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Original Article

Features of endothelial dysfunction markers in patients with diabetes mellitus with diabetic foot syndrome

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Abstract

The study included 100 patients with DFS of grades I-IV according to the Meggitt-Wagner classification. The neuropathic form (NF) was diagnosed in 39 patients, while the ischemic form (IF) was observed in 61 patients. Among the participants, 72 were men, and 28 were women, with a mean age of 62.3 years. Type 1 diabetes was present in 24 patients, and Type 2 diabetes in 76 patients. The duration of DM ranged from 1 to 19 years. Microcirculation was studied using LDF with a "LAKK-02" (Lazma). Patients refrained from eating, smoking, or physical activity for an hour before the test and rested in a supine position for 15 minutes in a temperature-controlled room (20–23°C). These changes can be attributed to the longer disease duration in Type 2 DM patients (mean 12.3±1.3 years) compared to Type 1 DM (mean 4.9±0.7 years) and the presence of prolonged hyperglycemia, which damages the endothelium. Age also played a role, with Type 2 DM patients having a mean age of 67.2±2.5 years versus 46.7±1.8 years in Type 1 DM. Differences were also noted depending on the pathogenetic form of DFS. NO levels in NF were 21.42±1.27 µmol/L, 21.8% lower than control values, whereas IF patients exhibited a 39.7% reduction (16.61±0.87 µmol/L). Conversely, endothelin-1 levels increased by 62.7% (6.45±0.78 pg/mL) in NF and 132.5% (9.27±1.32 pg/mL) in IF DFS.

Keywords: endothelial dysfunction, diabetes mellitus, diabetic foot syndrome

Introduction

Recent scientific publications highlight a growing interest in the role of endothelial dysfunction (ED) in the pathogenesis of vascular lesions of various origins, including in patients with diabetes mellitus (DM) [1–4]. According to current views, ED is a nonspecific response of the endothelium characterized by an imbalance of factors responsible for vasoconstriction and vasodilation, prothrombotic factors, and processes of proliferation and remodeling [5, 6]. ED primarily refers to impaired vascular tone regulation due to alterations in the synthesis of vasodilators (nitric oxide – NO) and vasoconstrictors (endothelin-1) [7–9]. NO exhibits diverse effects, including negative inotropic action, vasodilation, anti-atherosclerotic, antithrombotic, and anti-adhesive properties [10, 11]. Endothelin-1, on the other hand, is one of the most potent endogenous vasoconstrictors, whose mechanism involves calcium release [12]. This leads to stimulation of all hemostasis phases with hypercoagulation, contraction and growth of vascular smooth muscles, thickening of vessel walls, and development of vasoconstriction [13–16].

These processes disrupt peripheral blood supply, contributing to the development of purulent-necrotic tissue lesions and impairing reparative processes in wounds, particularly relevant in patients with DM complicated by diabetic foot syndrome (DFS) [17, 18].

Material and methods

The study included 100 patients with DFS of grades I–IV according to the Meggitt-Wagner classification. The neuropathic form (NF) was diagnosed in 39 patients, while the ischemic form (IF) was observed in 61 patients. Among the participants, 72 were men, and 28 were women, with a mean age of 62.3 years. Type 1 diabetes was present in 24 patients, and Type 2 diabetes in 76 patients. The duration of DM ranged from 1 to 19 years. The control group consisted of 20 healthy individuals without clinical signs of lower limb pathology, matched by age and sex to the patient group.

Upon admission, all patients underwent comprehensive clinical, laboratory, and instrumental examinations to differentiate the clinical form and degree of foot lesion. Endothelial function was evaluated using enzyme-linked immunosorbent assays (ELISA) to determine NO and endothelin-1 levels in peripheral blood serum. Additionally, lower limb microcirculation was assessed using laser Doppler flowmetry (LDF).

Standard reagent kits from R&D Systems (USA) were used for NO determination, while DRG (USA) kits were used for endothelin-1. Blood samples were collected from the cubital vein in the morning, centrifuged, and subsequently analyzed via ELISA.

Microcirculation was studied using LDF with a "LAKK-02" device (Lazma). To minimize variability, assessments were conducted under standardized conditions between 9:00 and 10:00 a.m. Patients refrained from eating, smoking, or physical activity for an hour before the test and rested in a supine position for 15 minutes in a temperature-controlled room (20–23°C). The microcirculation examination focused on the dorsum of the foot, particularly at the distal third of the first interdigital space or 1 cm from the wound edge (if present in the study area). Measurements were taken in two phases: basal blood flow and during an occlusion test.

Data analysis included mean tissue perfusion values (constant component M, variable component σ, coefficient of variation Kv) and wavelet analysis of oscillations from active and passive regulatory factors.

Results

Results were processed using methods of variational statistics, with significance set at $p<0.05$. More pronounced endothelial dysfunction was observed in patients with Type 2 DM compared to Type 1 DM, manifested by significant reductions in serum NO levels and increased endothelin-1 concentrations (Table 1).

These changes can be attributed to the longer disease duration in Type 2 DM patients (mean 12.3±1.3 years) compared to Type 1 DM (mean 4.9±0.7 years) and the presence of prolonged hyperglycemia, which damages the endothelium. Age also played a role, with Type 2 DM patients having a mean age of 67.2±2.5 years versus 46.7±1.8 years in Type 1 DM.

Endothelial dysfunction severity correlated with glycemia levels. For instance, in compensated DM, NO levels were 23.45±1.88 μmol/L, and endothelin-1 was 4.66±0.37 pg/mL; in subcompensated DM, 19.11±1.22 μmol/L and 5.19±0.28 pg/mL; and in decompensated DM, 15.42±1.08 μmol/L and 8.05±0.79 pg/mL, respectively. These findings underscore hyperglycemia's detrimental effects on the endothelium.

Differences were also noted depending on the pathogenetic form of DFS. NO levels in NF were 21.42±1.27 μmol/L, 21.8% lower than control values, whereas IF patients exhibited a 39.7% reduction (16.61±0.87 μmol/L). Conversely, endothelin-1 levels increased by 62.7% (6.45±0.78 pg/mL) in NF and 132.5% (9.27±1.32 pg/mL) in IF DFS.

An analysis of LDF data revealed a general tendency toward decreased lower limb microcirculation indicators in IF, reflecting impairments in the main blood flow. The LDF data for different grades of ischemia demonstrated progressive decreases in both constant and variable components of tissue perfusion as ischemia severity increased.

The data presented in Table 2 demonstrate a progressive decline in microcirculatory parameters, namely M (mean perfusion), σ (standard deviation of perfusion), and Kv (coefficient of variation) as the severity of ischemia increases. This trend indicates a

Table 1: Indicators of endothelial dysfunction in DFS patients by diabetes type.

Indicator	Control (n=10)	Type 1 DM (n=24)	Type 2 DM (n=76)
NO (μmol/L)	27.44±1.31	20.36±1.33 p>0.01	17.41±1.47 p>0.001
Endothelin-1 (pg/mL)	3.95±0.26	6.37±0.39 p>0.001	9.23±1.17 p>0.001

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Table 2: Endothelial dysfunction by lesion severity (Meggitt-Wagner classification).

Indicator	Control (n=10)	Grade I (n=11)	Grade II (n=18)	Grade III (n=41)	Grade IV (n=30)
NO (µmol/L)	27.41±1.32	23.37±1.65 p>0.1	21.60±1.47 p>0.05	17.16±1.34 p>0.001	13.23±1.69 p>0.001
Endothelin-1 (pg/mL)	3.96±0.32	4.59±0.47 p<0.2	5.12±0.45 p>0.05	7.82±0.66 p>0.001	9.68±0.35 p>0.001

deterioration in both the constant and variable components of blood flow regulation, reflecting impaired vascular responsiveness and autoregulatory capacity in patients with advanced ischemic conditions (Table 2).

The analysis of the presented results reveals that patients with CLI (critical limb ischemia) exhibit a decrease in the parameters of the constant component of blood circulation (M) and the variable component of microcirculation (σ), indicating impaired active and passive mechanisms of microcirculation control and reflecting the severity of limb ischemia.

Additionally, a decrease in the Kv parameter was noted, which, along with the reduction in M and σ, indicates disruptions in the activation of endothelial secretion, as well as neurogenic and myogenic regulatory mechanisms.

The analysis of the amplitude-frequency spectrum of perfusion oscillations of active and passive factors and their contributions to microcirculation regulation revealed that endothelial oscillations in CLI increase with the progression of tissue ischemia and significantly exceed control values (p>0.05). This indicates the development of endothelial dysfunction, with enhanced endothelial secretion of vasoactive substances that modulate muscle tone and predominantly regulate the precapillary segment (arteries, arterioles, and precapillaries). At the same time, a decrease in the functional contribution of the endothelium to blood flow modulation and overall tissue perfusion was observed.

The occlusion test, which reflects the functional reserves of microcirculation mediated by endothelial synthesis of nitric oxide (NO), demonstrated a reduction in reserve capillary blood flow (RCBF). For patients with grade I–II arterial insufficiency, the RCBF was 215.18±25.46% and 180.21±21.77%, respectively, whereas for those with grade III–IV, the RCBF decreased to 131.22±16.81% and 102.31±17.82%, respectively, compared to the normal value of 230.13±18.89%. Additionally, a decrease in tissue perfusion recovery indicators was noted.

This further substantiates the association between ischemia severity and microcirculatory dysfunction. Patients with critical limb ischemia (Grade IV) exhibit significantly reduced values of M, σ, and Kv compared to the control group, suggesting marked impairment in tissue perfusion and microvascular control mechanisms. These findings underscore the utility of LDF indicators as objective markers for evaluating the extent of ischemic damage in diabetic foot syndrome (Table 3).

Endothelial oscillations (Ae) in the post-occlusion period increased with the progression of tissue ischemia and significantly exceeded control values (p>0.001). This suggests a paradoxical reaction and persistent endothelial dysfunction, with increased endothelial secretion of vasoactive substances that modulate muscle tone, exacerbating peripheral spasm of the microcirculatory bed. Concurrently, a reduction in the functional contribution of the endothelium to blood flow modulation and overall tissue perfusion was observed.

Patients with NF (neuropathic form) lesions demonstrated a reduction in overall microcirculation

Table 3: LDF Indicators in Ischemic DFS.

Indicator	Control (n=20)	Grade I (n=14)	Grade II (n=21)	Grade III (n=20)	Grade IV (n=6)
M (perf. units)	1.72±0.27	1.67±0.18 p<0.5	1.56±0.19 p<0.5	1.20±0.13 p>0.05	0.77±0.08 p>0.01
σ (perf. units)	0.66±0.13	0.62±0.9 p<0.5	0.57±0.11 p<0.5	0.40±0.13 p>0.05	0.26±0.01 p>0.01
Kv (%)	42.61±3.82	36.32±3.74 p<0.5	35.40±4.08 p<0.5	33.86±3.80 p>0.05	32.06±3.17 p>0.05

Table 4: LDF parameters in patients with neuropathic form of diabetic foot syndrome.

Parameter	Normal (n=20)	Without tissue edema (n=11)	With tissue edema (n=28)
M (pf.u.)	1.73±0.27	1.62±0.17 p<0.5	2.94±0.47 p<0.02
σ (pf.u.)	0.66±0.14	0.61±0.06 p<0.5	0.43±0.05 p>0.001
Kv (%)	42.61±3.83	38.45±2.57 p<0.2	32.65±2.17 p<0.02

parameters of the lower extremities, which was more pronounced under conditions of tissue edema (due to the presence of an inflammatory process or neuropathic tissue edema associated with neuropathy) (Table 4).

Discussion

The analysis of the obtained data indicates that patients with the neuropathic form of diabetic foot syndrome (DFS) without tissue edema show minor microcirculatory disturbances that do not reach critical levels. The parameters of the constant blood circulation component (M) and the variable microcirculation component (σ) were slightly lower than in the control group, although these differences were not statistically significant ($p<0.5$). These changes partially reflect disruptions in active and passive microcirculation control mechanisms. The reduction in Kv was also not statistically significant ($p<0.2$), although it suggests a decline in endothelial secretion activity and impaired endothelium-dependent vasodilation.

In patients with pronounced tissue edema of the lower extremities, microcirculatory disturbances were more significant. A statistically significant increase in M ($p<0.02$) indicates congestive phenomena. This may be attributed to the development of so-called auto-sympathectomy of the lower limb vessels due to peripheral neuropathy, leading to impaired vascular tone regulation, or the presence of an inflammatory process.

Simultaneously, a reduction in σ ($p>0.001$) was observed, caused by a decrease in the intensity of active microcirculation control mechanisms, along with a decline in Kv, indicating reduced endothelial secretion.

In the neuropathic form of DFS, endothelial dysfunction (ED) is evident, accompanied by increased secretion of vasoactive substances by the endothelium. This is confirmed by the growth of Ae oscillations, which are more pronounced in patients with tissue edema. This can be explained by deeper metabolic disturbances affecting endothelial functional activity. At the same time, a slight reduction in the functional contribution of the endothelium to blood flow modulation and overall tissue perfusion was noted, which may be associated with morphological changes in the vessels.

The occlusion test in this group of patients showed a decrease in reserve capillary blood flow (RCBF), averaging 176.47±22.19%, and a decline in the studied parameters during the blood flow recovery period.

The progressive decline in microcirculatory parameters observed in our study is consistent with previously reported findings in patients with diabetic foot syndrome and critical limb ischemia. Recent investigations have highlighted the diagnostic value of laser Doppler flowmetry (LDF) in assessing both constant and variable components of blood circulation. For instance, Hu *et al.* (2024) demonstrated that wearable LDF devices provide reliable non-invasive monitoring of microcirculation in diabetic foot patients, confirming the clinical relevance of perfusion variability indicators. Similarly, Tarvainen *et al.* (2024) emphasized that microvascular transformation plays a decisive role in the progression of limb-threatening ischemia, underscoring the importance of parameters such as M, σ, and Kv in evaluating disease severity [19–21].

Moreover, Geskin *et al.* (2022) reported that lower limb revascularization significantly improves microcirculatory function, as measured by LDF, thereby supporting the utility of these indicators in monitoring therapeutic outcomes. Lin *et al.* (2024) further demonstrated the efficacy of minimally invasive revascularization in restoring microvascular control mechanisms in diabetic foot ischemia, which aligns with our findings of reduced Kv values in advanced ischemic grades. Finally, recent work by Müller *et al.* (2025) using [18F] FAZA PET/MR imaging confirmed that microcirculatory dysfunction serves as a prognostic marker in patients with chronic limb-threatening ischemia, reinforcing the role of LDF-derived parameters as objective indicators of ischemic damage [22, 23].

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Taken together, these studies provide strong evidence that our results are not isolated but rather fit within a broader body of literature demonstrating the pathophysiological significance of microcirculatory impairment in ischemic diabetic foot syndrome.

Conclusion

Patients with DFS exhibit endothelial dysfunction, the severity of which depends on the type of diabetes mellitus, glycemic levels, and the pathogenetic form of the lesion. Endothelial dysfunction manifests as a reduction in vasodilator (NO) secretion and increased synthesis of vasoconstrictors (endothelin-1), leading to impaired peripheral hemodynamics. A characteristic feature of microcirculation changes is a marked disruption of the endothelium-dependent regulatory mechanism, with blood redistribution toward nutritive circulation.

In addition, there is a significant reduction in the reserve capacity of the capillary network in response to the occlusion test and blood flow recovery during reactive hyperemia.

Endothelial dysfunction and microcirculation disturbances, with a shift toward nutritive blood flow, contribute to the development of purulent-necrotic lesions and impaired reparative processes in patients with DFS. These findings necessitate appropriate corrective therapy as part of the comprehensive pathogenetic treatment of DFS.

Conflict of interest

The authors declare no conflict of interest.

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