Effect Of Epidermal Growth Factor On Chronic Leg Ulcers With Anti Septic Dressing: A Study In Bangabandu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh

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Abstract

Introduction: Lots of developments and researches are being done in the quest for ideal wound dressings. Epidermal growth factor dressings are one of these new developments and stimulates the cell growth, proliferation and differentiation by binding to its EGFR. There are not so many studies to quantify the rate of healing applied to the chronic non healing ulcers. **Objective:** The present study is being done to compare the rate of healing of epidermal growth factor dressings vs. antiseptic dressings. **Materials And Methods:** This is a randomized, prospective and comparative study done in the Department of Vascular Surgery, Bangabandu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh From June 2017 to May 2018. Thirty patients with chronic non-healing ulcers and divided into two groups A and B each containing fifteen patients. Group A were dressed with epidermal growth factor and group B were dressed with normal saline. Though the exact mechanism of action of dressing with antiseptic is unknown. **Results:** This study is done to evaluate the effects of healing in chronic non healing ulcers as evidenced by amount of reduction in ulcer size done by epidermal growth factor dressings and anti-septic dressings for a period of fourteen days in 30 patients, 15 patients with EGF and 15 patients with normal saline. Patients subjected to topical EGF 0.01% GEL dressings were classified under study and those who underwent conventional antiseptic wound dressing were classified as control. Antiseptics are commercially available in pharmacies. Epidermal growth factor is available in the commercial trade name of REGEN-D90 and applied over the ulcers. Patients are evaluated daily from day zero to day 14. On fourteenth day, it is observed by visual analog scale that, there is significant decrease in the size of ulcer and formation of granulation tissue in patients who were dressed with epidermal growth factor when compared to patients dressed with normal saline. **Conclusion:** There is significant difference in decrease in size of the ulcer between epidermal growth factor dressing and normal saline dressing. The cost effectiveness, availability, decreased hospital stay and ease of application makes epidermal growth factor a better choice for treating chronic non healing ulcers.

Keywords: Wound healing, Epidermal growth factor, REGEN-D, Ulcers.

I Introduction

The prevalence of leg ulcers is probably between 0.18% and 1 % of the population. And is likely to increase as the average age of the population rises.

The cost of treating chronic wounds is enormous." The working capacity of the patient is often reduced; in our society no data is available but approximately 2 million work days are lost annually in the United States because of leg ulcers but in our country we have no specific data or papers like that. In addition, there are numerous psychosocial squeal. Chronic wounds, especially non healing types are one of the most common surgical conditions encountered by a surgeon. For example, around 70% of chronic leg ulcers are caused by venous disease and compression therapy is the gold standard treatment, yet a U.S. study found only 17% of patients with venous leg ulcers received compression, and Australian studies found 40–60% of venous leg ulcers in Australia did not receive adequate compression. A number of reasons have been identified as contributing to this evidence-practice gap, including lack of information and skills, difficulties with access to evidence based guidelines, the costs and lack of reimbursement associated with specialist wound care and treatments such as compression bandaging, limited access to specialist multidisciplinary teams, poor communication and limited evidence on effective assessment, referral and treatment pathways of care to manage this chronic condition. The peculiarity of a chronic wound is that inspite of daily dressing with expensive local applications, the wound does not heal. This problem is especially seen in diabetic ulcers, venous ulcers and pressure ulcers. Thus to treat these wound is a constant challenge for the surgeon. The notion that wounds should be kept dry, although still held by a considerable number of surgeons, is steadily losing ground. We now know that wounds develop granulation tissue when treated with dressing which allow moist wound healing. During the last two decades a wide variety of innovative dressings have been introduced. People have tried various non-conventional topical therapies in wound healing, such as Normal saline, Aloe Vera, collagen, gentian violet, benzyl peroxide, impregnated gauze, insulin, Mercurochrome, oxygen therapy, sugar and vinegar. Studies have also shown that topical EGF promotes healing of decubitus ulcer, venous ulcer, pressure ulcer & leprosy ulcer and was found to be of superior in the management. The present study was conducted to assess the efficacy of topical epidermal growth factor dressing as compared to conventional antiseptic wound dressing in healing process in non healing ulcers.

II Materials And Methods

This is a randomized, prospective and comparative study done in the Department of Vascular Surgery, Bangabandu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh From June 2017 to May 2018. Thirty patients with chronic non- healing ulcers and divided into two groups A and B each containing fifteen patients. Group A were dressed with epidermal growth factor and group B were dressed with normal saline. Regen-D 150 is considered an epidermal growth factor and is a new generation therapy for diabetic foot ulcers. It contains vitamins, minerals and amino acids that help stimulate cell growth and help nourish skin cells. It is indicated for topical healing of neuropathic diabetic foot ulcers. Regen-D 150 Gel should be applied topically to the full ulcer area. Regen-D 150 Gel may encounter unwanted side effects such as:

1. Irritation, burning or stinging sensation of the skin
2. Dry or flaking skin
3. Thinning or sensitive skin at application site
4. Discoloration of the skin
5. Darkened pigmentation

Inclusion Criteria:

1. Patients between 20 to 50 years of age of both sexes.
2. Admitted patients of chronic non healing ulcers of diabetic, varicose veins and any of non-malignant etiology.
3. Size 4x4 cm and above with no tendency of healing in past 2 months despite conventional treatment.

Exclusion Criteria:

1. Age <20 yrs. and >50 yrs.
2. Patients with deep vein thrombosis
3. Significant arterial insufficiency
4. Severe neuropathy
5. Renal insufficiency
6. Malignant ulcers
7. Parasitic ulcer

Visual Analog Scale: 10^{th} point scale is used in this study. A total of 10 grade scale is used. The percentage of new skin tissue covering is measured as 0 to 10,10 to 20,20 to 30,30 to 40.and 90 to 100. Greater the amount of percentage of skin coverage is given a greater scale. Maximum skin covering the entire wound as taken as 100 percent and is given a 10 point. For e.g: 90 to 100 is given a scale of 10 and 0 to 10 is given.

Statistical Analysis: Data will be analyzed by using graph pad prism software of 6.01 version. Data was summarized by Mean ± SD for continuous data, median ± IQR (Inter Quartile Range) for score data and percentages for categorical data. The comparison between different days within the group was done by repeated measures one way analysis of variancy test and followed by post hoc multiple comparisons test for continuous data. The comparison between two groups was done by T test / MANN WHITNEY U TEST / for continuous data. The association between variables was done by Fischer's exact test / chi square test for categorical data. All P values less than 0.05 where considered as statistically significant.

III Results

The 30 patients admitted for the study were divided into two equal and comparable groups. Patients subjected to topical EGF 0.01%GEL dressings were

classified under study and those who underwent conventional antiseptic wound dressing were classified as control. Out of the fifteen patients in the test group, 6 were males and 9 were females' whereas in the control group 11 were males and 4 were females. In this study of chronic ulcer about 30 patients were under observation. All the patients were subjected to detailed history examination and basic investigation. About 56.67% of the patients were males and 43.33% were females. There was no significant effect of sex on the treatment outcomes between the test and control groups. (p value0.139-not significant). [Table & Figure-1].

Table-1: Sex wise distribution of patients (N=30)

Groups	Male	Female	Total	P-value
Control	11	4	15	0.139
Test	6	9	15	
Total	17	13	30	

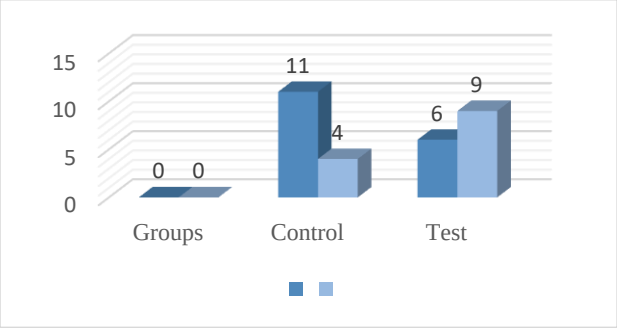

Figure-1: Sex wise distribution of patients Groups.

Table-2: Age wise distribution of patients (N=30)

Groups	N	Minimum	Maximum	Mean	SD	P-Value
Control	15	37	59	46.9	6.8	0.017
Test	15	31	51	40.7	6.4	

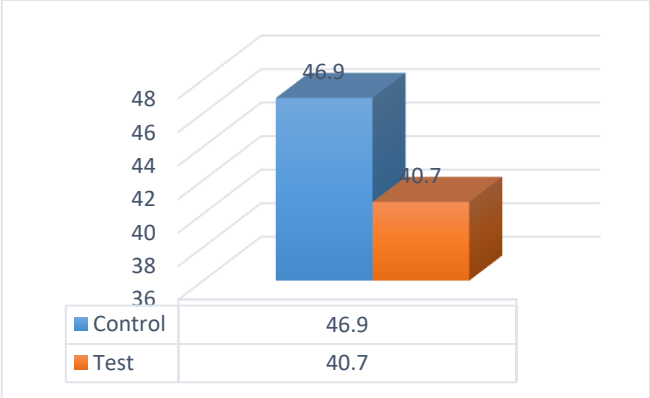

Figure-2: Age wise distribution of patients.

As per the inclusion criteria, patients were enrolled from age group 20 to 60 years. The minimum age of the minimum age of the patient was 31 years and maximum age of the patient enrolled was 59 years. In test group patients were in the range of 31 to 51 years with a mean of 40.7±6.4 years. In control group, the mean age was 46.9 with a standard deviation of 6.8 years, range being 37 to 59 years. There was no statistical significance with regard to age as p value was not significant.i.e. 0.017. [Table-2 and Figure-2].

Table-3: Cause wise distribution of ulcer (N=30)

Groups	Diabetic	Post Burn	Traumatic	Venous	Total	P-Value
Control	6	3	3	3	15	0.852
Test	5	3	5	2	15	
Total	11	6	5	5	30	

The maximum number of patients reported was of diabetic etiology, 11 out of 30, corresponding to 36.67%, followed by traumatic (26.67%), post burn (20%) and venous pathology (16.67%). The cause did not have any statistical significance with p value 0.852. [Table-3].

Table-4: Table showing mean distribution of ulcer area when treated with antiseptic (control) over two weeks (N=30)

Duration (in days)	N	Minimum	Maximum	Mean	SD	P-Value
0	15	12.00	35.00	20.00	8.40	<0.0001
2	15	11.20	33.80	19.20	7.90	
3	15	11.00	32.50	18.60	7.70	
5	15	9.60	30.00	17.50	7.40	
7	15	8.80	27.50	16.50	6.80	
9	15	8.30	25.00	15.40	6.10	
11	15	7.50	22.40	13.90	5.40	
14	15	6.00	20.00	13.10	5.20	

Whereas, the control group received antiseptic dressings. On day 0, the mean area 20.00 cm^2 with a standard deviation of 8.40 cm^2, range being 12.00 to 35.00 cm^2. After two weeks, the ulcer reduced to 13.10 cm^2 with a standard deviation of 5.20 cm^2, range being 6.00 to 20.00 cm^2 [Table-4]. On comparing the two groups, the reduction in ulcer area was significant in test group compared to control group. (P value<0.0001).

Table 5: Table showing mean distribution of ulcer area when treated with epidermal growth factor over two weeks (N=30)

Duration (in days)	N	Minimum	Maximum	Mean	SD	P-Value
0	15	10.00	60.00	32.10	16.60	<0.0001
2	15	9.80	45.00	26.10	11.00	
3	15	9.40	40.00	22.20	8.90	
5	15	8.00	29.30	19.10	7.30	
7	15	7.00	24.50	15.30	5.20	
9	15	6.00	24.10	13.10	4.80	
11	15	5.00	20.50	10.50	4.20	
14	15	4.00	18.00	8.80	3.70	

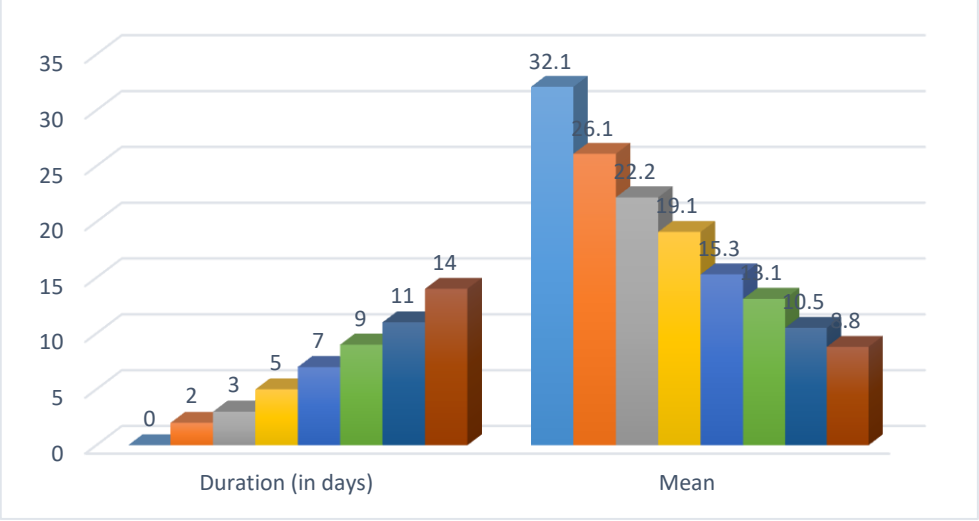

Figure-3: Figure showing mean distribution of ulcer area when treated with epidermal growth factor over two weeks.

The test group received topical EGF for a week of two weeks. On day 0, the mean area of the ulcers was 32.10 cm^2 with a standard deviation of 16.60 cm^2. (Range 10.00 to 60.00 cm^2) [Table-5 and Figure-3]. After two weeks, the ulcer area reduced to 8.80 cm^2 with a standard deviation of 3.70 cm^2, Range being 4.00 to 18.00 cm^2 [Table-6].

Table-6: Comparison between two groups (N=30)

Duration (in days)	Groups	N	Minimum	Maximum	Mean	SD	P value
0	Test	15	10.00	60.00	32.10	16.60	0.018
	Control	15	12.00	35.00	20.00	8.40	
2	Test	15	9.80	45.00	26.10	11.00	0.058
	Control	15	11.20	33.80	19.20	7.90	
3	Test	15	9.40	40.00	22.20	8.90	0.253
	Control	15	11.00	32.50	18.60	7.70	
5	Test	15	8.00	29.30	19.10	7.30	0.555
	Control	15	9.60	30.00	17.50	7.40	
7	Test	15	7.00	24.50	15.30	5.20	0.594
	Control	15	8.80	27.50	16.50	6.80	
9	Test	15	6.00	24.10	13.10	4.80	0.275
	Control	15	8.30	25.00	15.40	6.10	
11	Test	15	5.00	20.50	10.50	4.20	0.061
	Control	15	7.50	22.40	13.90	5.40	
14	Test	15	4.00	18.00	8.80	3.70	0.015
	Control	15	6.00	20.00	13.10	5.20	

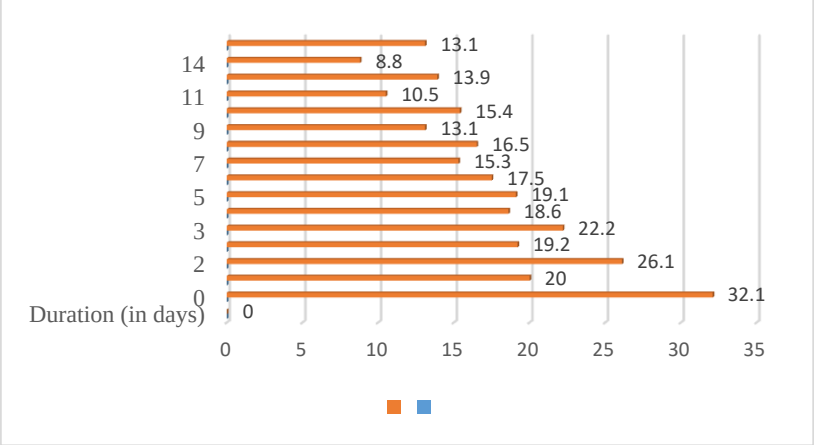

Figure-4: Comparison between two groups Duration (in days).

Table-7: Comparison between two groups (Point scale) (N=30)

Groups	N	Minimum	Maximum	MEDIAN	IQR	P value
Control	15	1	5	3	4 to 2	<0.0001
Test	15	4	8	7	8 to 6	

Epidermal growth factor is available in the commercial trade name of REGEN-D90 and applied over the ulcers. Patients are evaluated daily from day zero to day 14. On fourteenth day, it is observed by visual analog scale that, there is significant decrease in the size of ulcer and formation of granulation tissue in patients who were dressed with epidermal growth factor when compared to patients dressed with normal saline. On comparing the two groups, the reduction in ulcer area was significant in test group compared to control group (P value<0.0001) [Table-7].

IV Discussion

The history of wound healing is as old as the history of mankind. The earliest medical writings deal extensively with wound care. Seven of the 48 case reports included in the Edwin Smith Papyrus (1700 BC) describe wounds and their management. Empirically, the ancient physicians of Egypt, Greece, India and Europe developed gentle methods of treating wounds by removing foreign bodies, suturing, covering wounds with clean materials and protecting injured tissue from corrosive agents.[1] The theory of the "three healing gestures" was formed more than 4000 years ago, with earliest writing recorded on a clay tablet from 2200 BC. These gestures have survived over time, evolving into varying forms of today's same basic themes. The Greeks belief of dry healing came from Hippocrates, at a time when the only function of dressings was thought to be the protection of the wound from injury.[3] Antoine[4], a Belgian Surgeon, was largely responsible for the development and proper use of debridement. Antoine's philosophy was that all war wounds were most likely to be infected and therefore should be debrided. Antoine Depage quoted, "The debridement by opening widely the contused center, decompresses the tissues strangulated by the constrictions of fascia.

The surgeon tries to prevent septic and serious complications, to place the wound in most favorable conditions for healing and suturing". Physiologically, wound healing requires an orchestrated integration of complex biological events including cell migration, cell proliferation, extracellular matrix deposition, revascularization, and reestablishment of tissue integrity[5] .Growth factors involved in these events include EGF, PDGF, FGF, transforming growth factor-b (TGF-b), granulocyte colony stimulating factor (G-CSF), and keratinocyte growth factor (KGF)[52,53]. EGF was discovered by cohen in 1962 .[6]Multiple previous studies have reported that EGF treatment In particular, is associated with increased collagen and glycosaminoglycan content in experimental tissue granulation models .[7]EGF is known to act as a potent mitogenic factor for fibroblasts and epithelial cells.[56] Laatoet al. have shown stimulatory effects of EGF on wound healing due to increased proliferation of collagen-producing fibroblasts.[8]Brown et al. Have demonstrated that application of EGF-containing cream stimulates wound healing. They also demonstrated that the use of cream as a drug delivery vehicle further prevents wound desiccation and reduces the risk of bacterial infection.[9] Nanney reported that EGF interacts with the EGF receptor on epidermal cells and fibroblasts.[59] And several other studies have shown that EGF stimulates epithelial cell growth across the wound surface, enhances epidermal regeneration, and accelerates epithelialization. Though, only a few studies have reported clinical outcomes for diabetic foot ulcers treated with EGF, the results are promising .[10] Hong et al. reported complete healing in 76% (52/68) of chronic Diabetic foot ulcers patients treated with topical recombinant human EGF (rhEGF) applied with an advanced dressing in their observational study .[11] Tsang et al. found that rhEGF cream decreased the median time to complete healing of DFUs in a single-center trial .[12]Optimal concentration and dose of rhEGF for enhancing Diabetic foot ulcers healing, remains controversial.Tsang MW et al reported that 20 of 21 Diabetic foot ulcers completely healed following treatment with locally applied 0. 04% rhEGF cream .[12]However, they suggested that 0.02% rhEGF cream did not offer significant benefits over conventional ulcer management. In contrast, Hong JP et al reported complete Diabetic foot ulcers healing in 52 of 68 patients who received topical wound treatment with low-concentration rhEGF (0.005%) .[11]Kwang Hwan Park et al reported 60 of 82 DFU patients experienced complete ulcer healing within 12 weeks of initiating treatment with twice daily application of 0.005% rhEGF plus multimodal wound management.[13]Kwang Hwan Park et al studied 167 adult patients at six medical centers who were randomized to receive routine wound care plus either topical spray treatment with 0.005% rhEGF (n = 82) or an equivalent volume of saline spray (n = 85) twice a day until ulcer healing or for up to 12 weeks. They concluded that more patients in the rhEGF group significantly had complete wound healing compared to placebo (73.2% versus 50.6%, respectively; P = .001). Wound healing velocity was faster in the rhEGF group (P = .029) regardless of HbA1c levels. The rhEGF group had a shorter median time to 50% ulcer size reduction (21 versus 35 days; hazard ratio = 3.13, P < .001) and shorter time to complete ulcer healing (56 versus 84 days; hazard ratio = 2.13, P < .001)[13] According to previous reports, adverse events during rhEGF treatment have been generally mild to moderate and easily manageable. Tiaka et al. previously reported that skin irritation was the most common adverse event following topical application of EGF, with more adverse events observed at higher doses of EGF versus lower doses.[14] Fernandez-Montequı´net al. reported that 8 (7.9%) of 101 patients receiving EGF treatments experienced SAEs, including severe infection, cellulitis, renal failure, myocardial infarction, and pneumonia, but these SAEs were not believed to be EGF treatment-related.[15] In another preliminary study using spray-applied 0.005% rhEGF for the treatment of Diabetic foot ulcers, Tuyet et al. found that minor over-granulation was observed in one of 28 patients (3.7%), but no skin allergic reactions was reported.[16] Kwang Hwan Park et al reported 6 cases (7.3%) with serious adverse events (SAEs) in the EGF treatment group, but these SAEs were not considered to be EGF treatment-related and were comparable with 7 cases (8.2%) of SAEs in the placebo group .These results support the safety of rhEGF in the treatment of Diabetic foot ulcers.[13] Christman et al. reported that HbA1c was significantly associated with wound healing rate.[17]Vella et al. suggested that HbAlc was an important biomarker in predicting wound healing time.[18] However,Kwang Hwan Park et al reported, HbA1c had no association with wound Healing. Regardless of HbA1c level, healing velocity, time to achieve a 50% reduction in ulcer size, and time to complete ulcer healing of the rhEGF group was significantly faster than those of the placebo group.[13] Previously, several studies showed that faster healing of diabetic wound would decrease serious complications of Diabetic foot ulcers.[19] Veves et al. reported that incidences of osteomyeli-tis and major/minor amputation was significantly decreased by cell therapy in a randomized 12-week trial of 208 patient with diabetic foot ulcers [20] Kwang Hwan Park et althere was no case of osteomyelitis or amputation in both groups during the study period.However, the rate of superficial wound infection at studied ulcer was lower in the rhEGF group .[13] Although our study was not initially powered to investigate DFU complications as primary or secondary endpoint, it is encouraging and indicate that spray-applied rhEGF can help preventing superficial and deep wound infection that finally leads to lower limb amputation. Prabakar A, et al. reported that the rate of healing of ulcers less than 5 cm in the EGF treated group was significantly greater than in the control group. The rate of healing of ulcers moe than 5 cm in the EGF treated group was also significantly greater than in the control group. Overall, the rate of healing of ulcers in the EGF group was compared with the control group. Rate of healing

in EGF group was 86.67% compared to 66.67% in the control group.[11] Vimal Ramachandran et al noticed that the decrease in ulcer size was more evident in the first 15 days when compared to the next 15 days. During this time the ulcer size has reduced more than 50% as compared to the conventional group in which the decrease in size was less than 25 % for most ulcers. In our study we also noted that as compared to the first day, on the 30th day the ulcer healing in terms of size ranged from 54-81.5% in the EGF group as compared to the conventional group in which the decrease in size ranged from 34-47%.

V Conclusion

In this study of 30 patients with non healing ulcer about 56% of patients were male and 54% were female. Most of patients were between 41-60 years of age. With maximum clustering between 41-50 years of age. The study group received 0.01% epidermal growth factor dressing. It showed that a positive response towards complete healing of chronic non healing ulcer when compared to conventional (antiseptic) applied to the ulcer. Only drawback is the high cost for dressing as commercially available comes in thousand rupees. My study is based on those principles but available resource in limited setup. Since non healing ulcer has multi factorial origin, multi disciplinary approach with holistic view forms the backbone for the management of non healing ulcer.

References:

1. Madden JW. Wound healing: the biological basis of hand surgery. ClinPlast Surg.1976; 3(1):3-11.

2. Jeter KF, Tintle TE. Wound dressings of the nineties: indications and Contraindications. ClinPlast Surg. 1991 Oct; 8(4):799-816.

3. Cohen IK. Lessons from the history of wound healing. ClinDermatol. 2007 Jan- Feb; 25(1):3-8.

4. Helling TS, Daon E. In Flanders fields: the Great War, Antoine Depage, and the Resurgence of debridement Ann Surg. 1998; 228(2):173-81.

5. Falanga V. Wound healing and its impairment in the diabeticfoot. Lancet 2005;366:1736–43

6. Bennett SP, Griffiths GD, Schor AM, Leese GP, Schor SL.Growth factors in the treatment of diabetic foot ulcers. Br JSurg 2003; 90:133–46.

7. Dinh T, Braunagel S, Rosenblum BI. Growth factors in woundhealing: the present and the future? Clin Podiatr Med Surg2015;32:109–19.

8. Cohen S. Isolation of a mouse submaxillary gland proteinaccelerating incisor eruption and eyelid opening in the new-born animal. J BiolChem 1962; 237:1555–62.

9. Kwon YB, Kim HW, Roh DH, Yoon SY, Baek RM, Kim JY, et al.Topical application of epidermal growth factor accelerateswound healing by myofibroblast proliferation and collagensynthesis in rat. J Vet Sci 2006;7:105–9

10. Ghatak S, Maytin EV, Mack JA, Hascall VC, Atanelishvili I,Moreno Rodriguez R, et al. Roles of proteoglycans andglycosaminoglycans in wound healing and fibrosis. Int J CellBiol 2015; 2015:834893.

11. Laato M, Kahari VM, Niinikoski J, Vuorio E. Epidermal growthfactor increases collagen production in granulation tissue bystimulation of fibroblast proliferation and not by activation ofprocollagen genes. Biochem J 1987;247:385–8.

12. Brown GL, Curtsinger L, Jurkiewicz MJ, Nahai F, Schultz G.Stimulation of healing of chronic wounds by epidermalgrowth factor. PlastReconstrSurg 1991; 88:189–94 (discussion95-6).

13. Nanney LB. Epidermal and dermal effects of epidermalgrowth factor during wound repair. J Invest Dermatol1990; 94:624–9.

14. Shen C, Sun L, Zhu N, Qi F. Kindlin-1 contributes to EGF-induced re-epithelialization in skin wound healing. Int J MolMed 2017; 39:949–59.

15. Hong JP, Jung HD, Kim YW. Recombinant human epidermalgrowth factor (EGF) to enhance healing for diabetic footulcers. Ann PlastSurg 2006;56:394–8 (discussion 9-400)

16. Tsang MW, Wong WK, Hung CS, Lai KM, Tang W, Cheung EY,et al. Human epidermal growth factor enhances healing ofdiabetic foot ulcers. Diabetes Care 2003; 26:1856–61.

17. Kwang Hwan Park, Seung Hwan Han, JoonPio Hong, Seung-Kyu Han. Topical epidermal growth factor spray for the treatment of chronic diabetic foot ulcers: A phase III multicenter, double-blind, randomized, placebo-controlled trial,.Diabetic research and clinical practice 142(2018)335-334

18. Tiaka EK, Papanas N, Manolakis AC, Georgiadis GS. Epidermalgrowth factor in the treatment of diabetic foot ulcers: anupdate. PerspectVascSurgEndovascTher 2012; 24:37–44.

19. Fernandez-Montequin JI, Valenzuela-Silva CM, Diaz OG,Savigne W, Sancho-Soutelo N, Rivero-Fernandez F, et al.Intra-lesional injections of recombinant human epidermalgrowth factor promote granulation and healing in advanceddiabetic foot ulcers: multicenter, randomised, placebo-controlled, double-blind study. Int Wound J 2009;6:432–43

20. Tuyet HL, Nguyen Quynh TT, Vo Hoang Minh H, ThiBich DN, Do Dinh T, Le Tan D, et al. The efficacy and safety ofepidermal growth factor in treatment of diabetic foot ulcers: the preliminary results. Int Wound J 2009; 6:159–66.