The efficacy of platelet-derived wound healing formula (PDWHF) in diabetic foot ulcers: Revisited

Hany M Abdel Aziz,[a] MSC; Basim M Zaki,[b] MD; Nahed S Boughdadi,[b] MD; Karim El-Lamie,[b] MD; Ashraf Maher,[b] MD; Iman Kamel,[c] MD

a) Department of Plastic Surgery, El-Helmeya Military Hospital, Cairo, Egypt.
b) Department of Plastic Surgery, Ain Shams University, Cairo, Egypt.
c) Department of Clinical Pathology, El-Mataria Teaching Hospital, Cairo, Egypt.

Abstract

Background: Diabetic ulcer of the foot is a major health problem with a high socioeconomic impact and may finally progress to amputation.

Methods: Forty patients suffering diabetic foot ulcers with history of non-healing for 8 weeks at least were subdivided into two groups (20 patients each). The first group (control) was treated with local antibiotic ointment and the second group (study) was treated with twice weekly local platelet gel application for 10 weeks. The aim of the study was to clinically evaluate the impact of platelet gel on healing of diabetic foot ulcers and to verify the effect of topical platelet gel therapy on the serum level of platelet derived transforming growth factor beta (TGF-ß).

Results: Analysis of ulcers' criteria showed that patients treated with platelet gel twice/week had a highly significant increase in rate of re-epithelialization compared to patients treated with topical antibiotic ointment. Serum TGF-ß was found significantly increased in the patients treated with platelet gel in comparison to the control group. There was no correlation between the level of TGF-ß and ulcer criteria at the beginning of the study, however; there was a negative significant correlation between its level and ulcer surface area, volume and Gilmen index in the study group after 10 weeks.

Conclusion: Platelet gel can be accepted as a valuable alternative in the wound healing pharmacology.

Introduction:

Lower extremity ulcers represent a major concern for patients with diabetes and those treating them, both from the quality of life and the economic standpoint, which is known as "The burden of diabetic foot ulcers".[1] The causes of these ulcers are multifactorial; vascular, neuropathic, infectious, cellular and biochemical abnormalities which all contribute to the problem.[2]

The wound healing process is, in large part, regulated by the ordered production of cytokines and growth factors that control cellular migration and proliferation within the wound milieu. Growth factors are mostly larger proteins than cytokines and, unlike the latter which are responsible for inflammation and immune response, growth factors influence cell proliferation, angiogenesis and protein synthesis.[3] These growth factors are synthesized and secreted by many types of activated cells involved in tissue repair including platelets, inflammatory cells, fibroblasts, epithelial cells and vascular endothelial cells.[4]

Inflammatory mediators like growth factors, cytokines, and nitric oxide (NO) are released by activated cells during the process of injury and have a crucial role in orchestrating the process of healing. The platelet derived wound healing formula (PDWHF) is an autologous concentrate of eight growth factors: PDGF-AA, PDGF-BB, PDGF-AB, TGF ß-1, TGF ß-2, vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and insulin like growth factor (ILGF-I). These growth factors are stored within platelet alpha granules

and initiation of their release begins within 10 minutes of blood clotting.[3-6]

After 1 hour of injury, 95% of these factors are released, and the platelets then continue to synthesize and release additional growth factors over the next 7 to 8 days.[4,5] When examined individually, the growth factors within PDWHF produce a multitude of effects. PDGF is a potent mitogenic and chemotactic factor for both fibroblasts and osteoblasts. In vivo studies have shown PDGF to stimulate bone formation and consistently enhance wound fill.[7,8] TGF stimulates the proliferation of osteoblast precursor cells, has a direct stimulatory effect on bone collagen synthesis, and also decreases bone resorption by inducing apoptosis of osteoclasts.[9] TGF-ß appears to be the major factor responsible for the formation of granulation tissue and the synthesis of proteins of the extracellular matrix and thus has deserved to be called a "wound hormone".[10] ILGF-I has been shown to enhance the differentiation of osteoblasts by increasing the expression of type I collagen as well as the rate of bone matrix apposition.[10-13] VEGF is a potent angiogenic cytokine that promotes endothelial cell proliferation and migration, leading to increased vascular ingrowth.[4,14] Finally, EGF has demonstrated the ability to speed wound epithelialization and reduce scar formation.[4,15,16]

Of the myriad of cytokines that have been investigated in terms of wound healing, TGF ß-1 has undoubtedly the broadest effects. Despite the vast number of reports documenting the actions of TGF-ß, both in vitro and in vivo, controversy remains as to its endogenous role. The paradoxical actions of TGF-ß are best appreciated in inflammation, dependent upon the state of differentiation of the cell and the context of action, TGF-ß acts in a bi-directional manner.[13] Understanding the nature of TGF-ß has led to the hypothesis that it may act as a therapeutic tool in some circumstances, and also act as a target for therapeutic intervention in others.[8,13] Latent TGFß-1, released in large quantities by degranulating platelets, is activated from its latent complex by proteolytic and non-proteolytic mechanisms[3] to influence wound healing from the initial insult and clot formation to the final phase of matrix deposition and remodeling.[4]

Using an ELISA technique on retrieved chronic wound fluid like diabetic foot ulcer or pressure sores, it was demonstrated that they lack essential growth factors.[6,7] Two theories have been proposed in order to explain such a phenomenon. The first is lack of synthesis of these growth factors as revealed by the decreased expression of mRNA and protein of several growth factors.[17] The other theory was that there is increased destruction of growth factors in the wound environment of diabetic patients which was supported by the increased levels of matrix metalloproteinases (MMPs) in diabetic wounds compared with that of acute wounds.[9,10] Moreover, it has been shown that macromolecular leakage, specifically fibrinogen, alpha macroglobulin, and albumin, leads to binding of these growth factors, making them unavailable for the process of healing. This process of trapping has been demonstrated in diabetic ulcers and venous stasis ulcers.[18]

Several studies have evaluated the "platelet derived wound healing formula" (PDWHF) as a source of growth factors in the clinical field.[13-16] However, conflicting data arose from these works regarding the effectiveness of applying the platelet gel in wound healing. In our study, we re-evaluated the effect of homologous platelet rich plasma on the rate of diabetic foot ulcer healing in comparison to the conventional method of local antibiotic ointment in chronic ulcer management. Moreover, the serum levels of TGF-ß in the studied patients were investigated, being one of the leading platelet derived growth factors involved in wound healing.

Materials and methods:
  Subjects:
A total of 40 patients with type II diabetes were enrolled in the present study, all presenting with foot ulcers persisting for at least 8 weeks. Patients were recruited from the plastic surgery out-patient clinic of Ain-Shams University hospitals and El-Helmeya military hospital. Twenty three patients (57.5%) were females, whereas seventeen patients (42.5%) were males. Full medical history, thorough local and general examination; radiological and laboratory assessment were performed to all patients enrolled in the study. Exclusion criteria included those patients receiving

corticosteroids, immunosuppressive agents, radiotherapy or chemotherapy. Chronic ulcers of etiological origin other than diabetes were excluded. Osteomyelitis of the underlying bones and associated limb ischemia were also excluded through plain x-rays and Doppler ultrasound respectively. Each patient signed an informed consent for the treatment before participating in the study. Many patients were excluded from the study as they refused to be subjected to blood withdrawal twice weekly.

Randomization and treatment:

Subjects were randomly divided into two groups (twenty patients each), the first group was assigned to topical antimicrobial agent (control group), while the second group was treated with topical platelet gel therapy (study group). The study team regularly followed up all patients of both groups twice weekly for a total of 10 weeks duration. Before the start of treatment, ulcers were subjected to surgical debridement to remove any necrotic tissue as well as culture and sensitivity tests to eradicate infection. Further debridement throughout the treatment period (10 weeks) was done as necessary for each case. Both groups were placed under the same medical conditions regarding control of the diabetic status, and were given instructions about the methods of pressure-relief (crutches, orthotic shoes, etc.) to be used during the treatment period in order to improve the reliability of the results.

Preparation and application of the platelet gel:

PDWHF is an autologous hemocomponent with adhesive properties obtained by associating activated hyper-concentrated platelets and cryoprecipitate. Preparation was initiated by drawing 20-50 mL of blood from the patient. The volume of drawn blood was determined by the size of the ulcer, whereas every 10 mL blood yields 1 mL platelet concentrate. Blood was drawn immediately before application and was not stored as instructed by previous researchers. Once obtained, the patient's blood was mixed with citrate anticoagulant in a ratio of 10:1, and then centrifuged at low speed centrifugation (500G) for 5 minutes in the MEDTRONIC®

device. This resulted in segregation of blood components into red blood corpuscles (in one chamber) which was discarded, and in the other chamber platelet poor plasma with a buffy coat containing white blood cells and platelet concentrate (platelet rich plasma). This was mixed with human thrombin derived from the platelet poor plasma in a ratio of 1:1 to form a gel in addition to activation of growth factors. Calcium chloride 10% (0.5 mL) was added to antagonize the anticoagulant effect of citrate. The platelet derived wound healing formula remains stable in an anticoagulated state for up to 8 hours after processing.[4,5] After cleaning the ulcer, the gel was applied locally using a dropper, followed by a period of 15 minutes before covering the ulcer with occlusive Opsite®.[6, 11, 14]

Laboratory investigations:

Preoperative assessment through complete blood count (CBC), fasting and 2 hours post-prandial blood glucose, glycated hemoglobin (Hb A1c), lipid profile, liver function tests (ALT and AST) and serum creatinine were done to all candidates participating in the study. Blood samples were collected for estimation of TGF-ß levels twice from all patients of both groups; once before the beginning of the study and the other after ten weeks of treatment. Serum was separated and stored at -20°C, and TGF-ß levels were measured using isoform-specific sandwich enzyme linked immunosorbant assay. Levels were compared to the reference range for human subjects (2.1 - 6.1 ng/mL) established by Wakefield et al.[19]

Evaluation of treatment:

Ulcers in both study groups were assessed clinically on a weekly-basis from the beginning of the study until healing or the elapse of ten weeks whichever came first. Assessment was done through measuring ulcers' dimensions using a measuring ruler; measurements were taken by centimeters approximated to the nearest one decimal point and the surface area was calculated. Photography was done at the beginning, throughout and at the end of the study period and comparison was performed. Results were compared to evaluate treatment advancement, healing progression and to detect

any difference. Adverse events - if present - were recorded at each visit.

Statistical analysis:

SPSS statistical software package (V. 17.0, Echosoft Corp., USA, 2008) was used for data analysis. Data were expressed as mean ± SD for quantitative measures. Comparison between two independent groups for non-parametric data was done using Wilcoxon Rank Sum test. Wilcoxon signed rank test was done for comparison between two dependent groups for non-parametric data. To study the degree of change due to follow-up, delta change (dC) was calculated for each patient. Ranked Speerman correlation test was done to study the possible association between each two variables among each group for non-parametric data. The probability of error at 0.05 was considered significant, while at 0.01 and 0.001 highly significant.

Operative management:

After the study period was conducted, operative coverage of the residual ulcer by a split thickness skin graft was performed in some of the cases. Two of the control group patients and three cases from the study group underwent grafting.

Results:

The forty patients enrolled in the study were randomly divided into two groups; control group and study group. The duration of the included ulcers in all subjects was 8-22 weeks with a mean duration 16.38 (± 7.32) weeks. Anatomical distribution of the included ulcers is demonstrated in **Table(1)**. Demographic criteria and ulcer criteria of both groups at the beginning of the study are documented in **Table(2)**. There was no statistically significant difference between any of the items studied between the two groups at the beginning of the study.

The clinical results comparison at the end of the 10th week is shown in **Table(3)**. There was a statistically highly significant difference in all ulcer criteria and statistically significant difference in TGF-ß serum level between the two groups after ten weeks of treatment. Statistical comparison between subjects' criteria at the beginning and after 10 weeks of treatment in the control and study groups is shown in **Tables(4,5)**, respectively. In the control group, there was no statistically significant difference in ulcer criteria between the beginning and end of the study period. While in the study group, there was a statistically highly significant difference in the ulcer length, width, surface area, circumference and Gilmen Index and a statistically significant difference in ulcer surface area and serum level of TGF-ß.

Correlation studies between TGF-ß serum levels and other various parameters at the beginning and end of the study in the disease control and study groups are demonstrated in **Tables(6,7)** respectively. In the control group, there was no correlation between TGF-ß levels and any of the ulcer criteria whether at the beginning or after ten weeks of treatment. In the disease study group at the beginning of the study, there was no correlation between TGF-ß and any of the ulcer criteria, while after ten weeks of treatment there was a significant negative correlation with ulcer surface area and Gilmen Index, and no significant correlation with ulcer length, width and circumference.

Table (1): Anatomical distribution of study ulcers.

Site of ulcer	Control Group (n=20)		Study Group (n=20)	
	No. of cases	% of cases	No. of cases	% of cases
Big toe	2	10	3	15
Lateral four toes	3	15	3	15
First metatarsal head	3	15	2	10
Second metatarsal head	2	10	2	10
Sole	3	15	2	10
Ankle	4	20	5	25
Amputated stumps	3	15	3	15

Table (2): Demographic criteria of enrolled subjects of both groups at the beginning of the study.

Criteria	Diseased Control Group (n=20)	Diseased Study Group (n=20)	Z	p	S
Age (yrs)	59.1 ± 11.92	57.9 ± 10.26	-1.429a	0.174	NS
Duration of diabetes (yrs)	18.4 ± 6.87	20.1 ± 7.35	-1.261a	0.136	NS
Hb A1c (%)	11.36 ± 5.74	12.13 ± 5.18	-0.961a	0.386	NS
Length of ulcer (cm)	6.61 ± 3.73	6.75 ± 4.01	-1.302a	0.107	NS
Width of ulcer (cm)	3.01 ± 1.17	2.98 ± 1.29	-0.672a	0.439	NS
Ulcer surface area (cm^2)	15.78 ± 11.69	15.56 ± 17.28	-0.928b	0.337	NS
Ulcer circumference (cm)	17.33 ± 8.60	17.34 ± 11.52	-0.617a	0.428	NS
Gilmen Index	0.121 ± 0.046	0.116 ± 0.072	-1.231a	0.193	NS
TGF-ß (ng/dL)	3.81 ± 1.94	3.97 ± 1.87	-0.743a	0.236	NS

NS: No Significance   S: Significant   HS: Highly Significant

Table (3): Demographic criteria of enrolled subjects in both groups at the end of the study (after 10 weeks of treatment).

Criteria	Diseased Control Group (n=20)	Diseased Study Group (n=20)	Z	p	S
Length of ulcer (cm)	6.43 ± 3.52	5.32 ± 3.91	-4.206	0.001	HS
Width of ulcer (cm)	3.01 ± 1.28	2.17 ± 1.15	-3.851	0.001	HS
Ulcer surface area (cm^2)	15.34 ± 12.21	13.46 ± 15.06	-3.231	0.001	HS
Ulcer circumference (cm)	17.37 ± 8.45	15.17 ± 10.53	-2.786	0.004	HS
Gilmen Index	0.113 ± 0.029	0.068 ± 0.106	-3.257	0.003	HS
TGF-ß (ng/dL)	3.87 ± 1.86	4.25 ± 2.61	-2.539	0.011	S

Table (4): Statistical comparison between subjects' criteria at the beginning and after 10 weeks of treatment in the control group (n=20).

Criteria	At the Beginning	After 10 weeks	Z	p	S
Length of ulcer (cm)	6.61 ± 3.73	6.43 ± 3.52	-0.919a	0.358	NS
Width of ulcer (cm)	3.01 ± 1.17	3.01 ± 1.28	-0.982a	0.326	NS
Ulcer surface area (cm^2)	15.78 ± 11.69	15.34 ± 12.21	-1.014b	0.311	NS
Ulcer circumference (cm)	17.33 ± 8.60	17.37 ± 8.45	-0.420a	0.675	NS
Gilmen Index	0.121 ± 0.046	0.113 ± 0.029	-1.114	0.126	NS
TGF-ß (ng/dL)	3.81 ± 1.94	3.87 ± 1.86	-0.976a	0.331	NS

Table (5): Statistical comparison between subjects' criteria at the beginning and after 10 weeks of treatment in the study group (n=20).

Criteria	At the Beginning	After 10 weeks	Z	p	S
Length of ulcer (cm)	6.75 ± 4.01	5.32 ± 3.91	-3.363a	0.001	HS
Width of ulcer (cm)	2.98 ± 1.29	2.17 ± 1.15	-3.420a	0.001	HS
Ulcer surface area (cm^2)	15.56 ± 17.28	13.46 ± 15.06	-3.409a	0.001	HS
Ulcer circumference (cm)	17.34 ± 11.52	15.17 ± 10.53	-3.409a	0.001	HS
Gilmen Index	0.116 ± 0.072	0.068 ± 0.106	-3.184a	0.001	HS
TGF-ß (ng/dL)	3.97 ± 1.87	4.25 ± 2.61	-2.645a	0.021	S

Table (6): Correlation between TGF-ß levels and other various parameters in the control group.

Parameter	At the beginning			After 10 weeks		
	r	p	S	r	p	S
Length of ulcer (cm)	0.451	0.106	NS	0.463	0.152	NS
Width of ulcer (cm)	0.583	0.061	NS	0.535	0.067	NS
Ulcer surface area (cm^2)	0.468	0.091	NS	0.512	0.065	NS
Ulcer circumference (cm)	0.542	0.024	NS	0.498	0.108	NS
Gilmen Index	0.474	0.088	NS	0.531	1.12	NS

Table (7): Correlation between TGF-ß levels and other various parameters in the study group.

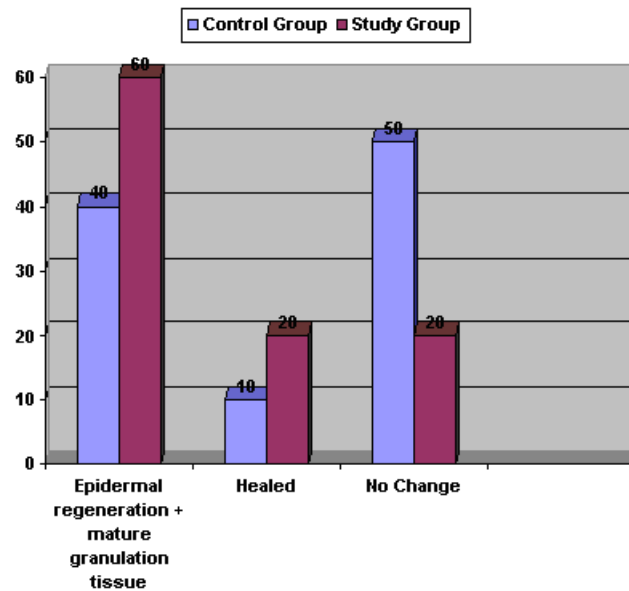| Parameter	At the beginning			After 10 weeks		
	r	p	S	r	p	S
Length of ulcer (cm)	0.438	0.115	NS	0.452	0.126	NS
Width of ulcer (cm)	0.528	0.059	NS	0.518	0.061	NS
Ulcer surface area (cm^2)	0.543	0.086	NS	0.608	0.016	S
Ulcer circumference (cm)	0.491	0.106	NS	0.498	0.108	NS
Gilmen Index	0.474	0.088	NS	0.624	0.013	S

Figure (1): Comparison between control group and study group regarding healing progression.

At the start of treatment   At the end of 10 weeks treatment

Figure (2): A case treated with platelet gel (pre and post-application) showing complete healing.

At the start of treatment   At the end of 10 weeks treatment

Figure (3): A case treated with platelet gel (pre and post-application) showing a healing ulcer.

Adverse events:

Complications in the first group included diffuse inflammation and discharge in three cases (15%), two of which were managed conservatively by antibiotics and the inflammation subsided while the third case was relieved by surgical drainage. The recorded complication among the treatment group was local infection in two cases (10%) managed conservatively.

The two cases in the control group who had undergone skin grafting showed partial graft loss on follow up, while the three operated cases in the study group ended with a 100% graft take.

Discussion:

Diabetes mellitus is one of the leading causes of impaired wound healing. Most chronic wound problems in diabetic patients involve the feet and the impact is enormous considering the number of patients that exist. To expand the significance of the problem one estimate suggested that admissions for foot infections constituted 20% of hospitalizations for patients with diabetes, and led to 50% of all non-traumatic lower limb amputations.[20]

The classic chronic ulcer in diabetes mellitus is a relatively small punctuate wound that lies on the plantar surface beneath a deformed metatarsal head where pressure is most exerted. In addition, the tips of the toes may develop pressure related ulcers due to clawing. There is often callus formation around the ulcer and once the wound develops, it often remains open for prolonged periods.[19,20] One of the major contributors to ulcer development is peripheral neuropathy, yet other factors such as ischemia, cellular, immunological as well as biochemical abnormalities all share in the aggravation of the ulcer.[2]

Biologic manipulation of the healing process of chronic diabetic foot ulcers through wound supplementation with growth factor agents is an appealing concept. Several clinical studies have evaluated a "platelet-derived wound healing formula" (PDWHF) or "platelet gel" prepared from patients' own blood. This releasate from autologous platelets contains PDGF, TGF-ß, VEGF, EGF, and platelet factor-4 among other factors important for the proper stimulation of wound healing.[3-6] The use of this autologous material did not require FDA scrutiny in that it is not viewed as a drug. However, slight differences in methods of preparation from center to center lead to variation in the nature of the material being evaluated in different trials.

Transforming growth factor-beta 1 (TGF-ß1), has controversial effects on wound healing. However, the ideal method for its administration to the wound site remains unknown. For most studies, the TGF-ß 1 was incorporated into phosphate-buffered saline, into DuoDERM hydroactive paste, and into a poly-ethylene oxide hydrogel.[11,13,21] The release of 125 I-labeled TGF-ß1 from carriers was measured in full-thickness wounds in rats and the wound healing was analyzed by histology and wound area measurements. The TGF-ß1 was released from all formulations at a different rate and in an active form as determined by growth inhibition assay. Wound size measurements and analysis of the amount of cellular influx, fibroplasia, and granulation tissue showed that a single dose (1 microgram/wound) of locally administered TGF-ß1 significantly ($P < 0.01$) enhanced the wound healing. This effect was most prominent with polyoxamer gel formulation, which provided the most sustained release of TGF-ß 1.[13]

Knighton and colleagues[13] had conducted a study over 32 patients complaining of lower extremity ulcers due to a variety of causes (vascular, neuropathic and traumatic). In the sixteen patients who received daily applications of PDWHF topically, by the end of the first 8-week period 81% of patients receiving the platelet gel had complete epithelialization of their wound compared with 15% in the placebo group. Atri and colleagues[14] also identified great improvements in wound healing using platelet gel preparation. At the end of their study period, all patients receiving daily application of platelet gel ended in a 100% wound closure after an average of 9 weeks. Steed and colleagues[15] also tested the platelet gel formula in a double-blind trial in 13 patients exclusively with diabetic foot ulcers for a treatment period of 20 weeks. During this period, 5 of 7 patients receiving platelet gel

healed completely whereas only 1 of the 6 patients in the placebo group healed.

Contradictory results arose from other investigators who used platelet gel on ulcers with different causes. Kurpski et al.,[21] and Senet et al.,[22] trials have showed no significant statistical differences between platelet gel and placebo in their studies. These investigators attributed their negative results to incomplete knowledge regarding the optimum dose of platelet gel. Those contradictory reports with the introduction of the recombinant technology which allowed the production of larger volumes of specific growth factors caused investigators to shift to other solutions for ulcer healing. Recombinant PDGF-BB (rhPDGF-BB), known commercially as Becaplermin (Regranex, Johnson & Johnson) has taken approval of FDA as the first and only growth factor in an ointment vehicle for chronic ulcers.

Contrary to wound supplement with recombinant growth factors, which is very expensive and necessitates scrutiny from FDA or other health organizations, in our study we find that the forgotten autologous platelet gel is still an available cheap solution. Nearly all patients were subjected to surgical debridement before the start of treatment and were selected according to the criteria mentioned before and then regrouped into control and treatment group. The control group was treated by conventional dressing and application of a local antimicrobial while the treatment group was treated with platelet gel application; a hemocomponent obtained by sampling whole blood from the same patient (20-50 ml) and then the whole blood was split into platelet concentrate and mixed with thrombin to form a sticky cloth. The thrombin activates the platelets, leading to growth factor release thus performing their functions on wound healing.[23]

In our study, platelet gel was applied to the ulcer(s) of the treatment group and then covered by an occlusive dressing (Opsite®) and the dressing was removed after 72 hours. The other group (control group) was treated by local antibiotic ointment with the same frequency of dressing to obtain a convincing comparison between both methods. It was shown that healing is more obvious among the treatment (platelet gel) group than the control (local antibiotic) group. Four cases in the study group have shown complete healing in comparison to two cases only in the control group. Twelve cases have shown healing progression in the study group; three of them showed an increase in the angiogenic appearance of the ulcer (healthy granulation tissue with bright red color) and underwent skin grafting with 100% graft take. On the other hand, eight cases in the control group have shown slow healing progress; two cases underwent skin grafting but showed partial loss on follow up.

Autologous platelet derived wound healing formula has many advantages; being autologous, there is no risk of blood transmitted diseases or hypersensitivity reaction in addition to being cheap, easily prepared and applicable. The PDWHF provides the ulcer with the necessary growth factors proved by many researches to be deficient in chronic ulcers. These growth factors may help in ulcer healing and proved to change the character of granulation tissue to be more healthy and thus if complete healing is not achieved, the ulcer may be ready for successful grafting.

The main drawback faced in this study was the limited number of dressing (twice weekly) because of the limited chance to have more than two samples per week from the patient. This resulted in appearance of infection and foul odor in some cases which may have been prevented if the dressings were more frequent. Homologous platelet rich plasma may have helped to perform daily dressing, but the patients may then be at risk of protracting blood transmitted diseases even if the donors were well-screened, since the blood may be withdrawn during the incubation period before seroconversion occurs.

Conclusion:

Diabetic ulcers are a nightmare for diabetic patients due to the economic impact and the fear of amputation as the last resort. This ulcer is better prevented than being treated from the medical, social and economic point of view. Yet once it occurs, it should be treated appropriately and efficiently as early as possible.

Patient care and early concern for medical advice are the clues for successful treatment.

Moreover, patient education and well selected supportive means (orthotic shoes, crutches) are essential for the proper management and decrease the rate of recurrence.

PDWHF as shown in this study proved to be a safe, readily available and cheap method for treatment of diabetic ulcers. It contains a mixture of several growth factors and showed promising effects once the wound was adequately debrided. Like hyperbaric oxygen and negative pressure therapy, PDWHF should be accepted as one of the established and efficient alternative wound healing modalities.

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