VIA MEDICA
ORIGINAL ARTICLE
ISSN 2450–7458

Anna Adamska, Stanisław Piłaciński, Dorota Zozulińska-Ziółkiewicz, Agnieszka Gandecka, Agata Grzelka, Aneta Konwerska, Agnieszka Malińska, Michał Nowicki, Aleksandra Araszkiewicz
Poznan University of Medical Sciences, Poland

Disturbances in angiogenesis and vascular maturation in the skin are associated with diabetic kidney disease in type 1 diabetes

ABSTRACT

Introduction. The skin, as one of the most accessible tissues, is frequently used for investigations of microcirculation and angiogenesis. The aim of this study was to assess the relationship between the dermal microvessel density (MVD) and maturity and the presence of diabetic kidney disease (DKD) in adults with type 1 diabetes (T1D). Skin as the most accessible organ served as a model for the study of angiogenesis.

Materials and methods. 148 consecutive T1D patients (87 men), median age of 41 [interquartile range (IQR): 31–49] years and diabetes duration of 21 (17–30) years, participated in the study. The patients were under the care of the Department of Internal Medicine and Diabetology, Poznan University of Medical Sciences. Diabetic kidney disease was diagnosed in patients with increased albuminuria and at least 10-year duration of diabetes or evidence of diabetic retinopathy. The skin biopsy was performed on distal part of lower leg, using a sterile, disposable 3 mm biopsy punch with plunger (Disposable Biopsy Punches, Integra™ Miltex®). In the immunohistochemical analyses, we used: anti-CD133, anti-CD34, anti-CD31, and anti-von Willebrand factor (vWF) autoantibodies. Microvessel density measurement in all specimens was performed using "hot spots technique". Slides were scanned using the MiraxMidi scanner (Carl Zeiss) and were viewed using CaseViewer (3DHISTECH Ltd. Budapest, Hungary). Data were analyzed using Statistica v. 13 software.

Results. In the study group 21 patients with diagnosis DKD+, as compared to 127 subjects withaout DKD–, had longer duration of diabetes [30 (IQR: 21–36) *vs.* 21 (16–28) years, $p = 0.002$], higher prevalence of hypertension [14 (67%) *vs.* 37 (29%), $p = 0.002$], lower estimated glomerular filtration rate (eGFR) [66 (55–88) *vs.* 94 (83–106) mL/min/1.73 m^2, $p < 0.001$]. Median MVD compared between groups with and without DKD, was similar for CD34+ vessels/1 mm^2 [123 (100–170) *vs.* 121 (100–170), $p = 0.775$], CD133+ vessels/1 mm^2 [79 (50–100) *vs.* 79 (63–93), $p = 0.823$], and for CD31+ vessels/1 mm^2 [29 (21–46) *vs.* 38 (17–58), $p = 0.454$]. Median MVD vWF+ vessels/1 mm^2 was lower in the group with than without DKD: 42 (25–54) *vs.* 54 (43–71), $p = 0.009$. The values given above were calculated for both layers of the dermis (papillary and reticular dermis). In multivariate logistic regression analysis presence of diabetic kidney disease was associated with lower median vWF+ MVD [odds ratio: 0.97 (95% confidence interval: 0.95–0.99), $p = 0.017$], with adjustment for age, gender, eGFR value, diabetes duration and presence of hypertension. MVD did not differ significantly between chronic kidney disease stages.

Conclusion. In patients with type 1 diabetes and diabetic kidney disease the disturbances in the angiogenesis and vascular maturation are present. The number of mature blood vessels (vWF+) in the skin is reduced. Disturbances in the angiogenesis occur at early stages of diabetic kidney disease. (Clin Diabetol 2019; 8, 5: 231–237)

Key words: type 1 diabetes, diabetes complications, diabetic kidney disease, microcirculation; microvessel density (MVD), von Willebrand factor (vWF)

Address for correspondence:
lek. Anna Adamska
Katedra i Klinika Chorób Wewnętrznych i Diabetologii
Uniwersytet Medyczny im. Karola Marcinkowskiego w Poznaniu
ul. Mickiewicza 2, 60–834 Poznań
Phone: 0048 61 224 52 70
e-mail: ania@adamska.info
Clinical Diabetology 2019, 8, 5, 231–237
DOI: 10.5603/DK.2019.0019
Received: 22.06.2019 Accepted: 11.07.2019

Introduction

Diabetic kidney disease (DKD) remains the most frequent cause of end-stage renal failure, despite progress in the treatment of diabetes. Traditionally, diagnosis of DKD is based on the presence of albuminuria and either at least 10-year duration of diabetes or evidence of retinopathy [1]. Hyperglycemia and glycemic fluctuations, hypertension, dyslipidemia, and smoking are modifiable and genetics, sex, age, age at onset, and duration of diabetes are non-modifiable risk factors of DKD [2]. According to previous studies, in some people with DKD unpredictable progressive increase of albuminuria and declining glomerular filtration rate (GFR) has been observed [3]. Due to small and insufficient number of early markers of diabetic nephropathy, the aim of this study was to search for new prognostic markers of renal injury [4].

Endothelial cells (ECs) are constantly subjected to mechanical damage and chronic exposures to destructive factors, that lead to characteristic cell changes (morphology) and death (apoptosis) [5]. Terminally differentiated, mature ECs are characterized by a low proliferative potential, so endothelial progenitor cells (EPCs) derived from the bone marrow are involved in the creation and repair of blood vessels [6]. During new vessel formation, characteristic antigens are expressed on the surface of the endothelium, while some other antigens disappear. EPCs have properties of embryonal angioblast and are characterized by expression of CD133 (cluster of differentiation 133) and CD34 (cluster of differentiation 34) (late EPCs) [7]. ECs are characterized by an expression of von Willebrand factor (vWF), CD31, CD34, but do not express immature markers as CD133 [8]. In our study we used immunohistochemical markers [CD133, CD34, CD31 (cluster of differentiation 31) and vWF] to determine the morphological changes observed in dermal microangiopathy in diabetic patients.

The skin, as the most accessible organ, served as a model for the study of microcirculation [9, 10]. The objective of this study was to assess the dermal microvessel density (MVD) and maturity in relation to the presence of diabetic kidney disease in adults with type 1 diabetes (T1D).

Materials and methods

Patients

The study group consisted of 148 (87 men) consecutive patients with type 1 diabetes, median age (IQR) of 41 (31–49) years and diabetes duration of 21 (17–30) years. The patients were under the care of the Department of Internal Medicine and Diabetology, Poznan University of Medical Sciences.

Inclusion criteria were: age ≥ 18 years, type 1 diabetes of at least 10-year duration, written informed consent of the patient to participate in the study. Exclusion criteria were: activated partial thromboplastin time (APTT) > 37 s, international normalized ratio (INR) > 1.1, platelet count < 100 G/mm^3, anticoagulant or antiplatelet treatment, skin disorders.

The research protocol was approved by a local Bioethics Committee (No. 1064/15). The study was carried out in accordance with the World Medical Association Declaration of Helsinki.

Data collection procedures

All patients participating in the study completed the questionnaire containing demographic data, duration of diabetes and method of treatment, comorbidities, medication use and smoking-related data. Then, anthropometric measurements (body mass, height, waist and hip circumference, body mass index (BMI) = weight (kg)/squared height (m^2) and blood pressure measurement (twice using a sphygmomanometer in a sitting position after 10 minutes of rest) were performed.

Laboratory tests

Blood samples (10 milliliters) were taken after 10 hours of fasting, after a period of rest, with minimum occlusion of the vein using an S-Monovette blood collection system. The serum concentrations of creatinine, total cholesterol, high-density lipoproteins (HDL) cholesterol, low-density lipoproteins (LDL) cholesterol, and triglycerides (TG) were measured using standard methods. Estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) study equation. Glycated hemoglobin A_{1c} (HbA_{1c}) was measured in venous blood with competitive turbidimetric inhibition immunoassay method on Cobas analyzer (Roche Diagnostics, Basel, Switzerland) and expressed in % and IFCC (the International Federation of Clinical Chemistry and Laboratory Medicine) units (mmol/mol), the values were calibrated with respect to Diabetes Control and Complication Trial (DCCT)/National Glycohemoglobin Standardization Program (NGSP).

The urinary albumin excretion was assessed based on a 12 h urine collection, with the simultaneous determination of the albumin/creatinine ratio in the morning urine. Urinary albumin excretion from 30 to 300 mg per day in two of the three urine collections and albumin/creatinine ratio > 30 mg/g in the morning urine sample was considered to be increased albuminuria. Diabetic kidney disease was diagnosed in people with increased albuminuria and a 10-year

duration of diabetes or shorter duration of the disease with coexistence of retinopathy [1]. DKD was divided into stages based on the result of the eGFR: stage 1 (eGFR ≥ 90 mL/min/1.73 m^2), stage 2 (eGFR 60–89 mL/ /min/1.73 m^2), stage 3 (eGFR 30–59 mL/min/1.73 m^2). In the investigated group there were no patients with more advanced chronic kidney disease (CKD) stages.

Biopsy procedure

The skin biopsy was taken on the distal part of the lower limb (10 cm above the lateral malleolus) after the skin was anesthetized with 2% lidocaine injections in sterile conditions. We have used a sterile, disposable 3 mm biopsy punch with a plunger (Disposable Biopsy Punches, Integra™ Miltex®). The biopsy was minimally invasive and did not require suturing. The excised tissues were fixed in Bouin solution for 24 hours at room temperature, and then embedded in paraffin blocks.

Immunohistochemistry

Paraffin-embedded tissue blocks were cut into 3- to 4-μm-thick sections on a semi-automatic rotary microtome (Leica RM 2145, Leica Microsystems, Nussloch, Germany). All of the immunohistochemical (IHC) analyses employed the StreptABComplex/HRP method modified by the use of biotinylated tyramine (Dako Catalyzed Signal Amplification System, Peroxidase, K1500, DakoCytomation A/S, Glostrup, Denmark). The endogenous peroxidase activity was blocked with 10% hydrogen peroxide. The staining IHC protocol included the following steps: 1) preincubation with the appropriate normal goat serum in phosphate buffered saline for 30 minutes at room temperature, 2) incubation with the specific primary antibody overnight at 4°C in a hybridization chamber, 3) incubation with the secondary antibody for 60 minutes at room temperature, and finally 4) antigen-antibody complexes staining using 0.5% 3-3' diaminobenzidine (DAB; Sigma Chemical Co., St. Louis, MO).

All of the sections from blood vessels samples from an individual patient were processed in the same IHC experiment. The specific primary antibodies were:

- anti-CD34 (Dako, Glostrup, Denmark; code M7165, diluted 1:30);
- anti-CD133 (Novus Biologicals, Littleton, CO, USA; code NB300–266, diluted 1:3000);
- anti-CD31 (Dako, Copenhagen, Denmark; code M0823, diluted 1:20);
- anti-vWF (Dako; code M0616, diluted 1:30).

All tissue sections were analyzed under an AxioImager Z.1 light microscope and selected pictures were taken with an attached AxioCam MRc5 digital camera (Carl Zeiss). The negative controls consisted of specimens incubated with non-immune IgG1 (X-0931, Dako, Gdynia, Poland) and sections for which the primary or secondary antibody was omitted.

Morphometric analyses

Microvessel density (MVD), defined as the mean number of blood vessels presented in 1 mm^2 of analyzed tissue, was calculated using the "hot spots technique". The histological preparation was viewed under a small magnification (20 ×) by selecting three areas with the highest number of blood vessels. Then, the vessel sections were counted under a magnification of 40× in a selected area. The arithmetic mean of the three "hot spots" was calculated for microvessel number and subsequently calculated to 1 mm^2. This procedure was applied separately for CD133, CD34, CD31, and vWF. Slides were scanned using the MiraxMidi scanner (Carl Zeiss) and were viewed using CaseViewer (3DHISTECH Ltd. Budapest, Hungary). All of the analyses were evaluated independently by two scientists on coded samples that included positive and negative controls.

Statistical analysis

Data were analyzed using Statistica v. 13 (StatSoft Inc., Tulsa, OK, USA), MedCalc v. 18.5 (MedCalc Software bvba, Ostend, Belgium). Patients with diagnosis of DKD (DKD+) and without DKD (DKD–) were compared using Mann-Whitney U test or Fisher exact test, as appropriate. Descriptive statistics and results of comparatory analyses are expressed as medians and IQR or numbers and percent. Kruskal-Wallis ANOVA was used to compare vWF+ MVD between stages of CKD (1, 2 and 3a). Multiple logistic regression was used to check the association between presence of CKD and vWF+ MVD, with adjustment for potential confounders (age, gender, diabetes duration and presence of hypertension). P value less than 0.05 was considered statistically significant.

Results

The study group there were 21 T1D patients (12 men) with DKD, median (IQR) age 44 (32–58) and 127 (75 men) individuals with T1D without DKD, median age 40 (30–49) years.

DKD+ patients as compared to DKD– subjects had longer duration of diabetes [30 (21–36) *vs.* 21 (16–28) years, $p = 0.002$], more often had hypertension [14 (67%) *vs.* 37 (29%), $p = 0.002$ and lower eGFR [66.1 (54.7–87.0) *vs.* 93.5 (82.5–106.2) mL/min/1.73 m^2, $p < 0.001$].

The median MVD determined by CD34 blood vessels per 1 mm^2 dermal biopsies was 123 (100–170) in DKD+ group and 121 (100–154), $p = 0.775$ in

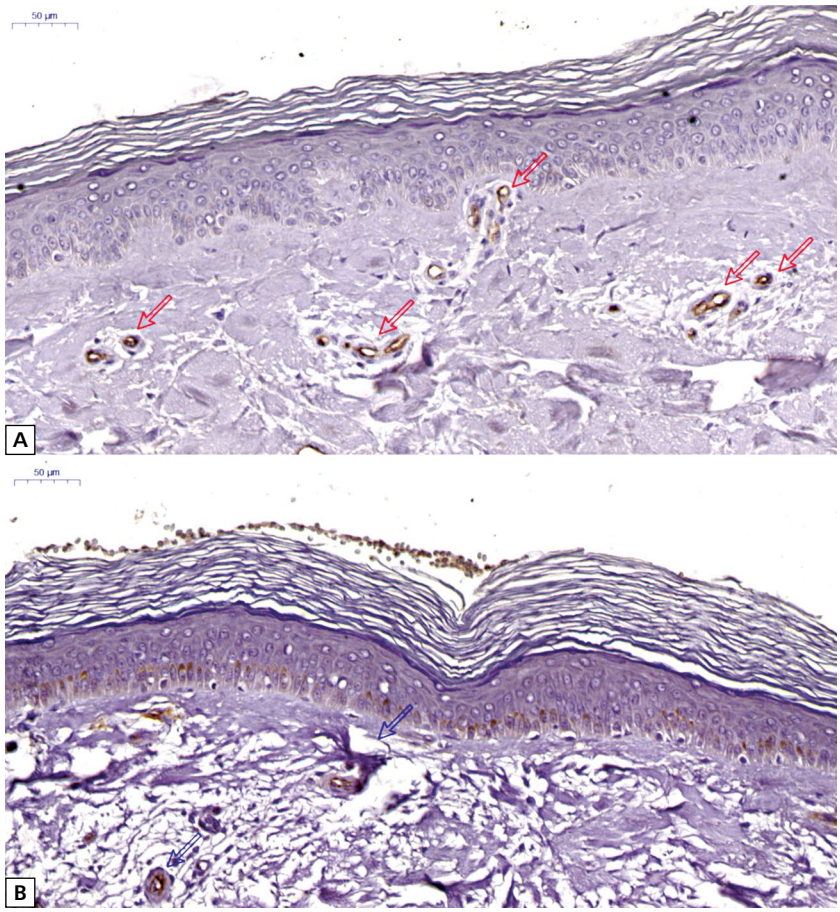

Figure 1A, B. Demonstration of microvessels density (MVD) in skin biopsies of adults with type 1 diabetes. Sections were stained by immunohistochemistry to show the MVD defined by von Willebrand factor (vWF) (**A**) were obtained from representative patient without diabetic kidney disease (DKD–) and (**B**) from patient with DKD+. Notice significantly lower microvessel density defined by vWF in patient with DKD+. Scale bar = 50 μm

DKD– group, defined by CD133 79 (50–100) *vs.* 79 (63–92), p = 0.823, by vWF 42 (25–54) *vs.* 54 (43–71), p = 0.009 and by CD31 29 (21–46) *vs.* 38 (17–58), p = 0.454 (Figure 1). The values given above were calculated for both layers of the dermis (papillary and reticular dermis). Comparison of patients DKD+ and DKD– is shown in Table 1.

The group contained 79 (52.7%) patients with CKD stage 1, 58 (39.2%) patients with CKD stage 2, 8 (5.4%) CKD patients with stage 3a and 3 (2%) patients with CKD stage 3b. There were no patients with CKD stages 4 and 5. Comparison of MVD defined by vWF+ in groups of patients depending on the CKD stage is shown in Table 2.

In multivariate logistic regression analysis presence of diabetic kidney disease was associated with lower median vWF+ MVD [odds ratio (OR): 0.97 (95% confidence interval {CI}: 0.95–0.99), p = 0.017]. In multivariate model the results were adjusted for age, gender, diabetes duration and presence of hypertension.

Discussion

Angiogenesis is involved in the pathogenesis of diabetic kidney disease. The abnormal new vessels present in glomerular capillary area (Bowman's capsule in the glomerular vascular pole) are dilated and the glomerular basement membrane is extremely thin. According to the study of Osterby et al. an "extra efferent arteriole" are detected in the early stages of diabetic nephropathy [11]. Hypertension may be another important driving factor in the progression of angiogenesis in diabetes [12]. Angiotensin-converting enzyme (ACE) inhibitors suppress angiogenesis in glomerulus [13]. One of the possible explanations is that these vessels can play the role as a "by-pass" to reduce intraglomerular pressure [11]. Morphological effect of neovascularization is glomerular hypertrophy while functional effect is temporarily excessive filtration (increased GFR).

New blood vessels are structurally and functionally immature, endothelial cells are swollen, the basement membrane is thin. All this leads to increased perme-

Table 1. Comparison of groups of patients with type 1 diabetes in relation to diabetic kidney disease (DKD). Patients with diagnosis of diabetic kidney disease (DKD+) and without diagnosis of diabetic kidney disease (DKD–)

Variables	DKD+ (N = 21)	DKD– (N = 127)	p value
Age (years)	44 (32–58)	40 (30–49)	0.226
Sex, female/male, N	9/12	52/75	1.0
Duration of diabetes (years)	**30 (21–36)**	**21 (16–28)**	**0.002**
Smoking, N (%)	7 (33)	36 (28)	0.61
Hypertension, N (%)	**14 (67)**	**37 (29)**	**0.002**
BMI [kg/m^2]	24.9 (22.8–29)	25 (22–29)	0.980
HbA_{1c} (%)	8.4 (7.0–9.3)	8.0 (7.3–8.9)	0.527
HbA_{1c} [mmol/mol]	68.3 (53–78.1)	63.9 (56.3–73.8)	
TG [mmol/l]	1.2 (1.0–1.7)	1.0 (0.8–1.4)	0.057
LDL-cholesterol [mmol/l]	2.6 (2.3–3.1)	2.6 (2.1–3.4)	0.709
HDL-cholesterol [mmol/l]	1.6 (1.3–1.9)	1.6 (1.4–2.2)	0.459
eGFR (MDRD) [ml/min./1.73 m^2]	**66.1 (54.7–87.9)**	**93.5 (82.5–106.2)**	**< 0.001**
MVD CD34+ [vessels/1 mm^2]	123 (100–170)	121 (100–154)	0.775
MVD CD133+ [vessels/1 mm^2]	79 (50–100)	79 (63–92)	0.823
MVD vWF+ [vessels/1 mm^2]	**42 (25–54)**	**54 (43–71)**	**0.009**
MVD CD31+ [vessels/1 mm^2]	29 (21–46)	38 (17–58)	0.454
CD34/CD31 ratio	4.1 (2.1–6.6)	2.7 (1.8–5.3)	0.148
CD133/CD31 ratio	3.0 (1.6–4.2)	1.7 (1.3–3.3)	0.093
vWF/CD31 ratio	1.4 (0.5–2.3)	1.3 (0.8–2.0)	0.583

Note: data are presented as median (interquartile range) or N (%); BMI — body mass index; CD — cluster of differentiation; eGFR — estimated glomerular filtration rate; HbA_{1c} — glycated hemoglobin A_{1c}; HDL — high-density lipoproteins; LDL — low-density lipoproteins; MDRD — Modification of Diet in Renal Disease; MVD — microvessel density; N — number of patients; TG — triglycerides; vWF — von Willebrand factor

Table 2. Comparison of microvessel density (MVD) defined by von Willebrand factor (vWF) in groups of patients depending on the chronic kidney disease (CKD) stage

CKD stage	N (%)	eGFR MDRD	MVD vWF+ [vessels/1 mm^2]
1	79 (52.7)	103.6 (96.1–114.9)	54.2 (41.7–70.8)
2	58 (39.2)	55.9 (53.1–57.5)	52.1 (37.5–70.8)
3a	8 (5.4)	55.6 (53.1–57.5)	47.9 (12.5–56.3)
3b	3 (2)	37.1 (31.1–37.8)	20.8 (0–41.7)

Note: data are presented as median (interquartile range) or N (%); p = 0.33 for comparison vWF+ MVD between groups with CKD stages 1,2, and 3a; p = 0.056 for comparison vWF+ MVD between groups with CKD stages 1,2, and 3 (combined); Kruskal-Wallis test; N — number of patients; eGFR — estimated glomerular filtration rate; MDRD — Modification of Diet in Renal Disease

ability and finally results in the extravasation of plasma protein [11]. Vascular endothelial growth factor (VEGF) plays the main role in this process. Vascular endothelial growth factor A (VEGF-A) is derived from podocytes and tubular epithelial cells and vascular endothelial growth factor receptor 2 (VEGFR-2) is expressed in glomerular and peritubular capillaries. Another phenomenon called "uncoupling of VEGF-A with NO (nitric oxide)" (a low NO bioavailability along with high VEGF), observed in the diabetic mice could potentiate the vascular permeability in the glomerulus [14]. The final effect of unfavorable processes is progression to renal fibrosis. Due to advancing fibrotic changes and loss of endothelial cells, podocytes, and tubular epithelial cells, the production of VEGF is decreased and the process progresses to the advanced stages of chronic kidney disease [15].

VEGF reflects the pathology of neovascularization. The next step in understanding of the mechanisms of the pathology of neovascularization induced by metabolic hypoxia is the possibility of assessing the maturity of the vessels. We evaluated the following markers of angiogenesis in the material from skin biopsy: CD34, CD133, CD31 and vWF. Previously, we have observed that MVD, assessed by CD34 (a marker of "late" EPCs) and CD133 (a marker of early EPCs), were significantly higher in patients with cardiac autonomic neuropathy

(CAN). Also, CD34 MVD was higher in patients with diabetic peripheral neuropathy (DPN), as compared to subjects without complications [16]. These results support the concept that angiogenic processes are involved in the pathogenesis of neuropathy and confirm the neurovascular nature of chronic diabetes complications. Interestingly, we did not find any relationship between markers of early ECs and the presence of DKD. In the animal model of kidney disease, local quantity of CD34+ capillaries were decreased with increasing severity of glomerular and tubulointerstitial lesions [17]. We did not find any data that confirm this phenomenon in patients with DKD.

CD133+ progenitor cells were used for the treatment in an animal model of acute kidney injury (AKI). CD133+ cells promoted the restoration of the renal tissue, limiting the presence of markers of injury and pro-inflammatory molecules, promoted angiogenesis and protected against fibrosis [18]. Moreover, in the previous study we have found a negative correlation between CD31 MVD and skin auto fluorescence (AF) [19]. ECs are capable of undergoing endothelial to mesenchymal transition under the influence of transforming growth factor-β_1. They lose the expression of such antigens as CD31, von Willebrand factor, and vascular-endothelial cadherin and initiate the expression of mesenchymal cell antigens [α-smooth muscle actin (α-SMA), extra domain A fibronectin, N-cadherin, vimentin, fibroblast specific protein-1] [20]. According to previous studies, endothelial-to-mesenchymal transition is associated with albuminuria in diabetic nephropathy. In these studies, a decreased number of CD31+ endothelial cells and increased α-SMA expression in glomeruli of diabetic mice was observed [21, 22].

Von Willebrand factor (vWF) is a glycoprotein that is most researched in the context of its role in haemostasis and von Willebrand disease (vWD). Quantitative and qualitative vWF changes can lead to an increased risk of bleeding, on the other hand it is regarded as risk factor for cardiovascular disease or venous thromboembolism [23, 24]. The study of Lenting et al. [25] showed that vWF plays a role in the angiogenesis, cell proliferation and inflammation. The storage of von Willebrand factor is a function of Weibel Palade bodies that are ultrastructural organelles found only in mature vascular endothelial cells [26]. We found a decreased number of MVD determined by vWF blood vessels per 1 mm^2 in DKD+ group. We did not observe that correlation in relation to other chronic diabetic complications. Interestingly, we found a decreased number of mature vessels despite the very early stages of CKD present in the vast majority of patients. Lack of the association between vascular markers and eGFR suggests that altered expression of vascular markers denote earlier pathology than decreased glomerular filtration. The progression of chronic kidney disease is a very complex process and different factors may be dominant, depending on stage [27]. We can only speculate if this lower expression of mature vessels is caused by the altered angiogenesis and disturbances of vessel maturation as mechanisms involved in the development of diabetic nephropathy [28]. The process seems to be different than in other diabetic complications. In the course of neuropathy, excessive angiogenesis expressed by the increased number of MVD CD34+ and CD133+ plays a dominant role. The reduction of eGFR and positive albuminuria are already indicative of significant glomerular pathology, considered to be an important risk factor for ischemic heart disease. Perhaps at this stage, a simple marker that is vWF can be used to further determine the cardiovascular risk in patients, as it is directly related to the diagnosis of DKD. The observed phenomenon seems to be interesting but requires further research to explain.

Conclusions

In patients with type 1 diabetes and diabetic kidney disease the disturbances in the angiogenesis and vascular maturation are present. The number of mature blood vessels (vWF+) in the skin is reduced. Disturbances in the angiogenesis occur at early stages of diabetic kidney disease.

Statement of competing interests

The above-mentioned authors declare that there is no conflict of interest.

Acknowledgments

The study was funded by the Poznan University of Medical Sciences (No. 502-14-02234382-41190) and scientific grant of Diabetes Poland.

REFERENCES

1. KDOQI Clinical Practice Guidelines and Clinical Practice Recommendations for Diabetes and Chronic Kidney Disease. Am J Kidney Dis. 2007; 49(2 Suppl 2): S12–154, doi: 10.1053/j.ajkd.2006.12.005, indexed in Pubmed: 17276798.
2. Harjutsalo V, Groop PH. Epidemiology and risk factors for diabetic kidney disease. Adv Chronic Kidney Dis. 2014; 21(3): 260–266, doi: 10.1053/j.ackd.2014.03.009, indexed in Pubmed: 24780453.
3. Macisaac RJ, Jerums G. Diabetic kidney disease with and without albuminuria. Curr Opin Nephrol Hypertens. 2011; 20(3): 246–257, doi: 10.1097/MNH.0b013e3283456546, indexed in Pubmed: 21422923.
4. Fowler MJ. Microvascular and macrovascular complications of diabetes. Clin Diab. 2008; 26(2): 77–82, doi: 10.2337/diaclin.26.2.77.
5. Sabatier F, Camoin-Jau L, Anfosso F, et al. Circulating endothelial cells, microparticles and progenitors: key players towards the definition of vascular competence. J Cell Mol Med. 2009; 13(3):

454–471, doi: 10.1111/j.1582-4934.2008.00639.x, indexed in Pubmed: 19379144.
6. Asahara T, Murohara T, Sullivan A, et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science. 1997; 275(5302): 964–967, doi: 10.1126/science.275.5302.964, indexed in Pubmed: 9020076.
7. Peichev M, Naiyer AJ, Pereira D, et al. Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. Blood. 2000; 95(3): 952–958, indexed in Pubmed: 10648408.
8. Sadler JE. Biochemistry and genetics of von Willebrand factor. Annu Rev Biochem. 1998; 67: 395–424, doi: 10.1146/annurev.biochem.67.1.395, indexed in Pubmed: 9759493.
9. Sandeman DD, Shore AC, Tooke JE. Relation of skin capillary pressure in patients with insulin-dependent diabetes mellitus to complications and metabolic control. N Engl J Med. 1992; 327(11): 760–764, doi: 10.1056/NEJM199209103271103, indexed in Pubmed: 1501652.
10. Khan F, Elhadd TA, Greene SA, et al. Impaired skin microvascular function in children, adolescents, and young adults with type 1 diabetes. Diabetes Care. 2000; 23(2): 215–220, doi: 10.2337/diacare.23.2.215, indexed in Pubmed: 10868834.
11. Østerby R, Hartmann A, Bangstad HJ. Structural changes in renal arterioles in type I diabetic patients. Diabetologia. 2002; 45(4): 542–549, doi: 10.1007/s00125-002-0780-2, indexed in Pubmed: 12032631.
12. Nakagawa T, Sato W, Kosugi T, et al. Abnormal angiogenesis in diabetic nephropathy. Diabetes. 2009; 58(7): 1471–1478, doi: 10.2337/db09-0119, indexed in Pubmed: 19564458.
13. Joles JA, Braam B, Verhaar MC. ACE inhibition and glomerular repair: restructuring or regeneration? Kidney Int. 2006; 69(7): 1105–1107, doi: 10.1038/sj.ki.5000237, indexed in Pubmed: 16609678.
14. Nakagawa T. Uncoupling of the VEGF-endothelial nitric oxide axis in diabetic nephropathy: an explanation for the paradoxical effects of VEGF in renal disease. Am J Physiol Renal Physiol. 2007; 292(6): F1665–F1672, doi: 10.1152/ajprenal.00495.2006, indexed in Pubmed: 17545302.
15. Kim BS, Goligorsky MS. Role of VEGF in kidney development, microvascular maintenance and pathophysiology of renal disease. Korean J Intern Med. 2003; 18(2): 65–75, doi: 10.3904/kjim.2003.18.2.65, indexed in Pubmed: 12872442.
16. Adamska A, Pilacinski S, Zozulinska-Ziolkiewicz D, et al. An increased skin microvessel density is associated with neurovascular complications in type 1 diabetes mellitus. Diab Vasc Dis Res. 2019 [Epub ahead of print]: 1479164119850831, doi: 10.1177/1479164119850831, indexed in Pubmed: 31144511.
17. Masum MdA, Ichii O, Elewa YH, et al. Local CD34-positive capillaries decrease in mouse models of kidney disease associating with the severity of glomerular and tubulointerstitial lesions. BMC Nephrol. 2017; 18(1): 280, doi: 10.1186/s12882-017-0694-3, indexed in Pubmed: 28870174.
18. Aggarwal S, Grange C, Iampietro C, et al. Human CD133 renal progenitor cells induce erythropoietin production and limit fibrosis after acute tubular injury. Sci Rep. 2016; 6: 37270, doi: 10.1038/srep37270, indexed in Pubmed: 27853265.
19. Adamska A, Araszkiewicz A, Pilacinski S, et al. Dermal microvessel density and maturity is closely associated with atherogenic dyslipidemia and accumulation of advanced glycation end products in adult patients with type 1 diabetes. Microvasc Res. 2019; 121: 46–51, doi: 10.1016/j.mvr.2018.10.002, indexed in Pubmed: 30312628.
20. Piera-Velazquez S, Jimenez SA. Endothelial to mesenchymal transition: role in physiology and in the pathogenesis of human diseases. Physiol Rev. 2019; 99(2): 1281–1324, doi: 10.1152/physrev.00021.2018, indexed in Pubmed: 30864875.
21. Peng H, Li Y, Wang C, et al. ROCK1 induces endothelial-to-mesenchymal transition in glomeruli to aggravate albuminuria in diabetic nephropathy. Sci Rep. 2016; 6: 20304, doi: 10.1038/srep20304, indexed in Pubmed: 26842599.
22. Liu F, Zhang S, Xu R, et al. Melatonin attenuates endothelial-to-mesenchymal transition of glomerular endothelial cells via regulating miR-497/ROCK in diabetic nephropathy. Kidney Blood Press Res. 2018; 43(5): 1425–1436, doi: 10.1159/000493380, indexed in Pubmed: 30212830.
23. van Schie MC, van Loon JE, de Maat MPM, et al. Genetic determinants of von Willebrand factor levels and activity in relation to the risk of cardiovascular disease: a review. J Thromb Haemost. 2011; 9(5): 899–908, doi: 10.1111/j.1538-7836.2011.04243.x, indexed in Pubmed: 21342431.
24. Smith NL, Rice KM, Bovill EG, et al. Genetic variation associated with plasma von Willebrand factor levels and the risk of incident venous thrombosis. Blood. 2011; 117(22): 6007–6011, doi: 10.1182/blood-2010-10-315473, indexed in Pubmed: 21163921.
25. Lenting PJ, Casari C, Christophe OD, et al. von Willebrand factor: the old, the new and the unknown. J Thromb Haemost. 2012; 10(12): 2428–2437, doi: 10.1111/jth.12008, indexed in Pubmed: 23020315.
26. Edgell CJ, Haizlip JE, Bagnell CR, et al. Endothelium specific Weibel-Palade bodies in a continuous human cell line, EA.hy926. In Vitro Cell Dev Biol. 1990; 26(12): 1167–1172, indexed in Pubmed: 2079463.
27. Tsai WC, Wu HY, Peng YS, et al. Risk factors for development and progression of chronic kidney disease: a systematic review and exploratory meta-analysis. Medicine (Baltimore). 2016; 95(11): e3013, doi: 10.1097/MD.0000000000003013, indexed in Pubmed: 26986114.
28. Fogo AB. Mechanisms of progression of chronic kidney disease. Pediatr Nephrol. 2007; 22(12): 2011–2022, doi: 10.1007/s00467-007-0524-0, indexed in Pubmed: 17647026.