



VACUUM-ASSISTED THERAPY FOR PATIENTS WITH DIABETES MELLITUS AND CHRONIC FOOT ULCERS

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Цель. Определить бактериальную нагрузку ран при использовании вакуум-ассистированной терапии у пациентов с сахарным диабетом и хроническими ранами стопы.

Материал и методы. Объектом исследования были пациенты с сахарным диабетом, у которых имелись хронические поверхностные (кожа, подкожная клетчатка) раны стопы с проявлениями легкой инфекции согласно классификации для определения наличия и тяжести инфекции IWGDF/IDSA. Критериями исключения были следующие: онкопатология, тяжелая сопутствующая патология, поражения костно-суставного аппарата. Пациенты лечились амбулаторно и не получали системную антибиотикотерапию. Проводилась терапия ран отрицательным давлением – 125 mm Hg в постоянном режиме. Первый этап работы выполнялся для определения уровня бактериальной нагрузки ран до и после 3 суток вакуум-ассистированной терапии (группа из 10 пациентов). Вторая группа (10 пациентов) была отобрана для определения динамики изменений бактериальной нагрузки ран после каждых 24 часов вакуум-ассистированной терапии, наблюдение проводилось на протяжении 96 часов экспозиции повязки. Уровень бактериальной нагрузки контролировали по изменению показателя колониеобразующих единиц в грамме ткани (КОЕ/г) биопсийного материала из ран.

Результаты. Средняя бактериальная нагрузка ран у пациентов первой группы после 3 суток терапии составляла $8,11 \pm 1,27$ lg КОЕ/г, что на 31,9% превышало исходный уровень ($p < 0,05$). При исследовании материала из ран пациентов второй группы установлено увеличение средней бактериальной нагрузки ран через 24, 48, 72 и 96 часов после начала терапии, на 10,8%, 16,4%, 38,9% и 58,6% соответственно ($p < 0,05$).

Заключение. Использование вакуум-ассистированной терапии у пациентов с сахарным диабетом и хроническими ранами стопы с проявлениями инфекции не обеспечивает необходимого контроля уровня бактериальной нагрузки, поэтому метод необходимо применять в комплексе с системной антибиотикотерапией.

Ключевые слова: вакуум-ассистированная терапия, диабет, рана, бактериальная нагрузка, инфекция

Objective. To determine the bacterial load of wounds at the application of vacuum-assisted therapy for patients with diabetes mellitus and chronic foot ulcers.

Methods. The object of the research was patients with diabetes mellitus with chronic superficial (skin, subcutaneous tissues) wounds of the foot with the signs of mild infection according to the classification for determination of presence and severity of infection of IWGDF/IDSA. Oncopathology, heavy concomitant pathology and lesions of the osteoarticular apparatus were the criteria of exception. Patients were treated as out-patients and did not get system antibiotic therapy. Therapy of wounds was conducted by negative pressure – 125 mm Hg in the continuous mode. The first stage of work was conducted to find out the level of the bacterial load of wounds before and 3 days after the vacuum-assisted therapy (the group was of 10 patients). The second group (10 patients) was selected to determine the dynamics of changes of the wound bioburden level after every 24 hours of vacuum-assisted therapy, the observation was performed during 96 hours of the bandage exposure. The level of the bacterial load was controlled according to the changes of index of colony-forming units in the gram of tissue (CFU/g) of the wound biopsy material.

Results. The average bioburden level of wounds for the 1st group patients after the removal of bandage was 8.11 ± 1.27 lg CFU/g, this exceeds the initial level by 31.9 % ($p < 0.05$). The investigation of the material from the wounds of the 2nd group patients established a considerable increase of the average bioburden level of wounds in 24, 48, 72 and 96 hours after the beginning of the therapy, 10.8 %, 16.4 %, 38.9 % and 58.6 % accordingly ($p < 0.05$).

Conclusions. In patients with diabetes mellitus and chronic wounds of the foot with signs of infection, vacuum-assisted therapy does not provide essential control of the bioburden level and should be used in combination with systemic antibiotic therapy.

Keywords: vacuum-assisted therapy, diabetes, wound, bacterial load, infection



инфекции, которые лечились амбулаторно и не получали системной антибиотикотерапии. Установлено, что для избежания инфекционных осложнений вакуум-ассистированная терапия не должна использоваться без системной антибиотикотерапии.

What this paper adds

For the first time the bacterial loading of the wounds had been studied at application of vacuum-assisted therapy for patients with diabetes mellitus with the foot chronic superficial wounds with mild infection signs. These patients were treated as out-patients and did not get system antibiotic therapy. It has been established that to prevent infectious complications, the vacuum-assisted therapy should not be used without systemic antibiotic therapy.

Introduction

Centuries have passed from the first mention of the use of negative pressure wound therapy in medical practice, but in the form familiar to us, the technique has been widely used over the past 25 years [1]. There are more than two thousand publications on the feasibility and effectiveness of negative pressure wound therapy; more than 70 of these works are prospective randomized trials [2]. Despite the confirmation of the effectiveness of wound therapy by negative pressure with a large number of studies, the mechanism of action and optimal modes of using the method are not sufficiently studied and debatable. In 2008, the results of two retrospective analyzes of the literature were published, which did not reveal sufficient evidence to confirm the clinical and economic advantages of vacuum-assisted therapy of acute or chronic wounds compared to traditional methods [3, 4]. The results of a meta-analysis demonstrated in 2011 indicate a higher efficiency of the method compared to conservative agents in the treatment of patients with chronic wounds [5]. As for the duration of vacuum dressing exposure, the intervals between its replacement for various pathologies, an unambiguous solution and clear recommendations have not been developed. Each year new research results are published that cast doubt on the optimality of the schemes used for applying the method. According to published data, the interval between replacement of vacuum-assisted dressings in the treatment of wounds of various etiologies varies between 12 hours and 7 days [6, 7]. The question remains relevant and unexplored: does the use of vacuum-assisted therapy reduce the bacterial load of wounds or, on the contrary, increase it? The research by M.J. Morykwas et al., [8], L.C. Argenta et al. [9], convincingly demonstrated a decrease in the level of bacterial load in wounds when using vacuum-assisted therapy. Subsequently, in most studies, this effect was not noted, an increase in the level of bacterial contamination of wounds in the dynamics of treatment or the same level of bacterial load of wounds was noted, compared with wounds in the treatment of which the method was not used [3, 5, 10].

All patients with diabetes mellitus who have foot wounds should be regarded as patients with

probable infected wounds, and in the presence of more or less pronounced immunosuppression, characteristic to diabetes mellitus, the presence of a significant number of pathogenic microorganisms in the wound can quickly transform into a systemic infection [11]. That is why such patients need precise control of the wound state, and the increase in the level of bacterial contamination of the wound can have more formidable complications than in patients who have no diabetes. Nowadays there are no randomized clinical trials which results would reliably reflect changes in the bacterial load of the wound when using vacuum-assisted therapy in patients with diabetes mellitus and foot wounds who do not receive systemic antibiotic therapy.

Objective. To determine the bacterial load of wounds at the application of vacuum-assisted therapy for patients with diabetes mellitus and chronic foot ulcers.

Methods

The study was conducted during the period of March 2017 to February 2018 on the basis of the Lviv Regional Clinical Hospital, Department of Surgery No. 2 and the Department of Microbiology of the Danilo Galitsky Lviv National Medical University. The inclusion criteria for the study were the presence of diabetes mellitus and chronic superficial (skin, subcutaneous tissue) wounds of the feet with mild infections according to the classification for determining the presence and severity of IWGDF / IDSA infection [12]. The exclusion criteria were: oncopathology, severe concomitant pathology, lesions of the osteoarticular apparatus. Patients were treated as out-patients and did not receive systemic antibiotic therapy. The wounds were treated with negative pressure - 125 mm Hg in a constant mode, the NP32S HEACO apparatus, sterile dressing sets and HEACO containers were used. For tissue biopsies, local anesthesia was used if necessary. The sampling was carried out with the Dermo-Punch tool (Sterylab) with the diameter of 3.5 mm. Before the biopsy, the wounds were washed with sterile saline and dried with a sterile gauze swab. The first stage of the work was performed to determine the level of bacterial load of wounds before and after three days of vacuum-assisted therapy. The group of 10 patients was formed (5 women and 5 men,

average age 61 ± 5.3 years, $M \pm \sigma$). Biopsy of wound tissues for microbiological examination was performed before the start of vacuum-assisted therapy and three days after removal of the vacuum dressing.

The second stage of the work was to determine the dynamics of changes in the bacterial load of wounds after each day of vacuum-assisted therapy. For this, the second group was formed, which consisted of 10 patients (6 women, 4 men, average age 59 ± 4.9 years, $M \pm \sigma$). Material from the wounds was taken according to the method described above before applying a vacuum dressing and every 24 hours for 4 days, by perforation of the film with restoration of tightness after sampling.

Biopsy material was placed in a transport medium and delivered no later than 2 hours to the laboratory for inoculation. The weight of the biopsy was the weight difference of the eppendorfs, which were weighed on an analytical balance before and after sampling. Biopsies were ground in a sterile mortar with 1 ml of nutrient broth. From a successive series of ten-fold dilutions, 0.1 ml of homogenizate was inoculated on elective and differential diagnostic agarized and liquid storage media to isolate pure cultures. Cultivation of inoculation was carried out under aerobic and anaerobic conditions for 24-48 hours at 37°C .

The number of bacteria in 1 g of biopsy was determined by counting colony forming units (CFU/g) taking into account the weight of the biopsy, the amount of inoculation material and dilution according to the formula: $X = (10 \times N \times M) \div m$, where X is the number of CFU/g of biopsy, 10 – constant when inoculating 0.1 ml of homogenizate, N – number of colonies, M – dilution (10, 100, 1000 times), m – mass of biopsy. The number of microorganisms for statistical processing was expressed in decimal logarithms - lg CFU/g.

Statistics

Statistical analysis of the material was carried out using the computer program Statistica 8.0. and Microsoft Office Excel 2007 software.

Statistical indicators are presented in the format $M \pm \sigma$, where M is the arithmetic mean value, σ is the standard deviation.

To check the normality of the distribution of quantitative data of the samples, we used the Kolmogorov-Smirnov, Lilliefors and Shapiro-Wilk tests. Taking into consideration the limitations of each of the criteria, we did not consider the studied distribution normal if at least one of these criteria was significant ($p < 0.05$).

The reliability of differences between the two average values, in the normal distribution and in the comparison of dependent samples, was determined using a paired t-test (group 2).

The reliability of differences between the two average values, when comparing dependent samples and the absence of “normality of distribution”, was calculated using the Wilcoxon test (group 1).

The statistical difference between the studied parameters was considered significant at $p < 0.05$.

Results

As a result of the conducted studies, it was found out that the level of the bacterial load of wounds in patients before the vacuum-assisted therapy was from 8.4×10^4 CFU/g to 4.5×10^7 CFU/g. The average bacterial load in the group was 6.15 ± 0.75 lg CFU/g, which is significantly higher than the critical level of bacterial wound contamination [13]. An increase in the level of bacterial load was observed 3 days after the removal of the vacuum-assisted dressing in patients. The average bacterial load in the group after the dressing removal was 8.11 ± 1.27 lg CFU/g, which is 31.9% higher than the initial level ($p < 0.05$). The indicators of the number of microorganisms in patients vary from 2.3×10^5 CFU/g to 1.2×10^9 CFU/g.

Considering the results obtained, the second group of patients was created and the dynamics of changes in bacterial contamination of wounds after each day of vacuum-assisted therapy was determined. It permitted to establish the exposure time of the vacuum dressing, through which a rapid increase in the level of bacterial contamination of the wound begins.

As a result of the studies, it was found that bacterial contamination of wounds in the second group, before the application of the vacuum dressing ranged from 1.0×10^4 CFU/g to 2.6×10^6 CFU/g. The average bacterial load of the wounds was 5.19 ± 0.82 lg CFU/g, which is higher than the critical level of bacterial contamination of the wounds [13]. 24 hours after the application of the vacuum dressing, the average value of bacterial contamination of the wounds significantly increased compared to the number of microorganisms before the application of the dressing and amounted to 5.62 ± 0.68 lg CFU/g ($p < 0.05$). After 48 hours, their number increased and amounted to 6.04 ± 0.68 lg CFU/g ($p < 0.05$), after 72 hours – 7.21 ± 0.93 lg CFU/g ($p < 0.05$), and after 96 h – 8.23 ± 0.58 lg CFU/g ($p < 0.05$). After 24, 48, 72 and 96 hours, there was a significant increase in the average level of bacterial load of wounds in the group by 10.8%, 16.4%, 38.9% and 58.6%, respectively ($p < 0.05$). The results are presented in the figure.

Despite the fact that clinical manifestations were not an objective criterion for the study, it is advisable to note that in 2 of 10 patients of group I, during removal of the vacuum dressing, moderate

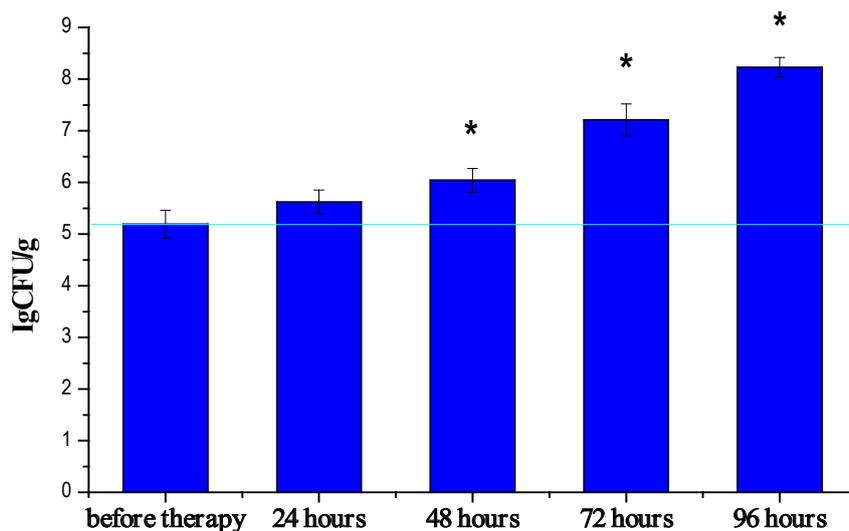


Fig. The bioburden level of wounds (Ig CFU/g) when applying vacuum-assisted therapy (* – $p < 0.05$ relative to the initial level).

hyperemia of the tissues around the wound was observed, and in 4 patients there was an unpleasant odor from the zone of application of the dressing. In patients of the second group, in which the dressing did not change for four days, hyperemia was noted in 3 patients, in 2 – mild maceration of the skin around the wound, and unpleasant odor was noted in 6 out of 10 patients.

Discussions

The wound healing process is extremely complex. It depends on the sequential interaction between several difficultly regulated factors, while little is known about the underlying pathomechanisms. An infection is one of the factors that impedes wound healing and is a major complication in patients with diabetes mellitus [14]. Obviously, it is impossible to draw peremptory conclusions based only on the nature of changes in the quantitative composition of the bacterial environment of wounds, but they must be taken into account in patients with manifestations of infection [15]. According to the IWGDF/IDSA classification, patients with diabetes mellitus and manifestations of mild infection are predominantly treated on an outpatient basis and are not under the daily supervision of medical personnel [12].

In patients with diabetes mellitus and chronic foot wounds who do not receive systemic antibiotic therapy, negative pressure wound therapy, despite the positive effect on wound healing, contributes to an increase in their bacterial load. As a result of the studies, a significant increase in bacterial contamination of wounds from the first day of exposure to a vacuum dressing is registered, which probably increases the risk of infectious complications. It is

not known due to what the increase in the bacterial load of wounds occurs; one of the possible reasons is a significant bacterial contamination of the polyurethane sponge [16]. A promising way to overcome the problem is the supplementation of vacuum-assisted therapy with solution instillation [17]. At the same time, the issues of changes in the population ratio of the qualitative composition of the bacterial environment, as well as the effect of systemic antibiotic therapy on the bacterial load of wounds during vacuum-assisted therapy in patients with diabetes mellitus and chronic foot wounds remain relevant and insufficiently studied [18].

Conclusions

Based on the fact that the study has shown a significant increase in the number of microorganisms when applying a vacuum dressing, which manifests itself already a day after its application, it can be argued that in patients with diabetes mellitus and chronic foot wounds with the signs of infection, the use of vacuum-assisted therapy does not provide the necessary monitoring the level of bacterial load, therefore, the method should not be used without systemic antibiotic therapy.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical aspects

The research was approved by the Ethics Committee of Danylo Halytsky Lviv National Medical University.

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