Evaluation of the efficacy and safety of topical epidermal growth factor Regen-D® in diabetic foot wounds: a randomised, parallel group phase III study

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Key words

- Diabetic foot
- Topical epidermal growth factor
- Wound treatment

Article points

1. Epidermal growth factor (EGF) plays an important role in wound healing.
2. This study evaluated a topical EGF gel for treating diabetic foot wounds.
3. Patients were randomised to receive standard therapy or standard care plus the EGF gel.
4. At the end of the study, wound closure rates were significantly higher in the EGF group.

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The authors designed a study to evaluate the safety and efficacy of topical epidermal growth factor (EGF) Regen-D® (Bharat Biotech), applied as a gel containing 150 µg total recombinant human EGF in the treatment of diabetic foot wounds. We enrolled 162 patients in a randomised, phase III prospective study. The patients were randomised into two groups: a control group, who received standard wound care, and a study group, who applied topical EGF to the wound twice a day, in addition to standard care. At the 4 month evaluation, the number of patients showing granulation and the number of healed patients were significantly higher in the study group than in the control group (*P*<0.001). In the total evaluation of all patients, the rate of wound closure was significantly higher in the study group (p<0.001). Our findings indicate that topical Regen-D® may be a beneficial agent for treating diabetic foot wounds.

The increasing incidence of diabetes is an important health problem worldwide. In Turkey, the prevalence of diabetes in adults has increased from 7.2% to 13.7% over the last 5 years (Satman et al, 2013). One of the most common and serious complications of diabetes is foot ulcers, with 12–25% of people with diabetes visiting healthcare facilities for foot problems during their lifetime (Lipsky, 2004; Cavanagh et al, 2005; Singh et al, 2005). Although the pathogenesis of diabetic foot ulcers is complicated, the key risk factors are peripheral neuropathy and vasculopathy related to hyperglycaemia (Ertugrul, 2010). In most populations, diabetic foot ulcers are completely neuropathic in about 35% of cases, predominantly ischaemic in 15%, or combined neuroischaemic in 50% (Armstrong et al, 2011).

Wound healing involves three basic processes: inflammation, proliferation and maturation (Singer and Clark, 1999). Long-term hyperglycaemia in people with diabetes causes non-enzymatic glycolysis of proteins, resulting in the formation of advanced glycation end-products. These products bind to macrophages and cause low levels of inflammation by inducing the release of various cytokines, mainly tumour necrosis factor alpha, and by increasing the levels of reactive oxygen species (Esposito et al, 2002; Duncan et al, 2003). This inflammation leads to apoptosis of fibroblast and endothelial cells found in diabetic foot ulcers due to mitochondrial damage (Acosta et al, 2008). Aberrations in other factors regulating wound healing, including production of extracellular matrix, growth factors and the receptors for these factors, result in the suppression of granulation tissue formation (Acosta et al, 2008). With diabetes, these pathological processes cause persistent wound inflammation, preventing progression to the proliferation stage, with its fibroblast activity, leading to the wound becoming chronic (Acosta et al, 2008). The elucidation of these processes has led to the development of new treatment modalities that use growth factors for the treatment of diabetic foot wounds.

Epidermal growth factor (EGF) is a 53-amino acid polypeptide that binds to cell membrane receptors

and exhibits cellular mitogenic activity (Cohen, 1962; Tsang et al, 2003). Physiological stimulation of EGF receptors leads to migration of endothelial cells and fibroblasts to the area of the ulcer, granulation, accumulation of extracellular matrix, maturation and de novo angiogenesis; wound contraction by myofibroblast activation and proliferation; and migration and proliferation of epithelial cells, leading to closure of the injured area (Berlanga-Acosta, 2011). In addition, EGF plays an important role in early-phase wound healing via keratinocyte proliferation and migration (Gibbs et al, 2000).

Currently, EGF is available for topical use in either a gel or a spray. In this study, the authors aimed to evaluate the efficacy and safety of topical EGF Regen-D® (Bharat Biotech), a gel containing 150 µg total recombinant human EGF, in the treatment of diabetic foot wounds.

Material and methods

Approval for this prospective, randomised, parallel group Phase III study was obtained both from the Clinical Research Ethics Committee of Dokuz Eylul University on 30 June 2016 (No. 2016/12–5) and Republic of Turkey, Ministry of Health, Turkish Drug and Medical Devices Agency on 08 August, 2016 (No. 93189304-514.03.01–106352). From January 1, 2017 to October 1, 2017, the authors enrolled 162 patients from 10 centres. The study finished on January 18, 2018, at the end of the follow-up period of the last patient.

The authors obtained written informed consent from all the patients enrolled in the study. For each enrolled patient, the authors collected age, sex, type and duration of diabetes, HbA_{1c}, history of hospitalisation and hypertension, lower extremity amputation, lower extremity infection, peripheral neuropathy, peripheral vascular disease, wound size and location, and use of negative pressure wound therapy, hyperbaric oxygen therapy or active wound dressing. Foot wounds were classified according to the Wagner classification (Wagner, 1981).

Study aims

The primary aim of this study was to investigate the efficacy of topical EGF formulation (Regen-D) on the development of granulation tissue or wound closure in people with diabetes with Wagner grade 1 or grade 2 foot ulcers.

Secondary aims of the study included the following:

- Compare the mean change in wound size between the two groups at 2 months and 4 months compared to baseline
- Evaluate the number of patients with complete wound healing compared to baseline
- Assess the safety of EGF in patients with a diabetic foot wound.

Study design

This was a randomised, controlled, parallel group, multicentre study evaluating the results of treatment with the recombinant human EGF formulation Regen-D compared with the standard wound care treatment. Patients were enrolled during a 10-month period. They underwent treatment for 2 months and then there was a 2-month follow-up period. After obtaining written informed consent, the study centres sent the completed randomisation form to a staff member who randomised the patients into two groups so that members of the study centres who had any contact with the study population could not influence the allocation. The staff member assigned equal numbers to each group.

The control group received standard wound care treatment based on their specific needs, including wound care dressing, negative pressure wound therapy, hyperbaric oxygen therapy, sharp debridement, revascularisation and offloading.

The study group received the same standard wound care as the control group, but also had topical EGF (Regen-D) applied to the wound twice a day.

Patients were evaluated at month one and month two of the treatment period and then 2 months after the termination of the treatment. The authors assessed the wound size (length, width and depth) development of the granulation tissue and percentage of wound closure. The authors also assessed whether there was any recurrence of the wound in patients with previously closed wounds.

Regen-D

Regen-D contains 150 mg recombinant human EGF as the bioactive compound, along with sodium propylparaben, mannitol and glycerol as the adjuvant components of the drug. Animal studies have shown that recombinant human EGF has

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wound healing effect (Boonstra et al, 1995; Berlanga et al, 2002; Sinha et al, 2003). The adjuvant components do not have any treatment affects. Propylparaben sodium is used as an antimicrobial or antifungal preservative, mannitol is a diluent and tonicity agent, and glycerol is used as a thickening agent in topical formulations (Armstrong, 2009; Forbes and Hulse, 2009; Pople and Singh, 2009). Only EGF has a treatment effect. This commercial formulation is packed in a 15 g tube. It is applied in a thin layer to cover the wound surface every 12 hours for 8 weeks. Because of the protein content of the product, it was stored at 4–8°C during transportation and application.

Inclusion criteria

Only patients who met all of the following criteria were eligible for inclusion in the study:

- Written informed consent form provided before evaluations
- Over 18 years old
- Type 1 or type 2 diabetes and foot ulcer
- Grade 1 or 2 foot wounds, according to the Wagner classification
- No wound infection (confirmed by negative culture) or an infected wound where infection was being managed
- The wound surface was clean and the necrosis had been removed.

Exclusion criteria

Patients who met any of the following criteria were excluded from this study:

- Under 18 years old
- No diabetes diagnosis
- Grade 3 or higher foot wounds
- Necrotic or infected wound
- Uncontrolled diabetes, renal insufficiency or insufficiently controlled hypertension
- Life-threatening or serious heart, liver or kidney failure, or severe immunologic disorder
- Use of another study drug for a period shorter than five half-lives, or <30 days since the completion of the anticipated pharmacodynamic effect (whichever was longer)
- Known hypersensitivity to any of the study drugs or drugs with similar chemical groups
- Malignant tumours of any organ system (except for skin basal cell carcinoma), treated or untreated, with or without local recurrence or metastasis in the last 5 years
- Pregnant or breastfeeding
- Women of childbearing age, if they do not use contraception during the duration of the study.

Statistical analysis

The normal distribution of continuous variables was evaluated using the Kolmogorov–Smirnov test. Descriptive statistics are presented as median (interquartile range [25th–75th percentile]) since none of the continuous variables showed normal distribution. Mann–Whitney U test was used for between-group comparisons of continuous variables. The descriptive statistics of categorical variables are expressed as number (%), and the chi-square test was used for between-group comparisons. A *P*-value <0.05 was considered statistically significant.

Results

A total of 162 patients from 10 sites were enrolled in the study between January 1, 2017 and October 1, 2017, with 81 patients each in the control (standard treatment) and study groups (Regen-D treatment in addition to the standard treatment). Of these, 145 patients completed the study (control group *n*=69, study group *n*=76). There were no statistically significant differences between the two groups in terms of demographic data (*Table 1*), except for the duration of the diabetic foot wound, which was significantly longer in the study group (*P*=0.023).

To evaluate the efficacy of EGF, the mean change in granulation tissue development and wound closure in patients with diabetic foot wounds was measured. As shown in *Table 2*, the authors found no significant difference between the control and study groups in terms of granulation development and wound closure at the end of the first month (control group *n*=66, study group *n*=75) or the second month (control group *n*=50, study group *n*=63).

At the 4-month evaluation, 56 patients from the control group and 60 patients from the study group were evaluated. The others patients did not attend the final evaluation. The number of patients who did not develop granulation was significantly higher in the control group than in the study group, while the number of patients showing granulation and the number of healed patients were significantly higher in the study group than in the control group.

Table 1. Patient demographic data.

	Control group (n=69)	Study group (n=76)	p-value
Sex (F/M)	17/52	22/54	0.559
Age (years)	60 (54–66)	61 (53–70)	0.846
Type of diabetes Type 1 Type 2	 5 (7.2) 64 (92.8)	 6 (7.9) 70 (92.1)	0.883
Duration of diabetes (years)	15 (5–24)	15 (10–21)	0.368
Duration of the diabetic wound (days)	90 (30–180)	120 (60–240)	0.023*
History of hypertension	36 (52.2)	43 (56.6)	0.595
History of anaemia	17 (24.6)	11 (14.5)	0.122
History of congestive heart failure	9 (13.0)	6 (7.9)	0.309
Antidiabetics in use Insulin Oral antidiabetics	 54 (78.3) 15 (21.7)	 57 (76.0) 18 (24.0)	0.747
Smoking history (current smoker)	20 (29.0)	25 (32.9)	0.611
History of hospitalisation due to diabetes	35 (50.7)	35 (46.1)	0.574
History of recurrent foot wound	40 (58.0)	51 (67.1)	0.256
Osteomyelitis in previous foot infection	23 (33.3)	20 (26.3)	0.355
History of previous debridement	42 (60.9)	39 (51.3)	0.247
History of previous amputation	33 (47.8)	26 (34.2)	0.096
History of previous vascular intervention	28 (40.6)	34 (44.7)	0.613
History of renal failure	9 (13.0)	9 (11.8)	0.827
Current dialysis treatment	4 (5.8)	3 (3.9)	0.604
Peripheral vascular disease Stage 1 Stage 2 Stage 3	 23 (33.3) 40 (58.0) 6 (8.7)	 30 (39.5) 45 (59.2) 1 (1.3)	0.107
HbA_{1c} (%)	7.6 (6.5–9.7)	7.6 (6.5–8.9)	0.989
Leukocyte count/mm^3	7.9 (6.5–9.3)	7.8 (6.8–9.6)	0.751
C–reactive protein (mg/dl)	5.9 (3.2–13.0)	4.1 (2.0–13.2)	0.180
Erythrocyte sedimentation rate (mm/h)	33 (17–62)	39 (22–59)	0.812
Data are presented as [median (25%–75%)] or as number (%). *Statistically significant			

In the total evaluation of all patients, the rate of unresponsiveness in the control group was higher than in the study group, and the rate of wound closure was significantly higher in the study group (*Table 2*).

The wound width was a secondary objective of the study. At baseline, there was no significant difference between the study group and the control group. The wound widths were significantly lower in the study group at the month 1, month 2 and end-of-study evaluations (*Table 3*).

No adverse events were reported in the treatment period other than pain at the application site (*n*=3 in the EGF group). Treatment was not interrupted in either group for any cause.

Discussion

In this study, the authors evaluated the efficacy and the safety of 15 g tube of Regen-D gel containing 150 µg of recombinant human EGF in patients with grade 1 or 2 diabetic foot wounds according to the Wagner classification, in comparison with patients receiving standard treatment.

Infection control and treatment of the wound

Table 2. Results of patient evaluations.

Month 1 evaluation	**Control group (*n*=66)**	**Study group (*n*=76)**	***P*-value**
No granulation	5 (7.6)	1 (1.3)	0.152
Existence of granulation	55 (83.3)	60 (80.0)	0.774
Wound closure	6 (9.1)	14 (18.7)	0.165
Month 2 evaluation	**Control group (*n*=50)**	**Study group (*n*=63)**	***P*-value**
No granulation	4 (8.0)	4 (6.3)	0.984
Existence of granulation	28 (56.0)	30 (47.6)	0.485
Wound closure	18 (36.0)	29 (46.0)	0.379
Month 4 evaluation	**Control group (*n*=56)**	**Study group (*n*=60)**	***P*-value**
No granulation	7 (12.5)	2 (3.3)	0.064
Existence of granulation	32 (57.1)	19 (31.7)	<0.001
Wound closure	17 (30.4)	39 (65.0)	0.006
End of study evaluation	**Control group (*n*=69)**	**Study group (*n*=76)**	***P*-value**
Wound	31 (44.9)	20 (26.3)	0.019
Wound closure	27 (39.1)	55 (72.4)	<0.001
No response	9 (13.0)	1 (1.3)	0.005
Recurrence	2 (2.9)	0 (0.0)	0.136

become more difficult in grade 3 and higher foot wounds, so wound healing before this stage is critical. In particular, superficial wound infections can spread to deep tissue and foot bones, resulting in minor (only foot area) or major (at a level above the ankle) amputations. Thus, if patients are treated appropriately in the early grades and wound closure is achieved, minor or major amputations can be avoided.

In a phase II trial conducted in India, Regen-D showed a statistically better (86% to 50%) rate of wound closure in patients with grade 1 and 2 foot wounds according to the Wagner classification (Viswanathan et al, 2006). In the same study, topical EGF shortened the treatment duration by 9 weeks. Regen-D treatment was compared with standard wound care treatment instead of placebo, and the wound closure rate with Regen-D treatment (72.4%) was significantly higher than with standard treatment (39.1%; $P<0.001$).

In a Regen-D phase IV study involving 135 patients with diabetic foot wounds, wound healing was observed in 96.3% of patients at the end of 6 weeks (Mohan, 2007). Wound healing was defined as wound closure and wound granulation

In the present study, the rate of wound closure and wound granulation was found to be 93.6% at 2 months, but there was no significant difference compared to the standard treatment. However, this rate had reached to 96.7% at 4 months, with a statistically significant difference compared to the standard treatment.

In a meta-analysis of 26 studies involving 2,088 patients, Sridharan and Sivaramakrishnan (2018) evaluated the efficacy of topical growth factors together with the comparison of standard treatment for diabetic foot ulcers. In this multivariate analysis, the relative odds ratio for the human recombinant topical EGF was 5.72, for platelet-rich plasma 2.65 and for human recombinant platelet-derived growth factor 1.97. The authors concluded that, in early-grade diabetic foot wounds, topical EGF therapy is more efficacious than standard treatment or other growth factors. Notably, the common feature of all these studies is the benefit of topical EGF in early-grade foot wounds (Wagner grade 1 and 2).

In the present study, no side-effects other than pain were observed in both the study and the standard groups. Only three patients in the standard group and three from the study group

Table 3. Between-group comparisons of wound surface widths.

	Control group	Study group	*P*-value
Beginning of the treatment	6.91 (3.27–14.92)	5.56 (2.10–11.17)	0.063
First month	5.04 (2.44–14.49)	1.78 (0.61–6.64)	<0.001
Second month	1.61 (0.74–4.43)	0.76 (0.17–1.62)	0.003
End of the study	0.90 (0.33–4.45)	0.11 (0.00–0.89)	<0.001
Data are presented as median (interquartile range [25th–75th percentile])			

reported pain. In both groups, there was no need for discontinuation of treatment because of side effects.

A possible limitation of this study is the difference between the number of patients at the beginning and at the end of the study. At the beginning of the study, 162 patients (control group *n*=81, study group *n*=81) were enrolled, while 145 patients completed the study (control group *n*=69, study group *n*=76). This difference was because some of the patients did not return to the clinics to be evaluated.

EGF contributes to wound healing and can prevent diabetic foot wounds from becoming chronic. However, this is not the main treatment for diabetic foot wounds; the main therapies comprise treatment of infection, cleaning necrosis, and managing the circulation and blood flow to the wound in patients with vascular insufficiency. It is clear that supportive treatments alone will lead to significant cost increases and will not be beneficial to patients. The authors' findings indicate that Regen-D is a beneficial drug in the treatment of early-grade foot wounds in people with diabetes. ■

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Acosta JB, del Barco DG, Vera DC et al (2008) The pro-inflammatory environment in recalcitrant diabetic foot wounds. *Int Wound J* 5(4): 530–9

Armstrong DG, Cohen K, Courric S et al (2011) Diabetic foot ulcers and vascular insufficiency: our population has changed, but our methods have not. *J Diabetes Sci Technol* 5(6): 1591–5

Armstrong NA (2009) Mannitol. In: Rowe RC, Sheskey PJ, Quinn ME, eds. *Handbook of Pharmaceutical Excipients*. London and Chicago: Pharmaceutical Press and American Pharmacists Association, 424–8

Berlanga J, Prats P, Remirez D et al (2002) Prophylactic use of epidermal growth factor reduces ischemia/reperfusion intestinal damage. *Am J Pathol* 161(2): 373–9

Berlanga-Acosta J (2011) Diabetic lower extremity wounds: the rationale for growth factors-based infiltration treatment. *Int Wound J* 8(6): 612–20

Boonstra J, Rijken P, Humbel B (1995) The epidermal growth factor. *Cell Biol Int* 19(5): 413–30

Cavanagh PR, Lipsky BA, Bradbury AW, Botek G (2005) Treatment for diabetic foot ulcers. *Lancet* 366(9498): 1725–35

Cohen S (1962) Isolation of a mouse submaxillary gland protein accelerating incisor eruption and eyelid opening in the new-born animal. *J Biol Chem* 237: 1555–62

Duncan BB, Schmidt MI, Pankow JS et al (2003) Low-grade systemic inflammation and the development of type 2 diabetes: the Atherosclerosis Risk in Communities Study. *Diabetes* 52(7): 1799–805

Ertugrul MB (2010) Diyabetik ayak infeksiyonları. *Türkiye Klinikleri J Gen Surg-Special Topics* 3: 46–56 [in Turkish]

Esposito K, Nappo F, Marfella R et al (2002) Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: role of oxidative stress. *Circulation* 106(16): 2067–72

Forbes RT, Hulse WL (2009) Propylparaben sodium. In: Rowe RC, Sheskey PJ, Quinn ME, eds. *Handbook of Pharmaceutical Excipients*. London and Chicago: Pharmaceutical Press and American Pharmacists Association, 599–600

Gibbs S, Silva Pinto AN, Murli S et al (2000) Epidermal growth factor and keratinocyte growth factor differentially regulate epidermal migration, growth, and differentiation. *Wound Repair Regen* 8(3): 192–203

Lipsky BA (2004) Medical treatment of diabetic foot infections. *Clin Infect Dis* 39(Suppl 2): S104–14

Mohan VK (2007) Recombinant human epidermal growth factor (REGEN-D 150): Effect on healing of diabetic foot ulcers. *Diabetes Res Clin Pract* 78(3): 405–11

Pople P, Singh KK (2009) Glyceryl behenate. In: Rowe RC, Sheskey PJ, Quinn ME, eds. *Handbook of Pharmaceutical Excipients*. London and Chicago: Pharmaceutical Press and American Pharmacists Association, 288–90

Satman I, Omer B, Tutuncu Y et al (2013). Twelve-year trends in the prevalence and risk factors of diabetes and prediabetes in Turkish adults. *Eur J Epidemiol* 28(2): 169–80

Singer AJ, Clark RA (1999) Cutaneous wound healing. *N Engl J Med* 341(10): 738–46

Singh N, Armstrong DG, Lipsky BA (2005) Preventing foot ulcers in patients with diabetes. *JAMA* 293(2): 217–28

Sinha A, Nightingale J, West KP et al (2003) Epidermal growth factor enemas with oral mesalamine for mild-to-moderate left-sided ulcerative colitis or proctitis. *N Engl J Med* 349(4): 350–7

Sridharan K, Sivaramakrishnan G (2018) Growth factors for diabetic foot ulcers: mixed treatment comparison analysis of randomized clinical trials. *Br J Clin Pharmacol* 84:434–44

Tsang MW, Wong WK, Hung CS et al (2003) Human epidermal growth factor enhances healing of diabetic foot ulcers. *Diabetes Care* 26(6): 1856–61

Viswanathan V, Pendsey S, Sekar N, Murthy GSR (2006) A phase III study to evaluate the safety and efficacy of recombinant human epidermal growth factor (REGEN-D 150) in healing diabetic foot ulcers. *Wounds* 18:186–96

Wagner FW Jr (1981) The dysvascular foot: a system for diagnosis and treatment. *Foot Ankle* 2(2): 64–122