Platelet-rich plasma for the treatment of diabetic foot ulcer: a randomized, double-blind study

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Background
Diabetic foot ulcer is a major complication of diabetes mellitus. Over the recent years, great progress has made in the techniques of wound healing, among which autologous platelet-rich plasma (PRP) has attracted the most substantial attention. Platelets are known to start the wound healing process through the release of locally active growth factors. The evidence from studies of autologous PRP to support its use in wound healing is not robust, and further rigorously designed blinded trials are needed. The aim of the study was to evaluate the efficacy and safety of the autologous PRP for diabetic foot ulcer in a randomized control multicenter double-blind design.

Patients and methods
The study included 50 patients with diabetic foot ulcers, who were divided into two groups: PRP and platelet-poor plasma (PPP) groups. The PRP group was treated with autologous PRP in gel form as a dressing. The PPP group was treated with autologous poor plasma as a dressing. The frequency of dressing change for each group was twice weekly.

Results
The healing rate of the PRP group was found to be significantly higher than that of the PPP group. The healing rate per week of the PRP group was significantly higher than that of the PPP group. The rate of complete healing was significantly higher in the PRP group than that of the PPP group.

Conclusion
Autologous PRP is effective and safe for treatment of diabetic foot ulcer.

Keywords:
diabetic foot ulcer, platelet-poor plasma, platelet-rich plasma

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Introduction

Diabetes is a major health problem that is currently showing an alarming rise in its prevalence [1]. Diabetic foot ulcer is a major complication of diabetes mellitus, and is the major component of the diabetic foot [2]. Alvarsson *et al.* [3] reported that up to 88% of all lower leg amputation is related to diabetic foot ulcer. The goal of the diabetic foot ulcer treatment is to obtain wound closure as expeditiously as possible [4]. Accepted therapeutic objectives and standards of care for diabetic foot ulcers include wound debridement, pressure relief in the wound area, appropriate wound management (e.g. moist wound healing), infection management, ischemia management, medical management of comorbidities, and surgical management as needed [5]. Over the recent years, great progress has been made in the techniques of wound healing, among which autologous platelet-rich gel has attracted the most substantial attention [6]. Platelets are known to start the wound healing process through the release of locally active growth factors [7–10]. The growth factors are able to produce granulation tissue and to induce epithelialization by the production of neovessels, attraction of fibroblasts and mesenchymal cells, secretion of collagen fibers, and by proliferation of keratinocytes [11–14]. Platelet-rich plasma (PRP) may also curb inflammation by suppressing cytokine release [15]. PRP has also been demonstrated to be of some antimicrobial properties against microorganisms, such as *Escherichia coli*, MRSA, *Candida albicans*, and *Cryptococcus neoformans* [16]. The evidence from studies of autologous PRP to support its use in wound healing is not robust, and further rigorously designed blinded trials are needed [17].

The aim of the study was to evaluate the efficacy and safety of the autologous PRP for diabetic foot ulcer in a randomized control multicenter double-blind design.

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Patients and methods

This prospective, randomized, controlled, multicenter double-blind study was done in the Vascular Surgery Department of Sohag Faculty of Medicine and Vascular And Endovascular Units in Al Azhar Faculty of Medicine following approval by the Scientific Ethics Committee.

Inclusion criteria

(1) Type 1 or 2 diabetes controlled by either medication or insulin.
(2) Presence of a foot ulcer for at least 4 weeks to be considered chronic.
(3) According to University of Texas Treatment-Based Diabetic Foot Classification System: ulcers included in this study are of grade 1A (wounds without tendon, capsule, or bone involvement, and also without associated infection or ischemia) or grade 1C (wounds without tendon, capsule, or bone involvement, and also, without associated infection but with ischemia). Patients with ischemia are allowed to be included but with ankle-brachial index (ABI) of greater than or equal to 0.6.

Exclusion criteria

(1) Patient's blood vessels are noncompressible for ABI testing.
(2) ABI of less than 0.6.
(3) Evidence of gangrene in ulcer or on any part of the foot.
(4) History of peripheral vascular repair within 30 days of randomization.
(5) Patient has radiographic evidence consistent with diagnosis of acute Charcot foot.
(6) Patient has known or suspected osteomyelitis.
(7) Ulcer size area (length–width) of less than 2 cm^2.
(8) Diabetic foot ulcers that are clinically infected.
(9) Patients having symptoms or signs suggesting general infection (fever, foot pain, hotness, and redness around the ulcer).
(10) Ulcers that had exposed tendons, ligaments, or bone.
(11) Patient who is currently receiving or has received radiation or chemotherapy within 3 months of randomization.
(12) Screening serum albumin level of less than 2.5 g/dl.
(13) Screening hemoglobin (Hb) of less than 10.5 mg/dl.
(14) Screening platelet count of less than 100×10^9/l.
(15) Patient undergoing renal dialysis, has known immune insufficiency, liver disease, active cancer, nutritional, hematologic, collagen vascular disease, rheumatic disease, or bleeding disorders.
(16) Patient having inadequate venous access for blood draw.
(17) Patients who did not complete their follow-up protocol.

This study was performed on 50 patients. The patient provides a written informed consent before enrolment in the study. All eligible patients were randomized into two groups according to the randomization schedule.

Randomization and blinding procedures

The randomization schedule was generated using the SPSS program (SPSS Inc., Chicago, Illinois, USA). The number and the type of dressing are provided to each vascular research center once the eligible case present there.

Each eligible study participant was assigned to one of the wo treatment groups: the PRP group or the platelet-poor plasma (PPP) group by receiving the next available consecutive randomization number and type of dressing according to the randomization schedule.

Each one of the two vascular research centers share in this research by two participating surgeons

The first participating surgeon (blind surgeon) select the eligible patients, prepared all wounds by removing the necrotic tissue, documented the size, site, and grade of the wounds and continued to follow the wounds during the outpatient visits, regarding the wound size. This surgeon was blind to the type of dressing.

The second surgeon (the unblind surgeon) know the number of the study patients and the treatment group of this patient according to an electronically generated randomization schedule. He also knows the type of the applied dressing and prepare dressings for the patients.

Eligible patients

(1) PRP group (25 patients): the wounds in this group were covered with PRP as their dressing protocol.
(2) PPP group (25 patients): the wounds in this group were covered with PPP as their dressing protocol.

Procedures

General measures

In both groups, surgical debridement of the wounds was done to freshen the wound bed and remove all

necrotic tissue debris. The wound site, sizes (length, width, and depth), and grade were documented.

Platelet-rich plasma and platelet-poor plasma preparation

(1) Less than 20 ml of venous blood was drawn from the patients (depending on the wound size) into a tube containing an anticoagulant, to avoid platelet activation and degranulation.
(2) Then the blood was centrifuged, the first centrifugation is called 'soft spin' (1000 rpm) for 7–10 min which allows the blood separation into three distinct layers:
    (a) At the bottom of the tube, the red blood corpuscles (RBC) constitute 55% of total volume.
    (b) At the top of the tube, the acellular plasma layer is mainly made up of circulating plasmatic molecules (in particular, fibrinogen) and is low in platelets. It is designated PPP and constitutes 40% of the total volume.
    (c) Between the two, there is an intermediate PRP layer (5% of total volume) called the 'buffy coat'.
(3) Using a sterile syringe, the PPP, PRP, and some RBCs (i.e. the upper two layers and very minimal 'unavoidable' amount of bottom layer) were transferred into another tube without an anticoagulant.
(4) This tube underwent a second centrifugation (3000 rpm) for 10 min called 'hard spin.' This allowed the platelets (PRP) to settle at the bottom of the tube with very few RBCs.
(5) The acellular plasma (PPP) (80% of the volume) was found on the top.
(6) Most of the PPP was taken with a syringe and the remaining PRP was left in the tube.
(7) At the time of application, the remaining PRP was mixed gently with calcium chloride 10% (0.1 ml) in a Petri-dish and left to rest for 10–15 min until the gel was formed.

Dressing protocol

(1) PRP group (25 patients): The PRP was applied to the ulcer followed by Vaseline gauze and then sterile dressing. The frequency of change of dressing was twice weekly. The dressing protocol was performed for up to 12 weeks or stopped whenever healing occurred.
(2) PPP group (25 patients): PPP was applied to the ulcer followed by Vaseline gauze and then sterile dressing. The frequency of change of dressing was twice weekly. The dressing protocol was performed for up to 12 weeks or stopped whenever healing occurred. General rules regarding the use of offloading techniques for the prevention and healing of plantar foot ulcers in diabetic patients are provided by reducing plantar pressure at sites of ulceration.

Follow-up

Follow-up was twice per week for 12 weeks. The rate of healing of the ulcer was carried out by measuring the ulcer's dimensions (length, width, and depth) using metric tapes at initial visit and at each visit. Laboratory tests were performed for all patients in two groups every 4 weeks until the patients reach the endpoint.

Endpoints

The endpoints of the current analysis were ulcer healing or end of study occurred at completion of the week 12.

Statistical analysis

Data were analyzed using STATA (StataCorp LLC, College Station, Texas, USA) intercooled version 12.1. Quantitative data were represented as mean and SD. Data were analyzed using Student's *t*-test to compare the mean of two groups and paired *t*-test was used to compare data before and after producers in each group. Qualitative data were presented as number and percentage and compared using either χ^2-test or Fisher's exact test. The *P* value was considered significant if it was less than 0.05.

Results

Between July 2016 and January 2017, 50 patients with diabetic foot ulcer met the inclusion criteria and enrolled in the current series in one of the two groups according to the randomization schedule, 25 patients in each group.

Baseline characteristics of the study patients

There was no significant statistically difference regarding demographic data, risk factors, laboratory parameters, ABI, and wound variables at the baseline for each group which are summarized in Table 1.

The baseline characteristics of diabetic foot ulcer are shown in Table 2 and Fig. 1. The ulcer's initial length ranged from 2 to 6.5 cm, the initial width ranged from 1.5 to 3.2 cm, the surface area ranged from 4 to 9.6 cm^2 with an average of 7.3 cm^2, and the volume ranged from 1.2 to 3 cm^3 with an average of 1.97 cm^3 in the PRP group. The majority of wound sizes in the PRP group (21 out of 25) were in the range of both

less than or equal to 7.0 cm^2 in area and less than or equal to 2 cm^3 in volume. Only four cases in the PRP group had areas of greater than 7 cm^2 and a volume of greater than 2 cm^3.

The ulcer's initial length ranged from 2 to 6 cm, the initial width ranged from 1.5 to 3 cm, the surface area ranged from 4 to 9 cm^2 with an average of 7.08 cm^2, and the volume ranged from 1.4 to 3 cm^3 with an average of 1.90 cm^3 in the control group. The majority of wound sizes in the control group (23 out of 25) were in the range of less than or equal to 7.0 cm^2 in area and less than or equal to 2 cm^3 in volume. The remaining two cases in the control group had areas of greater than 7 cm^2 and a volume of greater than 2 cm^3. There were no statistically significant differences between the two groups regarding average length, width, surface area, and volume.

The ulcer healing rate in the PRP group is significantly faster than the control group. There was statistically significant difference between the PRP group and the control group regarding the ulcer healing rate per week (Table 3).

There was statistically significant difference between the PRP group and the PPP group regarding the rate of completely healed ulcer at 10th and 12th weeks; however, the difference was insignificant at the eighth week (Tables 4 and 5, and Fig. 2).

There were no statistically significant differences between the PRP group and the PPP group from the baseline to the endpoint laboratory shift in blood picture (Hb and platelet count) and blood chemistry (albumin) (Table 4).

Table 1 The baseline characteristics of the study patients

Variables	PRP group	PPP group	*P* value
Number	25	25	
Age	56.88	55.8	0.76**
Male sex	16	14	0.56**
Risk factors			
Hypertension (%)	72	68	0.76**
Smoker (%)	48	40	0.57**
HbA1c	8.8	8.5	0.38**
Blood picture			
Hb	11.96	12.1	0.69**
Platelet count	258.8	265	0.81**
Blood chemistry			
Albumin	3.7	3.8	0.35**
ABPI	0.8±0.13	0.82	0.60**
Wound			
Area	7.3±1.6	7.08±1.27	0.57**
Volume	1.97±0.57	1.9±0.46	0.63**
Wound site	Foot	Foot	
Foot			
Right	14	12	0.57**
Left	11	13	

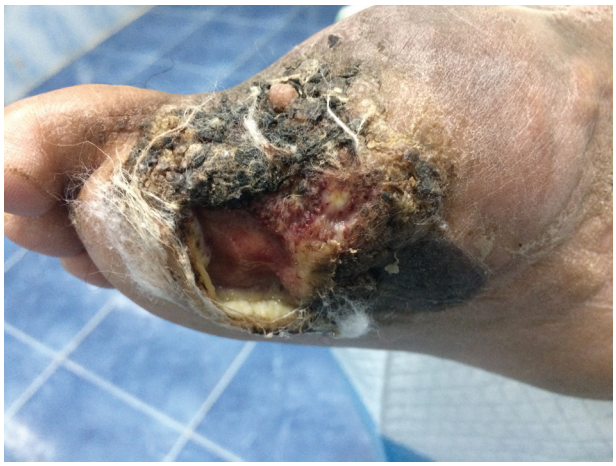
ABPI, ankle-brachial pressure index; Hb, hemoglobin; HbA1c, glycosylated hemoglobin; PRP, platelet-rich plasma; PPP, platelet-poor plasma. **$P>0.05$, not statistically significant.

Discussion

This is a prospective, randomized, controlled, double-blind multicenter trial on the use of PRP for the treatment of diabetic foot ulcer. The randomization in this study was generated using the SPSS program and the blindness involves the surgeon who takes the measurements and the patient to provide confidence in the results.

In this study, the majority of wound sizes in the PRP group (21 out of 25) and the PPP group (23 out of 25) were in the range of less than or equal to 7.0 cm^2

Figure 1



Predressing diabetic foot ulcer.

Table 2 Baseline criteria of diabetic foot ulcers

	PRP group			Control group			*P* value
	Mean±SD	Minimum	Maximum	Mean±SD	Minimum	Maximum	
Length	3.912±1.2 cm	2 cm	6.5 cm	3.88±0.87 cm	2 cm	6 cm	0.92**
Width	1.96±0.39 cm	1.5 cm	3.2 cm	1.88±0.38 cm	1.5 cm	3 cm	0.39**
Area	7.3±1.6 cm^2	4 cm^2	9.6 cm^2	7.082±1.27 cm^2	4 cm^2	9 cm^2	0.57**
Volume	1.973±0.57 cm	1.2 cm^3	3 cm^3	1.90±0.46 cm^3	1.4 cm^3	3 cm^3	0.63**

PRP, platelet-rich plasma. **$P>0.05$, not statistically significant.

in area and less than or equal to 2 cm^3 in volume. The remaining six cases, four in the PRP group and two in the PPP group, had areas of greater than 7 cm^2 and volume of greater than 2 cm^3. The results of various studies suggest that a wound size of less than 7.0 cm^2 is most common [18–20]. The average baseline area in the majority of wounds was similar to that reported in many literatures. Driver *et al.* [4] reported that the majority of wounds (35 out of 40) met the criteria of wound area of less than or equal to 7.0 cm^2 and volume of less than or equal to 2.0 cm^3. Lipkin *et al.* [21] reported in a tissue-engineered product study in healing of diabetic foot ulcer that ~70% of the ulcers were less than 6 cm^2. Another tissue-engineered product study in healing diabetic foot ulcer was done by Veves *et al.* [22]. Veves *et al.* [22] reported that the average wound size area in the graftskin group that included 112 patients was 2.97±3.10 and in the control group that included 96 patients was 2.83±2.45. In a large study that was done by Margolis *et al.* [23] included 26 599 diabetic foot ulcer patients, about 60% of which had an wound area of less than 6 cm^2 that matched the majority of wound areas in the current study.

In this study, the ulcer healing rate in the PRP-treated wound group is significantly faster than that in the PPP group (0.66±0.04 vs. 0.49±0.03). This result is similar to that reported in many literatures.

Saad Setta *et al.* [24] reported in a randomized trial on the use of PRP on chronic diabetic foot ulcer on 24 patients that the healing of ulcer by PRP is significantly faster than by PPP.

Table 3 Comparison between the two groups according the healing area over time

Time	PRP group	Control group	*P* value
First week	0.6388±0.009	0.4892±0.008	<0.0001*
Fourth week	2.5552±0.035	1.9568±0.030	<0.0001*
Sixth week	3.6168±0.152	2.9352±0.045	<0.0001*
Eighth week	5.1018±0.065	3.9256±0.046	<0.0001*
10th week	6.4786±0.042	4.892±0.078	<0.0001*
12th week	7.8±0	5.87±0.12	<0.0001*
Ulcer healing rate per week	0.66±0.04	0.49±0.03	<0.0001*

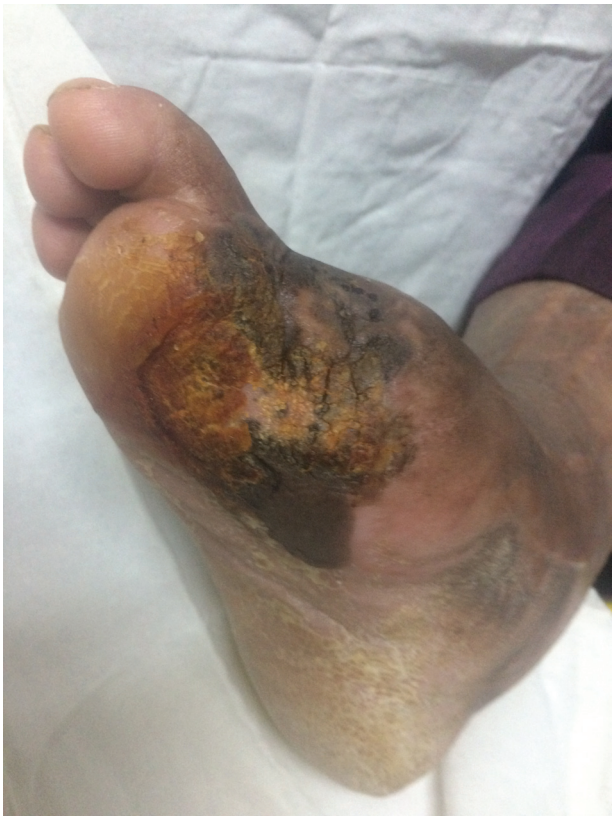
PRP, platelet-rich plasma. *$P<0.05$, statistically significant.

Table 4 Comparison between the two groups according to the rate of complete healed ulcer over time

Time	PRP group [*n* (%)]	Control group [*n* (%)]	*P* value
First week	0	0	
Fourth week	0	0	
Sixth week	0	0	
Eighth week	3 (12.00)	0	0.24**
10th week	11 (44.00)	1 (4.00)	0.002*
12th week	21(84.00)	13 (52.00)	0.02*

PRP, platelet-rich plasma. *$P<0.05$, statistically significant.
**$P>0.05$, not statistically significant.

Figure 2



Post dressing healed ulcer.

Table 5 Comparison of the laboratory investigation between PRP group and control group from the baseline to the endpoint

	PRP group		*P* value	Control group		*P* value
	Baseline	Endpoint		Baseline	Endpoint	
HbA1c	8.80±1.04	8.64±0.46	0.48	8.49±1.37	8.49±0.55	1.00**
Blood picture						
Hb	11.96±1.06	11.98±0.77	0.87	12.08±1.08	12.16±0.99	0.53**
Platelet count	258.80±31.27	255.00±16.32	0.44	261.00±32.91	261.20±30.87	0.91**
Blood chemistry						
Albumin	3.77±0.010	3.75±0.12	0.35	3.79±0.07	3.79±0.09	0.52**

HbA1c, glycosylated hemoglobin; PRP, platelet-rich plasma. **$P>0.05$, not statistically significant.

Kakagia *et al*. [25] reported in a randomized trial on chronic diabetic foot ulcers of 51 patients that the rate of healing for the combination of PRP and protease-modulating matrix statistical is higher compared with protease-modulating matrix alone.

In 2001, a retrospective controlled study by Margolis *et al*. [26] on the use of platelet releasates on diabetic foot ulcer of 26 599 patients showed statistically significant higher rate of healing at 20th week after treatment by platelet releasates (50 vs. 41%; $P<0.05$).

In 2010, a systematic review and meta-analysis of Villela and Santos [27] showed that there is scientific evidence regarding favorable outcomes especially the healing rate with the PRP group that reflects the effectiveness of the use of PRP for the treatment of diabetic ulcers.

In this study, the rate of completely healed ulcer in the PRP group was statistically significantly higher than the PPP group at 10th week and at 12th week [11 (44.00%) vs. 1 (4.00%)] and [21(84.00%) vs. 13 (52.00%)] consequently. The result in this study is similar to the result reported by Driver *et al*. [4], Ahmed *et al*. [29], and Jeong *et al*. [28].

Driver *et al*. [4] published a randomized double-blind trial on the use of PRP on chronic diabetic foot ulcers and found a statistically significant difference regarding the rate of complete healing after treatment of diabetic foot ulcer by PRP (81.3 vs. 42.1%, $P<0.05$).

In 2010, the prospective controlled study of Jeong *et al*. [28] on 100 patients with chronic diabetic foot ulcers founds a statistically significant higher rate of complete healing (79 vs. 46%, $P<0.05$) after treatment using blood bank platelet concentrates.

In 2017, Ahmed *et al*. [29] published a randomized controlled trial on the use of PRP on diabetic foot ulcer of 56 patients and found a statistical difference regarding the rate of complete healing after treatment by PRP (86 vs. 68%, $P<0.05$).The periodic laboratory tests that were done for patients in this study to measure Hb, hematocrit, platelet counts, and albumin showed that the frequent small amounts of blood collection (≤20 ml) that was done on each visit did not reduce these blood elements. The result in this study is agreeable with that reported by Driver *et al*. [4]. Driver *et al*. [4] reported that there were no statistically or clinically significant differences noted between the PRP gel and control from baseline to endpoint laboratory shifts in hematology, clotting factors, and factor V tests. Also, Driver *et al*. [4] reported that there were no clinical or statistically significant differences in chemistry test for sodium, potassium, chloride, bicarbonate, creatinine, or albumin. Serum glucose or glycosylated hemoglobin results showed that more patients shifted to high at endpoint in the PRP gel compared with the control group. These differences were not statistically significant or clinically meaningful.

Conclusion

The present study concludes that PRP is effective and safe for treatment of diabetic foot ulcer. PRP is effective where it significantly accelerates healing of diabetic foot ulcer and safe where it does not make significant changes on blood hematology or blood chemistry (albumin) in the patients.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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