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New Approaches in the Treatment of Diabetic Osteoarthropathy

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ABSTRACT

Background. The frequency of lower limb amputations in patients with diabetic foot syndrome complicated by osteoarthropathy is 15-20 times higher than in the general population and accounts for 50-70% of the total number of all non-traumatic amputations. At the same time, in patients with type 2 diabetes mellitus, at the time of diagnosis, from 30 to 50% of patients have signs of damage to the lower extremities of one degree or another.

Material. Under our supervision at the clinical base of the Tashkent Medical Academy there were 96 patients with osteoanthropic form of diabetic foot syndrome.

Results. Standard treatment without the use of immunomodulators reduces the severity of immune disorders, but the normalization of the studied indicators does not occur (with the exception of the level of immunoglobulin A). The inclusion of glutoxime in the treatment regimen leads to a preferential correction of the level of interleukin-1ß, interleukin-10 and oxygen-dependent bactericidal activity of neutrophils.

Conclusion. When using polyoxidonium, the highest degree of correction of phagocytosis and cellular adaptive immunity was noted: neutrophil stimulation index, oxygen-dependent bactericidal activity of neutrophils and CD8+ lymphocyte count.

Keywords: Diabetic osteoarthropathy, diabetic foot syndrome, immunocorrection

INTRODUCTION

Type 2 diabetes mellitus is recognized as one of the most important non-communicable diseases, the prevalence of which has acquired the character of a pandemic [1].

Late complications of diabetes mellitus, which adversely affect the ability of patients to physically active life, and in some cases lead to disability, include diabetic foot syndrome, which develops in 70% of patients. Purulent-destructive lesions of the bones of the foot develop in 4-10% of patients with diabetes and pose

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an immediate threat to amputation of part of the foot or lower limb, which leads to a sharp decrease in the quality of life and an increase in mortality of patients.

Treatment of diabetic foot syndrome is one of the urgent problems of surgery. Against the background of the introduction of new methods of surgical treatment and programs of combined antibiotic therapy, the percentage of disability of patients remains high and ranges from 8 to 13% [2].

Recurrences of osteoarthropathies in diabetic foot syndrome reach 20-30%, which leads to a high frequency of secondary amputations in 7.5-12.1% and functional inferiority of the limb in 10.3%-57% of cases [3].

It has been established that immune mechanisms play an important role in purulent complications of diabetic foot syndrome, participating in the formation of a patient's state of secondary immunodeficiency [4].

In conditions of immunodeficiency, treatment is not always radical, which causes repeated surgical interventions for osteomyelitis of the bones of the foot. The surgical intervention itself without appropriate immunocorrection often carries the risk of dissemination of the local infectious process [5].

The persistence of the pathogen, necrotic tissues in the area of inflammation and the resulting intoxication in osteomyelitis of the bones of the foot not only increases the load on the immune system but also seriously damages it. The proportions in the subpopulation composition of T- and B-lymphocytes are disturbed, and the activity of macrophages and neutrophils decreases [6].

The development of disorders is facilitated by both the immunogenicity of infectious agents and their toxins and morphological changes in tissues in the area of inflammation, which at a certain stage of the disease acquire the properties of autoantigens [7]. In this regard, immunocorrective and detoxification therapy becomes one of the most promising and pathogenetically substantiated directions in the complex treatment of such patients [8].

MATERIAL AND METHODS

Under our supervision at the clinical base of the Tashkent Medical Academy, there were 96 patients with osteoanthropic form of diabetic foot syndrome, aged 30 to 59 years. The control group consisted of 20 clinically healthy blood donors of the same age.

The list of general clinical research methods included a complete blood test, a study of the glycemic profile, and the level of glycated haemoglobin. All patients underwent bacteriological examination of wound exudate with the identification of microflora and assessment of its sensitivity to antibiotics.

Before surgical and conservative therapeutic measures, 10 ml of venous blood was taken from patients to study the immune status and wound discharge for bacteriological examination. The severity of the patient's condition was assessed on the APACHE II scale.

Group 1 patients (n=24) received standard treatment, which included mandatory transfer to insulin therapy with dosage adjustment depending on the glycemic profile, antibiotic therapy taking into account the sensitivity of the pathogen, anticoagulant, detoxification therapy, local treatment (dressings), surgical intervention (if indicated).

Patients of group 2 (n=24) in addition to standard treatment received polyoxidonium at a dose of 6 mg intramuscularly once a day daily, a total course of 10 injections.

Group 3 patients (n=24) received glutoxime at a dose of 20 mg intramuscularly once daily for 10 days in addition to standard treatment.

Group 4 patients (n=24) in addition to standard treatment received polyoxidonium at a dose of 6 mg and glutoxime at a dose of 20 mg intramuscularly once a day daily for 10 days.

Patients of each group were divided into 2 subgroups (A and B), depending on the form of osteoarthropathy, the presence of complications, and differences in the tactics of patient management. Subgroup A included 12 patients with fistulous form of osteomyelitis of the foot, and subgroup B of each group included 12 patients with phlegmon of the foot.

After the end of treatment, 10 ml of venous blood was re-taken from each patient in order to assess changes in the immune status. Patients were recruited into groups until statistically significant results were obtained. The differences were considered significant at a 95% probability threshold.

To assess the functional and metabolic activity of blood neutrophils, the phagocytic index (AF) was studied, i.e. the percentage of active phagocytes from the number of neutrophils counted; phagocytic number (PC) is the average number of latex particles absorbed by one phagocyte from among the counted polymorphonuclear

leukocytes. The usefulness of the phagocytic process was assessed by the completion of phagocytosis (CP) and the phagocyte activity index (PAI). The oxygen-dependent activity of bactericidal systems of neutrophils was studied in the test of reduction of nitroblue tetrazolium (NST-test). The neutrophil stimulation index was calculated as the ratio of diformazan-positive cells in the stimulated response (NST-stimulated) to diformazan-positive cells in the spontaneous nitro blue tetrazolium reduction reaction (NST-spontaneous).

The content of cytokines and immunoglobulins in the blood serum was determined by ELISA in the concentration range: for interferon-γ (IFNγ) 0-1000 pg/ml, tumor necrosis factor α (TNFα) 0-250 pg/ml, receptor antagonist interleukin 1 (RA IL-1) - 0-3000 pg/ml, interleukin 1ß (IL-1β) 0-250 pg/ml, interleukin 2 (IL-2) 0-500 pg/ml, interleukin 6 (IL-6) 0-300 pg/ml, interleukin 4 (IL-4) 0-100 pg/ml, interleukin 10 (IL-10) 0-500 pg/ml, immunoglobulin A (IgA) 0-4.2 mg/ml, immunoglobulin M (IgM) 0-3.2 mg/ml, immunoglobulin G (IgG) 0-24 mg/ml.

In order to assess the state of cellular immunity, the phenotypes of lymphocyte populations (CD3+, CD4+, CD8+, CD16+) were studied by immunofluorescence.

The degree of immune disorders and the coefficient of diagnostic value for immunological parameters were calculated. With the help of the diagnostic value coefficient, the formula of immune system disorders was determined by selecting from all the studied parameters the three leading ones that are most different from the normal level. The rating algorithm was established according to the magnitude of the degree of immune disorders, for which the studied parameters of the immune status were arranged in order of decreasing significance of differences from the specified values.

Statistical processing of the obtained data was carried out using the application packages "Excel" and "Statistica 8.0".

RESULTS

Upon admission, patients had decompensation and severe type 2 diabetes mellitus. The duration of diabetes mellitus varied in groups from 6 to 17 years, averaging 10.8±5.1 years. The duration of osteomyelitis of the foot was 6.7±1.8 years, and the number of relapses of the disease after a course of inpatient treatment was 1.8±0.7 per year.

From 28 to 36% of patients in each group were operated on earlier, they underwent autopsy and drainage of purulent foci on the foot, necrectomy, amputation of the toes. From 5 to 8% of patients in each group were operated repeatedly.

Attention is drawn to the fact that upon admission to the hospital, despite the presence of a focus of purulent inflammation, the temperature reaction was not observed in 39.8±18.5% of patients with fistulous form of osteomyelitis of the foot and in 29.2±12.7% of patients with phlegmon of the foot. In the remaining patients of subgroup A, a low-grade temperature in the range of 37.4-37.8 ° C in the evening was noted. In patients with phlegmon of the foot at the time of examination in the emergency department, the body temperature was 37.7-38.1 ° C.

At admission, patients of subgroups A in 44.9±13.2%, and in patients of subgroups B in 34.6±8.3% of cases, there was no leukocytosis and changes in the leukoformula. For the remaining cases, the leukocyte index of intoxication (LII) was calculated, which in patients with fistulous form of osteomyelitis of the foot was 3.7±0.5. In cases where osteomyelitis was combined with phlegmon or foot abscess, the LII was 4.9±0.8, which indicates a more pronounced endogenous intoxication.

X-ray examination of the foot revealed that the metatarsal bones were affected in 58.3%, the phalanges of the fingers in 62.5%, and the heel bone in 3.5% of cases. In the microbiological study of wound discharge, S. aureus was most often sown. In a significant percentage of cases, the growth of microflora was not revealed, which may indirectly indicate in favor of anaerobic microorganisms.

The study of immunity indicators showed that all patients showed a decrease in the phagocytic index and the number, completeness of phagocytosis, and the index of phagocyte activity. At the same time, the deviation from the norm of the phagocytic number and the index of phagocytosis activity corresponded to the III degree of immune disorders.

The oxygen-dependent activity of phagocytes according to the spontaneous test of nitro blue tetrazolium reduction was increased by 2 times, which corresponded to the III degree of immune disorders. An increase in the value of the NST test after stimulation with zymosan was not observed, which indicates a low functional reserve of neutrophils.

The content of lymphocytes did not differ significantly from the indicators of healthy individuals and corresponded to the first degree of immune disorders. Against the background of a decrease in the absolute and relative content of CD4+ cells (I and II

degrees of immune disorders, respectively) and CD16 + cells (I degree of immune disorders), the content of CD8+ lymphocytes was significantly higher than the values of healthy individuals (III degree of immune disorders). This led to the fact that the ratio of CD4+/ CD8+ lymphocytes was 3-3.5 times less than the control indicators (grade III immune disorders).

The study of blood serum in patients revealed a significant increase in the level of all the cytokines studied. Among the pro-inflammatory cytokines, the highest values of indicators compared to the control group were observed for IL-1β (44.1-47.5 times higher than normal) and IL-2 (37.9-45 times higher than normal). Taking into account the immune response identified in patients and the literature data on the sources of secretion of these cytokines, their hyperproduction may be due to increased activity of CD4 + cells as a compensatory reaction to a decrease in their content in the blood.

The concentration of anti-inflammatory cytokines was also increased (mainly for IL-4 - 13.5-14.6 above normal), but the degree of increase was less than that of pro-inflammatory. Moreover, the concentration of the receptor antagonist IL-1, which is a regulator of IL-1 activity, was increased by only 5-5.6 times compared to the norm.

In addition, an increase in the concentration of IL-2 (37.9-45 times) and IFNγ (31.4-32.9 times) compared to the control indicates the activity of type 1 helper T-cells. Among cytokines produced by type 2 helper T-cells, the highest concentration was observed in IL-6 (33.9-35.3 times higher than normal). The content of IL-4 was 13.5-14.6 times, and IL-10 was 9 times higher than the control. Of the 24 immunological parameters studied, 14 had grade III immune disorders and 5 had grade II. At the same time, the formula of immune system disorders before the start of treatment consisted of IL-1ß3+, IL-23+, TNFα3+.

At the end of the standard course of treatment, LII decreased in subgroup A from 3.7±0.5 and in subgroup B from 4.9±0.8 to 1.9±0.2 with normalization of the level of leukocytosis and leukoformula, there was a positive trend in the studied indicators of the phagocytic link of innate immunity, but their normalization did not occur. At the same time, there were no statistically significant differences between the indicators of subgroup A and B. There were statistically significant differences with the control group in all indicators, with the exception of CD3+ lymphocytes. A low CD4+/CD8+ ratio indicates a predominance of the suppressor effect of lymphocytes, and the absence of a significant difference between the values of the spontaneous and stimulated NST test indicates a low functional reserve of neutrophils.

Standard treatment led to a decrease in the level of both pro-inflammatory and anti-inflammatory cytokines, which, however, did not reach control values.

The imbalance of cytokines persisted, and although its severity decreased, it still corresponded to the III degree of immune disorders. The level of IgA did not change, the concentration of IgM exceeded the values of the control by 1.5 times, and the IgG index reached the values of healthy individuals.

After standard treatment, the number of indicators with II and III degrees of immune disorders remained at a high level (6 and 13, respectively). The formula of immune system disorders has not changed, which indicates a lack of effectiveness of standard pharmacotherapy.

After the course of treatment with glutoxime in both subgroups, the values of LII did not differ from those of healthy individuals (1.7±0.4 in subgroup A, 1.9±0.5 in subgroup B). The level of leukocytes in the blood decreased to control values (in subgroup A from 12.5±1.7x109/l to 6.5±0.9x109/l; in subgroup B from 14.3±1.9x109/l to 7.3±1.2x109/l), the composition of the leukocyte formula did not differ from that of healthy individuals. The studied immunity indicators did not have statistically significant differences in subgroups A and B. Phagocytic indicator and number, The completion of phagocytosis, the index of phagocyte activity, the index of neutrophil stimulation did not reach the control values. It was possible to achieve an effective correction of only the indicators of the NST test, which at the end of treatment did not statistically differ from the control. There were also differences in the cellular composition of lymphocytes with a predominance of CD8+ cells, as a result of which the CD4+/CD8+ index did not reach the indicators of healthy individuals. At the end of treatment, the level of CD16+ lymphocytes increased and did not statistically differ from the control values.

The treatment led to a significant decrease in the level of both pro-inflammatory and anti-inflammatory cytokines, which, however, did not reach the control values.

The level of IgA, IgM and IgG increased and reached normal values. Among pro-inflammatory cytokines, the highest values of indicators compared to the control group were maintained for IL-2 (4.9-4.1 times higher than normal in subgroups A and B, respectively) and

TNFα (3 and 3.4 times higher than normal in subgroups A and B, respectively). The concentration of anti-inflammatory cytokines also remained elevated (IL-4 was 3.9 and 4.1 times higher than normal in subgroups A and B, respectively).

When glutoxim was included in the treatment regimen, the number of indicators with grade III immune disorders decreased from 14 to 8, the number of indicators with grade II immune disorders increased from 5 to 6, and the formula of immune system disorders consisted of IL-23+, IL-43+, TNFα3+. The main targets of immunocorrection were IL-1ß, IL-10 and oxygen-dependent bactericidal activity of neutrophils (spontaneous NST test).

During treatment with polyoxidonium, LII decreased from 3.5±0.4 (subgroup A) and 4.8±0.6 (subgroup B) to 1.6±0.4 and 1.8±0.5, respectively. The level of leukocytes decreased to normal values (in subgroup A from 12.1±1.5x109/l to 7.2±0.8x109/l; in subgroup B from 15.2±1.9x109/l to 6.9±1.1x109/l), and the composition of the leukoformula did not statistically differ from that of healthy individuals.

At the end of treatment, immunity indicators in subgroups A and B had no statistically significant differences. When polyoxidonium was included in the treatment regimen, effective correction of the values of the NST test, phagocytic index and neutrophil stimulation index was noted. The phagocytic number increased by 2.4 times, the index of phagocyte activity by 2.9 times, but did not reach normal values. The level of CD4+ and CD16+ cells returned to normal, and the level of CD8+ cells decreased by 1.3 times, but did not reach normal values, so the CD4+/CD8+ index, although it increased by 2.5 times, continued to be statistically different from the control.

The treatment led to a significant decrease in the level of both pro-inflammatory and anti-inflammatory cytokines, which, however, did not reach the control values. The imbalance between the ratio of IL-1RA and IL-1β was preserved. The level of IgA did not change, and the content of other immunoglobulins reached the control indicators.

After a course of treatment with the use of polyoxidonium, the number of indicators with grade III of immune disorders decreased from 14 to 8, while there was also a decrease in the number of indicators with grade II of immune disorders from 5 to 4, and the formula of immune system disorders consisted of IL-43+, IL-1ß3+, TNFα3+. The target for immunocorrection was the indicators of phagocytosis and cellular immunity: ISN, oxygen-dependent bactericidal activity of neutrophils (spontaneous NST test) and the number of CD8+ lymphocytes.

With combination therapy with polyoxidonium and glutoxime, the studied indicators of phagocytosis and cellular immunity at the end of treatment did not have statistically significant differences from the values of the control group. The therapy led to a significant decrease and normalization of the level of both pro-inflammatory and anti-inflammatory cytokines, with the exception of IL-2, which exceeded the control values by 2 times.

DISCUSSION

The ratio of IL-1RA and IL-1β reached the value of the control group. The level of IgA, IgM and IgG increased and reached the indicators of healthy individuals. With combined treatment with the use of polyoxidonium and glutoxim, the number of indicators with grade III immune disorders, as well as with grade II, decreased to 1 [9].

The formula for immune system disorders included only IL-23+. Effective correction of both innate and adaptive immunity was noted, but the main targets were IL-1ß, TNFα and IL-2 [10].

When comparing the number of altered indicators of the immune status in this disease against the background of different pharmacotherapy regimens, it was found that the scheme of combined use of polyoxidonium and glutoxime drugs has a greater positive effect than their separate use, since it made it possible to reduce the number of remaining uncorrected laboratory parameters from 71% to 4%, compared with the indicators after the use of polyoxidonium - from 71% to 33% and after the use of glutoxime - from 71% to 41% [11].

When assessing the clinical efficacy of various immunomodulatory therapy regimens in the study groups in terms of the time of normalization of body temperature, the number of leukocytes, as well as the timing of fistula closure against the background of conservative treatment and healing of postoperative wounds, and the time of hospital stay, there were no statistically significant differences [12].

When studying the long-term results of treatment within 1 year after discharge from the hospital, exacerbation of osteomyelitis of the foot was observed significantly more often in patients of the group with standard treatment, and the least number of relapses was observed in patients of the group with the combined use of polyoxidonium and glutoxime - 2 times less often compared with the separate use of polyoxidonium and

glutoxime and 3 times less often, than in treatment without immunomodulators [13].

CONCLUSION

The development of osteomyelitis of the bones of the foot in patients with diabetic foot syndrome is accompanied by the formation of secondary immunodeficiency, which is manifested in low bactericidal activity and depletion of the functional reserve of neutrophils, an imbalance in the population composition of lymphocytes with a predominance of CD8+ lymphocytes, an increase in the level of pro- and anti-inflammatory cytokines with a violation of the ratio of interleukin-1β and the receptor antagonist interleukin-1, a decrease in immunoglobulin A. immunomodulatory therapy with polyoxidonium and glutoxime, all the studied parameters reach the normal level with the exception of interleukin-2, the content of which decreased by 19.3 times compared to the initial value.

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Consent for publication - The study is valid, and recognition by the organisation is not required. The authors agree to open the publication.

Availability of data and material - Available

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