SURGERY

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CHARACTERISTICS OF IMMUNOREACTIVITY IN PATIENTS WITH VARIOUS FORMS OF DIABETIC FOOT ULCER

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ABSTRACT

The article presents a comparative assessment of the levels of cytokines that regulate the differentiation of various macrophage phenotypes, as well as immunological responses in patients with diabetes. We examined and treated 245 patients who were allocated to two groups. The first group comprised neuropathic patients (comparison subgroup 1A without DFU, n=30 and subgroup 1B with DFU, n=80). The second group included patients with neuroischemic type 2 diabetes (comparison subgroup 2A without DFU, n=35 and subgroup 2B with DFU, n=100). As an indicator of hyperglycemia, the glycated hemoglobin level (HbA1c) was used, which was determined on the DCA 2000 device by the method of latex immunoagglutination inhibition using the "Hemoglobin A1c Reagent kit". The concentration of cytokines TNF-α, IFN-γ, IL-6, and OPG in blood serum was determined on the first day of hospitalization before surgery using enzyme immunoassay test systems (OOO "Cytokine", St. Petersburg) in accordance with the manufacturer's instructions. An imbalance of cytokines was revealed, indicating a shift in immunomodulatory responses, aggravation of metabolic and vascular disorders in tissues, accompanied by an inadequate course of the inflammatory and reparative process and the formation of a diabetic foot ulcer.

Keywords: diabetic foot ulcers (DFU), complications of diabetes mellitus, tumor necrosis factor, interleukin-VI, gamma-interferon, osteoprotegerin

RELEVANCE OF THE RESEARCH

Among patients with diabetes aged 25-75 years, lower extremity damage occurs in the form of diabetic foot syndrome in 20-80% of cases , and it is complicated by diabetic foot ulcers (DFU) in 25% of cases [1,2]. DFU is a serious complication of the disease, as they are difficult to heal due to a combination of internal and external factors. The body's immune response to injury (immunoreactivity) is critical to determining the rate and outcome of the healing process, including wound cleansing, scarring of the tissue defect, and restoration of foot function [3]. In the management of all phases of the inflammatory-reparative process in damaged tissues, as shown in both preclinical and clinical studies, an important role belongs to the innate and adaptive immune systems. The innate immune system consists of monocytes/macrophages, basophils, natural killer cells, granulocytes, resident mast cells, and dendritic cells. Recently, the effectiveness of regenerative immunotherapy based on the so-called "immunocentric revolution" or "macrophage-centric approach" has been recognized [4]. According to this concept, impaired wound healing in diabetes is associated with an increase in the number and long-term

persistence of wound monocytes/macrophages of the pro-inflammatory M1 phenotype [5,6]. Differentiation and activation of M1 phenotypes are provided by interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α) [7]. Also, in conditions of metabolic dysregulation in patients with neuropathic and neuroischemic forms of DFU, IL-6 and osteoprotegerin (OPG) may be additionally involved as inducers of M2 macrophage differentiation [8]. In this regard, it seems relevant to study the balance of cytokines involved in the regulation of M1 and M2 macrophage phenotypes in the DFU area.

The aim of the study was to study the pathogenetic features of the cytokine regulation of the macrophage aspect of the inflammatory and reparative process in patients with neuropathic and neuroischemic forms of DFU.

MATERIALS AND METHODS

We examined and treated 245 patients divided into two groups: those with neuropathic (comparison subgroup 1A without DFU, n=30 and subgroup 1B with DFU, n=80) and neuroischemic type 2 diabetes (comparison subgroup 2A without DFU, n=35 and subgroup 2B with DFU, n=100). The neuropathic nature of DFU was diagnosed on the basis of verification of peripheral sensorimotor neuropathy with intact (slightly impaired) main blood flow with an ankle-brachial pressure index on both legs of 0.9. The cases of neuroischemic DFU included patients with foot skin defects accompanied by obvious disorders of the main blood flow (ankle-brachial pressure index <0.5) and signs of peripheral sensorimotor neuropathy. Patients of the groups 1 and 2 had similar area (mean 3.1±1.7 cm^2 and 3.0±1.4 cm^2, respectively) and depth of the foot tissue ulcer (mean 2.7±1.5 mm and 2.9±1.3 mm, respectively). As an indicator of hyperglycemia, the glycated hemoglobin level (HbA1c) was used, which was determined on the DCA 2000 device by the method of latex immunoagglutination inhibition using the "Hemoglobin A1c Reagent kit". The concentration of cytokines TNF-α, IFN-γ, IL-6, and OPG in blood serum was determined on the first day of hospitalization before surgery using enzyme immunoassay test systems (OOO "Cytokine", St. Petersburg) in accordance with the manufacturer's instructions. The control group consisted of 15 healthy volunteers. Mathematical processing of the obtained results was carried out using the STATISTICA 6.0 and MedStat software.

RESULTS AND DISCUSSION

The percentage of glycated hemoglobin (HbA1c) in the blood samples of patients in the present study was chosen as an indicator reflecting the level of glucose in the blood during the previous months of the disease. In patients of the 1A and 2A subgroups, the hyperglycemic status was similar - HbA1c ≥ 6.4%, while in the 1B and 2B subgroups, the HbA1c values exceeded those in the comparison groups and amounted to 9.4±0.3% and 10.6±0,5%, respectively ($p<0.05$).

Patients of the 1A and 2A subgroups had a similarly increased level of serum pro-inflammatory TNF-α (by 23.04% and 43.94%, respectively) compared to the control. However, in patients with both forms of DFU, the increase in this indicator was significantly higher than in the control and such in subgroups without DFU. In 1B and 2B subgroups, it exceeded the control by 75.90% and 88.36% ($p<0.05$) (table). The IFN-γ cytokine status had a different picture: it was significantly lower in patients without DFU (by 32.55% and 43.6%, respectively, $p<0.05$), and in patients of 1B and 2B subgroups it was not significantly different from the control.

Diabetic patients had significant differences for serum IL-6 concentrations compared with the control group. Thus, in 1A and 2A subgroups, the indicator significantly exceeded the control one by an average of 2 times, while in patients with DFU in 1B and 2B subgroups it was 3.48 and 4.13 times higher than that in the control. Overproduction of IL-6 can result in the failure of macrophages to properly respond to infection [9]. Multidirectional changes were observed for the OPG level in patients with DFU and without ulcerative defects. In 1B subgroup, this indicator exceeded the control by 35.8% and in 2B subgroup - by 53.8%. In the absence of a foot ulcer in patients of 1A and 2A subgroups, the OPG level was lower in patients with neuropathic form of the disease (by 18.5%, $p<0.05$), while in patients with neuroischemic form it did not differ significantly from the control. (Table)

Table. Serum cytokines level in patients with various forms of DFU

Cytokine, pc/ml	Control, n=15	Neuropathic form		Neuroischemic form	
		1A, n=30	1B, n=80	2A, n=35	2B, n=100

TNF-α	4,21±0,53	5,18±0,09*	7,38±0,10	6,06±0,13*	7,93±0,12
IFN-γ	1,72±0,11	1,16±0,09*	2,05±0,11	0,97±0,55*	1,88±0,15
IL-6	6,05±0,14	9,13±0,24*	15,04±1,33*	10,44±0,76*	25,03±1,76*
OPG	22,90±0,97	18,66±1,04	31,10±1,58*	21,47±1,15	35,21±1,12*

*Note. * - significant difference in comparison with the control group is <0.05.*

Changes in the content of TNF-α and OPG may reflect the direct relationship of these cytokines in maintaining the inflammatory M1 phenotype of macrophages in wound tissues. Both cytokines play an important role in endothelial dysfunction, on the one hand, and in the differentiation of various macrophage phenotypes, on the other hand. The revealed high OPG level in patients with DFU can be an indicator of endoth elial cell dysfunction and damage to the cardiovascular system [10]. At the same time, high concentrations of TNF-α in patients with and without DFU most likely reflect a trend towards an increase in the number and/or activity of M1 macrophages in the wound, or are a manifestation of a cytokine imbalance that exacerbates vasodilation disorders and enhances apoptosis of endothelial cells indirectly through the vascular cell adhesion molecule 1 and E-selectin. In addition, TNF-α itself implements immunological reactions which result in vascular and metabolic disorders (15), therefore, a decrease in its serum level is aimed at leveling these reactions. Dysregulation of both monocytes/macrophages and imbalanced macrophage phenotypes can lead to impaired or delayed healing [11,12].

Identified mild changes in the content of serum IFN-γ of patients with DFU may be due to a decrease in the number of IFN-γ-producing cells, which occurs in type 2 diabetes [13]. On the other hand, inhibition of IFN-γ secretion slows down the release of NO by macrophages, which exacerbates vascular disorders in patients with DFU, and also reduces the phagocytic activity of mononuclear cells [14,15,16].

CONCLUSION

Patients with DFU have an imbalance of cytokines that regulate the differentiation of various macrophage phenotypes, which causes a shift in immunomodulatory reactions, exacerbates metabolic and vascular disorders in tissues, and is accompanied by an inadequate course of the inflammatory and reparative process in the form of a diabetic foot ulcer.

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