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Efficacy and safety of treatment of diabetic foot using intralesional infiltration of epidermal growth factor in Libyan patients

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ABSTRACT:

Previous studies have shown the effectiveness of intralesional infiltration of Epidermal Growth Factor in enhancing development of granulation tissue in high grades of diabetic foot. In our study the aim was to examine the clinical effects of the administration of Epidermal Growth Factor up to formation of > 75% of the area of the ulcer. 107 patients with neuropathic, ischemic or mixed diabetic foot ulcers or amputation residual ulcers. Patients were admitted to surgical department received standardized wound care. Average size of ulcer is 8 cm2, average number of infiltrations 4 (2;14). Adverse side effects were pain at site of infiltration, shivering, and chills. Our results showed that 80.3% of patients achieved 75% or more of functional granulation tissue in 2 weeks periods. Amputation was necessary in 1 case. Treatment of DFU with EGF is safe, effective and fast in formation of functional granulation tissue.

INTRODUCTION:

Prevalence of diabetes mellitus is rising worldwide and the number of diabetics are expected to reach from 171 million in 2000 to 366 million in 2030 (1). prevalence is estimated to reach 245,000 diabetics by the year 2030 in Libya (2).

People with diabetes are prone to develop lower extremity ulcerations and infections, both of which serve as major risk factors for limb amputation.

The annual incidence of DFU is more than 2% of diabetic patients (3) and increases if peripheral neuropathy is present.

At any given time, 2±3% of diabetics have an active foot ulcer, and 15% of all patients with diabetes will develop an ulcer during their lifetime(4).

The cost of diabetes foot damage is influenced by a number of factors including interventions to prevent foot ulcers strategies to heal these ulcers and shorten the time required for healing, management to prevent amputations in people who have developed ulcers, and care required by people with diabetes with a disability following an amputation.

In 2001, diabetes-related foot ulcers and amputations were estimated to cost US health-care payers 11 billion USD (5). Corresponding data from the UK estimated the total annual cost of diabetes-related foot complications at 456 million USD(6). Metabolic control, wound care, debridement, pressure relief, dressings and antibiotics are among the basic interventions for DFU management. New therapies are emerging to promote wound healing and to reduce amputations. These include recombinant human platelet- derived growth factor (7,8), low molecular weight heparin (9) and skin equivalents obtained by tissue engineering (10,11). However, these products have been only studied in relatively small, neuropathic-origin wounds.

Epidermal growth factor (EGF) plays an important role in the regulation of cell growth, proliferation and differentiation that can be useful to enhance wound healing (12). Topical application or subcutaneous injection of EGF produces skin keratinocytes and fibroblasts hyperplasia and hypertrophy, as well as corneous layer thickening (13,14). Exogenous EGF can also play a significant role in stimulating peripheral nerve regeneration (15). The availability of the growth factor on the deeper layers of the wound is an important issue to achieve an adequate efficacy. Intralesional injection of the growth factor could bring the active agent into the desired region (16).

OBJECTIVE

To study the healing effect of recombinant human epidermal growth factor (rhEGF) on diabetic foot ulcers in TMC.

METHODS AND MATERIALS

Patients (Type I and Type II Diabetes) admitted to Tripoli Medical Centre in general surgery department between (June and November) patients were of both genders with the diagnosis of diabetic foot problems. Patients were informed about the plan of management and treatment by intralesional infiltration of EGF and the possible adverse effects. For their participation all patients signed a well explained informed written consent. Patient were included if they had a Wagner's (2) grade 3 or 4 DFU that are more than 1 cm2. Exclusion criteria are pregnant and nursing mothers, patients with chronic uncontrolled debilitating diseases malignancies, psychiatric or neurological diseases. The patients have been evaluated on daily bases during their admission period and then followed up in outpatient department.

Laboratory tests were performed at baseline and thereafter whenever required, including blood cell count, haemoglobin, haematocrit, globular sedimentation rate, creatinine and aspartate aminotransferase, which were performed by routine clinical laboratory methods. Blood

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glucose was measured more frequently for the patients' metabolic control. Wound cultures were performed before and during therapy if necessary to monitor infections.

The patients received the standardized good wound care regimen and had a strict metabolic control and they were closely monitored.

Ulcers were cleansed daily using saline in case of contamination or infection. Sharp debridement was indicated whenever necessary to remove necrotic tissue. Saline-moisturized gauze dressing was used and the affected area was pressure off-loaded. Broad-spectrum antibiotics were used to treat infections, whereas metabolic control was managed with insulin alone or combined with oral hypoglycemic drugs. Evaluation consisted of baseline and weekly clinical examinations.

Data on demography, personal pathological history, type and duration of diabetes and its current treatment, peripheral neuropathy, peripheral vascular disease and wound examination were documented. Ulcers were classified in grades according to Wagner's classification. Patients were treated with intralesional injections of a lyophilized formulation of Heberprot-P containing 75 microgram (one vial) of EGF, three times a week on alternate days up The desired granulation formation.

Heberprot-P was administered together with good wound care. The product was dissolved in 8 ml of normal saline. This volume was distributed throughout the lesion at each administration. Using hypodermal needle EGF was infiltrated deep in the wound base and edges. Patient given 10 mg Loratadine to minimize the side effects.

After each infiltration session wound was dressed with wet to dry gauze and kept dressed the next day. Endpoint of primary efficacy was the outgrowth of a granulation tissue up to 75% or suitable to sustain spontaneous reepithelization. Local adverse events were monitored after each infiltration session. Systemic events and vital signs were monitored daily.

RESULTS

Patients demographic and baseline characteristics are shown in table1. A total of 107 patients recruited into this study. All were diabetic with diabetic foot ulcer. 22 patients are diabetic type I, 85 patients are of type II. 64 male and 43 females.

Baseline characteristics of the patients (table 1)

Character	Result
Age (mean)	59 years
Male	64
Female	43
Diabetes (years)	19 years
Duration of ulcer	4 months
Size of ulcer	8 cm^2
Type I	22
Type II	85

Mean age was 59 years with oldest patient is 85 years and the youngest is 30 years. Average period being diabetic is 19 years. Average time of evolution of ulcer is 4 months. average size of ulcer is 8 cm 2. According to classification of Wagner which is shown in table 2, 15

Table 2 Grade of ulcer acoording to Wagner's classification

grade	Number of patients
Grade 2	15
Grade 3	53
Grade 4	31
Grade 5	8

patients with grade II , 53 patients with grade III, 31 patients with grade IV, 8 patients with grade V, those grade V patients had some sort of amputation before starting treatment with EGF. 36 patients had ulcer at the site of toes, 21 had calcaneal ulcer, 40 patients with ulcer in the plantar region.

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Diabetes (years)	19 years
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Endpoint of treatment is having a healthy functional granulation tissue which is achieved by 75% or more of the area of the ulcer within 2 weeks. 86 patients representing 80.3% achieved 75% granulation within 2 weeks. Voluntary interruption was reported 3 cases. 13 cases had delayed healing more than 2 weeks. 5 patients had failed to heal.

DISCUSSION

This study showed good result of enhancement of wound healing and stimulation of growth of granulation tissue in a faster pace than regular treatment although basic primary wound care still a corner stone. Wound debridement, Offloading, Glycemic control, and treatment of vascular occlusion remain the corner stone of treatment of diabetic foot ulcers.

Our study showed that treatment with rh EGF is well tolerated with minor side effects.

A 75% granulation tissue was achieved in 80.3% of the cases by 2 weeks taking into consideration that these results are achieved in an advanced grade of diabetic foot ulcers mostly grade 3,4 according to Wagner's classification with some relatively big ulcers .

Other growth factors such as recombinant human PDGF-BB (becaplermin) have been used topically but in neuropathic and smaller lesions (17,18). In a meta-analysis of those studies, Smiell et al. concluded that within the setting of a comprehensive wound management program treatment with becaplermin gel at a dose of 100 microgram/g once daily increases the incidence of complete healing (19). However, 95% of the patients included in those trials had ulcers < 10 cm2 (median 1_4–3_5 cm2) and an adequate blood supply (defined as TcpO2 .30 mmHg) was a requisite for inclusion. On the contrary, this study treated more advanced, larger size ulcer and both neuropathic and ischemic wounds.

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Previous use of EGF topically, on DFU has been reported. A randomized, double-blind, placebo-controlled trial evaluated two doses of a rhEGF-containing cream in patients with DFUs compared with a protein-free calf blood derivative cream used as control. Healing rate was significantly enhanced by rhEGF 0.04% but not by the lower dose (0.02%). This trial also included much less severe ulcers given by Wagner's grades 1 and 2, equal or less than 4 cm2 size and only neuropathic (20). Another non controlled trial treated patients with grade 2–3, resistant to advanced dressing alone, neuropathic ulcers, mean size 4.8 cm2 with a topical formulation containing 0.005% rhEGF added to the dressing. In the treated patients, complete healing was noted in 76% (52/68) of patients within an average of 46 days (range from 2 to 14 weeks) (21). A limitation to the efficacy of topical formulations can be that the growth factor cannot steadily reach the deeper layers of the wound. Diffusion of the active agent is affected by necrotic tissue, sepsis, inflammation, and by the action of wound proteases (22).

It has been shown that chronic wounds have elevated proinflammatory cytokines, high protease activity, decreased levels of natural metalloproteinase inhibitors and diminished growth factor activity (23–24). The still active factor may be unavailable for biologic activity because of trapping or binding to molecules such as fibrinogen, macroglobulin or albumin (25,26). Additionally, the ever-present tissue level of bacteria in chronic wounds produce higher levels of proteases and other metalloproteinases that further degrade the growth factors and their receptors (27). These facts can contribute to explain the lack of efficacy of topical EGF and PDGF at lower doses. Intralesional injection of the growth factor could bring the active agent into the desired region and avoid the inactivating agents.

The results of our study confirms the effectiveness of the intralesional infiltration of rh EGF in advanced diabetic foot ulcers where 80.3% of the patients exhibited a productive granulation with only 5 cases failed to heal at any time. Treatment was well tolerated with mild side effects that are easily managed. One of the concerns of the use of rh EGF at concentrations much higher than physiological is that it could promote the development of malignant neoplasia. Classic experiments have shown that EGF promotes but doesn't initiate tumorigenesis. Nevertheless additional studies should be performed with a larger number of patients Another concern with the intralesional route of administration could be the risk of inoculating or spreading bacterial infection. This was minimized by the concomitant good wound care practices, broad-spectrum antibiotic coverage and adequate aseptic injection procedures. Infection control remains a critical problem for such advanced DFUs. Our study lacked control group since most of the patients had advanced diabetic ulcers and we wanted to give all of them the a possible chance for faster healing and abort the risk for amputation. Long term follow up study also lacked.

CONCLUSIONS

The intralesional application of Heberprot-P is effective to achieve objective response (≥ 75 % of granulation) at 2 weeks of treatment in patients with advanced diabetic foot, Wagner's grade 3 and 4 of any etiopathogenic, "pure" neuropathy as well as with ischemic component. The treatment have been shown a safety level, with few adverse events attributable to it. Pain in the site of injection, attributable to the procedure and shivering and chills associated with the EGF. The majority of the adverse events were of light or moderate intensity.

In summary, intralesional administration of rhEGF improved granulation tissue formation in both neuropathic and ischemic advanced DFU.

RECOMMENDATIONS

To continue the application of patients with diabetic ulcer. To continue the follow-up of the treated patients to know the efficacy and safety of the product in the long term.

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Figure 1

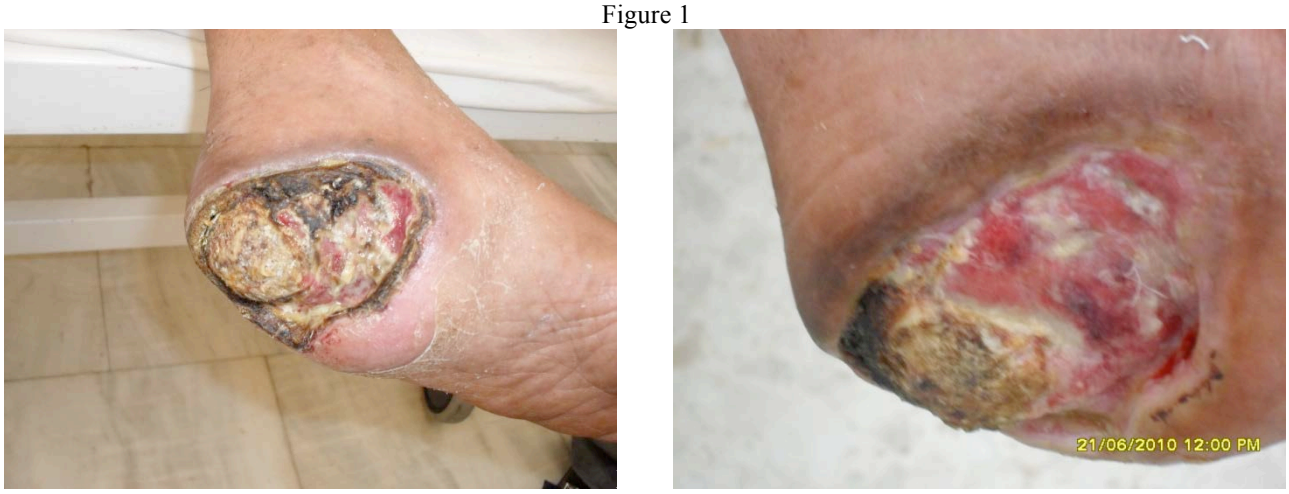

Figure 2

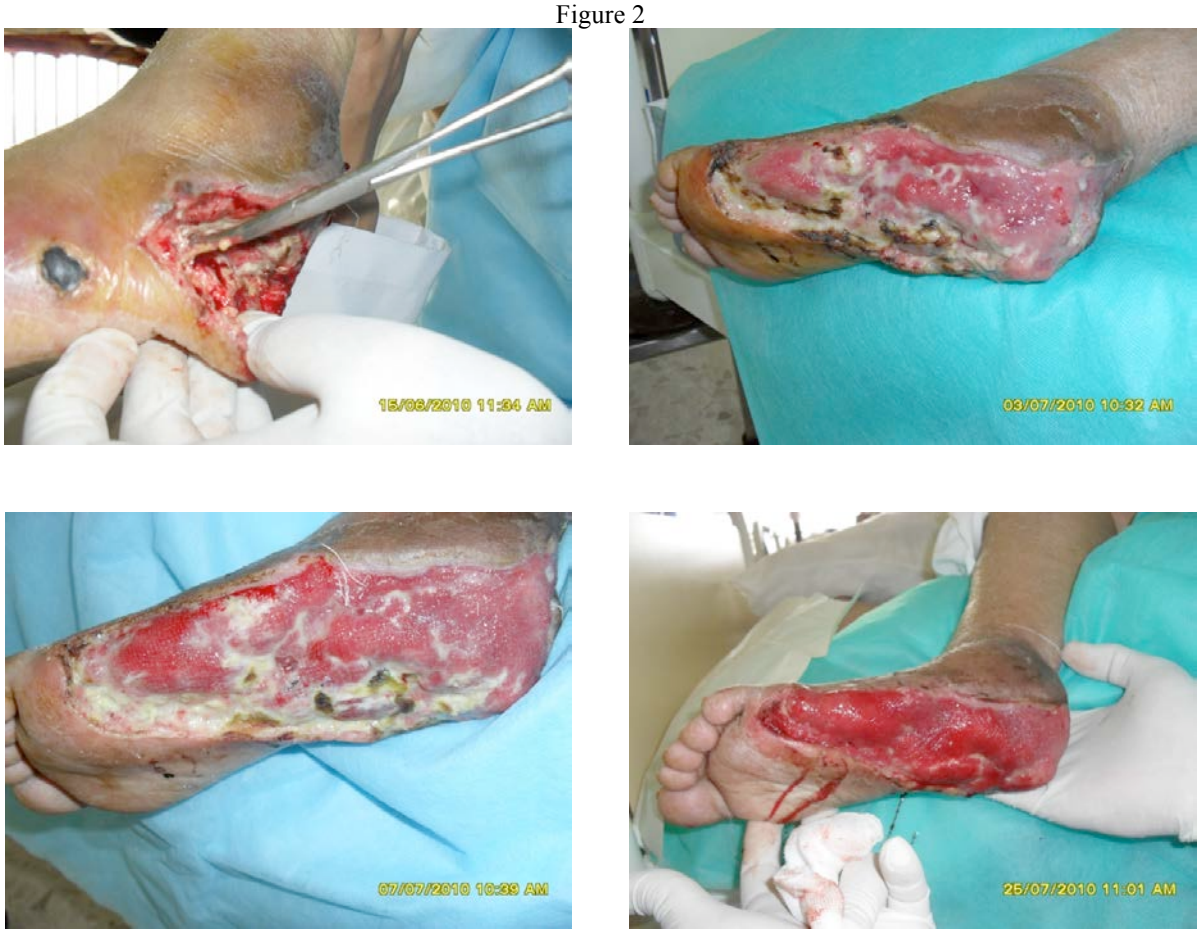

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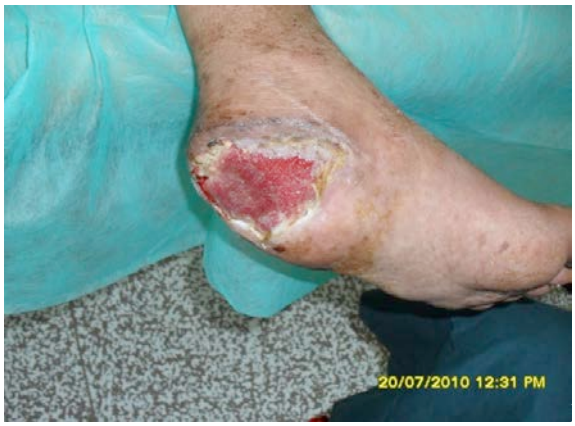
Figure 3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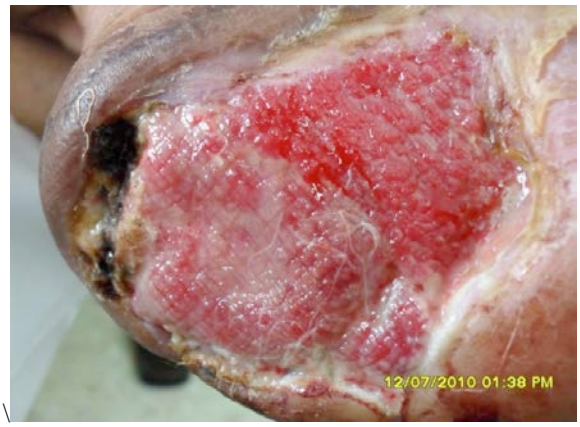
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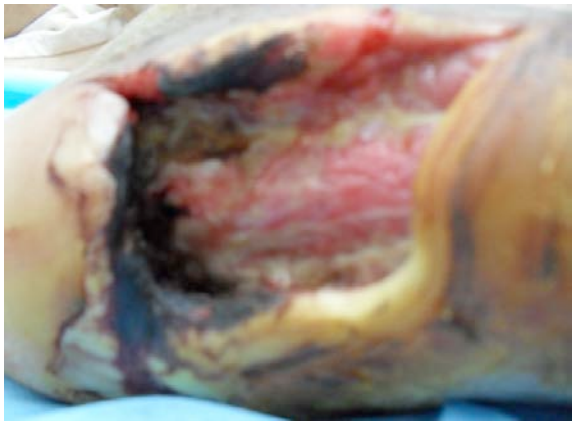

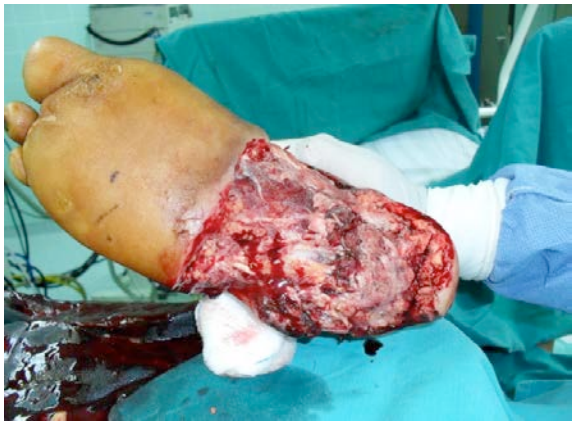


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