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Analysis of the Correlation between Microvascular Involvement and Neuropathy in Association with Metabolic Disorders in Case of Diabetic Leg Syndrome

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Abstract

Diabetic leg developing with vascular and neuropathic path mechanism is a late onset complication of diabetes mellitus. Connection and therapeutic manageability of the two mechanisms is in close correlation, but it is still an unsolved issue. In our study we performed complex examination of patients presenting with symptoms referring to polyneuropathy. With ENG analysis we determined the conduction velocity, amplitude and distal latencies. Examination of motor fibres was performed by stimulating the peroneal nerve, while sensory fibres were assessed through stimuli of the sural nerve and the peroneal nerve. Presence of macro vascular involvement was confirmed by ankle-brachial index test and by Duplex ultrasound. Microcirculation was assessed by laser-Doppler test, triggering the Venous-Arteriolar (VA) reflex. During the measurement we detected the metabolic status by monitoring cholesterol, triglyceride, LDL, HDL, A1C levels and parameters of renal function, which were then compared to the above specified factors. We performed our examinations in the age- and gender-specific control group. We examined 50 (34 males, 16 females) patients presenting with polyneuropathic complaints with average ages of 64, 62 years. Average duration of the disease being present was 15.38 years. Regarding the tests assessing microcirculation, when analysing the Venous-Arteriolar (VA) reflex within the control group, we detected significantly higher (*78% vs. 31.8%; p=0.001*) decrease in conduction velocity, compared to the diabetic patient group. We discovered significant correlation between the level of A1C characterizing the glycaemic control, and the decreased VA reflex response referring to microcirculatory disorder resulting from a neurogenic damage (R=0.322; p=0.024). We also found a similar association between increased triglyceride level and these pathological reflex response (R=0.35; p=0.015). ENG confirmed the presence of peripheral polyneuropathy (in 40% very severe, mixed type sensory-motoric neuropathy with axonal dominance, in 48% moderately severe, mixed type sensory-motoric neuropathy, in 6% severe sensory-motoric neuropathy with demyelinising dominance, and in 6% of them sensory-motoric neuropathy) in all cases. On the other hand we have not found a significant correlation between neuropathy confirmed by the ENG examination and the severity of the microcirculation disorder. Our results suggest that among macroangiopathic involvement of a diabetic leg, type and severity of the presenting neuropathy, the metabolic status of the patient and the observed microcirculation disorder, the latter one, the VA reflex measured by laser-Doppler is suitable for complex characterization of neuropathic, microcirculatory and metabolic status.

Keywords: Diabetes mellitus; Neuropathy; Microcirculatory damage

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Introduction

Based on WHO data, 420 million patients suffer from different types of diabetes mellitus worldwide [1]. Based on these data it is not an extreme statement that diabetes is the endemic disease of the 21st century, representing a significant public health issue. According to estimates, an undiagnosed diabetic patient can be associated with each discovered diabetic case [2]. In Hungary, the number of diabetic patients is around 700 000, its international prevalence is 8.3% [1], while in Hungary it is 7.5%. In 90% of cases Type II diabetes is present which manifests in adulthood, while in 10% of the instances it is Type I diabetes or other rare types (MODY, gestational diabetes, etc). Diabetes is a severe metabolic disease, according to WHO data, nearly 3.8 million people die of its diverse

complications annually. Treatment of diabetes and its complications puts a great burden on patients and society as well. Most frequently occurring diabetic complication is polyneuropathy resulting from chronic microangiopathy. Examination findings confirmed that diabetic neuropathy is an independent predictor or mortality [3]. In the development of diabetic neuropathy, microcirculation disorder, activation of hyperglycaemia-induced alternative metabolic pathways and metabolic disorder of neurons together play an important role. The developing microcirculation disorder affects the vasa nervorum, the basal membrane thickens, vasoconstriction, oedema and ischemia occurs. All of these changes occurring simultaneously lead to nerve damage developing through metabolic disorder of neurons. Hyperglycaemia causing oxidative stress triggers increased production of ROS, which activate the alternative metabolic pathways [4]. ROS activates other metabolic pathways as well. There are four main alternative pathways, which are the following: polyol metabolic pathway, non-enzymatic glycation of proteins; AGE production, the hexosamine pathway and the PKC pathway. These mechanisms also lead to ROS production, thus aggravating the vascular and nerve damage. Microcirculation disorder together with neuropathy results in development of diabetic leg syndrome, which is a late-onset, and one of the most severe complications of diabetes [5]. It affects 25% of diabetic patients. Diabetic leg syndrome and its complications put a great burden on hospital budget, since this is the most frequently occurring complication and it requires the most expensive treatment in diabetes [6]. In developed countries, one fourth, in some areas even half of hospitalized diabetic patients are treated due to diabetic leg, the average inpatient care needed is 25 days [3,6]. The amount of money spent on these treatments is equal to all expenses covering every other health costs of diabetic patients [7]. Diabetic leg syndrome is a complex clinical picture. In its development, primarily neuropathy (50%) and vasoconstriction (25%) play a role, which may lead to ischemia, and among one fourth of patients both aetiological factors can be identified. Three different types can be distinguished: neuropathic, ischemic and the mixed type. The purely ischemic/angiopathic form differs from the type developing only from neuropathy. Leg of patients with ischemic diabetic leg is cool, the skin is pale, livid, thin and hairless, and onychomycosis is often present. Tissue necrosis localized to the leg is acral. In mild cases patients complain about intermittent claudication as a symptom of peripheral arterial disease. Since neuropathy is not present, there are no sensory disorders and tendon reflexes can be triggered. The ankle-brachial index is also pathological and it is below 0.9 or frequently under 0.5. Modern method of determining local, tissue oxygenation is measuring the transcutaneous oxygen tension ($TcpO_2$). In case of ischemic aetiology we measure pathological values under 40 mmHg [8]. All these pathologies call our attention to an ischemic damage. Warm, oedematous leg is characteristic of the neuropathic type, pain or sensory loss is even present when resting. Symptom of the disease is the glove and tights-like synaesthesia, pain and sensory loss occurring on the fingers and toes, mainly on the feet, which extend into proximal direction on the affected limb. The peripheral pulse can be palpated, the ankle-brachial index is above 0.9 and the transcutaneous pO_2 is within the normal range, it is above 40mmHg. The autonomous neuropathy favours development of ulcers. Vasomotor and sudomotor functions are also disturbed. In case of mixed type ischemic and neuropathic symptoms occur simultaneously. It differs whether ischemic or neuropathic signs dominate. The most severe complication of a diabetic leg is occurrence of ulcers. Ulcers precede 85% of diabetic amputations. Amputations performed due to diabetic leg complications and diabetic ulcers represent up to 50% of non-traumatic major amputations in Hungary. These limb amputations could be avoided in 85% of the cases [3,9]. Wagner's classification is one of the most frequently used classification system in the diagnostic procedure of ulcers [10]. More than half of diabetic ulcers are infected by the time they are discovered. Osteomyelitis, abscess, gangrene may develop as complications. In these particular cases, saving the affected extremity is often impossible. Curing the complex clinical events associated with diabetic leg syndrome is only possible with multidisciplinary co-operation of different medical fields. In our study we aimed to analyse the association between microcirculation and neuropathy in connection with the current metabolic status. We also assessed whether microcirculatory dysfunction and nerve damage can be detected in patients having neuropathic complaints, and if there is any detectable correlation between the average duration of the disease presence and the severity of the microcirculatory disorder.

Patients and Methods

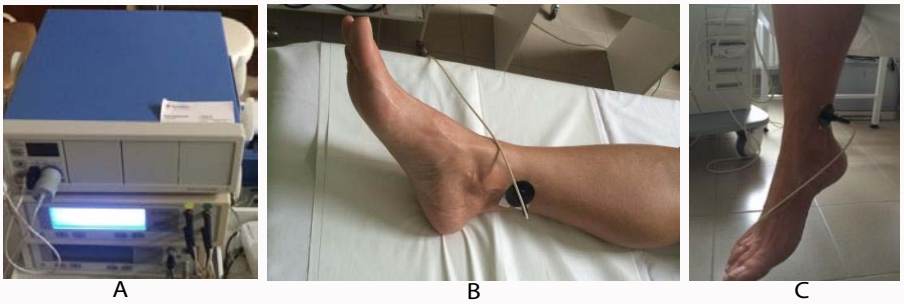
Patients

Altogether 50 Type II diabetic patients presenting with neuropathic complaints were enrolled for the study who received medical treatment at the Institute of Angiology, the Institute of Metabolic Diseases and the Institute of Vascular Surgery. Gender distribution among these 50 patients was the following: 36 males and 14 females. Average age of the patients was 64, 62 years (they were between the ages of 51 and 90 years). All patients enrolled for the study is suffering from Type II diabetes and average existence of the disease is 15, 35 years.

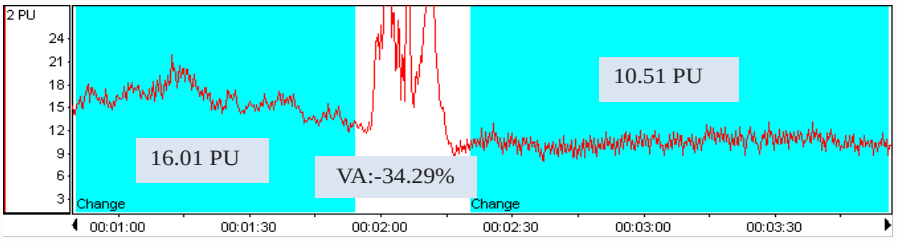
In all cases, neuropathic complaints affected the lower extremity, and in vast majority of them they appeared symmetrically on both lower limbs. Among the explored neuropathic symptoms of patients, limb numbness, spasms, pain, burning sensation and sensory loss were dominant. 6 of our patients had mixed type, neuro-ischemic ulcer on their lower shin, why 2 of our patients have developed pressure ulcer on the lower limb of neuropathic aetiology. Regarding the drug therapy of diabetes, our patients could be classified into 3 groups. 23 patients received oral anti-diabetic therapy, 13 received insulin treatment, while co-administration of an oral anti-diabetic and insulin was present in case of 14 of the patients. Besides anti-diabetic medication therapy we also analysed occurrence of late-onset, micro- and macroangiopathic complications of diabetes. Chronic macroangiopathic complications of diabetes can be associated with the accelerated, generalized atherosclerosis. Patients were screened for peripheral arterial diseases by analysing the ankle-brachial index, and if it was necessary we also performed arterial colour Doppler ultrasound examination of the lower extremity. We reviewed retrospectively all the documentations of patients available in an electronic form through the Med Solution system, paying special attention onto other cardiovascular (ischemic heart disease, TIA, stroke) and microangiopathic (neuropathy, retinopathy, nephropathy) complications. Among the traditional cardiovascular risk factors we examined hypertension and hypercholesterinaemia. We also performed our examinations in the age- and gender-specific control group. Ten persons (4 males and 6 females) belonged to the control group, the average age of whom was 45.4 years.

Methods

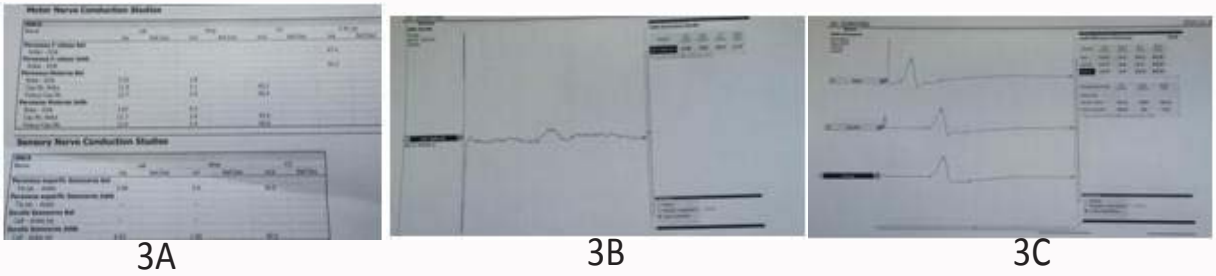
Determination of ankle-brachial index (ABI): We applied the determination of ankle-brachial index as a method for screening for peripheral arterial disease, which is of great specificity and sensitivity.



Picture 1 (A-C): Positioning of the lower extremity during VA examination (B-C), and the laser-Doppler flow meter (A).



Picture 2: Figure showing the changes detected during venous-arterial reflex test.



Picture 3 (A-C): ENG findings after analysing the sensory and motor fibres of the lower extremity, together with accurate data of conduction velocity 3A) amplitude 3B), and distal latency 3C).

The analysis was performed with a Continuous Wave (CW) Doppler.

Arterial colour doppler examination of the lower extremity: On patients, in case of whom the measured ABI was below 0.9 or above 1.3, we performed arterial colour Doppler examination (with Phillips, HD-11XE, 5-10 MHz linear transducer) in order to assess the vascular status of the lower extremity.

Analysis of microcirculation with laser-doppler flowmetry: Analysis of microcirculation was performed with the help of a laser-Doppler flowmeter (PeriFlux, 4000, Perimed, Sweden). The method of measurement is based on the Doppler-principle. Based on the Doppler-principle, the He-Ne monochrome laser light emitted by the device is partially absorbed in tissues, but on the other hand a significant portion is reflected. Frequency of the light wave reflected from red blood cells travelling through vessels is altered, since RBCs are moving. This change is proportionate to the number of RBCs found in the examined tissue sample and to their average velocity. The change in the frequency is proportionate to the number of RBCs found in the examined tissue sample and to their average velocity. The reflected laser light is perceived by the detector and from this we are able to calculate capillary flow of the examined tissue sample. The flow is not determined and expressed in actual units, but in Perfusion Units (PU). Resting flow rate of the skin is influenced by multiple factors. One of the most important factors is the external temperature, as a result of which thermo-regulating shunts are opening or closing [11-13]. Besides detecting resting flow rate, different provocation tests may provide wider range of information.

Provocation Tests - Venous-Arterial Reflex

In order to detect the presence of diabetes-induced microangiopathy we performed a provocation test, we examined the venous-arterial reflex. Examination of the Venous-Arterial reflex (VA response) is non-invasive, neither local warming of the skin, nor restraining the limb is necessary. Venous-arterial reflex response is a peripheral, sympathetic axonal reflex. It is well known that in healthy individuals, if the lower limb is brought into vertical position from horizontal, the flow will decrease by more than 50% due to vasoconstriction of pre-capillary arterioli. The reflex is decreased in diabetic microangiopathy, and it is almost completely lost is diabetic neuropathy [14-16]. Our tests were performed under standardized conditions, by providing constant external temperature (23 degree Celsius). The laser-Doppler device (PeriFlux 4001, Perimed, Sweden) emits a monochromatic light beam, which penetrates the skin through a 1 mm^2 surface, 1-1.5 mm deep, thus it enables us to analyse a 1-1.5 mm^3 sized tissue sample. The test probe is fixed 2cm above the medial malleolus or to the metatarsophalangeal joint of the first toe. The examination was performed in both lower extremities. During the first section of the examination, resting flow rate was measured

for 2 minutes, or until the perfusion has not changed for a minute at least, after the patient lay 10 minutes on the back, still in that supine position. After that we asked patients to hang their legs from the knee beside the examining table. Changes in flow rate were detected with the same method detailed above. After that, based on the resting flow rate and the flow rate values measured after hanging the leg, the degree of flow decrease can be determined with the help of special software. Decrease in flow rate is considered to be pathological, if it is below 50% (Picture 1, A-C).

We indicated the resting flow rate (16.01 PU), the decrease in flow rate detected after the leg was brought into a vertical position, as well as the calculated VA. The artefact caused by the change is illustrated within the white box (Picture 2).

Electroneurography - ENG

Electroneurography of the lower limb was performed in case of each patient in the Electrophysiology laboratory of Neurology Clinic at DE Clinical Centre, to confirm the presence of polyneuropathy. Based on the study protocol, analysis of motor fibres was done by stimulating the peroneal nerve, while analysis of sensory fibres was examined by stimulating the sural and the peroneal nerves (Picture 3, A-C).

Laboratory Tests

Determination of general laboratory parameters, blood picture, renal functions (urea, creatinine, eGFR), blood glucose, glycaemic control described by A1C values, as well as analysis of the lipid profile (triglyceride, cholesterol, HDL-C, LDL-C) was performed in Institute of Laboratory Medicine at DE Clinical Centre.

Statistical Methods

Data obtained during the tests were processed with the help of SPSS software. If average values were shown, we also indicated the standard deviations (-+ SD: standard deviation). We applied the Kolmogorov-Smirnoff test to assess normality. In case of data with normal distribution we used paired t-test and the Pearson correlation. Findings were considered to be significant if $p < 0.05$.

Findings

Chronic complications of diabetes mellitus, excluding diabetic neuropathy

Among the 50 patients enrolled, 9 suffered from diabetic nephropathy, 4 of them presented with retinopathy and there were 3 patients in case of whom retinopathy and nephropathy was present concomitantly. Complications affecting large vessels could be observed in 56% of our patients. Regarding the incidence, we can conclude that peripheral arterial disease was the most common macroangiopathic complication of diabetes. PAD could be diagnosed in 16 patients (Figure 1). Ischemic heart disease was present in case of 9 patients. Stroke suffered prior to the study period was present in medical anamnesis of 3 patients.

ENG findings

The ENG examination of lower extremities confirmed the presence of neuropathy in peripheral blood vessels in case of all our patients. In case of 20 patients, a very severe, mixed type sensory-motor neuropathy was described with axonal dominance. In case of 24 patients, the presence of moderately severe, mixed type sensory-motor neuropathy was confirmed. Signs of a severe sensory-motor neuropathy with demyelinating dominance were observed in case of 3 patients, while 3 patients were diagnosed with mild sensory mono-neuropathy.

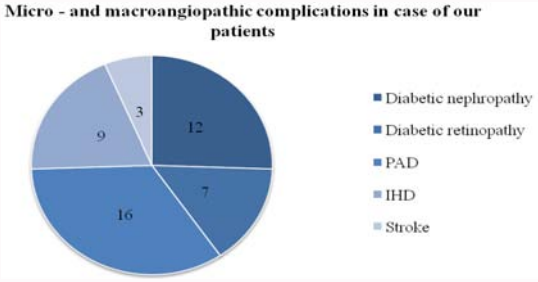


Figure 1: Chronic complications of diabetes mellitus in our patient group, excluding diabetic neuropathy.

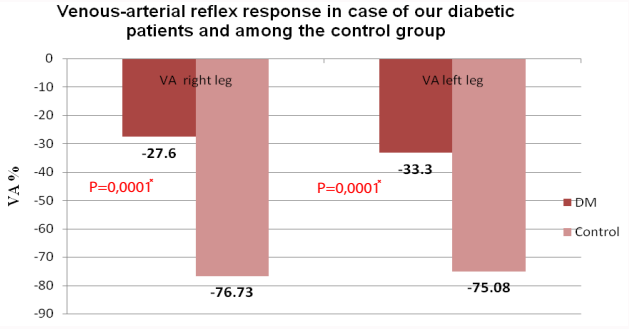


Figure 2: The venous-arterial reflex response of lower extremities in case of our patients and among the control group.

Findings of venous-arterial reflex tests characteristic of the degree of microcirculatory damage were compared to the severity of the presenting neuropathy. The venous-arterial reflex response did not show significant correlation to the severity of neuropathy, but our results showed that more severe neuropathy is associated with more severe damage to microcirculation.

Glycaemic control and lipid parameters

For describing glycaemic control, we analysed A1C values. The avarage value of A1C was 7.5 % (SD: ± 1.257). In case of 5 patients the A1C value did not exceed 6.1%, and the value was not over 15% in neither of the cases. Regarding the lipid parameters the average triglyceride level was 2.6 mmol/l (SD: ± 3.359), and the average cholesterol level was 4.6 mmol/l (SD: ± 1.25).

Findings of analysis of microcirculation

Venous-arterial reflex test was performed on both lower extremities, in case of all patients. The provocation test showed pathological findings at least on one lower limb in all patients examined and the decrease in flow rate did not exceed 50%. In case of two of our patients, the reflex response was so damaged, that instead of vasoconstriction we observed vasodilatation, which resulted in increased flow. On the whole we can conclude that microcirculatory dysfunction and damage could be confirmed in case of all patients enrolled in our study.

In our control group, the flow rates detected were in all instances above 50%, on both lower extremities.

In the diabetic group the avarage value of VA on the right side was -27.6 % (SD: ±19.58), while n the left side was -33.3% (SD:

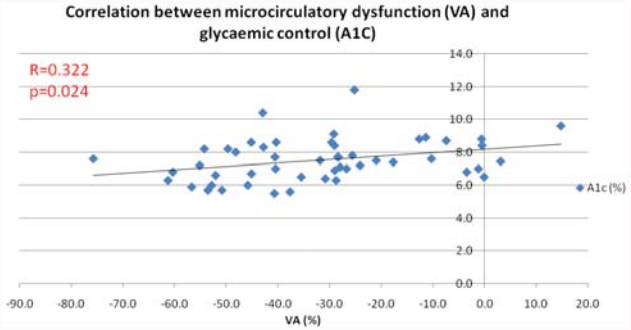


Figure 3: Correlation between microcirculatory dysfunction and glycaemic control.

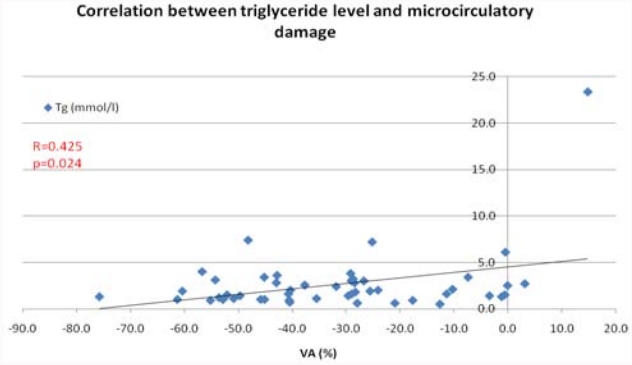


Figure 4: Correlation between triglyceride level and microcirculatory damage. Significant correlation could have been detected between the triglyceride level and the venous-arterial reflex response

±19.75). As for the non-diabetic control group the right side avarage value of VA was -76.73% (SD: ±5.99) , and ont he left side was -75.08 % (SD: ±4.55). We demonstrated that within our patient group, the decrease in flow rate emerging as a result of venous-arterial reflex was significantly lower compared to the findings obtained in the control group, regarding both lower limbs (p=0.0001) (Figure 2).

The Association between Microcirculatory Damage and Glycaemic Control

We were looking for a connection between glycaemic control and microcirculatory dysfunction. Our examinations revealed significant correlation (p=0.0011, R=0.472) between A1C values and the venous-arterial reflex response. In case of a higher A1C values, the decrease in flow rate was more significant (Figure 3).

Connection of Microcirculatory Damage and Lipid Parameters

Findings of analyses performed to examine microcirculation were compared to other laboratory tests results characterizing metabolic parameters. Among lipid parameters, significant correlation (p=0.024, R=0.425) was detectable between triglyceride levels and venous-arterial reflex response. The cholesterol (p=0.318, R=0.147), LDL (p=0.54, R=-0.094) and HDL levels (p= 0.61, R= 0.158) were not in significant association with microcirculatory damage (Figure 3).

Discussion

Diabetes mellitus affects hundreds of millions of people worldwide, who mainly suffer from Type II diabetes. Diabetes is not only associated with disorder of glucose metabolism, but its complications may also cause damage to multiple organ systems. The most frequently occurring chronic microangiopathic complication is the development of diabetic neuropathy. Path mechanism of diabetic neuropathy is not clearly elucidated yet. Microcirculatory dysfunctions, hyperglycaemia-induced activation of alternative pathways and metabolic disorder or neurons together play a role in its development. The developing microcirculatory disorder also affects the vasa nervorum, the basal membrane thickens, vasoconstriction, oedema and ischemia occurs. The total of these changes causes nerve damage through metabolic disorder of neurons. Aim of our study was to assess pathological components of diabetic leg syndrome, as well as to look for associations between these pathological processes. We performed our examinations on patients suffering from Type II diabetes, presenting with neuropathic complaints. Findings of preliminary examinations of 37 patients were published recently in the Journal of Vascular Diseases [17]. Our most important finding was the observation that the decrease in flow rate of the lower extremity detected during a venous-arterial reflex response measured by a laser-Doppler flowmeter was significantly lower compared to results obtained in the age- and gender-specific control group. Based on these findings it can be concluded that venous-arterial reflex response test is a trustworthy method to detect microcirculatory damage in Type II diabetic patients.

Farkas and colleagues analysed venous-arterial reflex response among patients with Type I and Type II diabetes mellitus. They concluded that the incidence of pathological responses was significantly higher in both groups, compared to the control group [18]. Golster and colleagues examined the microcirculatory dysfunction with laser-Doppler flowmetry among Type I and Type II diabetic patients, by determining the venous-arterial reflex response. The examined population consisted of patients suffering from Type I diabetes, between the ages of 10-21 years, and of the control group. They determined that the decrease in flow rate was significantly lower in diabetic patients, than in the control group. They also demonstrated that in patients with poor glycaemic control (HbA1c>7.5) the decrease in flow rate was significantly lower compared to those who had proper glycaemic control (HbA1c <7.5%) [19]. Yosipovitch and colleagues also assessed venous-arterial reflex response in young, Type I diabetic patients. According to their study, the venous-arterial reflex response was significantly decreased in the diabetic group, even without a presenting neuropathy, compared to the control group [20]. In our patient group, the presence of peripheral neuropathy could have been observed in each case. On the other hand, no significant correlation was detectable between severity of the peripheral neuropathy and the pathology of the venous-arterial reflex response. These findings suggest that microcirculatory damage precedes development of neuropathy, but severity of the developing neuropathy is influenced by multiple other factors as well. Findings of our microcirculatory tests were compared metabolic parameters of our patients. We demonstrated that microcirculatory damage shows significant correlation to short-term (3 months) glycaemic control characterized by A1C level. We were also able to detect a significant correlation between triglyceride level and severity of the microcirculatory damage. We have not found relevant data in the international scientific literature, whether anyone analysed the connection of venous-arterial reflex response and metabolic parameters among patient suffering from Type II diabetes. But on the other hand, Velcheva and colleagues examined the association between haemorheological parameters, cerebral blood flow and

venous-arterial reflex response among Type II diabetic patients. They also demonstrated that the decrease in flow rate observed during the venous-arterial reflex response was significantly lower, compared to the control group. In addition they also determined that increased blood viscosity is associated with decreased cerebrovascular blood flow, as well as with decreased venous-arterial reflex [21]. Our findings suggest that with better glycaemic control and by keeping lipid parameters within the normal range – especially with an adequate triglyceride level – we can positively influence microcirculatory dysfunction and complications caused by diabetic microangiopathy. Our findings also suggest macroangiopathic involvement of a diabetic leg, type and severity of the neuropathy, metabolic status of the patient, and complexity of the microcirculatory disorder can be monitored well with the venous-arterial reflex response measured by a laser-Doppler. This work was supported by grants from GINOP 2.3.2-15-2016-00048 and the National Research, Development and Innovation Fund (K109712 and K120042).

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