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Pathophysiological Changes in Purulent-Necrotic Foot Lesions in Diabetes Mellitus and Optimization of Complex Treatment: An Experimental Study

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Abstract: Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia. In 2019, 463 million people worldwide were diagnosed with diabetes. According to forecasts, the number of people with DM is expected to reach 578 million by 2030 and 700 million by 2045. The increasing prevalence of diabetes has led to a rise in diabetic wound cases, significantly escalating the medical costs associated with wound treatment globally.

Key points: alloxan, purulent-necrotic foot model, amputation, X-ray endovascular procedures.

Introduction

To date, more than 537 million people worldwide are battling diabetes mellitus (DM), meaning that 1 in 10 individuals is affected [10]. This number is expected to rise to 643 million by 2030 and 783 million by 2045 [11]. Approximately 3 out of 4 people with diabetes live in low- and middle-income countries [10]. Experts also note that in developed countries, the number of diabetes cases doubles every 15 years, and this growth trend has not yet been halted [8].

In 2021, diabetes was responsible for 6.7 million deaths, equating to one death every five seconds. Alarmingly, many of these individuals were unaware that they had diabetes [9]. Diabetes can progress silently for years without showing any symptoms. Due to the severity of this issue, the World Health Organization (WHO) has declared diabetes as the epidemic of the 21st century [10].

In Uzbekistan, according to statistics from the Republican Specialized Scientific and Practical Medical Center of Endocrinology, more than 277,000 people are battling diabetes and its complications, including over 3,000 children [2].

The purulent-necrotic form of diabetes significantly increases the cost of treatment, and as the number of patients suffering from it continues to grow, the expenses for their treatment also rise [3]. Purulent-necrotic foot injuries due to diabetes can result from direct infection or spread through the bloodstream (hematogenous dissemination) [4]. The purulent-necrotic process is primarily caused by infection, with the most significant pathogens being Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli [5].

According to data from the Russian Federation, the cost of treating patients with the purulent-necrotic complications of diabetes amounts to 3.62 million rubles per patient. Half of this amount is allocated to primary medical care, while the remaining portion is spent on primary and repeated amputations as well as prosthetics [1].

Additionally, the average duration of inpatient treatment for wound care, minor amputation, and major amputation is 13.3, 20.5, and 59.6 days, respectively. The average annual cost per patient is $3,368 (for wound care only), $10,468 (for minor amputation), and $30,131 (for major amputation)

[10]. The long duration of treatment, high disability and mortality rates, prolonged hospital stays, and significant medical expenses not only severely impact the quality of life and physical and mental health of patients but also lead to substantial economic losses for society [12].

Currently, Russian researchers have identified several contributing factors to the increase in soft tissue surgical infections, including poor living conditions, chronic immune deficiencies, an aging population, and a worsening environmental situation [1]. In the Russian Federation, over 5 million cases of purulent-necrotic foot wounds unrelated to diabetes have been recorded [1]. In the United States and Western Europe, up to 10% of hospitalized patients suffer from purulent-necrotic wounds unrelated to diabetes, resulting in annual economic losses of $9–10 billion for the state [10].

The primary reason patients seek medical assistance is soft tissue surgical infections [15]. In five European Union countries—France, Germany, Italy, Spain, and the United Kingdom—1.3 million patients hospitalized with soft tissue surgical infections in 2004 had purulent-necrotic foot wounds as a complication of diabetes [8, 9]. According to expert estimates, approximately 700,000 patients with this pathology are recorded annually in the Russian Federation [1].

The purulent-necrotic form of diabetes is one of the most widespread and severe complications, affecting 15% of all diabetic patients. This condition leads to over 80,000 foot amputations in the United States every year [10]. Neuropathy, peripheral artery disease of the lower limbs, and infections are recognized as risk factors for the development of purulent-necrotic diabetic complications [9].

Research Objective

To improve pathogenetic approaches to the treatment of purulent-necrotic foot wounds in diabetic patients based on clinical-experimental studies.

A. *Materials and Methods*

Healthy rats were selected for the experiment. The study was conducted on 195 male white rats weighing 150-200 g, housed in the vivarium of TMA. The rats were kept in optimal conditions, with a 24-hour light cycle, a constant temperature of 23-27°C, and free access to water. All animals were provided with an ad libitum diet, meeting the nutritional standards for rodents (GOST R50258-92), along with daily drinking water.

All procedures and manipulations with the animals were carried out under general anesthesia in accordance with the European Community guidelines (86/609/EEC) and the principles of the Helsinki Declaration, following the "Rules for Working with Experimental Animals."

The experimental animals were divided into four groups:

Intact group – unaltered control group.

Control group – creation of an experimental model of purulent-necrotic foot wounds on the background of alloxan-induced diabetes.

Comparison group – traditional complex treatment (ozone therapy + Reosorbilact) for diabetic purulent-necrotic foot wounds.

Main group – complex treatment of alloxan-induced diabetic purulent-necrotic foot wounds using ozone therapy and Reomannisol.

After a 24-hour fasting period, the rats were weighed, and a 2% alloxan solution diluted in 0.9% physiological saline was administered intraperitoneally at a dose of 12.0 mg per 100 g of animal weight. No lethal cases were observed at this dose. A total of 165 rats continued in the study. Food and water were provided 30 minutes after drug administration. Blood glucose levels were assessed over three days

Result

In the control group, the concentration of Pseudomonas aeruginosa on day 1 of the experiment was 3.3±0.85×10⁶ CFU/mL. By day 3, it increased by 1.06 times ($p<0.001$) compared to day 1. On day 7, it decreased by 1.22 times ($p<0.001$), followed by reductions of 1.14 times ($p<0.001$) on days 10 and 14, and 1.65 times ($p<0.001$) on day 14. By day 21, the bacterial count had decreased by 2.2 times ($p<0.001$).

For Escherichia coli, the concentration on day 1 was 4.3±0.96×10⁶ CFU/mL. By day 3, it increased by 1.11 times ($p<0.001$). On day 7, it decreased by 1.07 times ($p<0.001$), followed by reductions of 1.23 times ($p<0.001$) on day 10 and 1.43 times ($p<0.001$) on day 14. By day 21, the bacterial count had decreased by 1.79 times ($p<0.001$) (Table I).

Dynamics of Bacterial Reduction in the Control Group

Control group	bacterial culture	
	Pseudomonas aeruginosa	***Escherichia coli***
Colony-Forming Units (CFU/ml)		
day 1	$3.3 \pm 0.85 \times 10^6$	$4.3 \pm 0.96 \times 10^6$
day 3	$3.5 \pm 0.72 \times 10^5$	$4.8 \pm 0.8 \times 10^4$
day 7	$2.7 \pm 0.65 \times 10^4$	$4.0 \pm 0.75 \times 10^3$
day 10	$2.9 \pm 0.5 \times 10^4$	$3.5 \pm 0.52 \times 10^3$
day 14	$2.0 \pm 0.44 \times 10^4$	$3.0 \pm 0.32 \times 10^2$
day 21	$1.5 \pm 0.2 \times 10^2$	$2.4 \pm 0.25 \times 10^2$

The data show a progressive decrease in bacterial count over time. For Pseudomonas aeruginosa, a significant reduction was observed from the first day of treatment, with a 1.27-fold decrease ($p<0.001$) compared to the pre-treatment period. By the 3rd day, the count further decreased by 1.13 times ($p<0.001$) relative to the 1st day, reaching a 3.5-fold reduction ($p<0.001$) by the 14th day.

Similarly, for Escherichia coli, a 1.11-fold reduction ($p<0.001$) was recorded on the 1st day, with a continuous decline reaching a 3.63-fold decrease ($p<0.001$) by the 14th day. By the 21st day, no bacterial strains were detected, indicating the efficacy of the applied treatment approach. Table II.

In the main group, Pseudomonas aeruginosa decreased by 1.57 times ($p<0.001$) on day 1, by 1.86 times ($p<0.001$) on day 3 compared to day 1, and by 3.41 times ($p<0.001$) on day 7. Escherichia coli showed a reduction of 1.34 times ($p<0.001$) on day 1 compared to the initial day, 2.78 times ($p<0.001$) on day 3, and 4.87 times ($p<0.001$) on day 7. Due to the local application of ozonized physiological saline and the systemic reduction of intoxication with Reomannisol, no bacteria were detected on days 10, 14, and 21.

Dynamics of Bacterial Reduction in the Comparison Group

comparison group	bacterial culture	
	Pseudomonas aeruginosa	***Escherichia coli***
Colony-Forming Units (CFU/ml)		
before treatment	$4.2 \pm 0.9 \times 10^6$	$4.0 \pm 1.6 \times 10^5$
day 1	$3.3 \pm 0.8 \times 10^6$	$3.6 \pm 1.2 \times 10^4$
day 3	$2.9 \pm 0.77 \times 10^5$	$2.5 \pm 0.84 \times 10^3$
day 7	$2.3 \pm 1.3 \times 10^4$	$2.1 \pm 0.47 \times 10^3$
day 10	$2.0 \pm 0.36 \times 10^2$	$1.5 \pm 0.33 \times 10^2$
day 14	$1.2 \pm 0.23 \times 10^1$	$1.1 \pm 0.24 \times 10^1$
day 21	-	-

Dynamics of Bacterial Reduction in the Main Group

main group	bacterial culture	
	Pseudomonas aeruginosa	***Escherichia coli***
Colony-Forming Units (CFU/ml)		
before treatment	$4.1 \pm 1.2 \times 10^6$	$3.9 \pm 0.92 \times 10^5$
day 1	$2.6 \pm 0.91 \times 10^5$	$2.9 \pm 0.64 \times 10^4$
day 3	$2.2 \pm 0.67 \times 10^4$	$1.4 \pm 0.45 \times 10^2$
day 7	$1{,}2 \pm 0{,}37 \times 10^2$	$0.8 \pm 0.11 \times 10^2$
day 10	-	-
day 14	-	-
day 21	-	-

We also studied the activity of cytokines responsible for inflammation and wound healing in blood plasma. After monocytes migrate to the site of inflammation and differentiate into tissue macrophages, they synthesize and release cytokines. These cytokines consist of a combination of pro-inflammatory and anti-inflammatory cytokines (Table 3.2.4).

In the control group, analysis of IL-1β, IL-2, and IL-10 levels on day 1 revealed a 1.74-fold decrease ($p<0.001$), a 1.43-fold decrease ($p<0.001$), and a 5.65-fold increase ($p<0.001$), respectively, compared to the baseline group. By day 3, we observed further reductions of 1.46-fold ($p<0.001$), 1.37-fold ($p<0.001$), and 1.35-fold ($p<0.001$). On day 7, IL-1β exhibited a 1.21-fold ($p<0.001$) increase, indicating a heightened inflammatory response, suggesting that the process was advancing towards a purulent-necrotic phase. Meanwhile, IL-2 decreased by 1.34-fold ($p<0.001$), while IL-10 remained almost unchanged.

On days 10 and 14, IL-1β levels reached critical values, increasing by 1.49-fold ($p<0.001$) and 1.68-fold ($p<0.001$), respectively. IL-2 continued its downward trend, decreasing by 1.39-fold ($p<0.001$) and 1.51-fold ($p<0.001$). IL-10, however, exhibited a compensatory peak, rising by 1.83-fold ($p<0.001$), correlating with IL-1β levels. By day 21, IL-1β and IL-10 returned to normal values, while IL-2 remained elevated at 0.363±0.00 pg/mL.

Dynamics of Changes in Systemic Inflammatory Response Syndrome Indicators Over Time

Days	Table Column Head		
	Interleukin-1β, pg/ml	***Interleukin-2, pg/ml***	***Interleukin-10, pg/ml***
	0,122±0,00	0,344±0,01	0,122±0,00
control group			
1	$0{,}07 \pm 0{,}00^{aaa}$	$0{,}240 \pm 0{,}01^{aaa}$	$0{,}69 \pm 0{,}00^{aaa}$
3	$0{,}083 \pm 0{,}00^{aaa}$	$0{,}25 \pm 0{,}01^{aaa}$	$0{,}09 \pm 0{,}00^{aaa}$
7	$0{,}148 \pm 0{,}00^{aaa}$	$0{,}256 \pm 0{,}01^{aaa}$	$0{,}117 \pm 0{,}00^{aaa}$
10	$0{,}182 \pm 0{,}00^{aaa}$	$0{,}247 \pm 0{,}00^{aaa}$	$0{,}19 \pm 0{,}00^{aaa}$
14	$0{,}206 \pm 0{,}00^{aaa}$	$0{,}227 \pm 0{,}00^{aaa}$	$0{,}224 \pm 0{,}00^{aaa}$
21	$0{,}128 \pm 0{,}00^{aaa}$	$0{,}363 \pm 0{,}00^{aaa}$	$0{,}127 \pm 0{,}00^{aaa}$
Comparison group			
1	$0{,}072 \pm 0{,}00^{aaa}$	$0{,}217 \pm 0{,}01^{aaa}$	$0{,}067 \pm 0{,}00^{aaa}$
3	$0{,}103 \pm 0{,}00^{aaaбб}$	$0{,}294 \pm 0{,}00^{aaaбб}$	$0{,}182 \pm 0{,}00^{aaa}$
7	$0{,}154 \pm 0{,}00^{aaaббб}$	$0{,}402 \pm 0{,}00^{aaaббб}$	$0{,}156 \pm 0{,}00^{aaaббб}$
10	$0{,}148 \pm 0{,}00^{aaaббб}$	$0{,}390 \pm 0{,}01^{aaaббб}$	$0{,}139 \pm 0{,}00^{aaaббб}$
14	$0{,}136 \pm 0{,}00^{ббб}$	$0{,}353 \pm 0{,}01^{aaaббб}$	$0{,}123 \pm 0{,}00^{aaaббб}$
21	$0{,}072 \pm 0{,}00^{aaa}$	$0{,}217 \pm 0{,}01^{aaa}$	$0{,}067 \pm 0{,}00^{aaa}$
Main group			
1	$0{,}072 \pm 0{,}00^{aaa}$	$0{,}223 \pm 0{,}01^{aaa}$	$0{,}070 \pm 0{,}00^{aaa}$
3	$0{,}135 \pm 0{,}00^{aaaббб}$	$0{,}342 \pm 0{,}00^{aaaбббccc}$	$0{,}174 \pm 0{,}00^{aaaбббccc}$
7	$0{,}146 \pm 0{,}00^{aaaббб}$	$0{,}382 \pm 0{,}01^{aaaбббccc}$	$0{,}165 \pm 0{,}00^{aaaбббccc}$

Days	Table Column Head		
	Interleukin-1β, pg/ml	***Interleukin-2, pg/ml***	***Interleukin-10, pg/ml***
10	$0,124\pm0,00^{\text{бббссс}}$	$0,345\pm0,00^{\text{аббссс}}$	$0,123\pm0,00^{\text{аабббссс}}$
14	$0,072\pm0,00^{\text{ааа}}$	$0,223\pm0,01^{\text{ааа}}$	$0,070\pm0,00^{\text{ааа}}$
21	$0,135\pm0,00^{\text{аааббб}}$	$0,342\pm0,00^{\text{аааббссс}}$	$0.174\pm0.00^{\text{аааббссс}}$

Note: a - Statistically significant compared to the unchanged group (a - $p<0.05$; a a - $p<0.01$; a a a - $p<0.001$). b - Statistically significant compared to the control group (b - $p<0.05$; b b - $p<0.01$; b b b - $p<0.001$). c - Statistically significant compared to the comparison group (c - $p<0.05$; c c - $p<0.01$; c c c - $p<0.001$).

IL-1β is a key cytokine that appears in plasma upon the initial exposure to pathogenic bacteria or in autoimmune conditions such as type 2 diabetes mellitus. During disease progression, IL-1β levels decrease but exhibit a secondary surge during the healing phase, which can be interpreted as a residual immune response. IL-10 acts as an antagonist to pro-inflammatory cytokines, modulating the immune response by suppressing inflammation and playing a crucial role in wound healing. Throughout the disease course, IL-10 levels mirrored those of IL-1β, increasing during inflammation and normalizing as the wound healed.

In the comparative group, IL-1β, IL-2, and IL-10 demonstrated an initial upward trend on day 1 compared to the control group, except for IL-10, which showed a significant decline of 10.29-fold ($p<0.01$). This suggests that ozonated physiological saline and Reosorbilact possess immunomodulatory properties, likely exerting their effects by interacting with cytokine receptors to suppress inflammation in the early phase. By day 3, IL-1β increased by 1.24-fold ($p<0.001$), which we attribute to the stimulatory effect of these treatments on immune cell activity. IL-2 increased by 1.17-fold ($p<0.001$), while IL-10 levels also rose, reaching a 2.02-fold ($p<0.001$) increase.

On day 7, all cytokines exhibited critical elevations compared to the control group: IL-1β by 1.04-fold ($p<0.001$), IL-2 by 1.56-fold ($p<0.001$), and IL-10 by 1.33-fold ($p<0.001$). By day 10, cytokine levels (except IL-2) began to decline, indicating the therapeutic efficacy of the treatment regimen. IL-1β and IL-10 decreased by 1.23-fold ($p<0.001$) and 1.36-fold ($p<0.001$), respectively, while IL-2 remained elevated at 1.58-fold ($p<0.001$). By day 14, cytokine levels aligned with those of the baseline group, except for IL-2, which remained elevated at 0.353±0.00 pg/mL. This suggests a sustained heightened immune response against bacterial toxins and other infectious agents.

In the main experimental group, IL-1β levels remained unchanged on day 1 compared to the comparative group, while IL-2 showed a slight increase of 1.02-fold ($p<0.001$), and IL-10 exhibited no significant changes. This suggests that Reomannisol possesses potent immunomodulatory properties. By day 3, IL-1β increased by 1.31-fold ($p<0.001$), while IL-2 rose by 1.16-fold ($p<0.001$). In contrast, IL-10 levels declined slightly by 1.046-fold ($p<0.001$), likely due to the wound transitioning into the healing phase.

By day 7, IL-1β reached its highest critical point at 0.146±0.00 pg/mL, while IL-2 and IL-10 levels decreased relative to the comparative group, measuring 0.382±0.01 pg/mL and 0.165±0.00 pg/mL, respectively. These findings indicate that Reomannisol exhibits stronger immunomodulatory effects than Reosorbilact and significantly contributes to wound healing.

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