator of cardiovascular disease. It is present in individuals with frank type 2 diabetes, in those with increased insulin resistance (e.g. obese patients), in those at high risk for developing type 2 diabetes (i.e. impaired glucose tolerance or metabolic syndrome), and in patients with former gestational diabetes [1]. Endothelial dysfunction is thought to contribute to the development of cardiovascular disease through dysregulation of the vascular tone, growth, thrombogenicity, and inflammation. Several inflammatory and hemostatic biomarkers of endothelial dysfunction have been shown to be associated with cardiovascular disease, including C-reactive protein (CRP) [2], interleukin-6 (IL-6) [3], fibrinogen [4], fibrin D-dimer [5], plasminogen activator inhibitor-1 [6], and cellular adhesion molecules [7]. The factors associated with endothelial dysfunction in diabetes include activation of protein kinase C [8], overexpression of growth factors and/or cytokines [9], and oxidative stress [10].

Endothelial function can be evaluated using a physiological measurement of blood flow coupled with blood level determination of selected compounds thought to reflect endothelial cell function. These compounds include endothelin [11], the von Willebrand factor (vWF) [12,13], thrombomodulin [14], selectin, adhesion molecules (VCAM and ICAM) [15], and t-PA as well as its inhibitor PAI-1 [16].

Pentraxin 3 (PTX3) is a cytokine-inducible molecule expressed in different tissues, the levels of which increase in response to a variety of inflammatory

conditions. Recently, it has been linked to the serum glucose levels and some comorbidities in type 2 diabetes [17]. Two inflammatory biomarkers are known: the short pentraxin CRP and the long PTX3, both of which belong to the superfamily of pentraxins that have been found to play a role as markers of thrombosis and atherogenesis in the general population. CRP is mainly produced in hepatocytes after stimulation by cytokines, especially IL-6. The long PTX3 is an acute-phase reactant, which is considered to be more closely related to cardiac injuries such as myocardial infarction than CRP [18]. PTX3 is a multimeric inflammatory mediator mainly produced by dendritic cells, macrophages, activated leukocytes, and endothelial cells, but not by the hepatocytes, in response to primary inflammatory stimuli such as IL-1 and tumor necrosis factors, but not IL-6 [19]. Rolph *et al*. [20] reported a strong expression of PTX3 in human advanced atherosclerotic lesions and suggested that PTX3 may be directly involved in the pathogenesis of atherosclerosis [21]. Moreover, in the general population, the PTX3 levels measured within the first day from the onset of myocardial infarction along with other markers, including CRP, emerged as the only independent predictor of mortality in the first 3 months [22]. A study by Witasp *et al*. [23], revealed that the increased SAT *PTX3* (subcutaneous adipose tissue PTX3) mRNA expression is associated with increased circulating PTX3 levels, higher ADMA (circulating asymmetric dimethyl arginine) levels, and endothelial dysfunction in the uremic milieu.

Flow-mediated dilation (FMD) is at present used in an increasing number of studies as a measure of endothelial function. It has been suggested that it may help in risk stratification and is based on the evidence that endothelial dysfunction is related to the unfavorable levels of cardiovascular risk factors, the presence of vascular damage, and the future risk of cardiovascular disease [24]. It is a noninvasive method comprising measurement of brachial artery diameter changes induced by an increase in shear stress as a result of local endothelial release of nitric oxide [25].

This study was conducted to investigate the utility of measuring plasma long PTX3 levels in the early detection of endothelial dysfunction compared with vWF activity and flow-dependent arterial dilatation (well-known markers of endothelial dysfunction) in early diabetic nephropathy.

Patient selection

This is a cross-sectional observational comparative study that was carried out at the Diabetes, Endocrinology and Metabolism center, Faculty of Medicine, Cairo University, between 2009 and 2011. Fifty patients with type 2 diabetes (20 men and 30 women, age range 42–61 years) and 20 healthy controls (nine male and 11 female) of matched age and sex were included in the study. All patients provided informed consent. The study was approved by the ethical committee of the internal medicine department. The patients' diagnosis and standardization were based upon American Diabetes Association criteria. All patients were subjected to thorough history taking and physical examination to assess the age, sex, duration of diabetes, and any associated medical conditions, with special emphasis on exclusion of hypertension, clinical cardiovascular disease, cancer, and abnormalities in kidney function.

Anthropometric measures

The BMI was calculated using the Metric Imperial BMI Formula.

Methods

Venous blood was withdrawn from all participants and serum was extracted to assess the fasting plasma glucose level (after an 8-h overnight fast), lipid profile, serum cholesterol level, triglyceride level, and HDL cholesterol level. The LDL cholesterol level was estimated using the Friedewald equation, HbA1c and serum creatinine.

Estimated creatinine clearance (eC_{Cr})

Creatinine clearance was calculated according to Cockcroft–Gault formula [26]:

$$eC_{\mathrm{Cr}}=\frac{(140-\mathrm{age})\times\mathrm{body\ mass\ in\ kilogram}\times(0.85\ \mathrm{if\ female})}{72\times(\mathrm{serum\ creatinine\ in\ mg/l})}.$$

The urinary albumin excretion rate

The urinary albumin excretion rate (UAER) was calculated as follows: 24-h urine collection was performed three times, and the average was taken as a representative of each participant [27].

Plasma vWF activity

The plasma vWF activity was measured using an enzyme-linked immune sorbent assay for quantitative determination of the vWF activity in citrated human plasma [28]. A monoclonal capture antibody specific for the portion of vWF that binds platelets is coated onto 96-microwell polysterene plates. Diluted patient plasma is then incubated in the wells, allowing any available antigen to bind to the microwell surface. The plates are washed to remove unbound proteins and the bound antigen is quantitated using a horse radish peroxidase-conjugated anti-human vWF detection antibody. After incubation, a chromogenic substrate of tetramethylbenzidine and hydrogen peroxide is added to develop a colored reaction whose intensity is measured in optical density (OD) units using a spectrophotometer at 450 nm. The vWF activity, in relative percent concentration, is determined against a curve derived from the reference plasma provided with the kit.

Plasma long PTX3

The plasma long PTX3 level was estimated using the quantitative sandwich enzyme immunoassay technique [29]. A streptavidin-coated plate is incubated with a biotinylated monoclonal antibody specific for PTX3. The plates are then washed and pretreated standards and samples are added to the wells. Any PTX3 present is

Table 1 Characteristics of the control and study groups

	Control group ($N=20$)	T2 diabetes with no microalbuminuria ($N=25$)	T2 diabetes with microalbuminuria ($N=25$)	P
Age (years)	51.20 ± 5.37	50.24 ± 4.38	52.04 ± 4.92	0.430
Men [*n* (%)]	9 (45)	10 (40)	10 (40)	0.93
Women *n* (%)	11 (55)	15 (60)	15 (60)	
Diabetes duration (years)	–	2.46 ± 1.43	5.72 ± 2.26[a]	0.0001
BMI (kg/m^2)	30.3 ± 4.04	38.8 ± 5.25[b]	40.1 ± 5.25[b]	0.0001
SBP (mmHg)	115 ± 9.31	116.2 ± 10.33	114.4 ± 10.83	0.820
DBP (mmHg)	69 ± 7.37	70.2 ± 8.35	71.4 ± 9.63	0.648

Data are represented as mean ± SD.
DBP, diastolic blood pressure; SBP, systolic blood pressure.
[a]Significant difference between both diabetic groups.
[b]Significant difference when compared with the control group.

bound by the immobilized biotinylated antibody. An enzyme-linked conjugate specific for PTX3 is added to the wells, and after another wash to remove any unbound conjugate, a substrate solution is added to the wells and color develops in proportion to the amount of PTX3 bound. The color development stops and the intensity of the color is measured. The results are available in the form of standard curves that were generated for each set of samples assayed.

Flow-mediated dilation of the brachial artery

FMD of the brachial artery was performed using images that were digitized from the video output of the ultrasound machine using a frame grabber under control of a custom software. The examination requires the patients to be supine, at rest, in a quiet air conditioned room. A cross-section of the brachial artery is analyzed, and consequently, after a baseline measurement, a cuff, which can be placed either above or below the transducer position, is inflated to suprasystolic pressure to produce ischemia in the forearm. The cuff is deflated after a few minutes (usually 5), thus causing a reactive hyperemia, which in turn produces a shear stress stimulus that induces the endothelium to release NO, a vasodilator [30].

Statistical analysis

Numerical data were presented as mean (± SD) and median (25–75th) when appropriate, whereas categorical data were presented as frequency (%). Differences among the groups were detected using analysis of variance and Kruskal–Wallis tests. Correlations between different variables were detected using Spearman's correlation. Statistical significance was considered at a P value less than 0.05.

Results

The characteristics of the study group and controls

Table 1 shows the characteristics of the studied participants ($n=70$). There was no significant difference in terms of age and sex distribution among the participants in the three groups. Blood pressure showed no significant difference between the diabetic groups and the control group. The duration of diabetes was significantly higher in the diabetic group with microalbuminuria than in patients with a normal UAER ($P=0.0001$). The BMI was significantly higher in the diabetic groups when compared with the control participants ($P=0.0001$).

Biochemical data among the participants in the study groups

The levels of fasting blood sugar (FBS), HbA1c, plasma cholesterol, triglycerides, and LDL cholesterol were significantly higher in the diabetic groups compared with the control participants, but no difference was found between the two diabetic groups (Table 2). No significant difference in HDL level cholesterol level, serum creatinine level, and creatinine clearance was detected among the three groups. The UAER was 0.01 ± 0.09 mg/24 h in diabetic group I and 0.22 ± 0.05 mg/dl/24 h in diabetic group II.

vWF activity, long PTX3 levels, and FMD in the different studied participants

The mean vWF activity was significantly higher in the two diabetic groups than in the group of control participants, but with no significant difference between group I and II ($P=0.0001$) (Table 3).

Long PTX3 levels were significantly elevated in the diabetic patients with microalbuminuria compared with the diabetic patients with no microalbuminuria and the control participants ($P=0.0001$). There was no significant difference between the diabetic patients with no microalbuminuria and the control participants.

The FMD of the brachial artery was significantly diminished in the diabetic patients with microalbuminuria (group II) than in diabetic patients with no microalbuminuria (group I) and the control participants ($P=0.0001$). There was no significant difference between the diabetic patients in group I and the control participants.

Correlation between long PTX3 levels and the different parameters of the study

There was significant positive correlation between long PTX3 levels and the duration of diabetes in the group of diabetic patients with no microalbuminuria (Table 4 and Fig. 1).

There was also a significant positive correlation between long PTX3 levels and vWF activity in diabetic patients with microalbuminuria(Table 5 and Fig. 2).

Table 2 Biochemical data among the study groups

	Control group ($N=20$)	Diabetic patients with no microalbuminuria ($N=25$)	Diabetic patients with microalbuminuria ($N=25$)	P
FPG (mg/dl)	84.6 ± 16.9	168.2 ± 45.1[a]	200.1 ± 66.1[a]	0.0001
HbA1c (%)	3.67 ± 0.67	7.62 ± 1.29[a]	7.96 ± 2.12[a]	0.0001
Serum creatinine (mg/dl)	0.65 ± 0.33	0.76 ± 0.28	0.83 ± 0.34	0.177
Creatinine clearance (ml/min/1.73 m^2)	108.1 ± 15.1	98.2 ± 12.3	92.8 ± 14.0[a]	0.012
Urinary albumin excretion rate (mg/24 h)	–	0.01 ± 0.09	0.22 ± 0.05[b]	0.0001
Cholesterol (mg/dl)	127.4 ± 33.9	198.4 ± 39.1[a]	219.9 ± 48.7[a]	0.0001
Triglycerides (mg/dl)	83.0 ± 28.1	140.8 ± 38.7[a]	156.2 ± 31.4[a]	0.0001
HDL-c (mg/dl)	43.9 ± 6.7	44.9 ± 11.0	41.3 ± 7.7	0.345
LDL-c (mg/dl)	93.2 ± 22.3	144.8 ± 36.5[a]	143.4 ± 38.2[a]	0.0001

Data are represented as mean ± SD.
FPG, fasting plasma glucose; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol.
[a]Significant difference when compared with the control group.
[b]Significant difference between both diabetic groups.

Table 3 Von Willebrand factor activity, long pentraxin 3 levels, and flow-mediated dilatation in the different study groups

	Control groups	Diabetic patients with no microalbuminuria	Diabetic patients with microalbuminuria	P
vWF activity (%)	4.0 (2.7–7.2)	20.2 (14.7–32.3)[a]	16.3 (10.3–21.4)[a]	0.0001
PTX3 (ng/ml)	1.15 (1.0–1.3)	1.20 (1.0–1.75)	2.3 (1.25–3.65)[a,b]	0.0001
FMD	0.901 ± 0.04	0.627 ± 0.05	0.433 ± 0.059[a,b]	0.0001

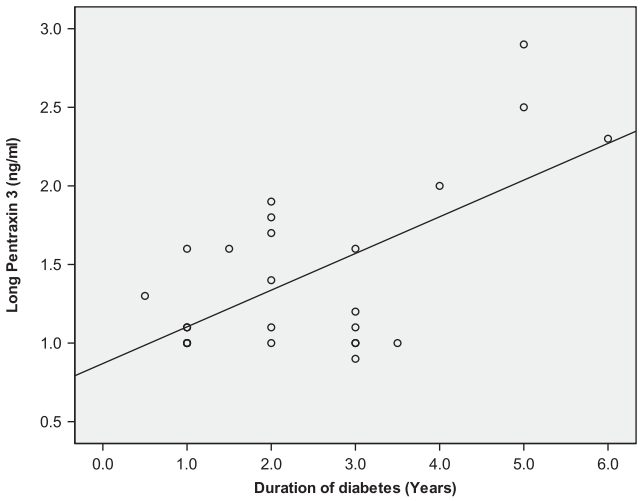
FMD, flow-mediated dilatation (mean ± SD); PTX3, long pentraxin 3 (median (25–75th); vWF, von Willebrand factor activity.
[a]Significant difference when compared with the control group.
[b]Significant difference between both diabetic groups.

Table 4 Correlation of long pentraxin 3 levels with all data in diabetic patients with no microalbuminuria

	Long pentraxin 3	Correlation
Age	–	$r=0.131$, $P=0.532$
Duration of diabetes	Positive	$r=0.621$, $P=0.001$
BMI	–	$r=0.41$, $P=0.844$
Waist circumference	–	$r=0.018$, $P=0.932$
FBS	–	$r=0.118$, $P=0.573$
HbA1c	–	$r=0.279$, $P=0.177$
Creatinine	–	$r=-0.188$, $P=0.367$
Creatinine clearance	–	$r=0.032$, $P=0.880$
Urinary albumin excretion rate	–	$r=0.006$, $P=0.979$
Cholesterol	–	$r=0.105$, $P=0.616$
Triglycerides	–	$r=-0.067$, $P=0.718$
HDL	–	$r=0.500$, $P=0.11$
LDL	–	$r=-0.067$, $P=0.749$
FMD	–	$r=-0.058$, $P=0.784$
Von Willebrand factor activity	–	$r=-0.146$, $P=0.487$

FBS, fasting blood sugar; FMD, flow-mediated dilatation.

Figure 1



Significant positive correlation of long pentraxin 3 levels with the duration of diabetes in diabetic patients with no microalbuminuria.

On correlating the long PTX3 levels with all the study data in all diabetic patients in the study (both groups I and II) (Table 6), a significant positive correlation was revealed with respect to the duration of diabetes (Fig. 3), FBS (Fig. 4), HbA1c (Fig. 5), cholesterol (Fig. 6), triglycerides (Fig. 7), and vWF activity (Fig. 8). Moreover, there was a significant negative correlation between long PTX3 levels in both diabetic groups and creatinine clearance (Fig. 9) and FMD (Fig. 10).

The significant correlation shown in Table 6 and Figs 3–8, when all diabetic patients were gathered and a trend line was drawn, may not be very sensitive statistically. The Scatter diagrams showed wide variation in the recorded values and in addition to the small number of observations might reflect a higher variation among all patients. Inclusion of a greater number of patients in future studies could prove the abovementioned hypothesis.

Discussion

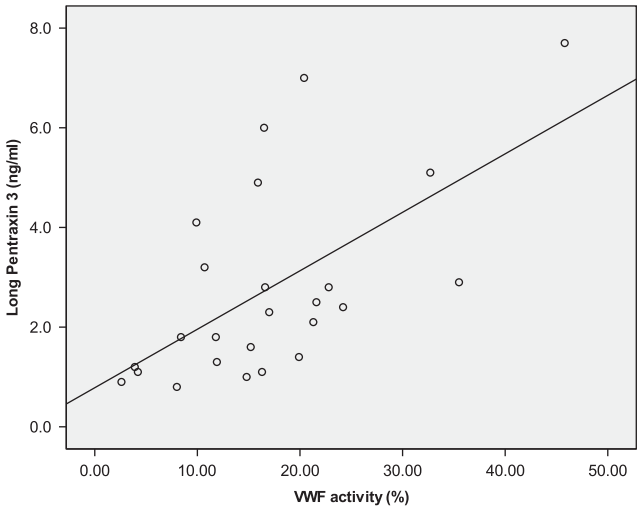
Nephropathy is a common microvascular complication among patients with type 2 diabetes mellitus and is a major cause of kidney failure. It is often present at the

Table 5 Correlation of long pentraxin 3 levels with all data in diabetic patients with microalbuminuria

	Long pentraxin 3	Correlation
Age	–	$r=-0.269$, $P=0.194$
Duration of diabetes	–	$r=0.002$, $P=0.991$
BMI	–	$r=0.77$, $P=0.714$
Waist circumference	–	$r=0.033$, $P=0.874$
FBS	–	$r=-0.076$, $P=0.717$
HbA1c	–	$r=-0.108$, $P=0.606$
Creatinine	–	$r=-0.112$, $P=0.595$
Creatinine clearance	–	$r=-0.259$, $P=0.211$
Urinary albumin excretion rate	–	$r=-303$, $P=0.142$
Cholesterol	–	$r=0.170$, $P=0.418$
Triglycerides	–	$r=-0.047$, $P=0.825$
HDL	–	$r=0.079$, $P=0.708$
LDL	–	$r=-0.199$, $P=0.340$
FMD	–	$r=0.169$, $P=0.421$
Von Willebrand factor activity	Positive	$r=0.603$, $P=0.001$

FBS, fasting blood sugar; FMD, flow-mediated dilatation.

Figure 2



Significant positive correlation between long pentraxin 3 levels and vWFfactor activity in diabetic patients with microalbuminuria. vWF, Von Willebrand factor.

time of diabetes diagnosis after the kidney has been exposed to chronic hyperglycemia during the prediabetic phase. Detection of diabetic nephropathy during its initial stages provides the opportunity for early therapeutic interventions to prevent or delay the onset of complications and improve outcomes [31].

Microinflammation and subsequent extracellular matrix expansion are common pathways for the progression of diabetic nephropathy. In recent years, many researchers have been convinced that the inflammation pathways play central roles in the progression of diabetic nephropathy, and the identification of new inflammatory molecules may link to the development of new therapeutic strategies [32].

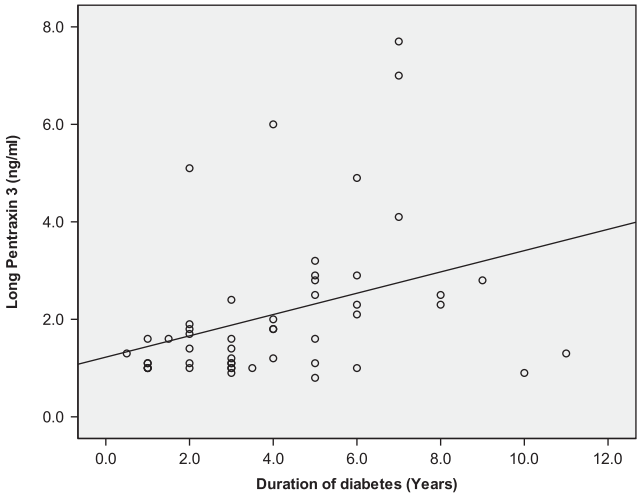
Aiming at tracing the early inflammatory markers of diabetic nephropathy, our study involved estimation of the levels of two markers, vWF activity and long

Table 6 Correlation of long pentraxin 3 levels with all data in group I+II

	Long pentraxin	Correlation
Age	–	$r=-0.439$, $P=0.952$
Duration of diabetes	Positive	$r=0.427$, $P=0.002$
BMI	–	$r=0.24$, $P=0.066$
Waist circumference	–	$r=0.183$, $P=0.130$
FBS	Positive	$r=0.262$, $P=0.028$
HbA1c	Positive	$r=0.237$, $P=0.048$
Creatinine	–	$r=0.038$, $P=0.754$
Creatinine clearance	Negative	$r=-0.289$, $P=0.015$
Urinary albumin excretion rate	–	$r=0.178$, $P=0.215$
Cholesterol	Positive	$r=0.359$, $P=0.002$
Triglycerides	Positive	$r=0.243$, $P=0.043$
HDL	–	$r=0.38$, $P=0.755$
LDL	–	$r=0.067$, $P=0.583$
FMD	Negative	$r=-0.397$, $P=0.001$
Von Willebrand factor activity	Positive	$r=0.400$, $P=0.001$

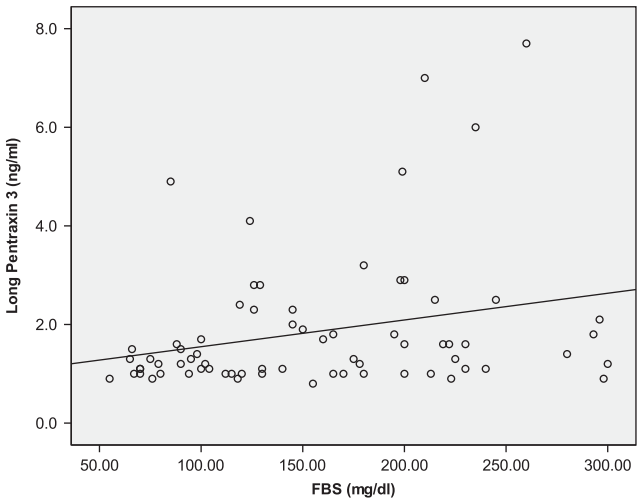
FBS, fasting blood sugar; FMD, flow-mediated dilatation.

Figure 3



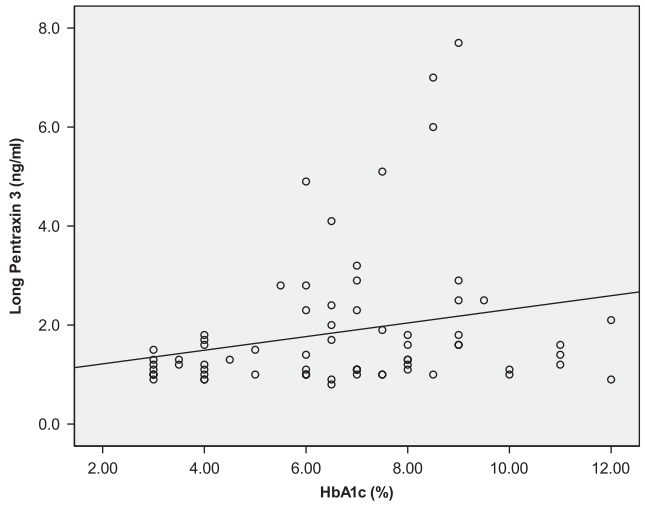
Significant positive correlation between long pentraxin 3 levels and the duration of diabetes in both diabetic groups.

Figure 4



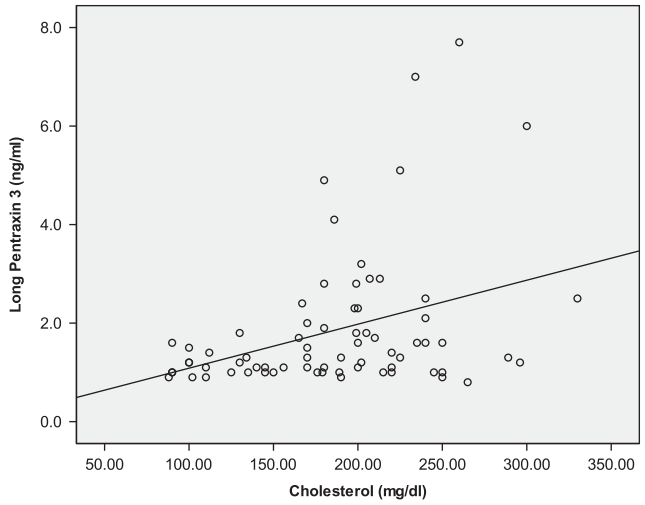
Significant positive correlation between long pentraxin 3 levels and FBS in all the studied groups. FBS, fasting blood sugar.

Figure 5



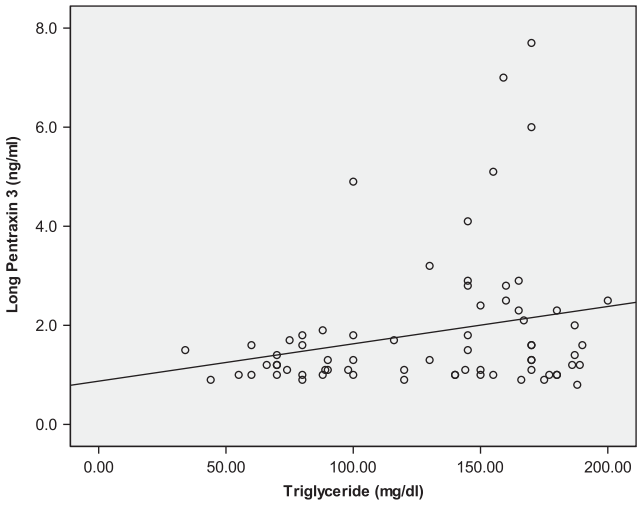
Significant positive correlation between pentraxin 3 levels and HbA1c levels in all the studied groups.

Figure 6



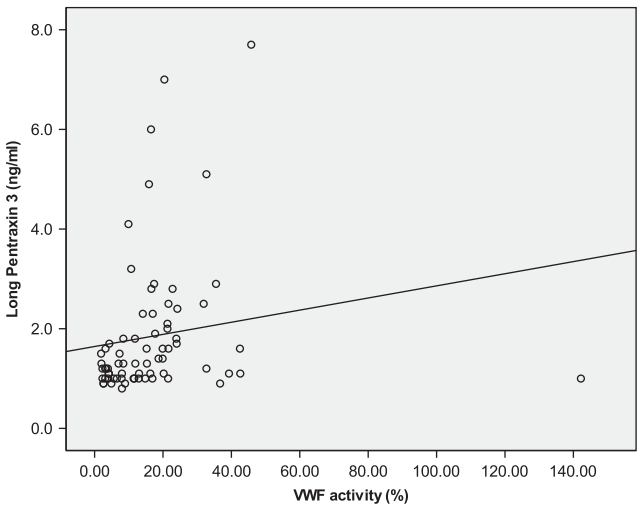
Significant positive correlation between long pentraxin 3 levels and cholesterol levels in all the studied groups.

Figure 7



Significant positive correlation between long pentraxin 3 levels and triglyceride levels in all the studied groups.

Figure 8



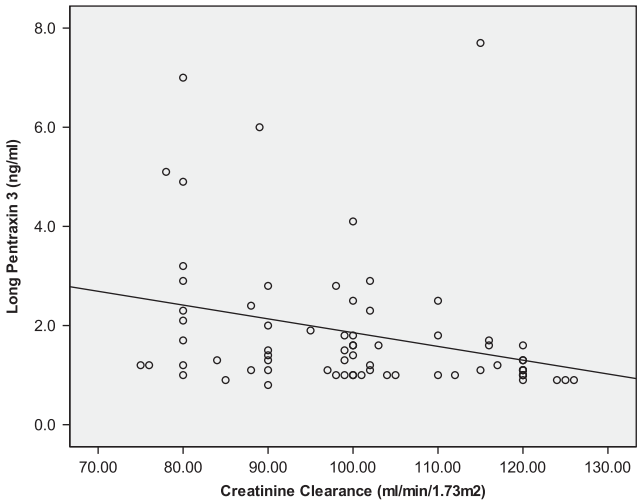
Significant positive correlation between pentraxin 3 levels and vWFfactor activity in all the studied groups. vWF, Von Willebrand factor.

PTX3, together with determination of the endothelial function by FMD in three groups of participants (two groups of diabetic patients, with group I comprising diabetic patients with no microalbuminuria and group II comprising diabetic patients with microalbuminuria, and a third group comprising control participants). All participants were subjected to clinical evaluation involving estimation of their BMI, waist circumference, and blood pressure. The FBS level, HbA1c level, lipid profile, creatinine clearance, UAER, vWF activity, long PTX3 level, and FMD were determined.

Our study data revealed that the plasma vWF activity was significantly higher in patients with type 2 diabetes with or without microalbuminuria than in control participants, with no significant difference between the two diabetic groups. These findings are similar to those reported by Vischer and colleagues, who traced the elevation in plasma vWF activity in diabetic patients with microalbuminuria and overt diabetic nephropathy. They also found an elevation in the level of propeptide vWF:AgII (but not vWF activity) in patients with type 1 diabetes with a normal UAER and suggested that concomitant determinations of plasma vWF and propeptide are useful tools to assess endothelial activation *in vivo* [33].

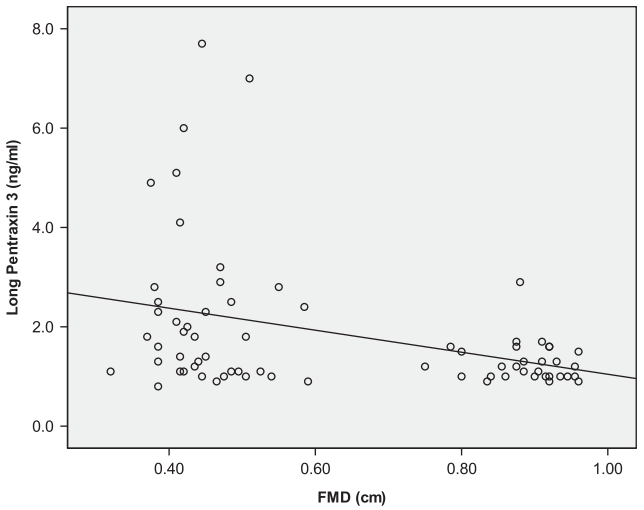
In our study, we found that the FBS and the HbA1c levels were higher than the levels set by American Diabetes Association and American association of clinical endocrinologists American association of clinical endocrinologists. This poorly controlled glycemic status in both diabetic

Figure 9



Significant negative correlation between long pentraxin 3 levels and creatinine clearance in all the studied groups.

Figure 10



Significant negative correlation between long pentraxin 3 levels and FMD in all the studied groups. FMD, flow-mediated dilatation.

groups might provide another explanation for the high vWF activity and endothelial dysfunction, which is in accordance with the results of Muntean *et al.* [34], who found an increase in the plasma levels of vWF in type 1 diabetes patients with a poor metabolic control in the absence of overt cardiovascular disease.

Moreover, the high BMI and the dyslipidemia observed in our study are additional factors that explain the endothelial dysfunction and high vWF activity in both groups with T2 diabetes. Nakano *et al.* [35] reported that hypercholesterolemia may enhance the endothelial dysfunction, and a reduction in plasma cholesterol levels (after using a lipid-lowering agent) could improve the vascular function in patients with type 2 diabetes. Calles-Escandon *et al.* [36] reported an impaired endothelial function in obese patients, which improved after weight reduction.

The duration of diabetes in our participants was significantly higher in patients with diabetic nephropathy than in those with a normal UAER; however, contrary to patients with type 1 diabetes in whom the duration of diabetes is determined accurately, the duration of diabetes in patients with type 2 diabetes cannot be determined accurately and vascular complications may be present at the time of diagnosis. Therefore, the influence of the duration of diabetes could not be confidently assessed in our study.

In our study, type 2 diabetic patients with microalbuminuria and a normal glomerular filtration rate had significantly higher PTX3 concentrations and significantly lower FMD compared with the control group and the group of diabetic patients with a normal urinary albumin excretion. There was no significant difference in PTX3 concentrations between normal individuals and diabetic patients with a normal urinary albumin excretion. Although the difference was not significant, the lower FMD in diabetic patients with a normal UAER, compared with controls, may be suggestive of the presence of endothelial dysfunction in these patients. This might be supported by the presence of significantly elevated levels of vWF activity in diabetic patients with a normal UAER, compared with controls, observed in our study.

Albuminuria and inflammation help predict cardiovascular events. PTX3, an inflammatory mediator produced, among others, by endothelial cells, may play a role in atherogenesis in patients with albuminuria. The development of albuminuria in patients with diabetes increases the risk for cardiovascular events by two to eight times [37] through mechanisms that could involve low-grade inflammation, endothelial dysfunction, and impaired insulin sensitivity [38].

The diabetic patients with microalbuminuria in our study who had elevated PTX3 levels showed a normal glomerular filtration rate. These results are in accordance with those reported by Suliman *et al.* [39], who found significantly elevated levels of plasmapentraxin 3 in patients with type 2 diabetes and proteinuria with a normal renal function when compared with healthy volunteers. Similar results were found by Yilmaz *et al.* [40], who detected elevated serum PTX3 levels and an impaired FMD in patients with stage 1 diabetic chronic kidney disease. In addition, they found that short-term ACE-inhibitor treatment significantly improved FMD and normalized PTX3 levels, CRP levels, and urinary protein excretion.

In addition, Stehouwer and colleagues found that microalbuminuria is linearly associated with impaired endothelium-dependent flow-mediated vasodilation in elderly individuals with and without diabetes. Theoretically, endothelial dysfunction could cause albuminuria both directly by increasing glomerular pressure and glomerular basement membrane permeability and indirectly by influencing mesangial cell and podocyte functions in a paracrine manner, for example, through inflammatory mechanisms involving PTX3 [41].

In our study, in patients with diabetes (group I + II), there was a significant positive correlation between PTX3 levels and vWF activity and a significant negative correlation between PTX3 levels and FMD. Moreover, there was a significant positive correlation between PTX3 levels and the duration of diabetes, fasting plasma glucose level, HbA1c level, cholesterol level, and triglyceride level; all of these factors may result in endothelial dysfunction in T2 diabetes.

In type 2 diabetic patients with microalbuminuria, although there was a significant positive correlation between PTX3 levels and vWF activity, no correlation was present between PTX3 levels and FMD. There was also no correlation between PTX3 levels and FMD or vWF activity in patients with a normal UAER.

Moreover, we did not find a significant correlation between PTX3 levels and UAER in the diabetic patients groups (I, II, and I + II), which is in disagreement with the results of Suliman *et al.* [39], who found an independent positive association between PTX3 levels and the UAER in patients with T2 diabetes with albuminuria and normal renal functions; in addition, they reported a significant negative and independent correlation between FMD and both PTX3 levels and UAER.

Despite the apparent difference between our results and those of Suliman and colleagues, in our study, PTX3 levels were significantly elevated and the FMD was significantly lower in group II (diabetes with microalbuminuria) than in group I (diabetes with a normal UAER), denoting that the inflammation represented by elevated serum PTX3 levels and microalbuminuria are both temporally related and are predictors of cardiovascular events represented in our study by the significantly lower FMD, which is independent of dyslipidemia and the degree of glycemic control, as no correlation was found between the latter two parameters and PTX3 levels in patients with microalbuminuria.

The utility of FMD measurements in randomized controlled trials as a primary outcome and an alternative for cardiovascular events has been suggested and is related to the unfavorable levels of cardiovascular risk factors because of the presence of vascular damage and the future risk of cardiovascular disease. PTX3 levels may directly reflect the inflammatory status. It has been reported that PTX3 levels are elevated in chronic kidney disease [21], myocardial infarction [22], atherosclerosis, vasculitis, and eclampsia.

Conclusion

Endothelial dysfunction in T2 diabetes exists even before the development of microalbuminuria, as reflected by the increased vWF activity in these patients due to many factors such as hyperglycemia, hyperlipidemia, and obesity.

In T2 diabetes, microalbuminuria is associated with elevated plasma vWF activity and plasma PTX3 levels, with a significant positive correlation between these two parameters. This may reflect the role of inflammation and endothelial dysfunction in atherogenesis and the increased risk of adverse cardiac events in these patients, as evidenced by the significantly impaired FMD of the brachial artery.

Therefore, elevated PTX3 levels may be added as a novel cardiovascular risk factor in T2 diabetes with microalbuminuria, and further studies are warranted to determine the cutoff level acting as a cardiovascular risk factor.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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الملخص العربى

مستوى البنتراكسين الثالث الطويل كعلامة على الخلل الوظيفي في الخلايا المبطنة في مرض اعتلال الكلية السكري المبكر.

هذا البحث يعمل على دراسة فائدة قياس مستوى البنتراكسين الثالث الطويل في الاكتشاف المبكر للخلل بوظيفة الخلايا المبطنة بالمقارنة لنشاط معامل الفون ويلبراند. والتدفق المعتمد على توسعة الشرايين في مرضى اعتلال الكلية السكري المبكر.

وقد تمت الدراسة على ثلاث مجموعات:

المجموعة الاولى: مرضى اعتلال الكلية السكري غير مفرزين للزلال في البول.

المجموعة الثانية: مرضى اعتلال الكلية السكري المفرزين للزلال في البول.

المجموعة الثالثة: اشخاص اصحاء.

وكانت النتائج كالآتي:

في المجموعة الثانية وجد ان مستوى البنتراكسين الثالث الطويل كان مرتفعا وان التدفق الدموي المعتمد على توسعة الشرايين كان منخفضا بالمقارنة بالمستويات المحددة في المجموعة الاولى والثالثة.

وجد ايضا ان نشاط معامل الفون ويلبراند كان مرتفعا في مرضى اعتلال الكلية السكري بمجموعتيه الاولى والثانية بالمقارنة للمجموعة الثالثة.

ودلت النتائج ايضا على وجود ارتباط ايجابي بين البنتراكسين الثالث الطويل ونشاط معامل الفون ويلبراند وايضا مع طول مدة الاصابة بمرض السكر ومقياس السكر الصائم للمرضى, الهيموجلوبين الجليكوزيلاتي, الكوليسترول والدهون الثلاثية. في حين هناك ارتباط سلبي مع التدفق الدموي المعتمد على توسعة الشرايين في جميع مرضى البوال السكري في المجموعتين الاولى والثانية.

في مرضى المجموعة الثانية كان هناك الارتباط ايجابي واضح بين البنتراكسين الثالث الطويل ونشاط معامل الفون ويلبراند.

من هذه النتائج نستدل على أن استخدام مستوى البنتراكسين الثالث الطويل في الدم يعد عامل مفيد للاستدلال على الخلل الوظيفي للخلايا المبطنة في مرضى اعتلال الكلية السكري.