Efficacy of topical application of beta urogastrone (recombinant human epidermal growth factor) in Wagner’s Grade 1 and 2 diabetic foot ulcers: Comparative analysis of 50 patients

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Abstract

Introduction: Diabetes mellitus is growing at epidemic proportions world wide and associated with this is an increase in incidence of diabetic foot ulcers. For better understanding and ease of management, diabetic foot ulcer severity is often classified using the Wagner system. In recent times, various treatment modalities have been put to test for getting early wound healing, including growth factors like human epidermal growth factor. Materials and Methods: The present study was conducted in the Department of Surgery, Dayanand Medical College and Hospital, Ludhiana. The patients were divided into two groups of 25 patients each. Group 1 was the study group and patients in this group received topical application of beta urogastrone (rhEGF) gel. Group 2 was the control group and patients in this group received betadine dressing. The patients were followed up after every two weeks for eight weeks. Results: The age and sex were comparable in both groups. Mode of onset was either spontaneous or posttraumatic or following debridement. Initially in group A, 12 patients each had serous and seropurulent discharge respectively. 1 patient did not have any discharge. In group B, 15 patients had sero purulent discharge, 9 patients had serous discharge and 1 patient had purulent discharge. Initially, 13 patients in group A and 15 patients in group B had granulation tissue. Mean size at the beginning of the study in group A was 19.56 sq cm and 21.20 sq cm in group B. Two patients from group A had incomplete healing at the end of the study as compared to 14 patients from group B. Conclusions: The application of rhEGF shortens the wound healing time significantly and the mean closure was significantly higher in the EGF group compared with placebo.

Key words: Diabetic foot, growth factors, wound healing

INTRODUCTION

The prevalence of diabetes mellitus and its associated complications is growing at epidemic proportions world-wide. Foot ulcers are one such complication observed in one in six people with diabetes. Unfortunately, these foot ulcers do not heal easily, are difficult to treat and are more prone to secondary infections.¹ Neuropathy, high plantar pressure, poor glucose control and duration of diabetes contribute to the severity of foot ulceration² and are the most common underlying cause of non-traumatic lower extremity amputations.³

Severities of diabetic foot ulceration are classified using the Wagner system.⁴ Grade 1 ulcers are superficial ulcers involving the full skin thickness, but no underlying tissues. Grade 2 ulcers are deeper, penetrating down to ligaments and muscle, but not involving bone or abscess formation. Grade 3 ulcers are deep ulcers with cellulitis or abscess formation, often complicated with osteomyelitis. Ulcers with localized gangrene are classified as Grade 4 and
those with extensive gangrene involving the entire foot are classified as Grade 5.

Novel therapeutic approaches such as topical use of honey, collagen, cryopreserved fibroblast implants or growth factors are being evaluated to treat diabetic foot ulcers. Human epidermal growth factor (hEGF) is one such factor, which plays a significant role in the regulation of cell growth, proliferation and differentiation. Moreover, recombinant hEGF (rhEGF) (beta-urogastrone) has been developed using the recombinant deoxyribonucleic acid (DNA) technology to explore its therapeutic application. In the present study, we have to evaluate the efficacy of beta-urogastrone (rhEGF) dressings in Wagner’s Grades 1 and 2 diabetic foot ulcers.

MATERIALS AND METHODS

The present study was conducted in the Department of Surgery, Dayanand Medical College and Hospital, Ludhiana, India. Patients were divided into two groups of 25 patients each. Group I was the study group and patients in this group received topical application of beta-urogastrone (rhEGF) gel. Group II was the control group and patients in this group received betadine dressing. Patients were followed-up after every 2 weeks for 8 weeks.

Inclusion criteria
Wagner’s Grades 1 and 2 diabetic foot ulcers, Age 18-65 years, ulcer size between 2 and 50 cm² in the area and ankle brachial index > 0.75.

Exclusion criteria
Wagner’s Grade 3 or more comorbid gastrointestinal tract, hepatic, renal, hematological or immunological diseases, hypersensitivity to any of the ingredients of the gel, uncontrolled diabetes or diabetic coma.

On the 1st day of treatment, the wound surface was cleaned with normal saline. In some patients, surgical intervention (amputation or debridement) was performed to remove any slough or necrotic tissue over the wound surface before initiating the treatment. In Group I, beta-urogastrone (rhEGF) was applied. Beta-urogastrone is commercially available in the market in gel form in 15 g concentration. It was applied as a thick layer and a dry sterilized gauze piece was placed over the dressing and covered with a bandage. The beta-urogastrone dressing was kept over the wound surface for a maximum period of 8 weeks. In Group II, dressings were performed with betadine daily.

The observations of the treatment were recorded after every 2 weeks. At each inspection, the following points were recorded:

- Type of wound discharge, i.e., purulent/seropurulent
- Amount of wound discharge, whether increasing or decreasing
- Granulation tissue formation as percentage of the total surface area
- Changes in the size of wound as the largest transverse diameter and largest vertical diameter
- Presence of slough, if any – as a percentage of the total surface area
- Any cellulitis in the surrounding area.

The patients were randomly allotted to the two groups. Statistical analysis for analyzing the outcome of the study was carried out with the help of the t-test and Chi-square test.

Before the start of this study, approval from the ethics committee of the institution was taken. Patients were explained in detail about the treatment protocol and due consent was taken in their vernacular language.

RESULTS

A total of 50 patients having diabetic foot ulcers, irrespective of their etiology and associated diseases, were included in this study. The primary efficacy end points in the present study were set to be achieved on (1) complete healing of the ulcer, (2) split skin grafting/suturing of the ulcer, (3) surgical intervention of the involved non-healing part or (4) completion of 8 weeks of treatment.

The age of the patients in our study ranged from 26 years to 82 years with a mean of 58.80 and 55.84 years in Groups I and II, respectively. In Group I, 21 patients (84%) were males and 4 patients (16%) were females while in Group B, 23 patients were males (92%), and 2 patients were females (8%).

In Group I, 6 patients had spontaneous onset of the diabetic foot ulcer. In 4 patients, the onset was traumatic (including trivial trauma). In 7 patients, the ulcer was formed following debridement, whereas amputation was the etiological factor in 8 patients. In Group II, 11 patients had spontaneous onset, 2 patients suffered a trauma, 6 patients underwent debridement and 6 patients had an amputation as the mode of onset.

In Group I, 9 patients underwent debridement, 9 patients required an amputation, whereas 7 patients did not undergo any surgical intervention. In Group II, 13 patients underwent debridement, 6 patients required an amputation and another 6 did not undergo any surgical intervention.

Initially in Group I, 12 patients had serous and seropurulent discharge. One patient did not have any discharge. In
Group B, 15 patients had seropurulent discharge, 9 patients had a serous discharge and 1 patient had purulent discharge. In Group I, the number of patients with seropurulent discharge decreased from 9 to 3 from the 2nd week to 4th week with the discharge becoming gradually serous. In comparison, during the same duration, in Group II, the number of patients with seropurulent discharge remained constant at 16 ($P = 0.0495$).

At the conclusion of the study, Group I had only 3 patients left with two having seropurulent and one having serous discharge. While in Group II, 9 patients had seropurulent discharge, 5 patients had a serous discharge, whereas 1 patient had a non-discharging ulcer ($P = 0.009$).

Prior to the initiation of the therapy, 24 patients’ in Group I and all patients in Group II had discharging ulcers. In the 2nd week post-therapy, 16 patients in Group I and 5 patients in Group II showed a decreasing trend ($P = 0.008$). In Group I, 3 patients were resistant to the treatment in comparison to 15 patients in Group B ($P = 0.007$). In both the groups, 4 patients showed an increase in the amount of discharge. In the 4th week of therapy, 14 patients from Group I and 8 patients in Group II had a decreasing trend. A total of 13 patients were still resistant to the treatment in Group II ($P = 0.007$). In the 6th week of therapy, 16 patients from Group II showed a decrease in the amount of discharge. In the 8th week of therapy, only 3 patients were left in Group I out of which 2 patients showed an increase while 1 patient showed a decrease in discharge. In Group II, 9 patients showed a decreasing trend whereas 5 patients were still resistant to the treatment and 1 patient had an increase in the amount of discharge [Table 1].

A total of 13 patients in Group I and 15 patients in Group II had granulation tissue. In the 2nd week, 22 patients from Group I had an increase in granulation tissue, whereas 13 patients from Group II showed such a trend ($P = 0.041$). In the 4th week, of the 18 patients in Group I, 17 patients continued to show an increasing trend, whereas in Group II, 13 patients showed a similar trend. In the 6th week, of the 10 patients in Group I, 8 patients showed an increase and so did 13 patients from Group II. One patient showed a decrease in the surface area covered with granulation tissue. In the 8th week, of the 3 patients in Group I, two had an increase in the surface area covered with granulation tissue with 9 patients in Group II showing a similar trend ($P = 0.041$) [Table 2].

A total of 15 patients (60%) from Group I and 20 patients (80%) in Group II had surrounding cellulitis at baseline. By the end of this study, one patient (4%) from Group I and 2 patients (8%) from Group II showed a surrounding cellulitis.

Mean wound size at the beginning of the study was 19.56 and 21.20 sq. cm in Group I and II, respectively. In the 2nd week, 21 patients (84%) from Group I showed a decreasing trend, whereas 10 patients (40%) from Group II showed a decreasing trend ($P = 0.008$). Remaining patients in both groups were resistant to treatment. In the 4th week, 16 patients (64%) had a decreasing trend in Group I as compared with 9 patients (36%) in Group II ($P = 0.009$). One patient in Group II showed an increase in wound size. In the 6th week, 8 patients (32%) from Group I and 11 patients (44%) from Group II showed a decreasing trend ($P = 0.038$). Two patients (8%) in Group II showed an increase in wound size. Remaining patients in both groups were resistant to treatment. At 8 weeks, decreasing trend was seen in 2 patients (8%) from Group I and in 9 patients (36%) from Group II.

In Group I; 7 patients underwent grafting, 12 had completely healed ulcers [Figures 1-3], 2 patients had their wounds sutured, 1 patient required an amputation, incomplete healing occurred in one patient and one patient

### Table 1: Trends in wound discharge in both groups

| Weeks | Discharge   | Group I (%) | Group II (%) | Group I (%) | Group II (%) | Group I (%) | Group II (%) | Group I (%) | Group II (%) |
|-------|-------------|-------------|--------------|-------------|--------------|-------------|--------------|-------------|--------------|
|       | Serous      | 15 (60)     | 7 (28)       | 15 (60)     | 6 (24)       | 9 (36)      | 14 (56)      | 2 (8)       | 5 (20)       |
|       | Seropurulent| 9 (36)      | 16 (64)      | 3 (12)      | 16 (64)      | 1 (4)       | 5 (20)       | 1 (4)       | 9 (36)       |
|       | Purulent    | 0 (0)       | 2 (8)        | 0 (0)       | 1 (4)        | 0 (0)       | 2 (8)        | 0 (0)       | 0 (0)        |
|       | Nil         | 0 (0)       | 0 (0)        | 0 (0)       | 0 (0)        | 0 (0)       | 0 (0)        | 0 (0)       | 1 (4)        |

### Table 2: The trends in granulation tissue formation

| Weeks | Granulation | Group I (%) | Group II (%) | Group I (%) | Group II (%) | Group I (%) | Group II (%) | Group I (%) | Group II (%) |
|-------|-------------|-------------|--------------|-------------|--------------|-------------|--------------|-------------|--------------|
|       | Resistant   | 2 (8)       | 12 (48)      | 1 (4)       | 9 (36)       | 2 (8)       | 7 (28)       | 1 (4)       | 6 (24)       |
|       | Increasing  | 22 (88)     | 13 (52)      | 17 (68)     | 12 (48)      | 8 (32)      | 13 (52)      | 2 (8)       | 9 (36)       |
|       | Decreasing  | 0           | 0            | 0           | 0            | 0           | 1 (4)        | 0           | 0            |
was excluded from the study because of cardiovascular complication in the form of heart failure. In Group II; 5 patients underwent grafting, 3 patients had completely healed ulcers, 2 patients required debridement, 14 patients had incomplete healing and one patient got his wound sutured. In the 3rd week, 3 patients from Group I and two from Group II reached their end points. In the 4th week, 7 patients from Group I and 1 patient from Group II reached their end points \((P = 0.025)\). In the 4th week, 7 patients from Group I and 1 from Group II reached their end points \((P = 0.025)\). In the 6th week, 5 patients from Group I reached their end points, whereas none of the patient in Group II reached the end point \((P = 0.023)\). The majority of the patients in Group II reached their end point by 7th and 8th week, whereas in Group I, 19 patients had reached their end points by 6th week. Two patients from Group I had incomplete healing at the end of this study as compared with 14 patients from Group II \((P = 0.007)\) [Table 3].

**DISCUSSION**

There is no single universal dressing material that is suitable for the management of all types of wounds and a few are suitable for single wound in all stages of wound healing. The main goal of dressing selection is to produce rapid and optimal wound healing that is cosmetically acceptable, reduce pain, prevent/control wound infections, decrease the quantity of wound secretion, cause minimal distress to the patient and improve the quality of patient’s life.

hEGF plays a significant role in the regulation of cell growth, proliferation and differentiation. Recombinant hEGF (beta-urogastrone) produced by recombinant DNA technology acts by binding to epidermal growth factor receptor on the cell surface and stimulates the intrinsic protein-tyrosine kinase activity of the receptor, ultimately leading to DNA synthesis and cell proliferation. The present study was undertaken to evaluate the efficacy of rhEGF dressings in Wagner’s Grades 1 and 2 diabetic foot ulcers.

rhEGF therapy significantly decreased the amount of discharge in both groups; however, the effects were observed earlier in Group I compared with Group II. The decreasing trend in Group I was more evident in the 2nd and 4th weeks, whereas in Group II, a similar trend was delayed and observed at 6th week. Our results

| Time in weeks | No. of patients reaching end point (%) | \(P\) value |
|---------------|---------------------------------------|-------------|
|               | Group I | Group II |               |
| 3             | 4 (16)  | 2 (8)    | 0.884        |
| 4             | 7 (28)  | 1 (4)    | 0.025        |
| 5             | 3 (12)  | 1 (4)    | 0.3024       |
| 6             | 5 (20)  | 0 (0)    | 0.0225       |
| 7             | 2 (8)   | 4 (16)   | 0.3884       |
| 8             | 1 (4)   | 3 (12)   | 0.3024       |

**Figure 1:** Diabetic foot ulcer prior to start of therapy

**Figure 2:** Ulcer immediately after debridement

**Figure 3:** Ulcer healed completely after 6 weeks of application of recombinant human epidermal growth factor

**Table 3:** The time taken by two groups to reach end point
are consistent with the previous study reporting topical application of rhEGF ointment promoting wound healing.[13] The benefits following rhEGF application was attributed to increased rate of epidermal proliferation, accelerated wound contraction, myofibroblast proliferation and collagen deposition. Increase in collagen deposition contributes to decrease in wound discharge. rhEGF therapy also significantly promoted granulation tissue formation as observed in Group I, which is consistent with previously reported effects of rhEGF in promoting skin wound repair by enhancing granulation tissue formation.[14,15] The mean time of wound healing was (17.2 ± 1.3) and (20.5 ± 1.6) days in Group I and II respectively (P = 0.01).

Cellulitis in the surrounding tissue was seen only in 15 patients (60%) in Group I while cellulites was observed in 20 patients from Group II; hence, it is reasonable to conclude that rhEGF therapy significantly reduced incidence of cellulites. Significantly more number of patient's in Group I showed reduced wound size while the majority of the patient's in Group II had ulcers resistant to treatment. Consistent with our study rhEGF therapy was shown to accelerate wound contraction and closure.[15-17] Further we observed a significant increase in the rate of wound healing in rhEGF treated group which is again consistent with the previous reports in surgical[14] or burn[18] or ulcerated,[19] wounds.

CONCLUSIONS

The application of rhEGF significantly reduces time to wound healing and dressings in diabetic foot ulcers significant increase in granulation tissue formation and reduced wound discharge and cellulitis in the surrounding area. Our study supports further development of rhEGF therapy in addition to good foot care in management of diabetic foot ulcer therapy.

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