

*“ Role of Injection of Platelet Rich Plasma
in Promoting Healing Of Chronic Venous
Leg Ulcer versus Conventional
Compression Therapy ”*

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Abstract

Background: Venous leg ulcers (VLU) consider the commonest type of leg ulceration; venous insufficiency is the main cause of it. Intra lesion injection of Platelet-rich plasma (PRP) is a potential factor for promoting ulcer healing by the effect of growth factors (GF). The aim was to evaluate the efficacy and safety of (PRP) injection in chronic non-healing (VLU) versus conventional compression therapy.

Methods: a randomized prospective comparative study, in 42 patients were managed by dividing into two equal groups; Group (1) managed by PRP injection inside the VLU after preparing the ulcer for healing, and relieving the causes of non-healing ulcer (infection and necrotic tissues or venous insufficiency). Group (2) managed the ulcers by conventional compression therapy. All patients were assisted for 3, 6,9 and 12 months or until wound healing. The injection was performed weekly and assessed granulation tissue healing.

Results: The mean percentage of wound closed of PRF injection group was discovered significantly higher than conventional group. With statistically difference in rate of healing ulcer between the PRF group and control group. The mean of reduction in ulcer area in group (1) within 12 months was 1.2 ± 0.5 , and in group (2) was 3.9 ± 1.6 . the rate of improvement was 92.7% and 77.1% respectively (P value <0.05).

Conclusions: Injection of PRP inside chronic VLU showed better clinical outcomes in comparing with conventional compression therapy alone.

Keywords:

platelet-rich plasma, venous ulcer, compression, healing and wound.

Introduction:

Chronic tissue ulceration is known as breaks in the skin and subcutaneous layer that don't respond to standard trial of management and might need a prolonged time to cure, with high liability for recurrence. ⁽¹⁾ Venous leg ulcer (VLU) is considered the most prevalence form between different types of chronic leg ulcers such as venous, arterial, diabetic, pressure and traumatic ulcers. ⁽²⁾ Because of the uncontrolled elevated venous pressure or incompetent valves. ⁽³⁾

The compression therapy is the standard method of management for VLU after intervention to treat the venous reflux with or not minimal debridement. Although the traditional compression therapy can achieve up to 50% healing of VLU after one month, and up to 60% to 70% within 1.5 month. But, the resistance of healing the ulcer is about 30% and the possibility for recurrence up to 70% after five years of only traditional therapy. ⁽⁴⁾

The charges needed during the prolonged time of Conventional care of chronic ulceration are big, and should be considered a health issue with a great influence on the human life, productivity and social status in addition to the high resources consuming. ^(2, 5) it was noticed that, the prevalence of above 65 years old with VLU because of venous insufficiency not more than 2-3%. ⁽⁶⁾

The process of wound healing is consistent sequences of tissue repair associated with the interaction of mediators such as cytokines, proteolytic activity, chemokines and metalloprotease activity affecting growth factors and fibrin formation. ⁽⁷⁾

The natural healing process for any wound summarized in steps: inflammatory phase, proliferation, and regeneration and remodeling phase. When bleeding happen, stimulation and activation of the platelet then, release of GF which encourage the inflammatory and healing sequences. Different biomolecules are released by (alpha, delta and lambda) granules inside the platelets. The most numerous are α granules, about 10% of platelet size (50-80 α granules per platelet) and release ~300 soluble proteins ⁽⁸⁾

Platelets are anucleate fractions of megakaryocytes that arise inside the bone marrow and pass through bloodstream for 7–10 days. It has a wide protein component in their granules (dense granules, lysosomes, and mainly α -granules). During the healing phase, platelets are activated in the ulcer. GF and cytokines are released and connect to the fibrin matrix and to proteoglycans, with the consequent establishment of a pool that could be stimulated by proteinases. ⁽⁷⁾ Each GF stimulate several reaction pathways. Once GF has attached to receptors, a next messenger is stimulated and the signal keeps activated, even in absence of GF. Depending on GF, special types of proteins and adjustment in cell function happen. ⁽⁵⁾

The chronic ulcer occurs after prolonged tissue disruption and breakdown of healing process by disturbing the inflammatory phase. ⁽⁹⁾ Chronic venous insufficiency affects the main role of venous drainage system, with or without flow stasis, causing the incidence of chronic leg ulceration. ⁽¹⁰⁾

The predisposing factors that most probably affecting the process of healing ulcer could be A) Regional factors as septic ulcer, recurrent trauma, hypoxia and tissue necrosis. B) Systemic factors as chronic illness like diabetes, hypertension and malnutrition. C) Receiving steroids or immune drugs. ⁽¹¹⁾

The best and recommended treatment should provide efficacious and prompt healing of the ulcer, and less liability of recurrence. The updated guidelines advised that to face chronic unhealed venous ulcers should care about venous hypertension management. ⁽¹²⁾ Long saphenous laser and thermal ablation, ⁽¹³⁾ reticular veins sclerotherapy ⁽¹⁴⁾ with conventional compression.

Different ways of wound management are recommended to increase the rate of healing VLU, including different modalities of dressing by topical antimicrobial drugs, hyperbaric treatment, vacuum for suction wound wastes and secretions, negative pressure wound therapy, interrupted pneumatic compression, lasers and platelet-rich plasma (PRP). ^(15, 16)

In the past, the reflux of the venous system is related to valve incompetence. But, the updated studies of molecular and biological views of the venous disease mentioned that it's a complex disease affecting the inflammatory process and the synthesis of connective tissue. ⁽¹⁷⁾

Patient and Methods:

This is a randomized prospective comparative study, was conducted on 42 patients, with venous manifestations (CEAP 6) with chronic non-healing VLU for more than 6 weeks on traditional management. could be associated with venous insufficiency, with or not dermato-sclerosis. The study was done after approval from the Ethical Committee at Port Said university hospitals, Egypt at Omar Ibn El-Khattab hospital, and El-Rahma hospital in Port Said, Egypt for three years. All patients provided their informed written consent. The study for evaluation the role of PRP injection in VLU for promoting healing versus Conventional compression treatment.

Before intervention all patients in the study underwent, 1) full history taking, risk factors such as (smoking, diabetes mellitus, hypertension, ischemic heart disease and history of DVT). 2) Clinical picture: primary symptomatic of VVs with chronic non-healing venous ulcer (CEAP6), Body mass index (BMI). venous symptoms in the leg, skin pigmentation, and leg venous ulcer size were evaluated. 3) Routine laboratory and radiological investigations such as (Duplex ultrasound of venous system of the lower limb with the determination of GSV, SSV, and extra-

axial varicosities (patency and diameters) were measured. First, VLU with necrotic tissues was cleaned and debridement was done.

The eligible Patients with non-healing VLU for more than 6 weeks on standard treatment. All patients will be evaluated for venous insufficiency by ultrasound for assessment of venous and arterial system patency. Also, the Ankle/Brachial Pressure Index (ABPI) of involved patients in the study not less than 0.9 for good healing and granulation tissue formation.

All patients have any of the chronic illnesses as uncontrolled diabetes mellitus or hypertension, malignancy, cardiac, hepatic or renal failure or connective tissue diseases were excluded. Also, the pregnant or lactating mother. Patients anticoagulants, steroids, or immunosuppressive drugs. If the ulcer infected with necrosis reached to bone necrosis. Patients refusing to participate in the study or difficult to compliance because distance were all excluded from the study. The 1ry endpoints will be the complete wound healing. The rate of curing the ulcer within the decided period. The 2ry endpoint will be occurrence of any related complication.

Technique of the procedure:

History taking for each patient in the study. Venous system assessment will be done for all cases. Patients with venous insufficiency and valve incompetent were managed surgical or ablation for correction of the venous problem before included in the study. local assessment of the VLU. Investigate all parameters of the ulcer include length, breadth, depth, edge, margin, floor and base. Minimal debridement if needed. History of recurrent VLU and duration of curing before and if there was complication. Biopsy will be taken if suspicious of malignancy or other pathology.

Preparation of PRP

Under aseptic arrangements, aspiration of 20 mL of venous sample blood from the patient and to a test tube mixed with acid citrate dextrose in a ratio of 9:1 (blood: acid citrate dextrose). 15 minutes Centrifusion at 5000 rpm for separation the red blood cells (RBCs) from platelet and plasma. ⁽¹⁸⁾ The precipitated component of the plasma will be collected and re-centrifuged at 2000 rpm for 5 min. The lower component of about 1.5 mL will be gathered, and 10% calcium chloride will be merged to stimulate activation of PRP (0.3 mL for 1 mL of PRP). ⁽¹⁹⁾ Minimal surgical debridement of necrotic tissues in the ulcer floor before being ready for injection of activated PRP inside and was dressed in a non-absorbent dressing.

We should inject activated PRP immediately into the wound as 70% of GFs are presented in ten minutes and 90% in one hour. So, the activation of PRP should be

just before injected, to preserve growth factors (GFs). Because activated PRP has the ability to synthesize more GFs for 8 days until deactivated. PRP application was repeated weekly.

In both groups; Elastic compression using layer bandage will be wrapped till healing of the ulcer. Then, elastic stockings (class II) will be used. The participants will be evaluated after 3, 6, 9 and 12 months till complete wound healing.

Randomization

The randomization was conducted by a computer-generated random list and opaque, sealed envelopes denoting the assignment group. Cases were classified into two equal groups: MA with high tie group(n=25) and traditional surgery group (n=25).

All surgical procedures were done in fully equipped operating rooms under spinal anaesthesia, supine in position with continuous monitoring of vital parameters. All patients received prophylactic antibiotic with intravenous ceftriaxone (1 g).

Statistical analysis:

The assessment of the change in measurement of baseline ulcer during the study in 3, 6, 9 and 12 month and comparing each group. In addition to assessment rate of complete ulcers curing in both groups during the same duration. SPSS program Ver 22 (IBM Corp. USA) for data. Quantitative measures will be described by mean and standard deviation (SD), while qualitative measures will be using numbers and percentages. Categorical measures were in frequencies, percentages and variables comparison between groups was by Pearson's Chi-square and Fisher's Exact tests as appropriate. Shapiro Wilk test for Numerical variants. The (ANOVA) test was used for analysis and measuring the variance between both groups. Results of significance hold a (p -value ≤ 0.05).

Result:

The study included 42 patients complaining of persistent non-healing leg ulcers after exclusion of 9 patients because of: venous insufficiency and refuse intervention, comorbidities and unstable conditions and refuse to participate in the study.

The patients were given the same protocol of management as daily compression by double layer bandages therapy, cleaning and removal of necrotic tissue when needed. Division into two groups: **A)** (21 patients) injection of

autologous P-PRP which taken from blood aspiration from same patient. **B**) (21 patients) received the conventional compression therapy, in the same regimen of treatment. The selection method was by randomization resulted in division the included patients in two groups with no significant differences with consideration of ulcers' condition.

Assessment of the ulcer at the beginning of the study, then follow up the progression of improvement in the next visits till finished. Both groups have the same gender distribution ratio. 15 patients in each group were males (71.4%) with mean age of 43.9 ± 13.7 (in group A), and 45.1 ± 13.2 (in group B) years. With only 6 patients (3 in each group) were diabetic on insulin and controlled and distributed equal in both groups. The body mass index (BMI) 18 to 34 kg/m², with mean 25.7 ± 4.3 (in group A) and 26.9 ± 3.7 (in group B). 16 patients (divided in both groups) had done stripping intervention of great saphenous vein (GSV). 18 patients (divided in both groups) had history of deep venous thrombosis (DVT) and managed till complete recanalization before. 8 patients had history of recurrent VLU.

In relation to Doppler ultrasound, one patient had perforator incompetence and seven patients had superficial vein incompetence. The time of non-healing ulcers presented by the patients' pre-treatment ranged from 2 to 3 years. All characteristics of involved patients are mentioned in **table (1)**.

Characters	Group A (21 patients)	Group B (21 patients)	P value
	Injection PRP	Conventional compression therapy	
Age "mean \pm SD"	43,9 \pm 13.7	45.1 \pm 13.2	0.362
Male	15 (35.7%)	15 (35.7%)	0.237
Diabetes Mellitus	3 (14.3%)	3 (14.3%)	0.058
BMI (kg/m ²)	25.7 \pm 4.3	26.9 \pm 3.7	0.061
Smoking	11 (52.4%)	14 (66.7%)	0.083
Duration of the ulcer (yrs)	3.9 \pm 3.1	3.6 \pm 3.7	0.087
History of DVT	8 (38.1%)	10 (47.6%)	0.067
Stripped LSV before	8 (38.1%)	8 (38.1%)	0.059
Ulcer in gaiter area	18 (85.7%)	17 (81%)	0.056
Recurrent ulcer	4 (19.1%)	4 (19.1%)	0.631

Table (1): Characters of participating patients in the study.

Note: P value is significant if $P < 0.05$, calculated by using the ANOVA test.

Abbreviations: BMI, body mass index; DM, diabetes mellitus; DVT, deep venous thrombosis; GSV, great saphenous vein; and PRP, platelet- rich plasma.

Debridement was needed for 16 patients (38%) of both groups with no statistically significant differences. Surgical stripping of GSV for 10 patients (23.8%) and the reticular veins were injected by foam sclerotherapy in 7 patients (16.7%) using Aethoxysklerol 2% (polidocanol) (Kreussler Pharma, Wiesbaden, Germany) (**Table 2**).

Events		PRP injection (n = 21)	Compression therapy (n = 21)	<i>P</i> value
Supportive intervention	Stripping LSV	6 (28.6%)	4 (19.1%)	0.864
	Ablations	5 (23.8%)	6 (28.6%)	0.542
	Injection sclerotherapy	3 (14.3%)	4 (19.1%)	0.003*
Healing rate at the 1 st 3 months	Complete	13 (61.9%)	3 (14.3%)	0.004*
	incomplete	8 (39.1%)	18 (85.7%)	0.006*
Healing rate after one year	complete	18(85.7%)	10 (47.6%)	0.03*
	incomplete	2 (9.5%)	8 (39.1%)	0.326
	recurrent	1 (4.8%)	3 (14.3%)	0.18*
Duration of healing (median) / month		3	9	0.009*

Table (2): surgical intervention and healing follow up.

Ulcer area management

The eligible patients with basal ulcer area, $P = 0.635$; while, the improvement of the ulcer criteria, post treatment, were significantly different among the two groups during follow up time, $P < 0.001$. (Figure 3; Table 3).

Group		Base	3 months	6 months	9 months	12 month	P value
PRP injection	Mean \pm SD	16.5 \pm 6.	5 \pm 3.7	3.5 \pm 2.3	2 \pm 1.1	1.2 \pm 0.5	< 0.001
	Rate improvement (%)		70%	78%	87.8%	92.7%	
Compression therapy	Mean \pm SD	17 \pm 5.1	11 \pm 4.2	7 \pm 3.1	5 \pm 2.5	3.9 \pm 1.6	< 0.001
	Rate improvement		35.3%	58.8%	70.6%	77.1%	

Table (3) : Ulcer area reduction along the study period, Significant as P value < 0.001

The rate of healing in the PRP injection group is more superior to the other one along the schedule time of follow-up (Table 2). The study showed a high significant of complete healing ulcer after PRP injection in 18 patients (85.7%) than compression therapy in 10 patients (47.6%), $P < 0.003$.



Figure (1): A) right leg necrotic venous ulcer. B) post minimal debridement. C) start with PRP injection with dressing. D) healing of the ulcer.



A

B

Figure (2): A) multiple infected VLU.

B) healing after PRP injection.



A

B

Figure (3):A) non-healed VLU.

B) healed VLU after PRP injection.

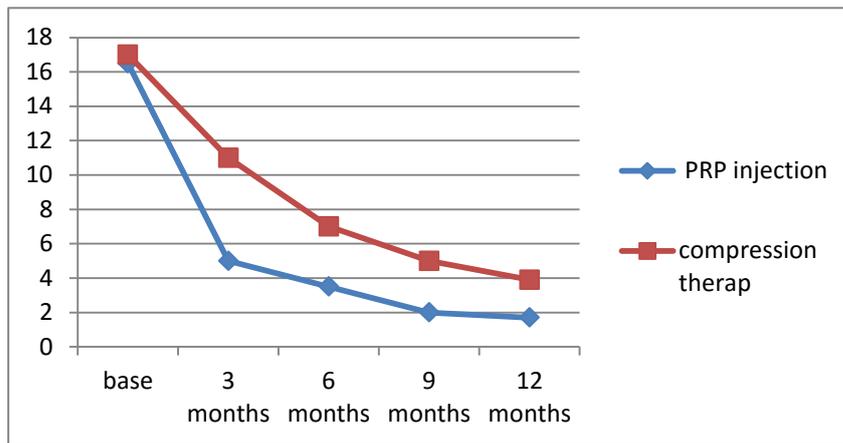


Figure (3): The ulcers improvement in both groups: groupA (PRP injection), and group B (compression)

The recurrence of leg ulcer after healing was detected in 4 patients (9.5%) in the study, as a new refluxed perforator was detected related to the ulcer area. (Table 2).

During the study, 4 patients (19%) in PRP injection group developed cellulitis with ecchymosis along the GSV, and 5 patients (31.8%) in compression therapy group which recovered by systemic and topical antibiotics.

Discussion

The supposition in this thesis is a sequel of more interest of utilization of regenerative medical substances for acceleration of VLU healing in neoteric medicine, where the traditional compression therapy couldn't afford satisfactory clinical outcome.

The traditional methods for management of chronic ulcers as surgical debridement, closed technique dressings, and local and systemic antibiotics if needed, usually has less effectiveness. Autologous PRP is a substitutional method ⁽²⁰⁾, helping to promoting the rate of healing and tissue repair in maxillofacial operations. ⁽²¹⁾ PRP has achieved great attention in clinical outcomes. ⁽²²⁾ And has participated comprehensively in tissue regeneration medicine along the last decades. ⁽²⁰⁾

The theory of using PRP injection to magnitude the rate of healing of chronic VLU is insufficient. Many studies mentioned about role of PRP in supporting healing of diabetic foot ulcers. ⁽²³⁻²⁶⁾ Different studies discussed the efficacy of PRP in healing of different ulcer types, with little mentioned about only role in VLU rate of healing. ⁽²⁷⁻²⁹⁾

For that, a prepared prospective randomized study including 42 patients to assess the outcomes within one year of injection PRP versus compression therapy in complete healing of chronic VLU.

The results of the research showed that PRP injection accomplished significantly more rapid ulcer healing and size reduction than compression therapy. However, there were significant difference concerning the recurrence and complication rates.

Anitua et al, recommended PRP application weekly, although, the preparation of the sample and frequency of using were not cleared in the study. But, in our study, we adopted the use of PRP weekly up to 6 weeks from the beginning of management. The scientific view of recommending PRP injection rather than other on account of reaching to the edges and depth to the base of the ulcer with appropriate consistency of platelets for maintaining of release of growth factors for more time. So, this is a more recommended way than applying regional PRP, because the last is less effective as the PRP might drop out the edges or adhesive to the gauze. The study detected a reduction in ulcer about 73% and 21%, in PRP injection and conventional compression groups, respectively ($P < 0.05$).⁽³⁰⁾

former researches mentioned comparison between applying of PRP and traditional compression therapy for healing of VLU, on the other side, no clear study had compared the PRP injection and compression therapy. There are Two studies for peri-ulcer injection with regional applying of PRP in chronic different types of leg ulcers.^(31, 32)

Because of the discrepancy in the studies around the role of PRP on VLU treatment. Aguirre et al mentioned that the PRP managed the ulcers healed in 8 weeks versus conventional group, with size reduction of about 82% and 24%, respectively, ($P < .001$). the consumed time for healing was 9.6 and 23.7 weeks respectively, ($P < .001.0$).⁽³³⁾

In Somani and Rai, randomised study for applying PR fibrin or saline dressing management for 4 weeks of treatment. Evaluation of healing process was detected in 55% and 0% of the ulcers, respectively. The decreasing of ulcer size was 85.5% and 42.7%, ($P < .001$), respectively.⁽³⁴⁾

Cardenosa et al, in their study, applying PRP and saline dressing for VLUs detected a size reduction of 67.7% and 11.1%, respectively, ($P = .001$).⁽³⁵⁾

Pravin *et al*, in a randomized comparative study for patients with nonhealing different types of ulcers, patients were managed with PRP and L-PRF weekly for 6

weeks. The result mentioned the more effective and rapid of L-PRF than PRP group. ⁽³⁶⁾

Another, autocontrolled prospective cohort study was done by Pinto *et al.* in a prospective cohort study using L-PRF therapy for chronic ulcers for not less than 3 months. The study concluded considerable elaboration in the healing process and improving symptoms. ⁽³⁷⁾

In addition to, Jorgensen, *et al.* in a prospective pilot study chronic ulcer with different causes not responding to conventional therapy. Using Leucopatch (Reapplix, Birkerod, Denmark), the ulcers were managed weekly. It was found a significant decrease in the ulcer area by 65% with Leucopatch. ⁽³⁸⁾

Size and duration of occurrence VLU are consider effective predictors of healing pathway: a less recommended in larger and more advanced course one. ^(35,39-41) Robson et al, mentioned that a better prognosis, in VLUs, if the ulcer area less than 15 cm² and course less than 1.5 year. ⁽⁴²⁾

Comparing with other researches that considered using PRP in different ulcers management as a safe method, with minimal complication of periulcer mild cellulitis ^(35,43,44)

In contrast, in other trials mentioned that, using PRP in management of chronic non-healing VLU is not recommended for promoting healing. ⁽²⁸⁾ Stacey et al, in a randomized study, patients in two groups, either platelet lysate application or placebo (conventional compression). Complete healing of ulcers was gained in 75% of cases in each side within 12 weeks. ⁽⁴⁵⁾

Conclusion:

Injection of PRP inside chronic VLU showed better clinical outcomes in comparing with conventional compression therapy alone. The results showed a significant reduction in the ulcer area.

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Conflicts of interest

There are no conflicts of interest.

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