ORİJİNAL ARAŞTIRMA ORIGINAL RESEARCH

DOI: 10.5336/dermato.2022-90463

The Effect of Intralesional Human Epidermal Growth Factor on Wound Healing in Chronic Diabetic Foot Ulcer: A Retrospective Analysis of Patient Results

Kronik Diyabetik Ayak Ülserinde İntralezyonel İnsan Epidermal Büyüme Faktörünün Yara İyileşmesine Etkisi: Hasta Sonuçlarının Retrospektif Analizi

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ABSTRACT Objective: Epidermal growth factor (EGF) plays an important role in wound healing. But, bioavailability of EGF is impaired in chronic diabetic foot ulcer (CDFU) due to increased protease activity. The study's objective was to evaluate the effect of intralesional human-EGF (h-EGF) on wound healing in patients with CDFU. **Material and Methods:** Twenty-eight patients with CDFU were included to study. 75 µg intralesional h-EGF thrice a week for 5 weeks were used to accelerate wound healing. Ulcer size was calculated by simple planimetric measurement. Specimens for culture were taken from all patients. Granulation response were evaluated with healing grade (HG) [HG-0 → no response, HG-I → minimal response, HG-II → partial response, HG-III → complete response] and complete wound closure. Pre- and post-treatment ulcers size were recorded to asses treatment outcome. **Results:** New granulation tissue over the wound (HG I-III) was present in 26 (92.8%) patients at the end of treatment. Complete wound healing (HG III) was achieved in 10 (35.7%) patients, complete wound closure was seen in 3 (10.7%) patients. Pre and post-treatment mean ulcers size were 23.9±18.5 and 6.5±4.8 cm2 (p=0.001). The most frequent adverse events of h-EGF were tremor (32.1%). Total of 47 causative bacterias (14 different types of bacteria) were isolated from 23 (87.2%) patients, whereas cultures were sterile in 5 (17.8%) of the patients. Primarily, Escherichia coli, Proteus mirabilis, Acinetobacter baumannii, Pseudomonas aeruginosa species were seen in rate of 19.1%, 17%, 12.7%, 10.6% respectively. **Conclusion:** Intralesional h-EGF is effective in the treatment of CDFU, has no serious side-effect and accelerates wound healing.

Keywords: Epidermal growth factor; diabetic foot; wound healing; adverse effect; culture

ÖZET Amaç: Epidermal büyüme faktörü [epidermal growth factor (EGF)], yara iyileşmesinde önemli bir rol oynar. Ancak artan proteaz aktivitesi nedeniyle kronik diyabetik ayak ülserinde (KDAÜ) EGF'nin biyoyararlanımı bozulur. Bu çalışmanın amacı, KDAÜ'lü hastalarda intralezyonel insan-EGF'nin [human-EGF (h-EGF)] yara iyileşmesi üzerindeki etkisini değerlendirmekti. **Gereç ve Yöntemler:** Çalışmaya KDAÜ'lü 28 hasta dâhil edildi. Yara iyileşmesini hızlandırmak için 5 hafta boyunca haftada 3 kez 75 µg intralezyonel h-EGF kullanıldı. Ülser boyutu basit planimetrik ölçüm yoluyla hesaplandı. Tüm hastalardan kültür için örnek alındı. Granülasyon yanıtı iyileşme derecesi (İD) ile değerlendirildi. [İD-0 → yanıt yok, İD-I → minimal yanıt, İD-II → kısmi yanıt, İD-III → tam yanıt]. Tedavi sonucunu değerlendirmek için tedavi öncesi ve sonrası ülser boyutu kaydedildi. **Bulgular:** Tedavi sonunda 26 (%92,8) hastada yara üzerinde yeni granülasyon dokusu (İD I-III) mevcuttu. On (%35,7) hastada tam yara iyileşmesi (İD III), 3 (%10,7) hastada tam yara kapanması görüldü. Tedavi öncesi ve sonrası ortalama ülser boyutu 23,9±18,5 ve 6,5±4,8 cm2 idi (p=0,001). h-EGF'nin en sık görülen yan etkisi tremordu (%32,1). Yirmi üç (%87,2) hastadan toplam 47 etken bakteri (14 farklı bakteri türü) izole edilirken, 5 (%17,8) hastada kültürler sterildi. Öncelikle Escherichia coli, Proteus mirabilis, Acinetobacter baumannii, Pseudomonas aeruginosa türleri sırasıyla %19,1, %17, %12,7, %10,6 oranında görüldü. **Sonuç:** İntralezyonel h-EGF, KDAÜ tedavisinde etkilidir, ciddi bir yan etkisi yoktur ve yara iyileşmesini hızlandırır.

Anahtar Kelimeler: Epidermal büyüme faktörü; diyabetik ayak; yara iyileşmesi; istenmeyen etkiler; kültür

Diabetes mellitus (DM) and chronic complication such as foot ulcer is getting increased in all of the populations worldwide. The annual incidence of chronic diabetic foot ulcer (CDFU) is 2.5 to 10.7%.[1-4] The probability of developing foot ulcers in diabetic patients during their lifetime varies between 15-25%.[5]

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Peer review under responsibility of Turkiye Klinikleri Journal of Dermatology.

Received: 10 Apr 2022 ***Received in revised form:*** 24 Aug 2022 ***Accepted:*** 25 Aug 2022 ***Available online:*** 31 Aug 2022

CDFU is one of the most burdensome complication of DM and is extremely difficult to be treated. Treatment of CDFU is a challenging task for healtcare professionals. CDFU leading to decrease patient's quality of life can last for months until healing, recurs at about 60% of patients, and are a significant cause of morbidity.[2,4] In such patients, sometimes amputation is unavoidable despite treatment. The annual incidence of amputation in patients with DM is 0.25% to 1.8%.[1-4] The 5-year mortality rate in patients undergoing lower extremity amputation due to diabetic foot ulcer is 40%.[5]

Despite often difficult to be treated, an understanding of underlying mechanism on how CDFU develops, showing specific attention and effort on how to manage these type of complication can be helped us for providing successful healing.

Neuropathy, ischemia or both and also infection play a major role in development of CDFU. Decreased muscle innervation and muscle atrophy due to peripheral neuropathy in diabetic patients cause foot deformities. Thus, certain foot areas are subjected to chronic high pressure. Ischemia developing secondary to peripheral arterial disease, reduces tissue oxygenation and causes foot ulceration. Because of cellular and humoral immune deficiencies in diabetics, foot wounds are prone to be infected especially in case of severe chronic hyperglycemia.[4,6]

Good wound care, effective debridement and medical treatment of ulcer, appropriate antibiotic therapy, meticulous off-loading therapy, optimizing foot blood flow with medication and revascularization if needed and well-regulated blood sugar are of paramount importance for ulcer healing.

Human epidermal growth factor (h-EGF) plays a crucial role in the regulation of cell growth, proliferation and differentiation.[7] h-EGF in wound healing process has a powerfull effect in terms of productive cells stimulation and migration, granulation tissue formation (wound covering), myofibroblast activation and proliferation (wound contraction), epithelial cells migration and proliferation (wound closure). Unfortunately, bioavailability of epidermal growth factor is impaired in CDFU.[4,8] This abnormality is mainly attributed to nonenzymatic glycation of growth factors as a result of hyperglycemia.[4,8]

The useful effects of topical EGF in wound healing and closure has been reported in a lot of studies.[9,10] But this beneficial effect is limited particularly in high grade ulcers because of an increased protease activity.[11] Intralesional application of h-EGF is able to overwhelm this limitation.[12,13]

We used intralesional h-EGF to accelerate wound healing in patients with CDFU in this study.

MATERIAL AND METHODS

PATIENT'S POPULATION, DEMOGRAPHIC AND CLINICAL DATA

Twenty-eight patients with CDFU who did not respond to standard medical treatment were included to our study in the period of March 2016 and December 2017.

The exclusion criteria of the study were limited to patients with ulcer grade (UG) V and infection grade (IG) IV and patients with active cancer, autoimmune disease, psychiatric illness, recent myocardial infarction, unstable angina, uncontrolled heart failure, chronic renal failure and receiving immunosuppressive or corticosteroids.

For all the patients, age, sex, duration of DM, hemoglobin A1c (HbA1c) level, routine laboratuary test results, ankle brachial index (ABI) measurement, data for peripheral vascular examination (manually or with doppler ultrasonography, angiography if needed), result of wound's culture, comorbite disease and adverse events of h-EGF application were recorded.

Additionally, localization, size and number of the ulcer, side of lower limb with ulcer, UG, and IG were recorded for all of the patients.

STUDY DESIGN AND ETHICAL APPROVAL

This is a retrospective single centre experimental clinical study. Approval of the Ethics Committee of the Pamukkale University Non-Invasive Clinical Research was taken for this study (approval date and decision number: February 22, 2021, E-601167-87-020-20629). Informed consent for h-EGF application

was obtained from all patients. This study was conducted in accordance with the principles of the Declaration of Helsinki.

ULCER'S CLASSIFICATION AND SIZE MEASUREMENT

Ulcer was graded with regard to Meggitt-Wagner classification system as follows;[14]

UG:

- UG I→ Superficial ulcer.
- UG II→ Deep ulcer to tendon, capsule or bone.
- UG III→ Deep ulcer with abscess, osteomyelitis or joint sepsis.
- UG IV→ Localized gangrene of forefoot or heel.
- UG V→ Gangrene of entire foot.

Ulcer size was calculated by means of simple planimetric measurement (greatest width X greatest length).[8] Size measurements of ulcer were done just before starting and after completing h-EGF treatment.

DIABETIC FOOT INFECTION CLASSIFICATION AND WOUND CULTURE

The infection was graded as follows according to the diabetic foot infection classification defined by Infectious Disease Society of America.[15]

IG:

- IG I→ No sign of infection.
- IG II→ Mild infection of skin and subcutaneous tissue only.
- IG III→ Moderate to severe infection of deeper structures.
- IG IV→ Severe wound infection along with systemic signs and symptoms.

Specimens for wound culture were taken from curettage, needle aspiration or infected soft and bone tissue materials according to the wound depth during debridement.

WOUND CARE, MEDICAL TREATMENT, DEBRIDEMENT AND VASCULAR PROCEDURES

Wound care was performed daily using saline and antiseptic solution. After cleaning the wound, saline-soaked gauze was placed over the wound. Then, sufficient sterile gauze was placed on the dressing and fixed with a rolled bandage. Dressing was done daily.

An empiric antibiotic regimen were used first. Appropriate specimen for culture were taken. Antibiotherapy was modified based on results of culture and sensitivity testing.

Metabolic control was made by insulin alone or combination with oral hypoglycemic drugs and diabetic diet.

A sharp debridement along with minor amputation whenever necessary to remove infected, necrotic soft and bone tissues was performed. During the treatment, repeated debridements were made when necessary.

Revascularization if needed was performed using interventional, surgical or hybrid vascular procedures.

h-EGF APPLICATION

Intralesional h-EGF (Heberprot-P 75, Heber Biotec, Havana, Cuba) treatment was made following the infection control by surgical debridement, wound care and antibiotic therapy.

Seventy-five µg intra and peri-lesional h-EGF 3 times per week in alternate days during 5 weeks were used in order to accelarate wound healing. h-EGF was dissolved within 5 mL sterile normal saline before injection. EGF solution was injected with insulin needle. This volume was given throughout the lesion in way of volume of 0.5 mL per injection, starting from the deeper zones in each application.

GRANULATION RESPONSE (HEALING GRADE)

Granulation response was considered as the ratio of the surface area covered by the granulation tissue to the initial area of the ulcer. The granulation response was briefly called as healing grade (HG) and was graded as follows;[5,7]

HG:

- HG 0 =HG of ≤25% → No response.
- HG I=HG of [26-50%] → Minimal response.
- HG II =HG of [51-75%] → Partial response.
- HG III=HG of ≥75% → Complete response.

Complete wound healing refers to more than 75% of the wound healing rate. Whereas, complete wound closure is defined as being covered the wound completely with skin, fibrous or epithelialized tissue without any discharge or dressing.

STATISTICAL METHODS

Continuous variables were stated as mean±standard deviation, while categorical variables were stated as number and frequency. Wilcoxon signed ranks test was used to compare the change of ulcer size of the patients in before and after treatment. p value of <0.01 was considered as statistically significant.

Statistical analysis of categorical variables with HG was performed using chi-square test. The relationship between HG and continuous variables was evaluated with the Kruskal-Wallis test. The reliability assessment and correlation analysis between HG and continous variables was made by Spearman correlation test.

The difference between the wound sizes before and after treatment was defined as Δ (delta) value. The ratio of this difference to the wound size before treatment was accepted as Δ ratio. This ratio is the same with "Healing Grade Ratio", also called as "Wound Healing Rate". The relationship between variables was evaluated with spearman correlation analysis. p<0.05 value was considered as statistically significant.

RESULTS

The demographic and clinical features of the patients are presented in Table 1. The mean age was 62.4±11.1 years. 78.6% of our patients were male. All had Type 2 DM and the number of patients receiving insulin was 20 (71.4%). The mean value of HbA1c was 8.6±1.9%. Ulcers was only 1 in 67.9% of patients. The ulcers were more often on the right lower extremity (57.2%) and were located on the toes (60.7%) and the sole (50%). Grade III ulcers were present in 43.2% and Grade III infections were present in 53.6% of the patients (Table 1).

According to culture results, a total of 47 causative bacterias (14 different types of bacteria) were isolated from 23 (87.2%) patients, whereas cul-

TABLE 1: Baseline demographic and clinical characteristics of the patients.

Parameters	$\bar{X}$±SD or %	Number (n)
Age (32-78), median 63	62.4±11.1	(28)
Gender		
• Female	21.4%	(6)
• Male	78.6%	(22)
History of diabetes mellitus (years)	7.8±5.3	
Insulin use	71.4%	(20)
Hemoglobin A1c	8.6±1.9	
Ulcer		
• Side		
o Right	57.2%	(16)
o Left	21.4%	(6)
o Both	21.4%	(6)
• Localization		
o Toe	60.7%	(17)
o Heel	14.2%	(4)
o Forefoot	7.1%	(2)
o Sole	50%	(14)
o Lateral/medial	10.7%	(3)
• Numbers		
o 1	67.9%	(19)
o 2	21.4%	(6)
o 3	10.7%	(3)
• Size (mean. cm^2) (Pre-treatment)	23.9±18.5	
Meggitt-Wagner's classification=UG		
• UG I	25%	(7)
• UG II	28.6%	(8)
• UG III	42.9%	(12)
• UG IV	3.5%	(1)
• UG V	-	
Diabetic foot IG		
• IG I	25%	(7)
• IG II	21.4%	(6)
• IG III	53.6%	(15)
• IG IV	-	
Serum creatinin		
• Value (mg/dL)	1.64±1.04	
• ≥2 mg/dL	17.8%	(5)
Ankle brachial index	0.74±0.25	
Peripheral arterial disease	60.7%	(17)
o Revascularization	46.4%	(13)
Minor amputation before starting ulcer's treatment	57.1%	16
Coronary artery disease*	42.8%	(12)
o Medical	17.8%	(5)
o Ptca/stent	7.1%	(2)
o Coronary artery bypass graft	17.8%	(5)
Serebrovascular disease	10.7%	(3)
The patients requiring hemodialysis during treatment	10.7%	(3)
Comorbidity (totally)	82.1%	(23)

*Only clinically stable patients were included in the study; SD: Standard deviation; UG: Ulcer grade; IG: Infection grade.

TABLE 2: Outcomes of wound specimens cultures.

Parameter	Features	%	Number
Culture's results			
● Positive		87.2%	(23)
o Single microorganism		35.7%	(10)
o Mix microorganism (2 or more)		46.4%	(13)
● Negative		17.8%	(5)
Number of types of microorganism growing in the cultures.			(14)
Total number of microorganism growing in the cultures.			(47)
Type of causative microorganism			
● Enterobacterales (O)		42.5%	(20)
o Entererobacteriaceae (F)		21.2%	(10)
■ Escherichia (G)			
• E. Coli (S)	Gram -, A	19.1%	(9)
■ Enterobacter (G)			
• E. Clocea (S)	Gram -, FA	2.1%	(1)
o Morganellaceae (F)		21.2%	(10)
■ Proteus (G)			
• P. Mirabilis (S)	Gram -, FA	17%	(8)
■ Morganella			
• M. Morganii (S)	Gram -, A	4.2%	(2)
● Pseudomonadales (O)		31.9%	(15)
o Moraxallacea (F)		21.2%	(10)
■ Acinetobacter (G)			
• A. Baumannii (S)	Gram -, A	12.7%	(6)
• A. Calcoaceticus (S)	Gram -, A	8.5%	(4)
o Pseudomonadaceae (F)		10.6%	(5)
■ Pseudomonas (G)			
• P. Aeruginosa (S)	Gram -, A	10.6%	(5)
● Lactobacillales (O)		14.9%	(7)
o Enterococcaceae (F)		12.7%	(6)
■ Enterococcus (G)			
• E. Faecium (S)	Gram +, FA	6.4%	(3)
• E. Faecalis (S)	Gram +, FA	4.2%	(2)
• E. Hirae (S)	Gram +, FA	2.1%	(1)
o Streptococcaceae (F)		2.1%	(1)
■ Streptococcus (G)			
• S. Pyogenes (GAS) (S)	Gram +, A	2.1%(1)	
● Bacillales (O)		8.5%	(4)
o Staphylococcaceae (F)		8.5%	(4)
■ Staphylococcus (G)			
• S. Aureus	Gram +, FA	6.4%	(3)
• Coagulase-S. (S)	Gram +, FA	2.1%	(1)
● Others		2.1%	(1)
• Corynebacterium striatum (S)	Gram +, A	2.1%	(1)

O: Order; F: Family; G: Genus; S: Species; GAS: Group A streptococcus.

tures were sterile in 5 (17.8%) of the patients. The most frequently isolated microorganisms were gram negative, aerobic or facultative anaerobic bacterias in rate of 74.4%. Primarly, *Escherichia coli, Proteus mirabilis, Acinetobacter baumannii, Pseudomonas aeruginosa* species were seen in rate of 19.1%, 17%, 12.7%, 10.6% respectively. More detailed information can be seen in Table 2.

The new granulation tissue over the wound was present in 26 (92.8%) patients at the end of the h-EGF treatment. Complete wound healing was achieved in 10 (35.7%) patients, complete wound closure was in 3 (10.7%) patients (Table 3).

There was a significant difference between the average ulcer size before and after h-EGF treatment ($p<0.001$) (Table 3). Mean Δ value and Δ ratio are also given in Table 3.

Comparative photos of ulcers before and after treatment can be seen in Figure 1 and Figure 2.

Recurrent ulcers were seen in some patients whose ulcer healed, and 3 patients have been done major lower limb amputation during the follow-up. Details about this can be found in Table 3.

The most common side effect of h-EGF administration were tremor (32.1%).The others are given in

TABLE 3: Change in wound size and wound healing after h-EGF treatment, the rates of recurrence after recovery and major amputation in follow up period.

Parameters	$\bar{X}$±SD, %	p value
o Mean ulcer's size (cm^2)		0.001
• Pre-treatment	23.9±18.5	
• Post-treatment	6.5±4.8	
o Ulcer's size difference (Δ value)	17.4 cm^2	
o Wound healing rate (Δ ratio)	64%	
	% value	**Number of patient (n)**
o Wound healing		
■ HG		
• HG0 (no response)	7.14%	(2)
• HG1 (minimal response)	7.14%	(2)
• HG2 (partial reponse)	50%	(14)
• HG3 (complete response)	35.71%	(10)
■ Complete wound closure	10.71%	(3)
o Recurrent ulcer*	25%	(7)
o Major amputation*†	10.71%	(3)

*Within 12 months after treatment; †Two of the 3 patients had peripheral arterial disease who could not be revascularized, the other patient had acute renal failure requiring hemodialysis; h-EGF: Human epidermal growth factor; SD: Standard deviation; HG: Healing grade.

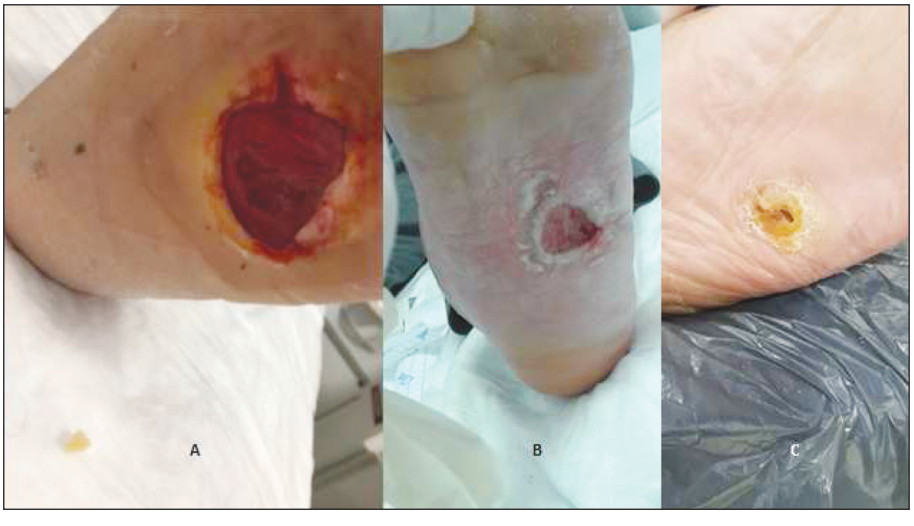

FIGURE 1: Comparative photos of ulcers for patient-1.
A) Just after debrided, **B)** During treatment, **C)** After treatment.

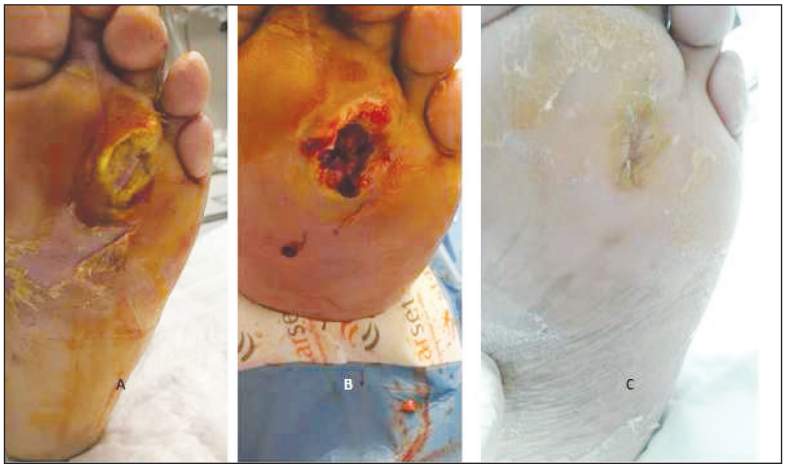

FIGURE 2: Comparative photos of ulcers for patient-2.
A) Before treatment **B)** Just after debrided **C)** After treatment.

Table 4. The treatment was not interrupted in any patient because of side effect.

Statistical relationship between HG and categorical variables can be seen in Table 5. There was a significant relationship between HG and revascularization (p=0.034). Although the average age was greater in the patients with no or less wound healing, there was no significant relationship between age and HG. Patients with complete wound healing had higher ABI value than the patients with less wound healing. However, there was no significant relationship between this 2 parameters. Statistical relationship between HG and continuous variables can be seen in Table 5.

Seventeen patients had peripheral arterial disease. Because of the contribution of revascularization to wound healing, 13 of these patients underwent revascularization procedures before starting h-EGF treatment. Therefore, there was a statistically significant relationship between peripheral arterial disease and revascularization (p<0.001). In addition, due to lower limb ischemia caused by peripheral arterial disease, there was a statistically significant relationship between peripheral arterial disease and ABI value (p=0.023).

There was a statistically significant relationship between age and minor amputation (p=0.043).

A statistically significant relationship was detected between ulcer grade and duration of DM (p<0.001). While there was no statistically significant relationship between HbA1c at admission and many variables, there was a significant relationship between HbA1c and the post treatment ulcer size (p=0.046).

The higher the ulcer grade, the greater the post treatment ulcer size (p=0.013).

As a result of the treatment, a statistically significant correlation was found between HG and post-treatment ulcer size, Δ value and Δ ratio. Wound healing rate was better in female (p=0.048) and in patient with better foot blood flow (higher ABI value,

TABLE 4: Adverse events of h-EGF applications.

Adverse events	%	Number
• Yes (+)	42.8%	(12)
• No (-)	57.2%	(16)
Non-serious*		
• Tremor	32.1%	9
• Nausea	10.7%	3
• Chills	7.1%	2
• Pain	14.2%	4
• Burning sensation	10.7%	3
• Others		
o (Mild hypotension plus nausea)	3.5%	1
Serious		
• Dizziness†	3.5%	1

*Non-serious adverse events in 12 patients. Some patients had 2 or more side effects; †Antihistamine were sufficient to stop dizziness and given prophylactically thereafter; h-EGF: Human epidermal growth factor.

TABLE 5: Statistical relationship between HG and variables.

Parameters	HG				p value
Categorical variables	HG 0	HG 1	HG 2	HG 3	
			Number of patients (n)		
• Gender					0.438
o Male	1	2	10	9	
o Female	1	0	4	1	
• Insulin use					0.581
o +	2	2	9	7	
o -	0	0	5	3	
• Number of ulcer					0.261
o 1	2	2	10	5	
o 2	0	0	4	2	
o 3	0	0	0	3	
• UG (Megitt-Wagner classification)					0.851
o I	0	0	4	3	
o II	1	0	4	3	
o III	1	2	5	4	
o IV	0	0	1	0	
• Minor amputation					0.336
o +	0	1	8	7	
o -	0	1	6	3	
• Peripheral arterial disease					0.534
o +	1	2	9	5	
o -	1	0	5	5	
• Revascularization					0.034*
o +	0	2	9	2	
o -	2	0	5	8	
Continuous variables			X±SD		
• Age	73.5±4.5	69.5±5.5	58.7±3.2	62.6±3	0.386
• Diabetes mellitus duration (years)	6±2	11.5±3.5	8.1±1.4	6.9±1.7	0.254
• Hemoglobin A1c (% value)	5.9±0.1	8.9±1.5	8.4±0.4	9.2±0.6	0.093†
• Ulcer size (pre-treatment, cm^2)	22±2.8	30±25.4	17.3±10.9	34.5±25.2	0.266
• ABI (pre-treatment, %)	0.8	0.70±0.2	0.73±0.08	0.75±0.07	0.888

*Statistically significant; †Statistically not significant, but close to significant; HG: Healing grade; SD: Standard deviation; UG: Ulcer grade; ABI: Ankle brachial index.

p=0.02). More details about the relationship between variables can be found in Table 6.

DISCUSSION

CDFU and its complications are increasing day by day and cause life-threatening consequences in diabetic patients.[1-4] Amputation is sometimes unavoidable for the patients with CDFU.[16-19] Unfortunately, the mortality rate increases considerably after amputation.[5] Because of this, optimal management of CDFUs and amputation prevention strategies has a vital importance for such patients.

The healing process in CDFU is hampered by some factors such as chronic inflammation, defects in fibroblast function, poor angiogenesis, and lack of cell migration.[20] EGF play a main role in wound healing. But, bioavailability of EGF is impaired in patients with CDFU.[4,8] Therefore, h-EGF is used to accelerate wound healing in CDFUs.

The effectiveness of EGF has been studied at different concentrations and by different administration routes. In CDFU, administration of h-EGF in addition to standard medical treatment is able to increase healing rates and to prevent foot amputations.[9,10,21,22]

TABLE 6: Correlation analysis between parameters.

Compared parameters	Correlation coefficient	p value
Age-minor amputation	+0.385	0.043*
Peripheral arterial disease-minor amputation	+0.338	0.079†
Peripheral arterial disease-UG	+0.232	0.235
Peripheral arterial disease-ABI	-0.429	0.023*
Peripheral arterial disease-revascularization	+0.602	0.001*
Revascularization-minor amputation	+0.517	0.005*
Duration of DM-UG	+0.689	<0.001*
Duration of DM-ulcer size (pre treatment)	+0.254	0.193
Duration of DM-HbA1c	+0.129	0.514
Duration of DM-insulin use	+0.425	0.024*
UG-insulin use	-0.458	0.014*
HbA1c-insulin	+0.064	0.747
HbA1c-ABI	-0.263	0.176
HbA1c-UG	+0.138	0.484
HbA1c-ulcer size (Pre treatment)	+0.116	0.558
HbA1c-peripheral arterial disease	+0.109	0.582
HbA1c-minor amputation	+0.063	0.752
HbA1c-revascularization	+0.231	0.237
Ulcer size (pre treatment)-age	+0.268	0.169
Ulcer size (pre treatment)-ABI	-0.359	0.06†
Ulcer size (pre treatment)-UG	+0.359	0.061†
UG-ABI	+0.044	0.825
Ulcer size (post-treatment)-age	+0.268	0.068†
Ulcer size (post-treatment)-HbA1c	-0.380	0.046*
Ulcer size (post-treatment)-ABI	+0.119	0.546
Ulcer size (post-treatment)-UG	+0.462	0.013*
Ulcer size (post-treatment)-revascularization	-0.432	0.022*
Ulcer size (post-treatment)-HG	-0.669	<0.001*
Δ value-HG	+0.474	0.011*
Δ value-UG	-0.226	0.247
Δ value-ABI	+0.438	0.02*
Δ value-peripheral arterial disease	+0.168	0.393
Δ value-revascularization	+0.147	0.457
Δ value-minor amputation	+0.304	0.115
Δ value-HbA1c	-0.266	0.172
Δ value-age	-0.044	0.823
Δ value-gender	+0.254	0.193
Δ ratio-HG	+0.806	<0.001*
Δ ratio-UG	+0.172	0.380
Δ ratio-ABI	+0.267	0.170
Δ ratio-peripheral arterial disease	+0.050	0.801
Δ ratio-revascularization	-0.222	0.257
Δ ratio-minor amputation	+0.143	0.468
Δ ratio-HbA1c	-0.308	0.111
Δ ratio-age	0.125	0.526
Δ ratio-gender	+0.377	0.048*

*Statistically significant relationship; †Statistically insignificant relationship although p value is close to 0.05; UG: Ulcer grade; ABI: Ankle brachial index; HbA1c: Hemoglobin A1c; HG: Healing grade.

Adequate amount of active growth factor need to be present in deeper layers of the wound to provide adequate healing. Since the diffusion of the active substance in topical EGF applications is affected by necrotic tissue, sepsis, inflammation and wound proteases, sufficient amount of active substance in the deeper layers of the wound can only be achieved by intralesional EGF applications.[20,23,24]

Administration of 75 µg intralesional EGF 3 times a week has been given satisfactory results in Stage III-IV ulcers.[20] In the study of Fernández-Montequín et al, complete wound healing at 5 weeks was accomplished in 73.9% and 50.0% of patients treated with 75 and 25 µg of EGF, respectively.[13] These results were also comfirmed by an extension of the study.[25] Complete wound closure achieved in 77.4%, 52.1%, and 56.2% of patients treated with 75 µg EGF, 25 µg EGF, and placebo, respectively, whereas time to complete wound closure was significantly faster in the 75-µg group.[25] The dose of 75 µg EGF has been consistently shown to achieve higher healing rates and shorter time to heal than the dose of 25 µg EGF and placebo.[25] For this reason, we also used 75 µg intra and peri-lesional h-EGF 3 times a week in alternate days during 5 weeks. In a cochrane systematic review, Marti-Carvajal et al. reported that growth factor would increase the rate of complete wound healing in patients with CDFU.[26]

In our study, the new granulation tissue over the wound was occured in 92.8% of the patients. Partial and complete wound healing was occured in 85.7% of the patients. Complete wound healing was achieved in 35.7%, complete wound closure was seen in 10.7% of the patients. The average value of wound healing rate reached at the end of 5 weeks in h-EGF treatment was 64%. We evaluate these rates as highly successful. Although there was a significant reduction in wound size at the end of the treatment, compared to the results of other studies, our complete wound healing rates were lower. This situation was attributed to the fact that our average wound size was larger and our minor amputation and sharp debridement rates were higher than the other studies reported in literature. Also, our patients were accompanied by a high rate of peripheral arterial disease. In addition, the duration of h-EGF treatment was lower than the

other studies. When evaluating the response to treatment, the main matter is not how many the complete wound healing was, but to what extent the wound has shrunk in a certain period of time. Briefly, wound healing rate is more important than complete wound healing. Even in the studies where 8 weeks of intralesional h-EGF treatment were applied, the complete wound healing process was up to 14 weeks.[25] Whereas, our complete wound healing rates were the results of 5 weeks of h-EGF treatment. Our patients who completed h-EGF treatment had higher rates of complete wound healing in 14 weeks.

The side effects of h-EGF administration are predominantly mild to moderate (65.6% mild, 28.6% moderate, and only 3.7% severe). Pain and burning sensation at the application site have been reported most frequently.[7] The most common side effect in our study was tremor. The pain was rarely seen due to possible neuropathy. The treatment was not interrupted because of any side effect.

In hyperglycemia induced by DM, decreased expression of genes regulating angiogenesis, namely vascular EGF and angiopoietin-1 decrease vascularization, increased expression of proinflammatory cytokines such as tumor necrosis factor-α, interleukin (IL)-6, IL-1α increase chronic inflammation and oxidative stress, an excess of proteases released by active neutrophils reduce bioavailability of EGF.[27]

Bioavailability of EGF is also impaired secondary to non-enzymatic glycation of growth factors as a result of hyperglycemia in patients with CDFU.[4,8] Therefore, to maintain well regulated blood sugar level during treatment is essential to increase the bioavailability of EGF and to accelerate wound healing. For this reason, blood sugar regulation is one of the most important and best-known topics to ensure good wound healing. However, in patients with CDFU, blood sugar levels are often uncontrollable or in poorly regulated condition. In our study, wound healing was paradoxically better in patients with higher HbA1c levels at hospital admission. This indicates that blood glucose regulation was not good before starting the treatment in these patients, but blood glucose level was adjusted well after hospitalization and wound healing was accelerated. Of course, well-regulated blood sugar level undoubtedly contributes significantly to wound healing.

In our study, there was a significant relationship between ulcer grade and duration of DM. This can be explained by an increase in chronic complications such as peripheral arterial disease and neuropathy as the duration of DM prolongs.[28]

Unlike other studies, the rate of peripheral arterial disease was higher in our study. In some studies even patients with peripheral arterial disease or ABI value below 0.7 has been excluded from the study.[10] Peripheral arterial disease was present in 17 (60.7%) of our patients. Revascularization procedures were performed in 13 (46.4%) of these patients. Our patients consisted of more complex patients and at the same time also had a higher ulcer grade compared to other studies.[20] In our study, there was no significant relationship between the presence of peripheral arterial disease and wound healing. However, wound healing was better in patients with higher ABI value. Considering these results, it is clear that revascularization procedures are well implemented in our patients and successful revascularization contributes significantly to wound healing.

In the majority of our patients, the wounds were observed to be infected and microbiological reproduction was present in the culture specimens taken from the ulcer. Fourteen different types of bacteria (47 causative bacterias) in 23 (87.2%) patients were isolated. The most frequently isolated microorganisms were gram negative, aerobic or facultative anaerobic bacterias. Primarily, *E. coli, P. mirabilis, A. baumannii, P. aeruginosa* species were seen in order of frequency. This bacterias being isolated are the microorganisms that are responsible for nosocomial infections and have a high rate of antibiotic resistance. The high bacterial diversity is likely related to the development of microbial biofilm that irreversibly attaches to the wound matrix.[29] Researchers have reported that bacterial biofilms and proteolytic enzymes on the wound surface inactivate h-EGF.[30,31] This situation delays wound healing. Therefore, infected foot wounds should be treated with curettage, effective and repeated debridement, good wound care and appropriate antibiotics.

Wound healing rate was significantly better in our female patients than in men. Studies reveal that estrogen can improve the age-related impairment in healing in both men and women, while androgens affect cutaneous wound healing negatively.[32]

Despite the treatment in patients with CDFU, ulcer recurrence and amputation are unavoidable complications. The most common cause of non-traumatic amputation is still CDFU. This leads to significant disability, morbidity and mortality if not treated well.[15,33] It has been reported that intralesional h-EGF is an effective and safe treatment method that protects the extremity from major amputation in patients with CDFU for whom known treatment methods have failed previously.[28] Gonzalez-Acosta et al. reported that intralesional EGF added to standard therapy was associated with a lower rate of major amputation (26.7% vs 8.3%) than standard therapy alone.[19] In the study of Kahraman et al., the long-term results of 34 CDFU patients who received an average of 18 doses of intralesional h-EGF were reported.[16] During the 5 year follow-up, 4 patients died secondary to diabetic complications. While 27 of the remaining 29 patients did not have ulcer recurrence [93.1% (27/29, no recurrent ulcer) vs 6.9% (2/29, recurrent ulcer +)], only 1 patient (3.4%) underwent minor amputation due to ischemic necrosis. In another study by Dumantepe et al., it was reported that complete wound closure was achieved in 16 (94.1%) of the patients, and ulcer recurrence was observed in only one patient (5.8%) in 1-year follow-up.[7] Amputation was required in our 3 (10.7%) patients at 1-year follow-up. In addition, it has been reported that 34% of patients with CDFU develop recurrent ulcers within one year.[16] Ulcer recurrence was observed in our 7 (25%) patients, at 1-year follow-up. The ulcer recurrence and amputation rates observed in our patients at 1-year follow-up were better than the patients being implemented standard treatment alone, indicating that h-EGF is effective in treatment.[34,35]

Despite many major advances in health care system for diabetic patients and the developments in new therapeutic methods, foot wounds continue to be a heavy toll over the quality of life of diabetic patients.

CONCLUSION

h-EGF in patients with CDFU is a good adjuvant treatment option to accelerate wound healing and to reduce ulcer recurrence and amputation according to standard medical treatments.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Şule Gökşin, Mustafa Çağdaş Çayır;* ***Design:*** *Şule Gökşin;* ***Control/Supervision:*** *Şule Gökşin;* ***Data Collection and/or Processing:*** *Şule Gökşin;* ***Analysis and/or Interpretation:*** *Şule Gökşin;* ***Literature Review:*** *Şule Gökşin;* ***Writing the Article:*** *Şule Gökşin;* ***Critical Review:*** *Şule Gökşin;* ***References and Fundings:*** *Şule Gökşin, Mustafa Çağdaş Çayır;* ***Materials:*** *Şule Gökşin, Mustafa Çağdaş Çayır.*

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