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Comparing the standard surgical dressing with dehydrated amnion and platelet-derived growth factor dressings in the healing rate of diabetic foot ulcer: A randomized clinical trial

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ABSTRACT

Aims: The management of diabetic foot ulcers is a challenging issue due to the pathophysiological background, delay in healing, and prevalence of diabetes. The purpose of this study was to compare the therapeutic effects of the three methods of diabetic wound care: surgical debridement and dressing, dressing with dehydrated amnion powder, and dressing with platelet-derived growth factor gel.

Methods: In this multi-arm parallel-group randomized trial, 243 patients with a minimum 4-week medical history of diabetic foot ulcers with Wagner's grades 1 and 2, no infection, and adequate tissue blood flow were randomly assigned to one of three 81-person groups: surgical debridement (the standard method), dehydrated amnion dressing, or platelet-derived growth factor dressing. The follow-up period lasted 12 weeks. The percentage area reduction (PAR) was measured as the final target. SPSS version 25 was used to perform statistical analysis on the data.

Results: All three study groups were comparable in terms of the type of ulcer, the area of ulcer, Wagner's grade, the period, and the ulcer's size. The PAR in the surgical debridement, platelet-derived growth factor, and dehydrated amnion groups were 7.4%, 14.8%, and 49.3% in week 4; 20.1%, 35.8%, and 79% in week 6; 43.7%, 56.8%, 86.4% in week 8; and 50%, 61.7%, and 87.6% in weeks 10 and 12, respectively. The observed differences were statistically significant ($p < 0.05$) over the entire period.

Conclusion: The study concluded that dehydrated amnion dressing, when compared to platelet-derived growth factor dressing and surgical debridement, resulted in better-improved healing in diabetic foot ulcer patients.

1. Introduction

The prevalence of diabetes for all age groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The total number of people with diabetes is increasing, from 171 million in 2000 to 366 million by 2030. Aside from acute complications such as hypoglycemia, diabetic ketoacidosis, eye diseases, renal impairment/failure, and hyperosmolar coma, one of the most annoying complications of diabetic patients is foot ulcers. Approximately one-quarter of diabetic patients develop diabetic foot ulcers over the course of their lives [1].

Each year, more than one million amputations are caused by non-traumatic factors worldwide; approximately half of these are due to diabetic foot ulcers [2]. In diabetic patients, the ulcer is caused by a variety of factors, such as neuropathy, trauma, and deformity [3], and they are typically subjected to prolonged healing as well as an increased risk of infection, hospitalization, and amputation [4,5]. Diabetic foot ulcers are extremely difficult to treat, and conventional treatments are often ineffective [6,7]. Therefore, trying new techniques to treat foot ulcers is one of the top priorities in these patients.

New treatments with platelet-derived growth factor (PDGF) and granulocyte–macrophage colony-stimulating factor (GM-CSF) have been reported to be effective in wound healing [8]. PDGF is a growth factor involved in the formation of new blood vessels and vascular regeneration [8]. Microscopic examinations have revealed that PGDF is effective in exacerbating inflammation and increasing the presence of neutrophils, monocytes, and fibroblasts [9]. It promotes the production of new blood vessels and aids in wound healing. The FDA has approved PDGF-BB (Becaplermin), a topical human compound, to treat diabetic foot ulcers [10].

Another new treatment for wounds is the use of amniotic membrane.

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The amniotic membrane was first used as a dressing in a burn wound. Moreover, one of the most common medical applications of amniotic membrane dressing is the recovery of corneal and conjunctival injuries [13]. In the treatment of wounds, the presence of a primary matrix, known as scaffolds, is necessary. This matrix's function is to create an environment for cells to connect, grow, and differentiate, as well as to aid in wound healing. The inner surface of the placenta has a layer of epithelial cells with thick basal cells and abundant stroma but no blood vessels. This stroma is made up of a large extracellular matrix, collagen, active cells, and vital molecules involved in the process of cell regeneration [14]. By using this membrane or its derivatives as a dressing, the antimicrobial, analgesic, anti-inflammatory, and scar tissue reduction effects make an impact at the application site [15]. Today, the amniotic membrane is processed and marketed as a sterile dehydrated powder.

There are no similar studies in the Cochrane and PubMed databases that investigate the effect of platelet growth factors, dehydrated amnion, and standard diabetic foot ulcer care methods at the same time. Due to the large number of patients with diabetic foot ulcers and the risk of amputation, this study was necessary to help improve the treatment of these patients. Therefore, the purpose of this study was to compare the healing effects of two new methods of treating diabetic foot ulcers, namely, dehydrated amnion and PDGF dressings, versus surgical debridement.

2. Materials and methods

This prospective interventional study was conducted on all patients with chronic diabetic foot ulcers with Wagner's grades 1 and 2 (Fig. 1) referred to Imam Hossein Hospital in Tehran in the first 10 months of 2020.

Ethical approval: Prior to the study, informed written consent was obtained from the patients. Nonetheless, according to the Helsinki Declaration's ethical principles, their participation or non-participation in the research had no effect on their diagnosis and treatment. The ethics committee of Shahid Beheshti University of Medical Sciences approved the study (IR.SBMU.MSP.REC.1398.545). The trial was registered as IRCT20120215009014N352 in the Iranian Registry of Clinical Trials (IRCT).

Participants: Inclusion criteria were diagnosis of type 1 or 2 diabetes mellitus, diabetic foot ulcers measuring 1–10 cm in size in the plantar aspect of the foot or in the phalanges, no healing at least 4 weeks after onset, ulcers with Wagner's grades 1 and 2, no radiological evidence of osteomyelitis, age over 18, HbA1c<8% (DCCT), adequate tissue circulation (Ankle Brachial Index > 0.7 or tri-phasic or biphasic blood flow in color Doppler ultrasound), and no malnutrition (albumin > 3 g/dl, total protein > 6 g/dl). If patients had more than one wound, the largest wound was used as index ulcer for inclusion in this study.

Exclusion criteria included being over the age of 75, wound duration>52 weeks, evidences of active Charcot's foot, having recently received chemotherapy or radiotherapy, having a suspected or known malignancy, having autoimmune connective tissue disease or kidney disease requiring dialysis, being on immunosuppressive drugs or drugs that affect tissue regeneration such as corticosteroids, and pregnant or breastfeeding women.

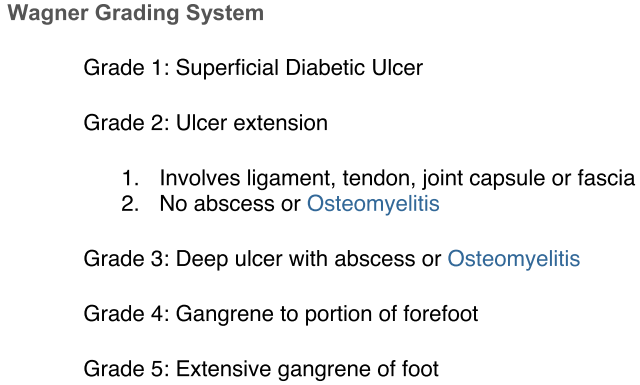

Fig. 1. Wagner Grading System.

Sample size: We conducted a multi-arm parallel-group randomized trial. The sample size was calculated using the G-Power 3.1.9.2 software for three groups with a significance level of<0.05 and a test power of 95%. Further, the 20% sample loss resulted in an estimate of 80 people in each group.

Randomization: During the implementation of the study, 400 patients with diabetic foot ulcers who referred to our clinic were examined. Of these, 270 patients met the inclusion and exclusion criteria and were randomly divided into three groups. Nine patients were removed from each group due to lack of cooperation with the research group and finally 81 patients remained in each group (Fig. 2). Following a 2-weeks run-in period, randomization was performed using a random table, with each patient labeled with a random number by a blind research assistant. One case in the surgical debridement group was lost to follow-up due to aggravation of infection and need for amputation.

Procedures: In the first session, a detailed history was taken from each patient regarding the duration and type of diabetes, duration of ulcer and prior treatment taken for ulcer any other co-morbid condition. We examined the involved foot to determine the ulcer location, size, shape, depth, any discharge, tenderness or rise in temperature and then photographed the foot ulcers, and drew ulcer area on a transparent sheet. Its size was measured and calculated by multiplying its largest length by its largest width. We do this in the day of randomization, prior to any debridement or treatment. Also we took an in-depth culture of wounds and performed laboratory tests, such as serum creatinine and blood urea nitrogen level (BUN), hemoglobin, and albumin in the first session.

In the first group, the regular debridement and dressing method was performed daily. If necessary, the necrotic tissue was debrided, and the wound was irrigated with normal saline, and "wet to dry" dressing was performed every day. A "wet to dry" dressing is used to remove dead tissue from the wound [7]. A piece of gauze is moistened with normal saline solution. Then it is put on the wound and allowed to dry. After the dressing dries, the dead skin tissue sticks to the gauze and comes off the wound when the bandage is removed.

In the second group, after debridement and wound irrigation, we used a gel containing PDGF with the generic name Becaplermin 0.01% (Regranex, Smith & Nephew) daily. We used 15 g tubes and varied the gel amount applied depending on the size of the ulcer area [10]. We used the following formula to apply:

$$\text{Length of ulcer} \times \text{width} \div 4 = \text{Length of gel (cm)}$$

Sterile gauze was soaked in normal saline before being placed on the gel and then gently bandaged. After 24 h, the gel's remnants were washed with normal saline, and it was re-dressed with the gel and gauze soaked in normal saline.

In the third group, after debridement and washing, a powder containing sterile dehydrated amnion with the generic name AMOR (Royan, Iran) was sprinkled on the wound to completely cover it. Then, it was covered with saline-soaked gauze [13]. In this group, the dressing was changed every 7 days [15].

We examined the patients once a week. If necessary, necrotic tissue debridement was performed, and the size of the wound was measured using the abovementioned method. We also repeated the in-depth culture of wounds and laboratory tests, such as serum creatinine and BUN, hemoglobin, and albumin. If the patients had symptoms of infection like pus, erythema, swelling, or fever, they were given systemic antibiotics. During the treatment, all wounds were offloaded.

We used the following formula to calculate the effect on wound healing or percentage area reduction (PAR):

$$\text{PAR} = (\text{primary wound area - secondary area / primary area}) \times 100$$

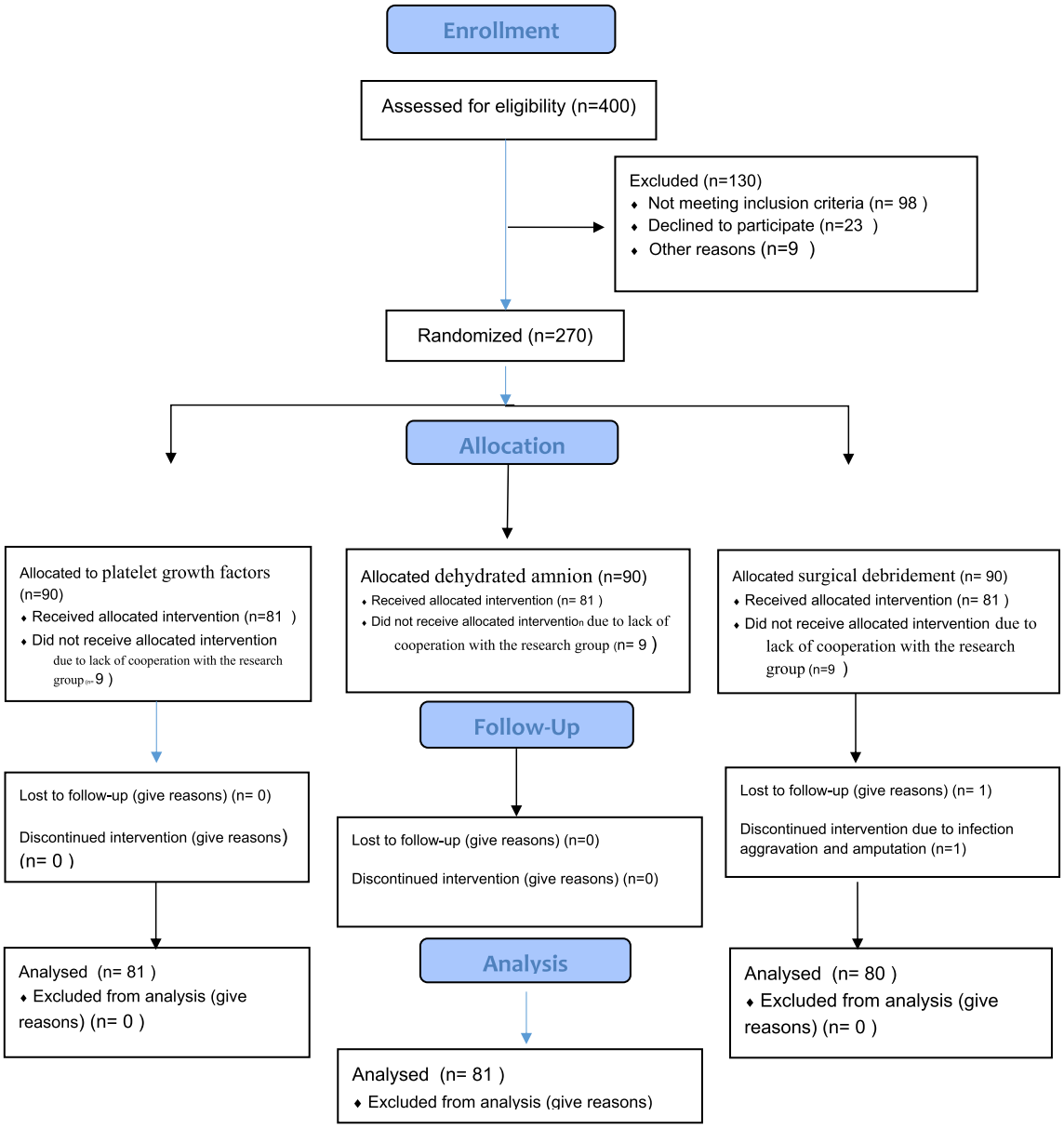

Fig. 2. Flow diagram of the study.

This was repeated in the fourth, sixth, eighth, and tenth weeks by a blinded assistant. In the case of healed wounds, we followed up with patients to ensure that the wound remained healed after 2 weeks per FDA recommendations.

Statistical analysis: Our primary endpoint was PAR, and no secondary endpoints were considered. Infection and side effects of the products were considered as safety endpoints. The collected data were entered into SPSS software version 25 (IBM, Inc., Armonk NY, USA) and displayed as mean ± SD or n (%). We used the Kolmogorov–Smirnov test for abnormal data distribution, the Mann–Whitney test to compare the mean of quantitative variables between two groups, and the Kruskal–Wallis test to compare three groups. In addition, to compare the frequency distribution of qualitative variables, the chi-square test was used. In all analyses, we considered a significance level of<0.05.

3. Results

In demographic analysis, there were 35 (43.2%) females and 46 (56.7%) males with a mean age of 60.2 ± 5.2 years in the surgical debridement group, 40 (49.3%) females and 41(50.6%) males with a mean age of 57.9 ± 5.8 years in the dehydrated amnion dressing group, and 29 (35.7%) females and 52 (64.3%) males with a mean age of 55.8 ± 5.6 years in the PDGF dressing group, which showed no significant difference between them (p-value > 0.05). The three groups did not differ significantly in wound type, wound location, Wagner's grade, wound duration, and wound size (Table 1).

On the other hand, the mean wound area before the intervention did not differ significantly between the three groups (Table 2). However, in the fourth week of the intervention, the wound area in the dehydrated amnion group with a mean size of 0.82 ± 1.41 cm^2 was significantly less than that the surgical group with a mean size of 2.43 ± 1.71 cm^2 (p-value = 0.003). In the sixth week of the intervention, the wound area in the two groups of dehydrated amnion and PDGF dressing was 0.16 ± 0.59 and 1.04 ± 1.66 cm^2, respectively, which was smaller than the surgical group with a mean of 1.81 ± 1.71 cm^2 (p-value = 0.027). In the eighth and tenth weeks, the wound area in dehydrated amnion group was significantly smaller than the surgical group (p-value = 0.009 and p-value = 0.007, respectively). In contrast, no significant difference in the

Table 1
Basic characteristics of the patients.

Characteristics	Surgical debridement (n = 80)	Dry amniotic dressing (n = 81)	Dressing with platelet derived growth factor (n = 81)	P value
Sex				
Female (n)	35(43.2%)	40(49.3%)	29(35.7%)	0.747
Male (n)	46(56.7%)	41(50.6%)	52(64.3%)	
Age; (year ± SD)	60.2 ± 5.2	57.9 ± 5.8	55.8 ± 5.6	0.127
BMI; (kg/m^2 ± SD)	30.5 ± 1.7	30.7 ± 2.1	31.7 ± 2.1	0.260
Duration of diabetes; (year ± SD)	10.8 ± 0.9	12.0 ± 1.2	11.4 ± 1.9	0.141
HbA1C; (%)	7.3 ± 0.3	7.4 ± 0.3	7.3 ± 0.2	0.981
Type of wound				
Traumatic (n)	64(79%)	58(71.6%)	58(71.6%)	0.884
Other causes (n)	17(21%)	23(28.4%)	23(28.4%)	
Wound location				
Plantar (n)	46(56.7%)	58(71.6%)	52(64.1%)	0.733
Dorsum (n)	35(43.2%)	23(28.4%)	29(35.8)	
Wagner's classification				
Grade I (n)	12(14.8%)	6(7.4%)	12(14.8%)	0.797
Grade II (n)	69(85.2%)	75(92.6%)	69(85.2%)	
Wound duration; (week ± SD)	6.4 ± 1.8	6.3 ± 0.8	6.0 ± 0.7	0.604
Wound size; (cm2 ± SD)	3.3 ± 0.5	3.4 ± 0.4	3.2 ± 0.5	0.335

mean wound area was found between the PDGF dressing and the surgical dressing at any of the follow-up times (Table 2).

In the fourth week of treatment, the PAR in the dehydrated amnion dressing group was 49.3%, which was higher than the PAR in the PDGF (14.8%) and surgical dressings (7.5%) groups (p-value = 0.017). This recovery trend was maintained in the sixth, eighth, and tenth weeks. At the end of the study (tenth week), the PAR was 87.6%, and 61.7% in the dehydrated amnion dressing group and the PDGF dressing group respectively, and 50% in the surgical dressing group (p-value = 0.011) (Table 3). We found no specific side effects with any of the methods and the safety profiles of all groups were similar.

4. Discussion

The present study found that dehydrated amnion dressing in patients with diabetic foot ulcers was associated with a PAR of 87.6% in 10 weeks. The PAR in patients with conventional surgical dressing was 50% and 61.7% in PDGF dressing in the same time. It demonstrates that dehydrated amnion dressing in diabetic foot ulcers can improve patient recovery in comparison with the two other methods, however, the use of PDGF is also satisfactory.

Treating diabetic foot ulcers is always a challenge for both patients and physicians. Treatment and healing of these chronic wounds are often brutal due to prolonged inflammation, stunted epidermal growth, infection with drug-resistant microorganisms, and microvascular complications [11]. Several clinical trial studies have been conducted to evaluate the efficacy of new treatments for diabetic foot ulcers, with varying results [12]. Our research findings show that using an amniotic membrane in the treatment of diabetic foot ulcers is one of the most effective methods.

In a *meta*-analysis study published by Paggiaro et al. [13] in 2018, it was indicated that wound healing in patients with amniotic membrane dressing was 32 days earlier and 2.32 times more than in patients with standard wound dressing. However, in the majority of other studies, no significant difference was observed with the standard group. In a comparative study, Zelen et al. showed that the dehydrated amniotic membrane dressing method's recovery rate was 92%, compared to the 8% for the standard method [14]. Their results with amniotic dressing are consistent with ours but disproportionate to our results with the surgical approach. The results of the *meta*-analysis of Haugh et al. in 2017 showed that in 311 patients with diabetic foot ulcers who were selected from 5 clinical trial studies, amniotic products increased the recovery rate by 2.74 times when compared to the conventional method [16]. This result is more consistent with the results of our study. In another clinical trial study, Lavery et al. used the amniotic membrane (Grafix) to treat diabetic ulcers [17]. They studied 50 patients, and reported that the average time for complete wound healing in the intervention group was 42 days, with a wound reduction rate of 62% on day

Table 3
Frequency distribution of PAR in the three groups of study.

PAR	Surgical debridement (n = 80)	Dry amniotic dressing (n = 81)	platelet derived growth factor (n = 81)	P value
Forth week	7.5%	49.3%	14.8%	0.017
Sixth week	21.2%	79%	35.8%	0.002
Eighth week	43.7%	86.4%	56.8%	0.019
Tenth week	50%	87.6%	61.7%	0.011

PAR: percentage area reduction.

Table 2
Comparison of mean wound area (cm^2) in the three groups.

Wound area(cm2 ± SD)	Surgical debridement (n = 80)	Dry amniotic dressing (n = 81)	Dressing with platelet derived growth factor (n = 81)	P^1	P^2	P^3
Before intervention	3.29 ± 1.51	2.75 ± 2.22	2.68 ± 1.76	0.839	0.178	0.285
Forth week	2.43 ± 1.71	0.82 ± 1.41	1.66 ± 1.84	0.104	0.003	0.164
Sixth week	1.81 ± 1.71	0.16 ± 0.59	1.04 ± 1.66	0.027	0.001	0.104
Eighth week	1.36 ± 1.65	0.15 ± 0.44	0.62 ± 1.29	0.210	0.009	0.178
Tenth week	1.02 ± 1.43	0.13 ± 0.3	0.37 ± 0.87	0.352	0.007	0.306
P^4	<0.001	<0.001	<0.001			

P1: Significance level obtained from independent *t*-test in comparison of mean wound area between two groups of dehydrated amnion dressing and platelet-derived growth factor dressing.
P2: Significance level obtained from independent *t*-test in comparison of mean wound area between two groups of dehydrated amnion dressing and surgical dressing.
P3: Significance level obtained from independent *t*-test in comparison of the mean wound area between the two groups of platelet-derived growth factor dressing and the surgical dressing.
P4: Significance level obtained from the analysis of variance test regarding the changes in the mean wound area from before intervention to the tenth week of after intervention in each group.

28. This result is also consistent with the results of our study.

The large extracellular matrix, collagen, active cells, and vital molecules found in the stroma layer of the epithelial layer of the inner surface of the placenta impress the cell regeneration and the wound healing process [14].

In addition to fibronectin and collagen, glycoproteins, and glycosaminoglycans, amniotic membrane with high levels of growth factors, neutrophils, and interleukin antagonists is effective in the treatment of chronic wounds. Amniotic membrane extract or powdered dehydrated amniotic membrane has the same beneficial properties of amniotic membrane. Its ease of use and application in deep and deformed wounds reduces the cost of treatment for the patient [18].

On the other hand, the results of studies show that the use of PDGFs can be effective in healing diabetic wounds. PGDF is effective in exacerbating inflammation and increase the presence of neutrophils, monocytes, and fibroblasts [9]. It promotes the production of new blood vessels and aids in wound healing. A study in India in 2013 showed that the rate of complete recovery was significantly higher in 19 patients who received platelet-derived growth factor (rh-PDGF-100ug/ml) compared to the standard method. Also, the mean area of wound at 4 and 6 weeks was significantly reduced in the PDFG group [19]. The results of a study by Rangaswamy et al. on 50 patients with diabetic foot ulcers showed a significant difference between the use of PDGF and standard treatment [20]. In a study on refractory diabetic wounds, 80% of cases treated with platelet gel improved, while only 40% of the control group improved [21]. In addition, a prospective study conducted in the United States demonstrated that using activated platelets was more effective than other standard treatments for diabetic ulcers [22]. According to other clinical trial studies, the recovery time and rate range between 12 and 20 weeks and 33% and 57.5%, respectively [23,24]. Becaplermin gel contains a large number of tissue growth factors. These factors include PDGF, transforming growth factor [TGF), epidermal growth factor (EGF), and insulin-like growth factor (IGF). TGF is effective in stimulating fibroblasts and endothelial cells. EGF stimulates appletization and angiogenesis. IGF is effective in wound healing and is involved in the proliferation and differentiation of osteoblasts [8]. Becaplermin gel stimulates angiogenesis. It acts as a chemotactic factor for monocytes, macrophages, and fibroblasts, resulting in increased collagen synthesis and tissue granulation. It is also useful for leukocyte accumulation and has an antibacterial effect [10].

In a study of 922 patients by Nagai et al. in 2002, they reported more complete wound healing with the use of Becaplermin 100 μg/g topical gel compared to the control group [10].

In our study, this product had a significant impact on wound healing, increasing the rate and speed of healing when compared to the standard surgical dressing, but it had a lesser impact when compared to amniotic dressing. The costs of the three methods used in this study did not differ significantly.

5. Conclusion

This study shows that in treating diabetic foot ulcers, dressing with dehydrated amnion or a gel containing PDGF has advantages over conventional debridement and dressing methods. When dehydrated amnion was compared to gel containing PDGF, it was discovered that dehydrated amnion dressing is associated with faster and better healing in diabetic foot ulcers. However, PDGF gel is also effective. Accessibility, economic factors, follow-up and referral durations, and the patient's desire to use the method can all influence the decision to use dehydrated amnion versus platelet-derived growth factor dressing.

6. Limitations

The current study had several limitations. First, due to problems caused by the method used in obtaining a medical history when admitting patients for surgery, a comparison of comorbidity data was not performed. As a result, we failed to define the secondary objectives in the study. Second, there was a low incidence of patient morbidity during the study, which was not in accordance with other studies.

Authorship: AMT and MT participated in and were involved in all stages of this research and writing the paper and accept public responsibility for the content.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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