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Low-grade risk of hypercoagulable state in patients suffering from diabetes mellitus type 2*

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Abstract: Objective: Diabetes, including type 1 and type 2, is associated with the hypercoagulable state. The aim of this study is to evaluate the concentration of selected hemostatic parameters and vascular endothelial growth factor-A (VEGF-A) in diabetic subjects. Methods: The study was conducted in 62 patients with diabetes. Group I consisted of 27 patients having uncontrolled diabetes with microalbuminuria and Group II included 35 well-controlled diabetic patients. The control group was made up of 25 healthy volunteers. In the citrate plasma, the concentrations of tissue factor (TF), tissue factor pathway inhibitor (TFPI), thrombin-antithrombin (TAT) complexes, and D-dimer were assayed. Serum concentrations of VEGF-A, lipid profile, creatinine, and plasma fasting glucose were measured and in the versene plasma the concentration of HbA1c was determined. Results: In the patients with uncontrolled diabetes, higher concentrations of TF, TFPI, and VEGF-A were observed, as compared with the well-controlled diabetics group and the control group. A significantly lower activity of antiplasmin was reported in patients from Group I as compared with the control group. In Group I, using the multivariate regression analysis, the glomerular filtration rate was independently associated with VEGF-A and dependently associated with total cholesterol. Conclusions: The study showed higher concentrations of TF and TFPI in the patients with uncontrolled diabetes with microalbuminuria, which is associated with rapid neutralization of the thrombin formation, since TFPI inhibits the complex of TF/VIIa/Ca^{2+}. The manifestation of the above suggestions is the correct TAT complexes and D-dimer, which indicates a low grade of prothrombotic risk in this group of patients, but a higher risk of vascular complications.

Key words: Diabetes, Extrinsic coagulation pathway, Angiogenesis, Glomerular filtration rate
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1 Introduction

In 2012, diabetes was claimed to have caused 4.8 million deaths in the world and 371 million people suffered from diabetes and it is expected to be a total of 522 million people in 2030 (Olokoba *et al*., 2012; Ruszkowska-Ciastek *et al*., 2014). It is considered that 80% of diabetic patients die from thrombotic episodes and 75% from the results of cardiovascular complications (Boden *et al*., 2007; Alzahrani and Ajjan, 2010; Chu, 2011; Kota *et al*., 2012). Diabetic patients without previous cardiovascular disease show a similar rate of myocardial infarction (MI), as compared with non-diabetic subjects who suffered from previous MI events (Alzahrani and Ajjan, 2010; Hess and Grant, 2011). Diabetes is also the most significant cause of end-stage renal failure worldwide. Clinically, glomerular hyperfiltration and microalbuminuria develop and, as a consequence, cause overt

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proteinuria and a reduced number of functioning nephrons, leading to renal failure (Kubisz *et al*., 2010; Veron *et al*., 2010).

Diabetes mellitus, including type 1 and type 2, is associated with the hypercoagulable state through increased thrombotic tendencies due to platelet hyperreactivity and an increased activation of prothrombotic coagulation factors, such as VII, VIII, X, XI, and XII, simultaneously with a decreased anticoagulant protein C level and the deterioration of the fibrinolysis process (Boden *et al*., 2007; Alzahrani and Ajjan, 2010; Breitenstein *et al*., 2010; Chu, 2011). These abnormalities, together with low-grade inflammation, lead to changes in the vessel architecture and the composition of the extracellular matrix (ECM) (Kota *et al*., 2012).

Tissue factor (TF), the physiological initiator of the coagulation cascade, which binds to factor VII, leads to the activation of factors IX and X, and causes thrombus creation. Damaged endothelium or rupturing of an atherosclerotic plaque leads to the exposure of TF to circulating blood and simultaneously to an initiation of the haemostatic system (Boden *et al*., 2007; Opstad *et al*., 2010; Hess and Grant, 2011). Tissue factor pathway inhibitor (TFPI) downregulates the TF-dependent blood coagulation cascade by inhibiting factor Xa (FXa) and TF/VIIa in a 2-stage approach. The first stage, in the inhibitory function of TFPI, involves reversible inhibition of FXa. The second step depends on binding TFPI/FXa to the TF/VIIa complex, hence inhibiting thrombin generation (DelGiudice and White, 2009; Chu, 2011). Moreover, TFPI is able to influence cell proliferation and a proper function of the endothelial cells (Opstad *et al*., 2010).

The fluctuation of the TF/TFPI ratio has been connected with the development of acute coronary syndrome, disseminated intravascular coagulation, sepsis, and diabetic vascular complications (El-Hagracy *et al*., 2010). However, there are some indications that, irrespective of the duration and the type of diabetes, the damage to the kidney can be avoided if proper glycemic control is preserved (Ghafoor *et al*., 2004).

TF is considered to be an important enzyme associated with plaque inflammation and arterial thromboembolism. Protease-activated receptors (PARs) on endothelial cells, co-expressed with TF, take part in processes such as inflammatory responses, atherosclerosis, tumor metastasis, and angiogenesis (DelGiudice and White, 2009; Chu, 2011). Moreover, a high level of TF/VIIa complex induces vascular endothelial growth factor (VEGF) production via PAR-2 signaling (DelGiudice and White, 2009; Chu, 2011; Kota *et al*., 2012). In a chronic hyperglycemic condition, angiogenesis is controlled in organ, tissue, and cell type-specific pathways. VEGF is crucial for maintaining the homeostasis of renal hemodynamics (Xu *et al*., 2012). However, VEGF overexpression in the glomerular podocytes is connected with an enhanced vascular permeability in glomerulus for macromolecules and an increased albuminuria and, consequently, proteinuria, leading to glomerular hypertrophy (Kubisz *et al*., 2010; Veron *et al*., 2010; Kota *et al*., 2012).

The study was designed to evaluate changes in selected coagulation, fibrinolytic and angiogenic parameters, especially TF, TFPI, thrombin activatable fibrinolysis inhibitor (TAFI), and VEGF-A in patients with diabetes mellitus type 2 (uncontrolled with microalbuminuria and well-controlled).

2 Subjects and methods

2.1 Subjects

Sixty-two type 2 diabetes patients were enrolled in the study. The patients were under the care of the Endocrinology and Diabetology Clinic of the A. Jurasz University Hospital, Poland. The first group (I) covered 27 patients with uncontrolled type 2 diabetes with microalbuminuria (10 females aged 46–72 years (mean 62 years) and 17 males aged 48–74 years (mean 63 years)). The qualification criterion for the patients involved in the study was the value of glycated hemoglobin (HbA1c) ≥59 mmol/mol (≥7.5%) and identifying considerable albuminuria. Albuminuria is defined as protein excretion of 30–300 mg/d in a timed overnight urine collection, in two of three measures after bacteriological urine examination and the exclusion of urinary tract infection or overt proteinuria. In these patients, glomerular filtration rate (GFR) was 40–60 ml/min per 1.73 m^2. GFR was measured according to the Modification of Diet in Renal Disease (MDRD) formula. The second group (II) consisted of 35 patients with well-controlled type 2

diabetes (17 females aged 47–75 years (mean 63 years) and 18 males aged 46–79 years (mean 64 years)). The diabetic compensation criterion in Group I was made up by an HbA1c value ≤48 mmol/mol (≤6.5%) and no vascular complications, assessed by determining the concentration of albumin in the urine (to exclude diabetic kidney disease) and an eye examination (to exclude the presence of diabetic retinopathy), as well as no symptoms of ischaemic heart disease, no symptoms of lower limb ischemia (measured by testing the pressure of the arteries of the lower limbs to calculate the ankle-brachial index: selected patients reported an index of 0.9–1.15), and no diabetic foot syndrome (clinical specific features in the patients are shown in Table 1) (Ruszkowska-Ciastek *et al*., 2015).

The medical history, collected by a physician to qualify the subjects for the study, allowed for obtaining the information on the general condition of the patients and the occurrence of co-existing diseases. All the patients received anti-hyperglycemic treatments (insulin, metformin, and sulfonylureas-derivatives), depending on the indication and all the patients also received statins and angiotensin-converting enzyme (ACE) inhibitor drugs.

The control group consisted of 25 healthy volunteers (12 females and 13 males, with a mean age of 52 years). The body mass index (BMI) range was (22.96±1.39) kg/m^2. The exclusion criteria for the controls were hypertension, hyperlipidaemia, hyperglycaemia, current smoking, acute and chronic infection, and cancer. For all patients, the exclusion criteria were receiving anticoagulant, thrombolytic therapy, and antiplatelet drugs, along with thromboembolic disease and pulmonary embolisms <6 months and surgery procedures <3 months (Table 1).

The patients from all the groups were informed about the aim of the study. They expressed their informed consent for participation in the study. The research was approved by the Bioethics Committee of the Collegium Medicum in Bydgoszcz, the Nicolaus Copernicus University in Torun (No. KB 366/2006), Poland.

2.2 Methods

Venous blood (4.5 ml) for the tests of TF, TFPI, TAFI-antigen (Ag), plasminogen, antiplasmin, thrombin-antithrombin (TAT) complexes, and D-dimer was collected in a fasting state into cooled tubes (Becton Dickinson Vacutainer® System, Plymouth, UK) containing 0.13 mol/L trisodium citrate (final blood anticoagulant ratio 9:1) after 30 min rest between 7:30 a.m. and 9:30 a.m. and after a 12-h overnight fast. The blood samples were immediately mixed and centrifuged at 3000*g* at 4 °C for 20 min. The platelet-poor plasma was divided into 200 μl Eppendorf-type tubes and then the samples were frozen at −80 °C (according to the manufacturer's procedures) until assayed within six months.

To determine VEGF-A, lipid profile, and creatinine concentrations, the blood was collected in a 4.5-ml tube without anticoagulants. It was centrifuged at 3000*g* for 20 min at 4 °C and subjected to further analytical procedures. To measure fasting glucose, blood was collected in a 4.5-ml tube with sodium fluoride ethylene diamine tetraacetic acid (EDTA). The plasma was centrifuged at 2000*g* for 10 min at 4 °C and subjected to further analytical procedures. In addition, 4.5 ml of blood was collected into tubes with sodium EDTA to determine the level of HbA1c, and versene plasma was obtained directly and subjected to further analytical procedures.

The concentration of TFPI was defined using the test of IMUBIND® total TFPI (American Diagnostica, Żory, Poland), TF was measured by the test of IMUBIND® TF (American Diagnostica, Żory, Poland), the concentration of TAT was determined by the test

Table 1 Selected clinical data for uncontrolled diabetic patients with microalbuminuria (Group I), well-controlled diabetes subjects (Group II), and the control group (Group III)

Group	Mean age (year)	Arterial hypertension	Triglycerides (mg/dl)	Cholesterol (mg/dl)			Mean duration of disease (year)	HbA1c IFCC (mmol/mol) (NGSP (%))	GFR (ml/min)
				LDL	HDL	Total			
I	63.0±6.4	27	119.0±12.3	131.0±22.9	37.0±7.7	193.0±17.2	12.6±7.0	67 (8.3)	54.0±15.0
II	64.0±5.2	25	106.0±11.1	116.0±16.5	47.0±5.2	191.0±11.3	8.1±3.0	48 (6.5)	82.0±18.0
III	52.0±3.6		108.0±7.4	92.0±21.1	57.0±11.6	186.0±8.1		<26 (<4.5)	80.0–120.0

LDL: low-density lipoprotein; HDL: high-density lipoprotein; IFCC: International Federation of Clinical Chemistry; NGSP: National Glycohemoglobin Standardization Program; GFR: glomerular filtration rate

of ENZYGNOST® TAT micro (Behring, Marburg, France), D-dimer was measured by the test of ASSERACHROM® D-DI (Diagnostica Stago, Asnieres, France), the concentration of TAFI-Ag was assayed by the test of TAFI-IMUBIND® TAFIa/ai (American Diagnostica Inc., USA), and the VEGF-A concentration was determined using the Quantikine VEGF Immunoassay (R&D Systems Inc., USA). The principle for all the methods was based on the reaction of enzyme-linked immuno sorbent assay (ELISA). The activities of antiplasmin and plasminogen, applying the chronometric method, were evaluated in an automated coagulometer CC-3003 apparatus and the reagents were produced by Bio-Ksel Co., Grudziądz, Poland. The parameters of lipid profile, fasting glucose, creatinine, and the HbA1c test were determined using the Abbott Clinical Chemistry Analyzer® Architect c8000 (Abbott Diagnostics Europe, Wiesbaden, Germany). Enzymatic and immunoturbidimetric methods were used to measure the concentrations of lipid profile, glucose, creatinine, and HbA1c, respectively.

2.3 Statistical analysis

The statistical analysis was performed using Statistica 10.0 software (StatStoft®). The Shapiro-Wilk test was used to assess the normality of the distribution. The data show different distributions from normal, thus the median (Me), lower quartile (Q1) and upper quartile (Q3) were used to present those values. To identify the significance of the differences between the groups, analysis of variance (ANOVA) Kruskal-Wallis post hoc was used. The multivariate regression analysis was accomplished in order to determine the associations between GFR, TF, TAFI-Ag, and selected parameters. Significance was defined as P-values of <0.05.

3 Results

Table 2 shows the selected parameters of the coagulation, fibrinolysis, and VEGF-A analyzed in the patients with uncontrolled diabetes with microalbuminuria (Group I), well-controlled type 2 diabetes patients (Group II), and in the control group (Group III). In the patients with uncontrolled diabetes, higher concentrations of TF (P=0.0434) and TFPI were observed (P=0.0012 and P=0.0119, respectively), as compared with the diabetic patients with well-controlled glycemia and control individuals. A significantly lower activity of antiplasmin was recorded in the patients from Group I than in the control group (P=0.0021). In Group I, there was noted a significantly higher level of VEGF-A, as compared with the group of patients with well-controlled glycemia and the control group (both P=0.0001).

In Group I, using the multivariate regression analysis, the GFR (normal values: GFR 80–120 ml/min per 1.73 m^2) was independently associated with VEGF-A (P=0.0448), when a high concentration of VEGF-A was accompanied by decreased level of GRF, which may indicate deterioration of the kidney function. Also GFR was dependently associated with total cholesterol (P=0.0215; Table 3).

Table 4 shows that the TF as a dependent variable was independently associated with triglycerides and HDL-cholesterol (P=0.0265 and P=0.0283, respectively) and dependently with the age (P= 0.0003).

Table 2 Concentrations of TF, TFPI, TAT complexes, D-dimer, TAFI-Ag, and VEGF-A, and activities of plasminogen and antiplasmin in the patients with uncontrolled diabetes with microalbuminuria (Group I) and well-controlled type 2 diabetes (Group II), as compared with the control group (Group III)

Group	TF (pg/ml)	TFPI (ng/ml)	TAT complexes (ng/ml)	D-dimer (ng/ml)	TAFI-Ag (ng/ml)	Plasminogen (%)	Antiplasmin (%)	VEGF-A (pg/ml)
I	226.49, 136.71/306.44	136.40, 91.44/165.60	2.45, 1.58/9.59	304.73, 240.98/431.17	33.91, 16.43/70.43	116.00, 105.0/129.0	96.00, 83.00/107.00	61.87, 42.67/109.72
II	154.04, 117.39/200.00	72.20, 63.30/97.62	1.90, 1.15/2.70	297.74, 217.69/437.83	36.77, 21.89/46.91	118.00, 106/126.0	106.00, 99.00/115.00	11.15, 7.22/17.06
III	164.28, 117.39/183.85	83.33, 68.96/94.78	2.49, 1.42/5.11	356.32, 199.66/588.93	34.74, 25.95/42.17	110.50, 100.00/115.00	115.00, 104.00/125.00	12.13, 9.18/16.07
P-value	0.0434*	0.0012*, 0.0119#	0.1778	0.842	0.7331	0.1002	0.0021#	0.0001*, 0.0001#

* Significant difference between Groups I vs. II; # Significant difference between Groups I vs. III. Values of the parameters are shown as medians and lower/upper quartile (Q1/Q3)

TAFI-Ag as a dependent variable was independently associated with HbA1c (P=0.0361; Table 5).

Table 3 Multivariate regression analysis (R^2=0.52, F=3.2, P<0.05) for dependent variable GFR in 27 patients suffering from uncontrolled diabetes type 2 with microalbuminuria

Parameter	Standardized regression coefficient	P-value
Constant		0.5007
Triglycerides	0.28	0.2556
LDL-cholesterol	–0.31	0.3568
Cholesterol	0.84	0.0215*
VEGF-A	–0.53	0.0448*

* Significant correlation at P<0.05

Table 4 Multivariate regression analysis (R^2=0.87, F=21.94, P<0.00001) for dependent variable TF in 27 patients suffering from uncontrolled diabetes type 2 with microalbuminuria

Parameter	Standardized regression coefficient	P-value
TFPI	0.35	0.1460
Age	2.13	0.0003*
Triglycerides	–0.79	0.0265*
HDL-cholesterol	–0.85	0.0283*

* Significant correlation at P<0.05

Table 5 Multivariate regression analysis (R^2=0.42, F=4.44, P<0.017) for dependent variable TAFI-Ag in 27 patients suffering from uncontrolled diabetes type 2 with microalbuminuria

Parameter	Standardized regression coefficient	P-value
Constant		0.00041*
HbA1c	–0.41	0.0361*
Anitplasmin	–0.21	0.2804

* Significant correlation at P<0.05

4 Discussion

Mechanisms for altered clot structure in diabetes are multifactorial: firstly, high plasma glucose can lead to increased glycations of fibrinogen and fibrin, which is associated with a more massive clot structure, which, in turn, favors resistance to lysis; secondly, elevated plasminogen activator inhibitor (PAI)-1 level reduces the fibrinolytic potential; thirdly, the non-enzymatic glycations of plasminogen and decreased activities of protein C act as the inhibitors of PAI-1 (Alzahrani and Ajjan, 2010).

This study has demonstrated that not only the plasma levels of TF but also TFPI were higher in type 2 diabetes mellitus (T2DM) patients; however, only uncontrolled with microalbuminuria. The TF expression by endothelial cells is found in trace amounts in the resting state; however, that synthesis is up-regulated in the presence of inflammation, which is commonly associated with diabetes type 2 (Alzahrani and Ajjan, 2010; Breitenstein *et al*., 2010). The TF level does not always correlate with the activity of TF, it is probably related to the simultaneous synthesis of the TFPI by the endothelial cells (Steffel *et al*., 2006). TFPI is the principal regulator in the initial phase of the coagulation pathway mediated by TF, which controls the creation of thrombin (Opstad *et al*., 2010).

Similar results to those reported in the present study were recorded by Lizakowski *et al*. (2007) in 57 patients with primary glomerulonephritis (36 with nephrotic syndrome and 21 without nephrotic syndrome). TF and TFPI concentrations in both groups were higher, as compared with the control group. They concluded that the data support the hypothesis concerning the activation of coagulation pathways in the patients with primary glomerulonephritis. These findings may be consistent with other studies reported in the group of 23 chronic ambulatory peritoneal dialyses (CAPD) patients, 24 subjects with nephrotic syndrome and 24 healthy volunteers, in which significantly higher concentrations of total, free, and truncated TFPI were observed in CAPD and chronic renal failure patients, as compared with the healthy volunteers (Malyszko *et al*., 2004). Boden *et al*. (2007) studied 18 T2DM patients and observed high TF levels, and suggested that the combination of hyperglycemia and hyperinsulinemia, common in poorly controlled patients with T2DM, contributes to a procoagulant state that is elevated partly in relation to underlying low-grade inflammation. In the study by Adams *et al*. (2008) patients with chronic kidney disease (CKD) reported a significantly higher TF antigen and factor VII coagulant (FVIIc) activity and significantly lower FX and antithrombin (AT) activity; however, no differences were observed in the concentration of TFPI antigen, as compared with the healthy controls, while dialysis patients also showed significantly higher TF antigen and TFPI activity, as compared with CKD patients. The authors summarized the results that the changes in blood coagulation

and its regulation were associated with the degree of renal impairment; however, they were independent of the endothelial function.

Pawlak *et al*. (2012) measured VEGF-A and the parameters of the haemostatic system in the mild-to-moderate and severe CKD patients. They noted in CKD patients, particularly those with severe kidney disease, higher VEGF concentrations. Also TF concentrations were significantly higher in the group with severe CKD, as compared with the group with the mild-to-moderate CKD and the control group. In contrast, TFPI concentrations were not significantly different between patients and controls. The elevated level of TFPI in the current study can be a secondary effect to the procoagulant state or to the damaged endothelium and decreased renal clearance can be a less common cause of TF and TFPI elevation.

Moreover, in the present study, a multivariate regression analysis was performed for the dependent variable TF in patients with uncontrolled diabetes type 2 with microalbuminuria. It was observed that TF could dependently be associated with age and independently with triglycerides and total cholesterol. An increased TF level with age was observed by El-Hagracy *et al*. (2010), which confirms a higher risk of the hypercoagulable state or endothelium damage with age. However, an inverse relationship between TF and triglycerides and total cholesterol is problematic to explain on the basis of the present results, perhaps the patients' treatment might have had a strong influence on the outcomes.

Thrombin-antithrombin is a sensitive indicator of thrombin formation. Oxidative stress in the course of induced diabetes contributes to the inactivation of antithrombin (Boden *et al*., 2007). In the present study, there were no significant differences between all the groups regarding the concentration of TAT complexes. The current results are similar with the study by Adams *et al*. (2009), who observed a higher concentration of TAT complexes for both CKD and dialysis patients, as compared with the controls, but the differences did not reach significant values.

Antiplasmin is an important inhibitor of plasmin. We observed a lower activity of AP in patients with uncontrolled T2DM, as compared with the healthy individuals. Perhaps antiplasmin is consumed during intense plasminogenesis or decreased synthesis of this enzyme in the liver.

Furthermore, multivariate regression analysis was made for dependent variable TAFI in patients with uncontrolled diabetes type 2 with microalbuminuria. TAFI was independently associated with glycated hemoglobin. This finding indicates that the stimulation of liver synthesis of TAFI (hypofibrinolysis) leads to HbA1c reduction. However, the present results are inconsistent with those reported by Sherif *et al*. (2014) who observed a positive correlation between TAFI and HbA1c in diabetic type 1 children and adolescents with microvascular complications.

In patients with uncontrolled type 2 diabetes with microalbuminuria in the present study, a significantly higher level of VEGF-A was recorded, as compared with the group of patients with well-controlled glycemia and the control group. Moreover, in patients with uncontrolled type 2 diabetes with microalbuminuria, the GFR, as a dependent variable, was independently associated with VEGF-A and dependently associated with total cholesterol.

In diabetic nephropathy, abnormal angiogenesis in the glomeruli as well as VEGF overexpression has been observed (Kanesaki *et al*., 2005). Poorly-controlled diabetes is strongly associated with an increased risk of the progression of vascular changes. The confirmation of this hypothesis is the association between VEGF-A and GFR, which was observed in the present study. VEGF is an initiator of angiogenesis and an endogenous regulator of a proper endothelial structure. An increased VEGF-A level with a simultaneous deterioration of GFR is a negative predictor of vascular instability.

Our findings are consistent with the research conducted by Mahdy *et al*. (2010) who evaluated the connection between VEGF and different micro- and macrovascular complications of diabetes type 2. The study revealed a significant increase in the serum VEGF in diabetic patients with micro- and macrovascular complications, as compared with the uncomplicated diabetic patients and the control individuals. Kowalski *et al*. (2011) obtained significantly higher levels of VEGF in the patients with metabolic syndrome with vascular complications, as compared with healthy individuals. Additionally, the type and severity of vascular complications did not correlate with the concentration of VEGF in these patients. Interesting results were reported by Kubisz *et al*. (2010) who studied the relationship between the VEGF

level and the parameters of endothelial dysfunction in patients with type 2 diabetes. The study group consisted of 84 diabetic patients (42 normoalbuminuric and 42 microalbuminuric patients). No significant differences were noted in the VEGF-A levels between patients with type 2 diabetes and accompanying microalbuminuria and the control group. However, there was a trend toward significance. Nevertheless, VEGF was significantly higher in the normoalbuminuric subjects, as compared with the controls. Also according to Kacso *et al*. (2012), in the normoalbuminuric diabetic patients, the VEGF-A concentration was higher than that in the control, and VEGF-A decreased in the presence of microalbuminuria. The results show a considerable divergence, which can be connected with the fluctuations of VEGF-A in the course of diabetic kidney disease, with a tendency to normalize over time. The verification of this thesis is the results recorded by Yuan *et al*. (2013) who observed no differences in the concentration of VEGF between healthy individuals and CKD stage 5 patients.

The studies by Chu *et al*. (2011) and El-Hagracy *et al*. (2010) and the current study confirmed the role of TF in microvascular diabetic complications. However, the results revealed an endothelial dysfunction instead of the procoagulant state. Additionally, VEGF also influences the hemostatic process by increasing the expression of TF, which was observed in the mild-to-moderate and severe CKDs, and those abnormalities are strongly associated with renal insufficiency (Pawlak *et al*., 2012).

In conclusion, serum VEGF significantly increased in the uncontrolled diabetic subjects, which is strongly related with an increased risk of the progression of vascular complications in those patients. The relationship between VEGF-A and GFR is confirmed. However, the proper control of hyperglycemia led to similar VEGF values, as compared with the control group. The study also showed higher concentrations of TF and TFPI in the patients with uncontrolled type 2 diabetes with microalbuminuria, which is associated with the rapid neutralization of the thrombin formation, since TFPI inhibits the complex of TF/VIIa/Ca^{2+}. The evidence of that hypothesis is correct TAT complexes and D-dimer, which indicates a low grade of prothrombotic risk in this group of patients. Finally, the current study suggests the role of TF in microvascular diabetic complications, which demonstrates a risk of an endothelial dysfunction, instead of the procoagulant state.

Compliance with ethics guidelines

Barbara RUSZKOWSKA-CIASTEK, Alina SOKUP, Tomasz WERNIK, Piotr RHONE, Krzysztof GÓRALCZYK, Kornel BIELAWSKI, Agata FIJAŁKOWSKA, Aleksandra NOWAKOWSKA, Elżbieta RHONE, and Danuta ROŚĆ declare that they have no conflict of interest.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5). Informed consent was obtained from all patients for being included in the study. Additional informed consent was obtained from all patients for which identifying information is included in this article.

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中文概要

题　目：2 型糖尿病患者高凝血状态的研究

目　的：评估糖尿病病人的止血参数和血管内皮生长因子的浓度。

方　法：62 例糖尿病患者分成两组：第一组包括 35 个血糖控制良好的糖尿病患者，第二组包括 27 个未控制血糖并伴有微蛋白尿的糖尿病患者。对照组由 25 名健康志愿者组成。测定血浆中组织因子（TF）、组织因子途径抑制剂（TFPI）、凝血酶抗凝血酶复合物（TAT）和 D-二聚体的浓度。同时测定血清中内皮生长因子 A（VEGF-A）、血脂、肌酐和血浆空腹血糖及糖化血红蛋白（HbA1c）的浓度。

结　论：研究表明，未控制血糖并伴有微蛋白尿的糖尿病患者具有更高的浓度的 TF 和 TFPI，这与凝血酶形成的快速中和有关。TAT 复合物和 D-二聚体的正确形成，能保证患者具有一个相对较低级凝血风险，但同时会带来更高的血管并发症的风险。

关键词：糖尿病；外源性凝血途径；血管生成；肾小球滤过率