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XRONİK XORALARIN VAKUUM-TERAPİYA ÜSULU İLƏ MÜALİCƏSİNİN KLİNİK-İMMUNOLOJİ ASPEKTLƏRİ

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Məqalədə aşağı ətrafların xronik xoralarının müalicəsindən alınan effektivliyin vakuum-terapiya (VAK-terapiya), cərrahi müalicə və hialuron turşusu preparatlarını özündə birləşdirən kompleks müalicə vasitəsilə artırılması məqsədilə aparılmış tədqiqat işi haqqında məlumat verilmişdir.

Aşağı ətraflarında xronik xoralar olan 52 xəstə tədqiq edilib. Xəstələrin əksəriyyəti (48 nəfər) qadın, yaşı 45-lə 75 arasında idi. Müalicənin effektivliyi xoraların klinik sağalma əlamətlərinə, mikrobioloji tədqiqatın nəticələrinə və immunoloji göstəricilərinə görə qiymətləndirilmişdir. Bütün xəstələrdə yaraların tam sağalmasına nail olunmuşdur. Xəstələrdən 67%-də köçürülmüş autodermotransplantat həyat qabiliyyətini davam etdirmişdir. 28% xəstədə ilkin transplantat hissəvi lizisə uğradığına görə, təkrar operasiyaya ehtiyac yaranmışdır. 3% xəstədə isə yara operativ müdaxilə olmadan sağalmışdır.

Beləliklə, xronik xoraların müalicəsinə müasir kompleks yanaşma problemi radikal şəkildə həll etməyə imkan verir. Buna T-helper hüceyrələrinin adekvat artımı fonunda immunitetin B-hüceyrə bölümünün azalması, T- və B-hüceyrə bölümləri arasında müvazinət halının bərpası ilə birgə dövr edən immun komplekslərin səviyyəsinin normallaşması sübut edir.

Açar sözlər: *xronik yara, VAC-terapiya, cərrahi müalicə, immun system*

Ключевые слова: *хроническая рана, VAC-терапия, хирургическое лечение, раневой процесс, иммунная система*

Key words: *chronic wound, VAC-therapy, surgical treatment, wound healing process, immune system*

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CLINICAL AND IMMUNOLOGICAL ASPECTS OF THE TREATMENT OF CHRONIC WOUNDS USING VAC-THERAPY

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Summary. *The article presents the results of a study conducted to improve treatment effectivenes of patients with chronic wounds of the lower extremities by using of complex treatment, which included VAC (Vacuum Assisted Closure) therapy, surgical treatment and hyaluronic acid preparations. The 52 patients with chronic wounds of the lower extremities were examined. Mostly they are women (48) aged 45-75 years. Evaluation of the treatment effectiveness was based on the study of clinical indicators of wound healing, microbiological studies and immunological data. Complete wound healing was achieved in all patients. In 67% of patients, the transplanted audoderm graft engrafted completely. In 28%, partial lysis was observed, which required repeated operations. In 3% of the wounds healed without surgery.*

A modern integrated approach can radically solve the problems of chronic wound healing, as evidenced

Introduction. Due to the increase in the number of patients with chronic diseases such as diabetes, cardiovascular and cerebrovascular diseases, cancer as well as local vascular diseases, infections and injuries [1,2,3], an increasing numerous of chronic wounds are observed. A chronic wound is commonly referred to as tissue defects with signs of a prolonged inflammatory process without a tendency to improve, provided adequate treatment for 4-6 weeks. Worldwide, there are more than 6.5 million patients with chronic wounds, and the total medical costs for their treatment exceed 25 billion US dollars per year [4]. According to etiology, there are categories of patients with the highest incidence of chronic wounds – these are diabetic foot ulcers, venous ulcers of the lower extremities and bedsores [5,6]. The high prevalence, high frequency of amputations and relapses of chronic wounds imposes a serious economic and social burden on the healthcare system [5,6,7]. However, innovations in clinical treatment and wound care are not enough to date. One of the new promising methods for the wounds treatment of various etiologies is the method of vacuum influence. Treatment of wounds using negative pressure (vacuum therapy, negative pressure wound therapy – NPWT, vacuum assisted closure – VAC) is one of the types of local treatment that is used to improve the course of the wound healing process [8, 9]. In recent years, significant progress has been made in the study and use of VAC therapy, however, the mechanisms of the influence of this method on various parts of the course of the wound process require further research.

The purpose of the study: to improve the results of treatment of patients with chronic wounds of the lower extremities by using complex treatment, including VAC-therapy, surgical treatment, hyaluronic acid preparations.

Materials and methods. In the period from 2018 to 2023 52 patients with chronic wounds of the lower extremities were treated and examined in the clinic of the department. Mostly they are women (48) aged 45 to 75 years. The area of the wounds ranged from 5 to 30 cm in diameter. Among the causes, 23 (44.3%) were complications of chronic venous insufficiency, in 13 (25%) the cause of wounds was mechanical trauma, in 8 (15.4%) animal and insect bites; in 5 (9.6%) – the consequences of acute purulent diseases and in 3 (5.7%) bedsores. Evaluation of the effectiveness of treatment was carried out on the basis of the study of clinical indicators of wound healing, microbiological studies and immunological data.

Microbiological studies were carried out after the treatment of the wound with a solution of "Betadine", excision of the area of the wound surface was carried out. The biopsy was ground in a mortar with sterile saline at the rate of 1:10. Tenfold dilutions were prepared and seeding was carried out according to the Gouldy method on plates with blood nutrient agar, followed by quantitative accounting of microbial contamination [10]. The identification of microorganisms was carried out using MALDI-TOF spectrometry (Bruker, Germany).

We studied various components of the immune system, in particular, its cellular immunity (total number of T- and B-lymphocytes, subpopulations of T-lymphocytes: CD3, CD19, CD4, CD8, CD16) by flow cytometry, humoral immunity - changes in the content of immunoglobulin classes: IgA , IgM, IgG in blood serum was determined by the method of radial immunodiffusion according to Mancini, (1965).

Mononuclear cells were isolated from patients' peripheral venous blood in a density gradient of 1.077 g/cm. The functional activity of granulocytes was assessed in the NBT-test by the reduction reaction of nitroblue tetrazolium. Circulating immune complexes (CIC) were determined by the method of V. Haskova [11]. Patients were examined upon admission to the hospital and on day 21 after autodermoplasty. The C-300 VAC system was used to treat wounds with negative pressure. During the first installation of the system, the device was adjusted to a constant aspiration mode with a negative pressure in the wound cavity of 80-110 mmHg vacuum dressings were used (hydrophilic polyurethane sponges with a pore size of 400–2000 μm with a transparent adhesive coating, connected by a drainage tube to a vacuum source apparatus). The first period of operation of the system lasted from 24 to 48 hours, the second - up to 3 days, the subsequent ones - up to 5 days on average. The system was remounted for the purpose of wound revision and during surgical treatment in the operating room.

Statistical data processing was carried out by determining the arithmetic mean (M) and its error (m). The significance of the difference between the mean values was determined using Student's t-test. Statistical data processing was carried out using a personal computer using STATISTICA 6.1 software (StatSoftInc., serial AGAR909E415822FA) and Microsoft Excel (Microsoft Office 2016 Professional Plus, Open License 67528927) using methods of descriptive and analytical biostatistics and multivariate methods of statistical analysis.

Results and its discussion. Comprehensive treatment began with VAC therapy, both to prepare wounds for surgery, and to reduce tissue edema, stimulate blood flow and cleanse

necrotic tissues. VAC therapy was also used after autodermoplasty. Introoperatively, the injection material for skin regeneration Lacerta 1.5%-1 ml was injected subcutaneously into the edges of the wound. After the end of the course of treatment with negative pressure in the postoperative period, dressings with ointments with hyaluronic acid were used: Cicatridina, Ialuset for 2-3 weeks. The treatment was carried out under the condition of adequate unloading of the limbs.

The use of VAC-therapy contributed to the rapid cleansing of wounds, reducing their area and depth, accelerated the formation of granulations and epithelization of the edges, and reduced the cost of wound care products. The duration of treatment of patients in the hospital averaged (38 ± 3) days. Complete wound healing was achieved in all patients. In 67% of patients, the transplanted audoderm graft engrafted completely. In 28%, partial lysis was observed, which required repeated operations. In 3% of the wounds healed without surgery. In 2%, a pronounced pain syndrome was observed during exposure to negative pressure, which forced them to abandon VAC-therapy and perform plastic surgery using a perforating propeller flap. Relapses were observed in 2 patients after 1 year and in 1 - 2 years after treatment.

Before starting antimicrobial therapy and during the course of antibiotic treatment, microbiological control was carried out. As a result of the study, 47 strains of pathogens were isolated. The main representatives among the isolated microorganisms were Staphylococcus aureus (15 strains), coagulase-negative staphylococci Staphylococcus haemolyticus (7 strains). Enterobacteria were found among Enterobacter cloaceae (6 strains) and Escherichia coli (7 strains); Enterococcus faecalis (6 strains), Proteus mirabilis (1 strain) and Acinetobacter baumanii (1 strain). The contamination of the wound in the examined patients at the time of the initial examination averaged $2.6\pm0.03x10^4$ CFU/ml. Purposeful antibacterial therapy was carried out against the background of VAC-therapy and complex surgical treatment. The course of treatment was carried out in accordance with the results of bacteriological cultures and the choice of drugs to which the greatest sensitivity was revealed. The VAC-therapy we used prevented further infection of the wound. Considering that bacterial infection is associated with the immunodeficiency state of the macroorganism, we studied some indicators of the immune status of the examined patients.

Studies have shown that all patients before treatment were found to have profound disturbances in the T- and B-cell links of immunity.

Analysis of the results of studies of T-cell and humoral immunity in patients with chronic wounds of the lower extremities is shown in Table 1.

Table 1. **State of cellular and humoral immunity in examined patients before and after treatment**

Indicators	Control values (n=25)	Before treatment (n=52)	After treatment for 21 days (n=52)
Leukocytes	5,35±0,21	7,81±0,79*	4,6±0,74
Lymphocytes % a.n.	28,71±0,81 1,61±0,07	20,12±1,67* 1,35±0,2	31,67±2,63 1,41±0,18
T- lymphocytes % CDZ+ a.n. $x10^9$ cel/l	50,88±0,68 0,76±0,04	31,23±2,74* 0,46±0,16*	51,33±2,64** 0,7±0,06
T- helpers % CD4+ a.n. $x10^9$ cel/l	38,71±0,52 0,53±0,03	21,31±1,69* 0,25±0,07*	32,5±2,38 ** 0,44±0,03
T- suppressors % CD8+ a.n. $x10^9$ cel/l	18,39±0,57 0,30±0,02	25,67±2,0 0,36±0,05	19,17±4,04 0,28±0,04
B- lymphocytes % CD19+a.n $x10^9$ cel/l	14,78±0,48 0,25±0,01	24,63±2,14* 0,43±0,09	14,17±1,4** 0,31±0,06
Tx/Tc	1,97±0,07	1,15±0,14*	1,67±0,2
CEC unit opt.	3,42±0,23	9,94±1,36*,	3,78±1,73**
IgA g/l	2,25±0,26	2,89±0,34	2,1±1,73
IgM g/l	1,53±0,1	1,04±0,16	2,03±0,35**
IgG g/l	12,72±0,42	17,58±1,32	13,21±0,91

Notes: *1. * – p<0.05 compared to control; 2. ** - p<0.05 between indicators before and after treatment*

Table 2. **The level of interleukins in the examined patients before and after treatment**

Indicators	Control values (n=15)	Before treatment (n=52)	After treatment for 21 days (n=52)
IL –8 pg/ml	26,0±2,62	219,63±15,87*	127,42±17,98*
IL- 4 pg/ml	18,5±1,67	82,62±12,49*	65,43±10,74*
IL1Ra pg/ml	750±186	1596,93±582,88*	1053,74±497,68*
IL – 2 pg/ml	14,50±0,93	658,39±48,49 *	455,54±47,38 *

Note: ** - p<0.001 compared to control*

In the studied patients, the number of phagocytic cells and their functional activity dropped sharply. The number of B-cells increased to the third stage of immune disorders, inversion of the second stage immunoregulatory subpopulations was observed, and the balance between T- and B- cell immunity was disturbed. The data obtained by us testify to significantly pronounced immune disorders and we did not determine a full-fledged immune response.

In all patients, we found an increase in the level of studied interleukins (Table 2).

Analysis of the content of the pro-inflammatory cytokine interleukin-8 showed that its growth was most pronounced in the examined patients. The degree of growth of interleukin-8 in blood serum demonstrates the degree of activity of the inflammatory process, since this chemokine, activating neutrophils, leads to their degranulation, the release of lysosomal enzymes and reactive oxygen metabolites, which have a damaging effect on the mucous membranes, increasing the damaging effect in the wound surface.

All patients had a high content of interleukin-1Ra. Inflammatory reactions are accompanied by a complex systemic response, mediated through interleukin-1. Violation of immune monitoring of the inflammation process contributes to the activation of mucosal antigens, an increase in the production of soluble mediators interleukin-2, interleukin-8, oxygen radicals and other biologically active substances by macrophages, leading to tissue damage, impaired penetration and the formation of a chronic focus of inflammation. It should be noted that for the progression of the inflamematory process, the imbalance in the production of interleukin-1 antagonists by phagocytes: the antagonist of its interleukin-1Ra receptors, which we observe in patients with chronic wounds of the lower extremities, is of great importance.

After complex treatment we observed positive dynamics of clinical and immunelogical parameters. So, after treatment, the indicators of T-cell immunity (CD3+, CD4+, CD19+) were restored, the level of CIC significantly decreased and the level of IgM increased. The level of the studied interleukins on the 21st day, despite a short period of time, showed a tendency to normalization, which indicates a positive trend in treatment with the use of VAC - therapy.

Features of the response of the immune system indicate the specificity of changes in immunity in patients with chronic wounds. Long-term stimulation with bacterial antigens modified by altered autologous tissue cells leads to a chronic inflammatory process and indicates an insufficient tension of the immune response in the studied patients.

Clinical example. Patient B., 64 years old. Admitted to the surgical department with a Diagnosis: Chronic wound of the left leg (Fig. 1).

She has been sicked for seven years after being bitten by unknown insects. She was treated on an outpatient basis. Objectively: on the inner surface of the left leg there is a wound 12x4 cm in size, covered with necrosis, serous-purulent discharge with a specific smell. Bacteriological examination isolated strains of Staph. aureus and Enterobacter cloaceae. She received VAC-therapy during 10 days in a constant mode with a pressure of 100 mm Hg. After the session, the wound was cleansed, covered with pink, juicy granulations with signs of active marginal epithelization (Fig. 2).

Fig. 1.*Patient B., 64 years old. Diagnosis: Chronic wound of the left leg. Upon admission to the surgical department*

Fig. 2. *Patient B., 64 years old. Dz: Chronic wound of the left leg. On the 10th day after VAC-therapy*

Complete closure of the wound was performed by performing a free autodermoplasty with a split perforated autodermograft 3 mm thick, 10x3 cm in size, followed by the continuation of VAC therapy for 3 days after the operation (P=90 mm Hg). Intraoperatively, Lacerta 1.5% -3 ml was introduced into the wound wall and the bottom of wound. The transplanted autodermal grafts fully engrafted (Fig. 3).

In the postoperative period, she received dressings with Ialuset ointment. She was discharged 15 days after operation. Examined three months later (Fig. 4).

Fig. 3. *Patient B., 64 years old. Dz: Chronic wound of the left leg. On the 7th day after the operation of autodermoplasty*

Fig. 4. *Patient B., 64 years old. Dz: Chronic wound of the left leg. Three months after discharge*

There are no complaints and wound has been completely healed.

Conclusion. The imbalance of immunoregulatory mechanisms plays an important role in the wound healing of lower extremities chronic wounds.

A modern integrated approach can radically solve the problems of chronic wound healing, as evidenced by decrease of the B-cell link of immunity against the background of an adequate increase in T-helpers and restoration of the balance between T- and B-cellular links accompanied with normalization of CIC index on the 21-th day after treatment.

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КЛИНИКО-ИММУНОЛОГИЧЕСКИЕ АСПЕКТЫ ЛЕЧЕНИЯ ХРОНИЧЕСКИХ РАН С ПРИМЕНЕНИЕМ VAC-ТЕРАПИИ

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Резюме. В статье представлены результаты исследования, проведенного с целью повышения эффективности лечения пациентов с хроническими ранами нижних конечностей путем применения комплексного лечения, включающего VAC-терапию (Vakuum Assisted Closare, вакуум-терапия), хирургическое лечение, препараты гиалуроновой кислоты. Обследовано 52 больных с хроническими

ранами нижних конечностей. В основном это женщины (48) в возрасте от 45 до 75 лет. Оценку эффективности лечения проводили на основании изучения клинических показателей заживления ран, микробиологического исследования и иммунологических данных. Полное заживление ран достигнуто у всех больных. У 67% больных пересаженный аудодермотрансплантат прижился полностью. У 28% наблюдался частичный лизис, что потребовало повторных операций. В 3% раны зажили без оперативного вмешательства. Современный комплексный подход позволяет радикально решить проблемы заживления хронических ран, о чем свидетельствует снижение В-клеточного звена иммунитета на фоне адекватного повышения Т-хелперов и восстановление баланса между Т- и В-клеточными звеньями с нормализацией показателя ЦИК.

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