

Treatment of diabetic ulcers with epidermal growth factor: case reports

Tratamento de úlceras diabéticas com fator de crescimento epidérmico: relatos de caso

DOI: <http://dx.doi.org/10.31011/1519-339X.2018a18n85.13>

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ABSTRACT

The aim is to describe the healing process of patients with diabetic ulcers treated with recombinant human epidermal growth factor (rhEGF), and colonization by *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Case study of patients with diabetic ulcers treated with rhEGF with outpatients in a university hospital. Data collection took place between April and August 2017, obtaining clinical characteristics, including measurement of areas and photographic records of the lesions, as well as collection of biological material by swab. The data analysis was done by descriptive statistics. The calculation of the rate indicated 100% healing in patient 1 and 30% in patient 2. In relation to the tissue present in the wound bed, there is an increase of granulation tissue and epithelization and a decrease of the shedding tissue. As for the exudate, there was reduction in the amount present in the wound at the end of the study. No clinical signs of infection were observed in the wounds. The results of the microbiological analyzes indicated that microbial colonization by *S. aureus* and *P. aeruginosa* was maintained in patient 1 and was minimized in patient 2. It concluded that the application of rhEGF was favorable in cicatrization of diabetic ulcers and in microbiological control.

Keywords: Diabetic Foot; Epidermal Growth Factor; *Staphylococcus Aureus*; *Pseudomonas Aeruginosa*; Nursing.

RESUMO

Objetiva-se descrever o processo de cicatrização de pacientes com úlceras diabéticas tratadas com fator de crescimento epidérmico recombinante humano (rhEGF), e a colonização por *Staphylococcus aureus* e *Pseudomonas aeruginosa*. Estudo de casos de pacientes com úlceras diabéticas tratadas com rhEGF acompanhados ambulatorialmente em um hospital universitário. A coleta de dados ocorreu entre abril e agosto de 2017, com a obtenção de características clínicas, incluindo mensuração das áreas e registros fotográficos das lesões, bem como, coleta de material biológico por *swab*. A análise de dados foi feita por estatística descritiva. O cálculo da taxa apontou 100% de cicatrização no paciente 1 e 30% no paciente 2. Em relação ao tecido presente no leito da ferida, nota-se um aumento do tecido de granulação e epitelação e uma diminuição do tecido de esfacelo. Quanto ao exsudato, houve uma redução na quantidade presente na ferida ao fim do estudo. Não foram observados sinais clínicos de infecção nas feridas, os resultados das análises microbiológicas indicaram que a colonização microbiana por *S. aureus* e *P. aeruginosa* manteve-se no paciente 1 e foi minimizada no paciente 2. Conclui-se que a aplicação do rhEGF foi favorável na cicatrização de úlceras diabéticas e no controle microbiológico.

Palavras-chave: Pé Diabético; Fator de Crescimento Epidérmico; *Staphylococcus Aureus*; *Pseudomonas Aeruginosa*; Enfermagem.

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INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disorder of heterogeneous etiologies, characterized by hyperglycemia and metabolic disorders of carbohydrates, proteins and fats, resulting from defects in insulin secretion and/or action⁽¹⁾. It is an epidemic that already affects about 246 million people worldwide, of which more than 12 million are Brazilians⁽²⁾.

Diabetic ulcers are one of the most common complications and, therefore, have become an alarming public health problem in Brazil. It basically results from neuropathies and peripheral arterial diseases, which often have a negative prognosis due to infections and amputations⁽³⁾, resulting in a high cost for health services⁽²⁾.

In this way, they require a multifaceted treatment with standard therapeutic components, including: metabolic control, application of topical coverages, debridement of necrotic tissues and measures to control infections⁽⁴⁾.

Among the coatings, it can be said that the use of recombinant human epidermal growth factor (rhEGF) on the wound can accelerate the rate of epidermal regeneration⁽⁵⁾. In this study, the product used has a bioidentical character of peptide, being composed of a chain of 53 amino acids and synthesized by a biotechnological process of fermentation, through the bacterium *Escherichia coli*.

Its mechanism of action involves the action at the cellular level through the interaction with tyrosine kinase type receptors, which determines the activation of a signaling cascade, resulting in a succession of biochemical changes. Its effects translate into proliferation of keratinocytes, stimulation of angiogenesis and activation of fibroblast function with increased levels of endogenous hyaluronic acid, collagen and elastin⁽⁶⁾.

However, is not clear the microbiological analysis of diabetic ulcers, especially those treated with rhEGF⁽⁷⁾. In general, it can be said that the bacteria most commonly found in chronic wounds are *Staphylococcus aureus* and *Pseudomonas aeruginosa*⁽⁸⁾.

Thus, knowledge about microbiological analysis and the possibility of greater effectiveness in the healing process of diabetic ulcers with topical application of rhEGF becomes clinically relevant.

The question that guided the present study was: how does the healing process occur in patients with diabetic ulcers treated with epidermal growth factor, as well as their colonization by *S. aureus* and *P. aeruginosa*?

Thus, this study aims to describe the healing process of patients with diabetic ulcers treated with recombinant human epidermal growth factor (rhEGF), and the colonization by *S. aureus* and *P. aeruginosa*.

CASE REPORTS

Two patients with diabetic ulcers were followed up in an outpatient clinic of a university hospital in Rio de

Janeiro between April and August 2017. Wounds were treated with a dressing composed of recombinant human epidermal growth factor (rhEGF) gel for twelve weeks. This gel was produced in the university pharmacy linked to the university hospital by pharmaceutical component of the team.

This study consists of a review of a research on the use of rhEGF in chronic wounds, which is being developed, both patients were part of the pilot study.

The data collection was performed by the research nurse, who recorded in a research protocol of sociodemographic and clinical variables of the patient, obtained by interviews conducted on the first day of care. The variables related to the healing process were measured weekly and included: type and degree of exudation; wound depth; types of lesional and perilesional tissues; presence of foul smell, pruritus and pain; presence and degree of edema. Photographic recording and measurement of the wound area - by sterile transparent acetate planimetry method⁽⁹⁾ - were performed at the first, sixth and twelfth visits.

During all nursing consultations, wound care involved cleaning the bed and the edges of the lesion with 0.9% saline solution and neutral soap, as well as conservative instrumental debridement⁽¹⁰⁾ with tweezers and sterile scalpel, followed by drying the adjacent skin with sterile gauzes and application of essential fatty acids. The lesion bed was always covered with EGF and occluded with sterile gauze and simple crepe bandage, fixed with adhesive tape.

For the analysis of the microbial colonization, the biological material of the wound was collected by swab⁽¹¹⁾. Upon arrival at the laboratory, the swabs collected and introduced into the transport medium were placed in 2.0 mL sterile saline (0.9%), vortexed, and about 0.9 mL was added in 1 mL Broth Tryptone (TSB) and then incubated at 35 ° C (\pm 2 ° C) for 24 to 48 hours.

After the TSB incubation period, the turbid tubes were seeded on saline mannitol agar and cetrinide agar to qualitatively investigate the presence of *S. aureus* and *P. aeruginosa*, respectively. In addition, 0.02 mL of the suspension obtained from saline swab processing was pipetted into these same solid culture media, aiming to quantitatively investigate the presence of these microorganisms in the samples. After incubation at 35 ° C (\pm 2 ° C) for 24 to 48 hours, the plates were analyzed for growth, quantity and characteristics of the colonies.

The dressing changes were performed daily by the patient at home, with the following nursing guidelines: protection of the dressing during the bath; daily dressing change after hand hygiene and with use of procedure gloves; resting; importance of controlling blood pressure and glycemic levels through the correct use of prescribed antihypertensive and oral hypoglycemic agents; adoption of hyposodic and hypoglycemic diet. All inputs needed to perform the dressing at home were provided by the research team.

The data analysis was done by simple descriptive statistics, with absolute and percentage frequency calculations.

This case study is part of the research project titled "Clinical and economic evaluation of the regenerative activity of the lesion through the use of EGF", which had its ethical aspects evaluated and approved by the Research Ethics Committee from Plataforma Brasil under opinion No. 2,189,183, highlighting that the patients signed the Free and Informed Consent Term (FICT) and authorizations for photographic registration.

Patient 1: Patient 1: Male, 51 years old, retired, elementary school incomplete and divorced. It reports being hypertensive, diabetic in daily use of antihypertensive and hypoglycemic agents, with diabetic ulcer since 2016. Lucid and oriented, communicative and cooperative, normal colored, eupneic, normotensive (PA = 120 x 80 mmHg) and afebrile throughout follow-up with hemoglobin glycated ratio of 6.2%. At the beginning of the follow-up he had a 1.8 cm² diabetic ulcer on the lateral dorsum of his right foot.

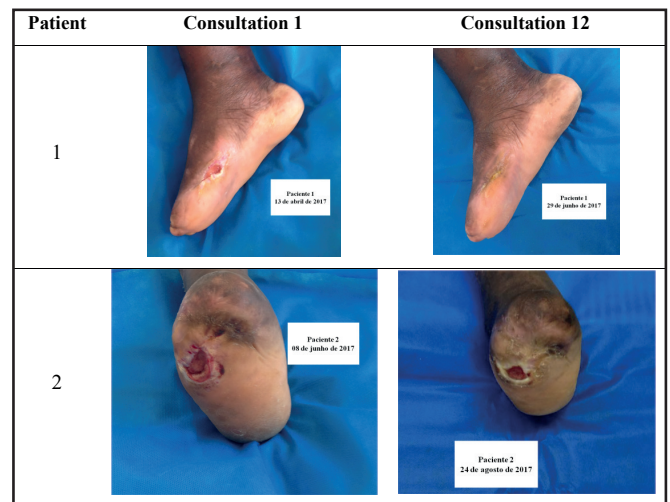
Patient 2: Male, 51 years old, retired, full high school and married. It reports being diabetic in daily use of hypoglycemic agents, with diabetic ulcer since 2014. Lucid and oriented, communicative and cooperative, normal colored, eupneic, normotensive (PA = 110 x 60 mmHg) and afebrile throughout the follow-up, with glycated hemoglobin of 8.8 %. At the beginning of the follow-up he had a 4cm² diabetic ulcer in the right foot plantar region.

Characteristics of wounds at the beginning and at the end of treatment are described in Table 1.

The patient 1 had complete wound healing. Patient 2, however, had a 30% reduction in the area of the wound (1.2 cm²), an increase in the presence of granulation tissue and a decrease in the shedding tissue in the lesion, besides an improvement in the quality of adjacent skin tissue.

There was no infection development in the lesion or adverse reactions to the product during the study. The photographs are shown in Table 2.

Table 2. Photographs of wounds at the beginning and end of treatment. Niterói, RJ, Brazil, 2017.



Source: survey data.

The first line of the board has the photos 1 and 2. The photo 1 presents the patient 1's lesion whose first consultation was performed on April 13, 2017. The

Table 1. Wounds' characteristics at the beginning and end of treatment. Niterói, RJ, Brazil, 2017.

Analysis Variables	Patient 1		Patient 2	
	Consultation 1	Consultation 12	Consultation 1	Consultation 12
Wound size	1,8cm ²	0 cm ²	4,0 cm ²	2,8cm ²
Type of Exudate	Serous	-	Serosanguineous	Serosanguineous
Amount of Exudate	Little	Absent	Moderate	Little
Granulation in bed	75%	0%	25%	75%
Epithelization in bed	25%	100%	25%	25%
Slough in bed	0%	0%	50%	0%
Edge	Hyperkeratinized	Epithelialized	Hyperkeratinized	Hyperkeratinized
Depth	Superficial	-	Partial	Superficial
Adjacent skin	Dry	Hydrated	Dry	Hydrated
Ache	No	No	No	No
Itching	No	No	No	No
Edema	No	No	No	No
Colonization by <i>S. aureus</i>	Present (10 ³ CFU)	Present (10 ⁴ CFU)	Present (10 ³ CFU)	Absent
Colonization by <i>P. aeruginosa</i>	Presente (10 ² CFU)	Present (10 ² CFU)	Absent	Absent

Note: colony forming units (CFU).

Source: survey data.

photographic record of the photo 2 was realized on June 29, 2017, and it refers to the same patient 1's lesion, however, twelve weeks of treatment later. On the second line of the board is possible to see the photos 3 and 4, which refer to patient 2's injury. The photo 3 is from his first consultation on June 8, 2017. The photo 4 refers to the same patient 2' lesion, however, after twelve weeks of treatment. This photographic record occurred on August 24, 2017.

DISCUSSION

The evaluation of the evolution of the wound healing process by second intention, as occurs with chronic lesions, should take into account as a matter of priority: the wound reduction rate, the improvement in the quality of the tissues present and the amount of exudate⁽¹²⁾. Both outcomes were obtained in the described cases. Differences in results (patient 1 achieved complete healing while patient 2 achieved a reduction of the area) can be attributed to the time of evolution, since patient 1 had about 12 months with the wound, while patient 2 had more than 36 months. It is also reiterated that a smaller lesion size also favors a more accelerated healing, as was the case of patient 1 (initially the lesion had 1.8 cm² of area).

It is known that topical treatment is not solely responsible for the evolution in wound characteristics. In this sense, it is worth highlighting the control of the underlying diseases - systemic arterial hypertension and diabetes mellitus, among the patients described⁽¹³⁾. It can be inferred that the best glycemic control of patient 1 (glycated hemoglobin of 6.2%) had a relevant role in its best outcome when compared to patient 2 (8.8% glycated hemoglobin).

In relation to the colonization by *S. aureus* and *P. aeruginosa*, it is possible to define that there is clinical infection in a wound when there is presence of purulent secretions or at least two signs or symptoms of inflammation (erythema, heat, sensitivity, pain and induration), according to the Infectious Diseases Society of America - IDSA⁽¹⁴⁾.

Both evaluated wounds remained free of these signs and symptoms throughout the follow-up period, indicating that rhEGF does not potentiate microbial colonization of wounds. Even because there was a maintenance of the absence of colonization by *P. aeruginosa* and control of colonization by *S. aureus* at the end of treatment in the patient's wound 2.

Regarding the quantitative microbial evaluation, it can be said that there was an increase in the number of CFUs characteristic of *S. aureus* after the follow-up period in the case of patient 1, which may be related to the fact that this microorganism is part of the normal microbiota of the whole human skin⁽¹⁵⁾, in view of the complete healing of this wound at the end of the treatment.

As limitations, although these cases indicate positive therapeutic responses from the topical application of rhEGF in diabetic ulcers, it is necessary the development of

randomized controlled clinical trials with more significant samples, for accurate measurement of the effectiveness of the product. Also, more robust microbiological analyzes will be relevant, that they must include molecular tests to verify the genetic characteristics of the strains present in the lesions, considering that those carried out in this work were based only on phenotypic evaluations of the colonies observed in the culture media.

CONCLUSION

The use of rhEGF in the outpatient follow-up of patients with diabetic ulcers is recommended, since complete healing (patient 1) and reduction of the wound area (patient 2) were obtained in the cases described in this study. It should be noted that no clinical signs of wound infection were observed, and microbiological analyzes indicated better control of microbial colonization by *S. aureus* and *P. aeruginosa*.

In order to establish results that contribute even more to its implementation in society and clinic, and in addition, that bring greater academic depth, it is necessary to carry out new studies with a larger sample number and methodological rigor, such as randomized clinical trials.

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