Autogenous leucocyte-rich and platelet-rich fibrin for the treatment of leg venous ulcer: a randomized control study

Asser A. Goda

Department of Vascular Surgery, Sohag Faculty of Medicine, Sohag University, Sohag, Egypt

Correspondence to Asser A. Goda, MD, Assis. Prof., Department of Vascular Surgery, Sohag Faculty of Medicine, Sohag University, 82511 Sohag, Egypt. Tel: +201093936161; e-mail: assergoda@yahoo.com

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Background
Venous ulcer (VU) is a serious health problem that has no satisfactory treatment. Platelet-rich fibrin (PRF) is one of regenerative medicines that promote wound healing by sustained release of growth factor (GF) and protein matrix for more than 7 days.
Aim
This study aims to evaluate the efficacy and safety of autologous leukocyte-platelet-rich fibrin (L-PRF) with venous leg ulcer.
Patients and methods
A randomized controlled study was conducted that included 36 patients with VUs. The eligible patients were enrolled in one of two groups (PRF and control groups) according to randomization schedule. Each group included 18 patients. The PRF group was treated with autologous L-PRF dressing, and dressing change was done once weekly. The control group was treated with conventional dressing of VU, but dressing change was done once in 2 days.
Results
The mean percentage of wound reduction of PRF group was found significantly higher than that of control group. There was a statistically significant difference between the PRF group and control group regarding the rate of completely healed ulcer at the fourth week for ulcer size less than 10 cm^2 and at the seventh week for ulcer size more than 10 cm^2.
Conclusion
Autologous L-PRF is effective and safe for treatment of venous leg ulcer.

Keywords:
platelet-rich fibrin, regenerative medicine, venous ulcer

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Introduction

Venous ulcers (VUs) constitute a serious public health problem. They interfere with the quality of life owing to their complications, which may result in significant morbidity [1]. VUs represent 70% of lower limb ulcers [2]. The treatment is often difficult and is generally associated with high recurrence rate [3–5]. Platelet concentrates have been widely used in regenerative medicine to promote wound healing as they contain growth factors [6]. Platelet-rich fibrin (PRF) is a second-generation platelet concentrate [7]. Leukocyte-platelet-rich fibrin (L-PRF) requires simplified processing without use of bovine thrombin and anticoagulants [8]. It helps in efficient cell migration and proliferation. It has supportive effect on immune system and also aids in hemostasis [7].

The aim of this study was to evaluate the efficacy and safety of autogenous L-PRF for treatment of VUs in randomized control multicenter double-blind design.

Patients and methods

This prospective, randomized, and controlled study conducted at Vascular Surgery of Sohag Faculty of Medicine included patients with VU between September 2016 and October 2017 after approval of the Scientific Ethics Committee.

Inclusion criteria

Inclusion criteria included patients with venous leg ulcers (VLUs).

Exclusion criteria

Exclusion criteria were as follows:

(1) Patients with peripheral arterial disease, characterized by ankle brachial index (ABI) less than 1.
(2) Osteomyelitis of leg bone.

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(3) Wound infection.

This study was performed on 36 patients. The patient provides a written informed consent before enrollment in the study. All eligible patients were randomized into two groups according to randomization schedule.

Randomization procedures

The randomization schedule was generated using SPSS program (SPSS Inc., Chicago, Illinois, USA). Each eligible study participant was assigned to one of two treatment groups, PRF group or control group, by receiving the next available consecutive randomization number, and type of dressing according to randomization schedule.

Eligible patients

(1) PRF group (18 patients): The wounds in this group were covered with PRF as their dressing protocol.
(2) Control group (18 patients): The wounds in this group were covered with conventional dressing.
    (a) NB: General rules regarding compression using elastic stocking or elastic bandage were followed.

L-PRF preparation

(1) A blood sample is taken without anticoagulant in 10-ml tubes, which are immediately centrifuged at 3000 rpm for 10 min.
(2) Coagulation starts during the centrifugation, and three parts quickly appear in the tube: a red blood cell base at the bottom, a cellular plasma as a supernatant (platelet-poor plasma), and the L-PRF clot in between. The latter is rich in fibrin, platelets (±95% of initial blood), and leukocytes (±50% of initial blood).
(3) A fibrin clot is then obtained from the middle of the tube, and is further transformed into a membrane, circa 1 mm in thickness, by careful compression between two gauzes.

Adressing protocol

PRF group (18 patients)

(1) The fibrin clot membrane was applied to the ulcer followed by paraffin gauze and secondary sterile dry dressing. Lastly, elastic bandage was applied.
(2) The frequency of change of dressing was once weekly. The dressing protocol was performed for up to 8 weeks or stopped whenever healing occurred.

Control group (18 patients)

Conventional dressing was applied to the ulcer using paraffin gauze that was applied to the ulcer followed by secondary sterile dry dressing. Finally, elastic bandage was applied.

The frequency of change of dressing was once in 2 days. The dressing protocol was performed for up to 8 weeks or stopped whenever healing occurred.

Follow-up

(1) The ulcer area was calculated at the initial visit by measuring the ulcer's dimensions (length and width) using metric tapes.
(2) The ulcer area was measured every week for all patients in each group, and the ulcer area reduction is calculated each week.
(3) The number of complete ulcer healing is detected in each group.
(4) Laboratory tests were performed for all patients in two groups at the initial visit and every 4 weeks until the patients reach the end point.

End points

The end points of the current analysis were ulcer healing or end-of-study occurred at completion of the week 8.

Statistical analysis

Data were analyzed by STATA, version 12.1 (Stata Statistical Software: Release 12; StataCorp LP, College Station, Texas, USA). Quantitative data were represented as mean and SD. Data were analyzed using student *t*-test to compare means of two groups. Qualitative data were presented as number and percentage and compared using either χ^2-test or fisher exact test. *P* value was considered significant if it was less than 0.05.

Results

During the study period, 36 patients with VUs met the inclusion criteria and were enrolled in the current series in one of the two groups according to randomization schedule, with 18 patients in each.

The baseline criteria of the study patients are shown in Table 1. There was no statistically significant difference regarding demographic data, risk factors, and laboratory parameters at the baseline for each group.

The mean ulcer area reductions in two groups are shown in Table 2 and Fig. 1. The mean ulcer area

reduction in PRF group with initial size less than 10 cm was 25.3, 53.4, 80.2, and 100% after 1, 2, 3, and 4 weeks, respectively. The mean ulcer area reduction in control group with initial size less than 10 cm was 12.3, 23.5, 47.2, 62.2, 78.3, and 100% after 1, 2, 3, 4, 5, and 6 weeks, respectively.

The mean ulcer area reduction in PRF group with initial size more than 10 cm was 16.3, 31.1, 49.4, 64.3, 79.5, 96.3, and 100% after 1, 2, 3, 4, 5, 6, and 7 weeks, respectively. The mean ulcer area reduction in control group with initial size more than 10 cm was 6.9, 14.4, 29.1, 43.3, 58.3, 71.6, 86.8, and 97.3% after 1, 2, 3, 4, 5, 6, 7, and 8 weeks, respectively.

There were no adverse effects detected from L-PRF dressing. There was a statistically significant difference between PRF group and control group regarding the mean ulcer area reduction either with initial length less than or more than 10 cm^2. The mean ulcer area reduction is significantly higher in PRF group than control group.

Table 1 The baseline characteristics of the study patients

Variables	PRF group	Control group	*P* value
N	18	18	–
Age (mean±SD) (years)	40.1±6.8	39.3±8.2	0.76
Sex (male)	10	9	0.74
Risk factors (%)			
Hypertension	16.7	22.2	1.00
Diabetes	11.1	5.6	1.00
Smoker	45.5	55.6	0.51
Blood picture			
Hb	10.8±0.9	11.2±1.2	0.35
Platelet count	275.5±29.98	280.8±25.5	0.57
Blood chemistry			
Albumin	3.8±0.08	3.9±0.12	0.03
Ulcer size (cm^2)			
<10	10	11	–
>10	8	7	–

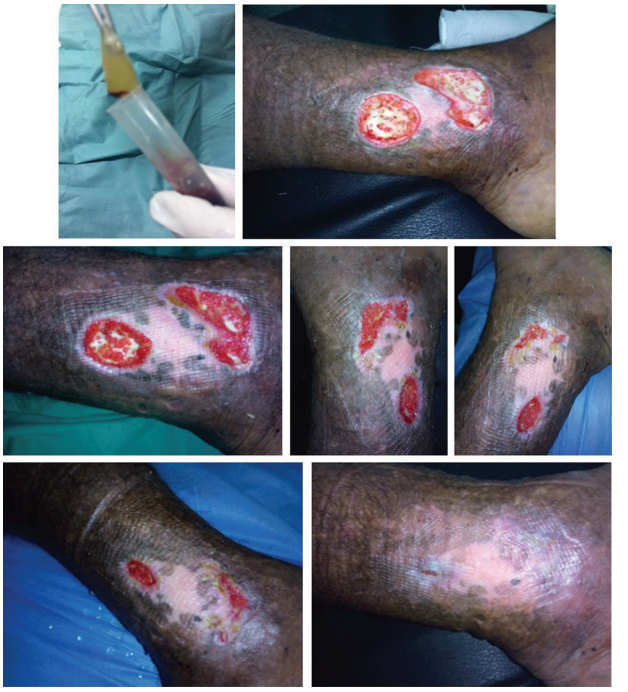
Hb, hemoglobin; PRF, platelet-rich fibrin; *P* value >0.05, not statistically significant.

The rate of complete healing of the ulcers in PRF group and control group is present in Table 3. The rate of complete closure in PRF group with initial size less than 10 cm^2 was 30% at the third week, and 100% at the fourth week, whereas in the control group with the same initial ulcer size (<10 cm^2), it was 0% at the third week and 9.1% at the fourth week.

The rate of complete closure in PRF group with initial size of more than 10 cm^2 was 50% at the sixth week and 100% at the seventh week, whereas in the control group with the same initial ulcer size (>10 cm^2), it was 14.3% at the sixth week and 42.6% at the seventh week.

There was a statistically significant difference between the PRF group and control group regarding the rate of completely healed ulcer at the fourth week for ulcer size

Figure 1



The mean ulcer size reduction in platelet-rich fibrin group and control group.

Table 2 The mean ulcer size reduction in platelet-rich fibrin group and control group

Groups	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week
<10 cm^2								
PRF group (%)	25.3	53.4	80.2	100	–	–	–	–
Control group (%)	12.3	23.5	47.2	62.2	78.3	100	–	–
P value	<0.0001	<0.0001	<0.0001	<0.0001	–	–	–	–
>10 cm^2								
PRF group (%)	16.3	31.1	49.4	64.3	79.5	96.3	100	–
Control group (%)	6.9	14.4	29.1	43.3	58.3	71.6	86.8	97.3
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	0.04	–

Hb, hemoglobin; PRF, platelet-rich fibrin; *P*<0.05, statistically significant.

Table 3 The rate of complete healing of the ulcers in platelet-rich fibrin group and control group

	Number of cases	Complete healing of the ulcers			
		3 weeks	4 weeks	6 weeks	7 weeks
<10 cm^2 [*n* (%)]					
PRF group	10	3 (30)	10 (100)		
Control group	11	0	1 (9.1)		
P value		0.09**	<0.0001*		
>10 cm^2 [*n* (%)]					
PRF group	8	0	0	4 (50)	8 (100)
Control group	7	0	0	1 (14.3)	3 (42.6)
P value				0.28**	0.03*

Hb, hemoglobin; PRF, platelet-rich fibrin; **P* value <0.05, statistically significant; ***P* value >0.05, not statistically significant.

Table 4 Comparison of the laboratory investigation between platelet-rich fibrin group and control group from the baseline to the end point

	PRP group		*P* value	Control group		*P* value
	Baseline	End point		Baseline	Endpoint	
Blood picture						
Hb	10.83±0.9	10.6±0.84	0.19	11.18±1.2	10.9±0.97	0.22
Platelet count	275.5±29.98	272.5±20.45	0.64	280.8±25.5	277.22±24.27	0.17
Blood chemistry						
Albumin	3.78±0.08	3.76±0.15	0.35	3.86±0.12	3.82±0.12	0.10

Hb, hemoglobin; PRF, platelet-rich fibrin; *P*>0.05, not statistically significant.

less than 10 cm^2 and at the seventh week for ulcer size more than 10 cm^2.

There were no statistically significant differences in the PRF group and control group from the baseline to the end-point laboratory shift in blood picture (hemoglobin and platelet count) and blood chemistry (albumin) (Table 4).

Discussion

The hypothesis for this study is a consequence of the increasing interest in the use of regenerative medicine for treatment of VLU in modern medicine, where the conventional therapy cannot provide satisfactory healing results.

PRF is an autologous platelet and L-PRF material and is an important advancement in regenerative medicine [6]. PRF is a second-generation platelet concentrate [8–10]. L-PRF is rich in fibrin, platelets, and leukocytes [11]. L-PRF release significantly large amounts of growth factors and matrix proteins [12] for a long period (>7 days) [13] owing to a specific polymerization and architecture of the fibrin matrix [14].

The use of PRF for treatment of VU is supported in many literatures. Anitue *et al.* [15] reported in a review of the use PRF on VLU that the PRF is 'promising'. Moreover, O'Connell *et al.* [16] proved that PRF was very efficacious in the treatment of VUs, and its efficacy in healing of VU was more than that of non-VU.

In this study, we used L-PRF for treatment of VLUs to evaluate its efficacy and safety, in prospective randomized controlled study. The baseline characteristics regarding demographic data, risk factors, laboratory parameters, ankle brachial index, and wound variables of both PRF group and control group are almost similar, with no statistically significant difference.

In this study, the mean ulcer area reduction is significantly higher in PRF group than control group. There was a statistically significant difference between PRF group and control group regarding the mean ulcer area reduction with either initial length of less than or more than 10 cm^2. The result in this study is similar to the results reported in many literature studies [6,7,17].

In a randomized controlled study done by Somani and Rai [6], 15 patients with chronic VLU who were not responding to available treatment modalities for more than 6-month duration were included. The ulcer size area was at least 1 cm^2 and less than or equal to 5 cm^2. The patients were randomly divided into two groups: PRF dressing group and saline dressing group. That study reported that the mean reduction in the area of the ulcer size in PRF group was 85.51%, and the mean reduction in the area of the ulcer size in the saline group

was 42.74%, which was statistically significant, with a $P<0.001$.

Moreover, Pravin *et al.* [7] included in a randomized, open-labeled comparative study 30 patients with nonhealing ulcers of various etiologies, and 22 of them were VUs. Fifteen patients were treated with PRP and 15 patients with L-PRF at weekly intervals for a maximum of 6 treatments. The study concluded that L-PRF is more efficacious and has a quicker healing rate than PRP.

Another, autocontrolled prospective cohort study was done by Pinto *et al.* [17] on the use of L-PRF therapy for chronic wounds refractory to standard treatment for at least 3 months. The study included 49 chronic wounds, where 32 of them were VLUs (17 of them were >10 cm^2 and 15 were <10 cm^2). After the L-PRF therapy, all 49 wounds showed significant improvements in the healing process and symptomatic relief.

Moreover, Jorgensen, *et al.* [18] included in a prospective, uncontrolled pilot study 16 lower extremity chronic wounds that had not responded to previous treatments. The wounds were of varying etiologies; six of them were VUs. The wound size ranged from 0.4 to 15.7 cm^2 (median: 2.3 cm^2). The ulcers were treated weekly with Leucopatch (Reapplix, Birkerod, Denmark). That study found the mean wound area decreased significantly by 65%, resulting in a median wound size of 0.9 cm^2 (range=0–9.6 cm^2) with Leucopatch.

In this study, there was a statistically significant difference between the PRF group and control group regarding the rate of completely healed ulcer at the fourth week for ulcer size less than 10 cm^2 and at the seventh week for ulcer size more than 10 cm^2. The result in this study is similar to the results reported by Somani and Rai [6], Pravin *et al.* [7], and Pinto *et al.* [17].

Somani and Rai [6] reported that the complete closure of the ulcers in the PRF group was five (55.55%) patients and in the saline group was no case.Pravin *et al.* [7] reported that ~100% healing of the ulcer was seen in 11 of the 15 ulcers in L-PRF and eight of the 15 ulcers in PRP at the end of the sixth treatment (73.3 vs. 53.3%).

Pinto *et al.* [17] reported that following the use of L-PRF therapy, all VLUs of less than or equal to 10 cm^2 showed full closure within a 3-month period. All wounds of patients with VLUs more than 10 cm^2 who continued therapy (10 wounds) could be closed, whereas in the five patients who discontinued therapy, improvement of wound size was observed.

In this study, there were no adverse effects detected from L-PRF dressing. There were no statistically significant differences in each group (PRF group and control group) from the baseline to the end-point laboratory shift in blood picture (hemoglobin and platelet count) and blood chemistry (albumin). Moreover, Jorgensen *et al.* [18] reported that there were no serious adverse events detected with the use of L-PRF for the treatment of recalcitrant chronic wounds.

Conclusion

Autologous L-PRF represents an effective and safe treatment modality that shows significant higher potential for healing of VLUs than conventional therapy. Autologous L-PRF should be added to our armamentarium for treatment of VLUs.

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Conflicts of interest

There are no conflicts of interest.

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