IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 21, Issue 12 Ser.4 (December. 2022), PP 62-67
www.iosrjournals.org

The study on effectiveness ofhuman epidermal growth factor in treating low grade diabetic foot ulcers, at a tertiary care hospital in Tamil nadu – A Randomized Control Trial.

AUTHOR

Abstract
Background: *Diabetic foot ulcers (DFU), a common complication of diabetes occurs due to pheripheral neuropathy in long standing disease. About 1 in 6 people with diabetes develop a foot ulcer at some stage. Diabetic wounds are relatively difficult to heal due to defective fibroblast activity, poor angiogenesis and lack of cell migration. In chronic wounds as in DFU, there is increased destruction or inhibition of growth factors due to elevated levels of proinflammatory cytokines and metallomatrix protein following repeated trauma and infection. The conventional treatment methods for diabetic foot ulcers includesmetabolic control, adequate wound debridement, treatment of comorbidities, revascularization and antimicrobial treatment of infections. Epidermal growth factor (EGF) helps in activating mesenchymal and epithelial cells, stimulating epidermal repair after injury. It also plays a significant role in angiogenesis, enzyme production, cell migration and proliferation thereby it helps in maintenance of the integrity and regeneration of the skin. Thus, the present study aimed to determine whether local application of a high concentration of human EGF (hEGF) might be effective in promoting wound healing of diabetic foot ulcers.*
Aim:*To evaluate the efficacy of epidermal growth factor in healing low grade diabetic foot ulcers (Grade I or II) of Wagner's classification and to compare the progression of wound healing in conventional dressing versus dressing with local application of recombinant human epidermal growth factor cream.*
Methodology:*The study was a single blinded randomized control study to assess the effectiveness of recombinant human epidermal growth factor cream (rhEGF) over the conventional method in treating low grade diabetic foot ulcers (Grade I or II) of Wagner's classification. Patients received either rhEGF or placebo intervention, in addition to standard diabetic foot management. The rhEGF and placebo treatments were administered as topical preparation. The study end point was the complete closure of the wound. Failure to heal was arbitrarily defined as incomplete healing after 8 weeks.*
Results:*Most of the study participants were in the age group of 51-65 years in both study and control groups. The study participants comprised of about 80% males and 20% females whereas in the control group 88% were males and 12% were females. Baseline disease characteristics like ankle brachial index, duration of diabetic ulcer, duration of diabetes, level of HbA1C, body mass index and mean wound size were almost similar in both study and control groups. The mean wound size were 9.7 sq.cm and 9.4 sq.cm in study and control group respectively. Participants from the study group had better wound closure compared to control group during the entire study period. The number of complete responders were higher in study group compared to control group.*
Conclusion:*The application of epidermal growth factor cream can significantly reduce the ulcer size and effectively promote healing of grade I and II diabetic foot ulcers.*
Key words: *Diabetic foot ulcers, Epidermal growth factor, Wagner's classification*

Date of Submission: 04-12-2022 Date of Acceptance: 16-12-2022

I. Introduction

The prevalence of type 2 Diabetes Mellitus (DM) continues to rise in all regionsaround the world. Thereasons for this rise are economic development, increasing sedentary lifestyle and increase in obesity due to unhealthy food consumption. In 2017, there were 425 million diabetic patients in the world and it was estimated that this number will reach 629 million with a 48% increase in 2045. [1] Untreated or inadequately treated DM can result inboth micro-vascular complications (including retinopathy, nephropathy, and neuropathy) and macro-vascular complications (including cardiovascular disease and insufficient blood flow to lower limbs). [2]

Diabetic foot ulcer (DFU) is a chronic complication of diabetes. Due to the neurological and vascular changes diabetic patients lose sensitivity in the extremities and have deformations in their feet, which increases the risk of having injuries leading to ulcers.[3]About 1 in 6 people with diabetes develop a foot ulcer at some

stage. The risk of developing ulcers in lower extremities is 15% during the course of diabetes. Also, the risk of amputation is 10-20% higher in diabetics (0.06 and 3.83 per 1000 diabetics) compared to non-diabetics with a high recurrence rate of 50% [4] Through conventional treatment, though the amputation rate of diabetic foot is reduced to a certain extent, diabetic wounds are difficult to heal. Additionally, these patients have atrophic skin changes, which facilitates the formation of fissures and the entry of germs, thus the incidence of wound infection is relatively high. [5,3]

Neuropathy is the main aetiology of diabetic foot ulcers (DFUs). The mechanisms include direct nerve damage by hyperglycemia and a decrease in blood flow to the nerves due to damaged small blood vessels. Physiologically, wound healing can be divided into three stages namely inflammatory, proliferatory and remodelling stage. Wound repair is characterized by a series of complex cellular and molecular events. The healing process of DFU is inhibited by local factors affecting all phases of recovery, such as abnormal neutrophil function in the late repair phase, defective fibroblast activity, poor angiogenesis and lack of cell migration. [3] In chronic wounds as in DFU, there is increased destruction or inhibition of growth factors due to elevated levels of proinflammatory cytokines and metallomatrix protein following repeated trauma and infection. [6]

The conventional treatment methods for diabetic foot includesmetabolic control, adequate wound debridement, treatment of comorbidities, revascularization and antimicrobial treatment of infections. [1] With the advancement of biomolecular technology, scientists have found that growth factors play an important role in promoting wound repair by stimulating chemotaxis, cellular proliferation, extracellular matrix formation and angiogenesis thereby promoting contraction and reestablishment of cellular integrity. [6]

Epidermal growth factor (EGF), a 6 kDa protein found in platelets, vascular and duodenal glands helps in activating mesenchymal and epithelial cells in readiness for proliferation and stimulates epidermal repair after injury. [7]It plays a significant role in angiogenesis, enzyme production, cell migration and proliferation thereby it helps in maintenance of the integrity and regeneration of the skin. It also activates epidermal and stromal cell division and migration and is a potent mitogen in keratinocytes. [4]By means of Northern hybridization specific for the content of mRNA of type I and type III procollagens, M. Laato et al. confirmed that EGF is a potent dose-dependent mitogen for the granulation fibroblast. [8]According to the recent guidelines of Wound Healing Society (WHS) in 2018, the effect of epidermal growth factors on DFU was unclear. Few recent small studies have found some benefit in healing with high risk of bias. [9]Therefore in the current study, we aimed to determine whether local application of a high concentration of human EGF (hEGF) might be effective in promoting wound healing of diabetic foot ulcers.

II. OBJECTIVES

1. To evaluate the efficacy of epidermal growth factor in healing low grade diabetic foot ulcers (Grade I or II) of
Wagner's classification.
2. To compare the progression of wound healing in conventional dressing and in dressing with local application of
recombinant human epidermal growth factor cream.

III. MATERIALS AND METHODS

The study was a single blinded randomized control study to assess the effectiveness of recombinant human epidermal growth factor cream (rhEGF) over the conventional method in treating low grade diabetic foot ulcers (Grade I or II) of Wagner's classification.Patients received either rhEGF or placebo intervention, in addition to standard diabetic foot management. The rhEGF and placebo treatments were administered as topical preparation.The study end point was the complete closure of the wound. Failure to heal was arbitrarily defined as incomplete healing after 8 weeks.

Study medication

The active drug was human epidermal growth factor cream (Regan-D 150G) and the placebo used was water based which did not include the active ingredient.

Inclusion criteria:

1. Patients who are able to understand and sign the informed consent.
2. Patients who were diagnosed with type 2 diabetes mellitus and subsequently developed diabetic foot ulcer.
3. Patients in the age group of 18 and 65 years with low grade diabetic foot ulcer (grade I or II) as defined by the Wagner Classification.
4. Ulcer size between 2 cm to10cm.

5. The diabetes should be under control by either oral hypoglycaemic drugs or insulin. The diabetic control was ensured by testing HbA1c.
6. Ulcer with adequate perfusion, as indicated by an ankle-brachial index (ABI) readings of ≥ 0.75.

Exclusion criteria:

1. Patients with ≥ Grade III diabetic foot ulcers by Wagner classification.
2. Uncontrolled diabetes mellitus, diabetic ketoacidosis or coma.
3. Pregnant women and nursing mothers
4. Those individuals who were receiving any treatment known to impair wound healing including corticosteroids, immunosuppressive drugs, cytotoxic agents, radiation therapy and chemotherapy.
5. Evidence of systemic or local infection, such as purulent drainage and osteomyelitis.

Comprehensive foot assessment was carried outby determination of Ankle Brachial Index (ABI), vibration perception threshold, and sensation assessment using 10-g monofilament and pin prick tests. Standard wound care was done by debridement of necrotic tissue and reduction of callus. Wound parameters such as exudates, signs of infection, presence of granulation tissues and eschar were documented. Wound swabs were taken if infection was suspected, and antibiotics were prescribed based on clinical judgment or on positive wound bacterial cultures. After adequate wound debridement and infection control (no edema, inflammation or exudation), the participants were randomly allocated into two groups by drawing lot.

A total of 50 patients were recruited. They were randomly allocated into two groups (study group and control group) with 25 patients in each group.Wound length and width was measured (in cm) using a measuring tape, from which the surface area of the wound was calculated using the formula for calculation of the regular geometric figure that best approximated to the shape of the wound.

Patients in both groups had their wounds washed with normal saline and dressed every day. Wound dressing consisted of sterile gauze and adhesive tape only. No disinfecting solution like betadine was used. In the study group, epidermal growth factor gel was applied as primary dressing and then covered with gauze, bandage and tape. Dressing was changed every day. In the control group, isotonic solution moistened gauze was applied over the wound area as primary dressing and then covered with bandage and tapes.

Patients were. evaluated four times on 1^{st} , 3^{rd} , 5^{th} and 8^{th} weeks. With each visit wound size and severity, the presence of granulation tissue, edema, erythema and infection were assessed.Complete healing was defined as full epithelialization of the wound with absence of discharge. Any clinical abnormal reactions or undesirable findings appeared during or after the administration of EGF was noted.The level of patient compliance was assessed at regular intervals. The patient was excluded during the study period if he/she missed 2 consecutive weekly clinical visits or any reporting of an adverse eventduring or after the administration of EGF.

Percentage wound closure was calculated using the formula:

$$\frac{\text{Initial wound size after 8 weeks} * 100}{\text{Initial wound size}}$$

The study outcomes were the rate of complete healing, the level of reduction in wound size by time.At the end of the study period of 8 weeks, the patients were categorized as follows:

1. Complete responder – complete healing
2. Partial responder – 50% or greater reduction in size
3. Non complete responder – less than 50% reduction in size
4. Non-responder – no reduction in size

Statistical analysis

Analysis was based on intention to treat. The rate of healing was calculated in percentage of wound completely healed wound after 8-weeks of treatment. The healing time was the duration of complete closure demonstrated in mean (standard deviation). The rate of wound reduction was presented in percentage compared to the initial size. The unpaired T test was used to compare the healing size of the study and control group. Response to treatment was assessed by comparing pre and post treatment using ANOVA. The P value less than 0.05 was considered as statistically significant.

IV. Results

Table 1: Baseline characteristics of study population

Characteristics	Study group (25)		Control group (25)	
	No of cases	Percentage (%)	No of cases	Percentage (%)
Age (in years)				
18-35	2	8%	0	0%
36-50	8	32%	6	24%

51-65	15	60%	19	76%
Sex				
Male	20	80%	22	88%
female	5	20%	3	12%

In our study, most of the study participants were in the age group of 51-65 years in both the groups. Most of the study participants were male in both the study and control groups.

Table 2: Baseline disease characteristics

Disease Characteristics	Study group (mean ± SD)	Control group (mean ± SD)
ABI ratio (Ankle Brachial Index)	1.09± 0.22	1.02 ± 0.16
Duration of ulcer (in weeks)	12.00 ± 5.56	11.48 ± 8.65
History of diabetes (in years)	10.11 ± 8.29	11.48 ± 7.68
HbA_{1C} (%)	7.97 ± 1.81	8.69 ± 1.99
BMI (kg/m^2)	25.69 ± 5.21	23.83 ± 3.17
Mean wound size (sq.cm)	9.7	9.4

Ankle Brachial Index ratio was assessed in both study and control group to ensure the absence of peripheral arterial disease. ABI ratio was found to be normal in both the groups.

Table 3: Comparison of mean wound size before and after intervention in both study and control group

Group	Average percentage of wound closure			
	1st week	**3rd week**	**5th week**	**8th week**
Study group	35.5	48.7	72.1	84.5
Control group	28.2	37.5	58.2	68.6

The mean wound size were 9.7 sq.cm and 9.4 sq.cm in study and control group respectively. Both the groups showed improvement in healing following intervention, but the healing was better among study participants compared to control group. The size of the wound at the 8th week was compared between study and control group using unpaired student T test. The P value was found to be less than 0.05, hence its statistically significant.

Table 4: Average percentage of wound closure in the study and control groups.

Group	Average percentage of wound closure			
	1st week	**3rd week**	**5th week**	**8th week**
Study group	35.5	48.7	72.1	84.5
Control group	28.2	37.5	58.2	68.6

Participants from the study group had better wound closure compared to control group during the entire study period.

Table 5: Healing response among the study and control groups

Response of the patients	1st week		3rd week		5th week		8th week	
	Study group	Control group	Study group	Control group	Study group	Control group	Study group	Control group
Complete responders	5	7	9	7	15	11	22	12
Partial responders	14	12	12	7	7	6	2	8
Non complete responders	6	4	3	9	2	5	1	4
Non responders	0	2	1	2	1	3	0	1

The number of complete responders were less during the beginning of the study. But this number had increased by 8th week where majority of the participants were complete responders both in study and control group. The number of complete responders were higher in study group compared to control group. The number of non-responders were minimal both in study and control group during the entire study period.

V. Discussion

Diabetes mellitus is a metabolic disease characterized by hyperglycemia. The incidence of diabetes has raised significantly over the past decade. Its prevalence in low and middle income countries is significantly higher compared to high income countries. Long standing disease can lead to various complications, affecting almost all systems of the body.Among the various complications, the foot ulcers are considered as the most preventable ones. Foot disease affects nearly 6% of individuals with diabetesmellitus and includes infection, ulceration or destruction of tissues of the foot**.** Among these, diabetic foot ulcer develops as aresult of neuropathy. It can impair the quality of life and affect social participation and livelihood.Around 0.03% to 1.5% of patients with diabetic foot require an amputation.The standard management of DFUs include wound debridement, dressings, glycemic control and antibiotics if needed. The reduced action of epidermal growth factors due to hyperglycemia leads to wound chronicity. The present study evaluates the enhancement of wound healing by external supplementation of epidermal growth factor.

This was a randomised control study done among 50 patients with grade I and II diabetic foot ulcer. The patients were grouped into study and control group with 25 participants in each group. The study participants was dressed with epidermal growth factor in addition to standard wound care. The control group was given standard conventional dressing care for the wound. Comprehensive foot assessment was carried out before the study to rule out any complication and ensure that the participants fulfilled the inclusion criteria.

In our study, most of the study participants were in the age group of 51-65 years in both study and control groups. Similar results was observed in a study done by Jawad Mohammad Akther et al. in rural region of Maharashtra which shows higher prevalence of DFU in the age group of 41-60 years. [10]In another study done by Ann Knowles, diabetic foot ulcer was more common among elderly people. [11]As age advances, the risk of peripheral neuropathy also increases which consequently leads to foot ulcers. In our study, the study participants comprised of about 80% males and 20% females whereas in the control group 88% were males and 12% were females. This male predominance might be attributed to increased exposure of outdoor activity in males compared to females which had resulted in foot trauma followed by ulceration.

The mean size of the wound was compared before and after intervention at 1st, 3rd, 5th and 8th week. The mean wound size before intervention was 9.7 sq.cm and 9.4 sq.cm in the study and control group respectively. The decrease in ulcer size was evident from fourth week onwards. The mean wound size measured at the end of 8th week was 1.5 sq.cm and 3.8 sq.cm in the study and control group respectively. The decrease in ulcer size was more than 50% in study group whereas, it was less than 25% in the control group at the end of follow up period. Compared to the pre intervention, the reduction in the mean wound size at the end of 8th week was 84.5% in the study group and only 68.6% in the control group.

In context of response to treatment, at the end of 8th week, 22 were completely healed, 2 were partial responders and 1 showed non complete response in the study group. Among the participants in control group, 12 were completely healed, 8 were partial responders, 4 were non complete responders and 1 showed no response to treatment. To conclude, 22 out of 25 diabetic foot ulcers completely healed with the application of topical epidermal growth factor for 8 weeks as a part of multidisciplinary approach.

The results of our study coincides with another study done by Vimal Ramachandran et al. (2017) which concluded that application of topical epidermal growth factor in addition to standard wound care shows better healing especially in terms of granulation tissue formation. [12] Meta-analysis done in 2018 showed that the use rhEGF together with standard wound care regimens significantly improved the healing rate compared to the placebo in DFU treatments. The efficiency was found with both intralesional and topical application. Further, increasing the frequency of application resulted in faster wound healing. [2] Our results was similar to studies conducted by American Diabetes Association which showed that the hEGF cream significantly enhances diabetic foot ulcer wound healing with reduction in the healing time. [6]Many studies done all over the globe had stated that epidermal growth factor stimulates the production of fibronectin, thus promoting the epidermal cell proliferation and migrating them to enhance wound closure. Also, it helped in the improvement of tissue nutrition and reducing inflammation. Also there was no serious side effects reported with the application of topical epidermal growth factor for diabetic ulcers. [2]

To conclude, therapeutic management of DFUs is a multidisciplinary approach. Proper wound care is the key component in treatment. A thorough foot examination is important to detect the disease early. Screening for peripheral neuropathy and peripheral arterial disease at the earliest can also help to identify patients at risk of foot ulcers. Diabetic foot can be prevented with good glycaemic control, regular foot assessment, appropriate footwear, patient education, and early referral for pre-ulcerative lesions. Also, newer and advanced topical

dressing with human epidermal growth factor can promote early healing with lesser complications when compared with conventional dressing.

VI. Conclusion

From our study, we can conclude that application of epidermal growth factor cream can significantly reduce the ulcer size and effectively promote healing of grade I and II diabetic foot ulcers. Compared to conventional dressing, the healing was significantly higher when dressing was done with EGF. It was also noted that ulcer healing in terms of mean wound size reduction was 84.5% in the study group, whereas only 68.6% in the control group.Thus diabetic wound management using topical application of epidermal growth factor along with standard wound care can significantly result in early healing with lesser complication compared to conventional dressing. Further studies are needed to define the optimal route, dose and also the possible use of other growth factors in the management of diabetic foot ulcers.

VII. Limitations

The limited sample size carries the risk of generalizability of the results to a broader group.Only gradeI and II ulcers were included in our study. Further grades should also be considered.The measurements were taken manually. This might affect the accuracy of results.The site of ulceration varied with the patients. Ulcers in different regions may have different aetiologies or aggravating factors, bringing bias when comparing the rate of healing.

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