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The Effect of Platelet Rich Plasma Dressing on Healing Diabetic Foot Ulcers

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Abstract

Background: Some of the studies confirmed the effectiveness of platelet rich plasma (PRP) in the treatment of diabetic foot ulcers (DFU). However, these studies had small sample size and used different methods such as PRP gel or PRP injections. The results are also contraversial.
Objectives: This study aimed to investigate the effect of PRP dressing on healing of DFUs.
Patients and Methods: A randomized, controlled trial was conducted on 50 patients with DFUs referred to Kashan's Shahid Beheshti hospital. Patients were randomly allocated to control (n = 25) and experimental (n = 25) groups. Data collection instrument consisted of two checklists; one for gathering demographic information and the other one included questions about ulcer characteristics and its treatment. After surgical debridement, ulcers depth and surface area were measured. Then, the ulcers of the control group were irrigated and dressed with sterile gauzes. However, in the intervention group, ulcers were dressed with sterile gauzes impregnated with PRP. Ulcers depth and surface area of all ulcers were measured on the days 0, 7, 14 and 21 after debridement. Independent-samples t-test, Mann–Whitney U and repeated measures analysis of variance were used to analyze data.
Results: At baseline, the mean ulcer depth were 15.08 ± 10.43 and 19.08 ± 14.01 mm in the control and intervention groups, respectively (P = 0.26), which decreased to 13.03 ± 14.1 and 4.560 ± 5.76 after three weeks (P = 0.04). Moreover, the mean ulcer surface area were 14.17 ± 8.52 and 12.791 ± 14.86 mm^2 in control and intervention groups respectively (P = 0.69), which decreased to 11.88 ± 13.65 and 2.68 ± 5.94 after three weeks (P = 0.03).
Conclusions: PRP dressing could significantly decrease the depth and surface area of DFUs in a three-week period.

Keywords: PRP Dressing, Ulcer Healing, Diabetic Foot Ulcer, Platelet Rich Plasma (PRP)

1. Background

Diabetic foot ulcers (DFU) are of the most serious and costly complications of diabetes (1). Studies in the United States estimated that each year more than 50000 to 60000 patients with DFUs undergo amputation (2). According to latest estimates, more than 7 million people with diabetes live in Iran (3) and 2 - 4% of them have diabetic foot ulcers (4). Today, management of diabetic foot ulcers is a big challenge (5). At present, two treatment protocols are used for these patients. The first method includes decreasing the pressure on the ulcer, debridement, infection treatment, managing ischemia and ulcer dressing. The second protocol involves using high pressure oxygen, negative pressures and artificial skin graft. Although both methods are costly and time consuming, their effectiveness is not satisfactory (6). Using platelet-rich plasma (PRP) is one of the new treatments for diabetic foot ulcers (7).

Most of growth factors affecting ulcer healing are secreted by platelets (8, 9). Therefore, vascular disorder around the diabetic foot ulcer would decrease transfer of blood cells and platelets to this area and then the speed of healing would decrease (10). However, it seems that PRP dressing would increase the level of growth factors and consequently lead to a faster and better ulcer healing (2). Nowadays, different forms of platelet products are used in the treatment of a variety of conditions (11). For instance, in a prospective, randomized, controlled trial, Driver et al. used autologous platelet-rich plasma gel for the treatment of diabetic foot ulcers and reported that PRP gel was effective and safe for use in the treatment of nonhealing diabetic foot ulcers (12). Moreover, in a case report, Mehrannia et al. reported the effective use of PRP in the treatment of non-healing DFUs (13). In a small study on six patients, Tran

et al. used activated PRP in the treatment of DFUs (14).

PRP is also used in many circumstances such as oral, maxillofacial, orthopedics, plastic and heart surgeries as well as wound treatment (11). Though using PRP in the treatment of DFU is very affordable, because it can be prepared from a small blood volume of patient (15). However, Jeong et al. did not recommend the use of autologous PRP in patients with anemia and those with unstable clinical conditions (16). In another study, Scevola et al. used allogeneic platelet gel for the treatment of pressure ulcer and reported that the method was only effective during the first two weeks of treatment (17). Moreover, in two review studies, Keshavarzi et al. (4) and Game et al. (18) referred to many problems in this field and concluded that these products are not sufficiently effective.

Although several previous studies confirmed the effectiveness of using PRP in the treatment of DFU, these studies were case reports or had small sample size and used different methods such as PRP gel (12) or injections (19) and no study is available on the use of PRP through simple routine dressings.

2. Objectives

Due to a formentioned controversies, this study aimed to investigate the effect of PRP dressing on healing of diabetic foot ulcers.

3. Patients and Methods

3.1. Design and Participants

This double blind randomized, controlled clinical trial was conducted on 50 patients with DFUs referred to Kashan's Shahid Beheshti hospital, from July 2014 to April 2015. Sample size was calculated using Kargar et al. investigation who studied the effect of platelet gel on the treatment of DFU. In that study, post-intervention mean $\pm$ standard deviation of the ulcer depth in control and experimental groups were 7.4 ± 1.6 and 5.3 ± 1.4, respectively (2). Accordingly, with a type I error probability of 0.05 and a power of 0.80, the sample size was determined as seven patients in each group. However, we recruited 30 experimental subjects and 30 control subjects to compensate probable attritions and achieve more reliable results.

Inclusion criteria were being able and willing to participate in the study, having a DFU in grades 1 or 2 (according to the Wagner classification system for DFU (20) and based on physicians' diagnosis), a hemoglobin level of 10 gr/dL and more, platelet count more than 100000 mm^3, receiving no immunosuppressive and contraceptive medications, having no known coagulopathy, immune deficiency, cancer, having no signs of ischemia around the ulcer, sepsis, osteomyelitis, deep vein thrombosis, limb paralysis, not receiving chemotherapy and lack of a history of spinal cord injury and stroke. Patient's decision to withdraw from the study and not completing the intervention were the study exclusion criteria.

After coordination with the hospital authorities, invitation letters were sent to diabetic patients with an active non-healing DFU to participate in the study. Among 100 patients who volunteered to participate and examined by a specialist in infectious diseases, 60 patients with inclusion criteria were selected and then using a random number tabulation were allocated into control (n = 30) and intervention groups (n = 30), Figure 1.

3.2. The Instruments

Data collection instrument consisted of two checklists. The first checklist consisted of questions on demographic information (i.e. patients' age, gender, occupation, marital status, participants, name, living location, residential address and phone number, income, weight, height, body mass index (BMI), education level, smoking habit, time passed from the diagnosis of diabetes, type of diabetes and the type of medical treatment used). In addition to fasting blood sugar (FBS) and HbA1c levels at the start of study, the second checklist included questions about wound characteristics and its treatments (i.e. the wound grade, the number of wounds, duration of ulcer, and its location, the need for debridement, need for early change of wound dressings, duration of wound healing, wound area and depth in frequent assessments during the study).

Content validity of the wound assessment checklist was confirmed by 5 experts in infectious disease. Reliability of the checklist was assessed through inter-observers method. For this purpose, the checklist was completed for 5 patients with DFU and for every patient by 3 researchers. Then, the agreement between the three observers was calculated (r = 0.95).

Demographic information was gathered at baseline and ulcer assessment was performed frequently during a three-week period.

3.3. The Procedure

3.3.1. Ulcer Assessment

According to the guideline for caring and treatment of DFUs (21), ulcer depth and surface area in the successive observations were used as measures for ulcer healing.

After ulcer grading by a specialist, all ulcers were surgically debrided of necrotic tissues by a surgeon. Then, ulcer depth and surface area were measured. According to the guideline for automatic colorimetric calibration of human

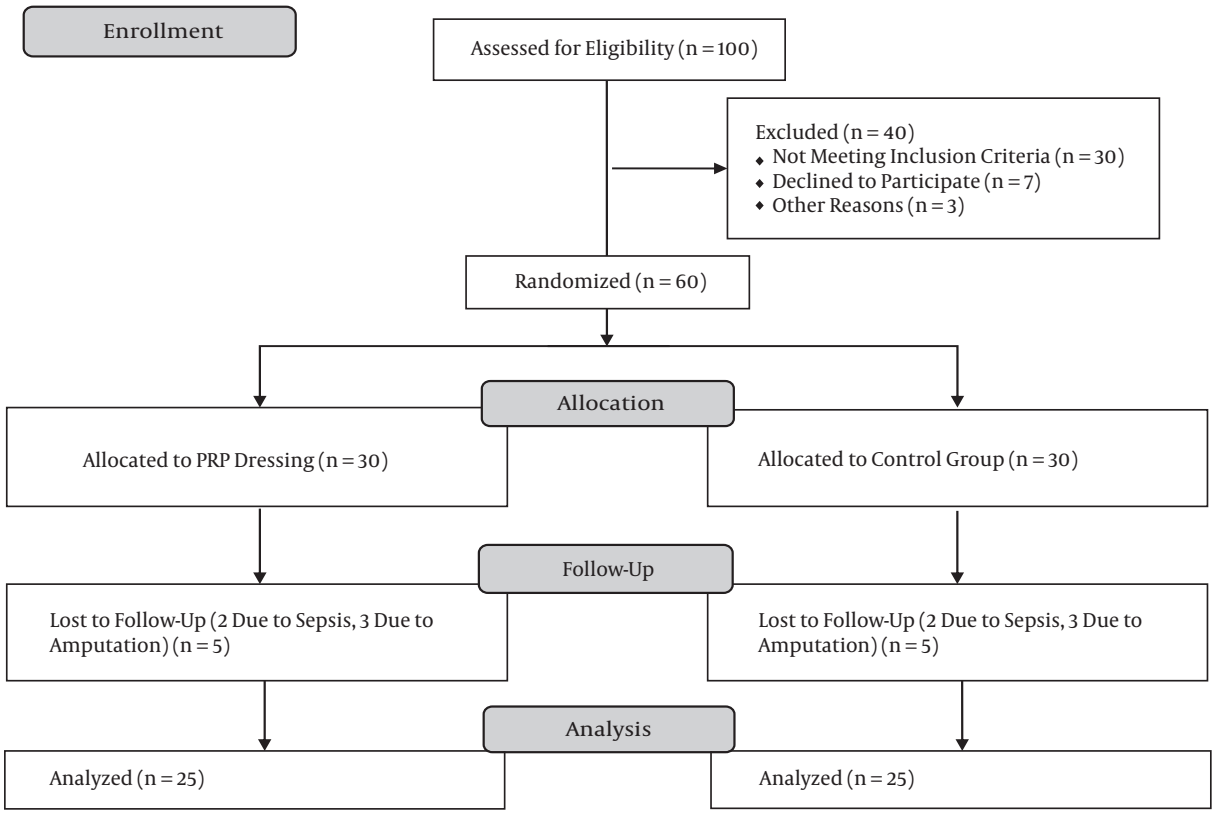

Figure 1. The CONSORT Flow Diagram of Patients in the PRP and Control Groups

wounds (22), a digital camera (Canon power shot A4000IS, zoom Lens 8 × 15, 16 mega pixel) was used to measure the ulcer surface area. The camera was calibrated by an expert in photography at the beginning of study and rechecked occasionally during the study. All photos were taken from a distance of 30 cm while a transparent, millimeter scaled ruler was placed near the ulcers. Then, an expert analyzed all photos using AutoCAD software version 19 (Autodesk, USA) to calculate ulcer surface area in square millimeters and the results were documented in the ulcer assessment checklists.

A sterile blunt metal millimeter scaled measure was used to determine ulcer depth. For this purpose, the tip of the metal measure was placed in the deepest point of the ulcer and then a piece of paper was placed perpendicular to the measure at the ulcer surface. Then, the measure was read in millimeters and the result was recorded in the ulcer assessment checklists.

Usually wound healing starts in the few first days after debridement and granulation tissue appears in 5 to 10 days or within 30 days after initial injury (23). Therefore, we observed and documented wound depth and surface area on days 0, 7, 14, and 21 after debridement.

3.4. Intervention

After debridement, the wounds of the control group were irrigated with normal saline and dressed with sterile gauzes. However, in the intervention group, the debrided ulcers were dressed with sterile gauzes impregnated with PRP. For this purpose, a piece of sterile gauze impregnated with PRP was placed on the ulcer surface and supported by two pieces of dry sterile gauzes and fixed using cotton bands. All dressings were changed every other day unless the signs of infection were observed. In these cases, the dressing was changed immediately and was documented. PRP dressing was performed only once at the beginning of study and subsequent dressings in both groups were performed similarly using normal saline and ordinary sterile cotton gauzes.

To avoid confounding factors, all patients were advised not to use any other material on the ulcer and avoid any dressing change without informing the researcher. All patients were also instructed about the sings of ulcer infection and asked to inform the researcher if any signs of in-

fection occurred. All dressing changes during the study were performed by the first researcher in hospital or in patients' house (if a patient was unable to refer to hospital).

3.5. PRP Preparation

Firstly, a thorough physical examination was performed to rule out anemia and other diseases. Then, using a sterile 50 mL syringe, 30 mL of whole venous blood was drawn from each patient to generate 5 mL of PRP with a concentration of 100000 platelets in mL. The whole blood was taken in a sterile Falcon tube containing 3 mL of sodium citrate (to prevent clotting). According to the guideline of the American association of blood banks (24), the samples were kept in a laboratory specimen collection box with inside temperature of 24°C and immediately transferred to the laboratory. In laboratory, samples were centrifuged at 200 rpm for 10 minutes (digital full-R5702 model, Eppendorf, Germany). Then, PRP accumulated on the upper surface of the tube was collected using a sterile pipette and transferred to another sterile Falcon tube. The prepared PRP was then kept in the specimen collection box with inside temperature of 24°C and transferred to hospital within 30 minutes. At patient's bedside, autologous PRP was placed in a sterile stainless receiver containing a sterile cotton gauze to prepare a PRP impregnated gauze to be applied on debrided DFU.

3.6. Ethical Considerations

This study was approved by the institutional review board and the ethics committee of Kashan University of Medical Sciences (approval number: 9321). The research objectives were explained to all patients and a written informed consent was obtained. All patients were also informed about voluntary participation and the right for withdrawal at any time. They also were assured that their anonymity would be protected and their personal information would be kept confidential.

3.7. Data Analysis

Data was analyzed using SPSS version 13 (SPSS Inc. Chicago, IL, USA). The findings were reported via absolute and relative frequency values, mean and standard deviation (SD). The categorical variables were analyzed by chi-squazre and Fisher's exact tests. On the other hand, variables with interval and ratio measurement scale were primarily assessed regarding their distribution using Kolmogorov-Smirnov test. Then, independent-samples t or Mann-Whitney U test was performed for analyzing these variables. Finally, the depth and area of DFUs between different measurement time-points were assessed using repeated measures analysis of variance. The level of significance was set at below 0.05.

4. Results

Overall, five patients in each group were lost from follow-up and finally 25 patients in each group entered the analysis. As presented in Table 1, the two groups were not significantly different for demographic and clinical variables.

Duration of diabetes mellitus in participants in experimental and control groups were respectively 13.48 $\pm$ 6.93 and 12.08 $\pm$ 6.64 years (P = 0.47). Moreover, the most common treatment used by participants for blood sugar control was insulin injection. Clinical outcomes are shown in Table 2, Figure 2.

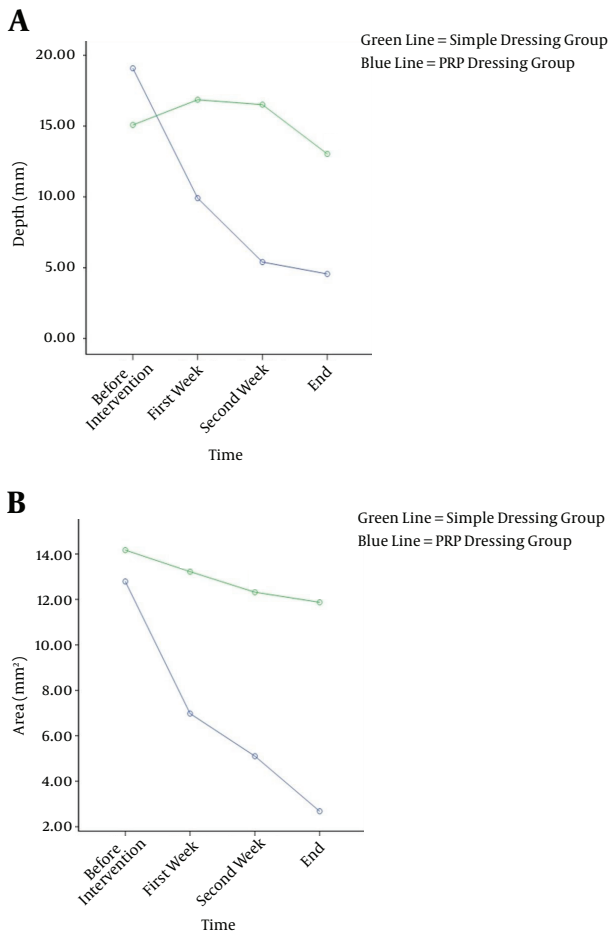

Figure 2. Variations of Ulcers Depth and Area in Both Study Groups

The frequency of complete wound healing after one month of treatments in the experimental and control groups were 36% and 40%, respectively (OR = 13.5, CI 95% = 1.56 - 117.14, and P = 0.005).

Table 1. Demographic, Laboratory and Clinical Characteristics of Patients in PRP and Simple Dressing Groups[a]

Characteristics	Group		P Value
	PRP Dressing	Simple Dressing	
Gender			0.51[b]
Male	20 (80.0)	18 (72.0)	
Female	5 (20.0)	7 (28.0)	
Location of residence			0.51[b]
City	20 (80.0)	18 (72.0)	
Village	5 (20.0)	7 (28.0)	
Economic State			0.93[b]
Low	8 (32.0)	8 (32.0)	
Middle	11 (44.0)	12 (48.0)	
High	6 (24.0)	5 (20.0)	
Education			0.74[b]
Illiterate	7 (28.0)	5 (20.0)	
Elementary school	7 (28.0)	7 (28.0)	
Intermediate	7 (28.0)	6 (24.0)	
High school or university	4 (16.0)	7 (28.0)	
Marriage			0.05[c]
Married	24 (96.0)	18 (72.0)	
Single	1 (4.0)	7 (28.0)	
Smoking			0.49[c]
Yes	2 (8.0)	0 (0)	
No	23 (92.0)	25 (100)	
Body Mass Index			0.65[b]
< 18.5	1 (4.0)	2 (8.0)	
18.5 - 25	9 (36.0)	11 (44.0)	
> 25	15 (60.0)	12 (48.0)	
Type of diabetes			0.35[c]
Type I	24 (96.0)	21 (84.0)	
Type II	1 (4.0)	4 (16.0)	
Drugs			> 0.99[c]
Insulin	21 (84.0)	20 (80.0)	
Oral agents	4 (16.0)	5 (20.0)	
Fasting Blood Sugar[d]	257.32 ± 95.06	216.20 ± 56.30	0.07[e]
HbA1C[d]	8.38 ± 1.03	7.86 ± 0.88	0.06[e]

[a] Values are expressed as No. (%) unless otherwise indicated.
[b] Chi-square test.
[c] Fisher's exact test.
[d] Values are expressed as mean ± SD.
[e] Independent t-test.

5. Discussion

This study showed that PRP dressing could significantly increase the rate of healing of DFUs, so that both outcome variables (i.e. the mean depth and surface area of ulcers) were significantly decreased three weeks after using PRP dressing in the intervention group. However, neither the ulcer depth nor the ulcer surface area of the control group changed significantly during the study. Our results are consistent with the findings of Driver et al. (12) who studied the effect of autologous PRP gel in the treatment of DFUs. Moreover, Lacci and Dardik showed the effectiveness of PRP on wound healing. Although, the healing time in the Lacci and Dardik study (15) was longer than that of the present study. Mehrannia et al. reported a single case of non-healing DFU that was successfully treated by injection of PRP inside and around the peripheral skin (13). Moreover, Tran et al. reported six cases of DFU that completely closed 7 weeks after two injections of PRP at the wound bed (14). Conversely, in another study, Maghsoudi et al. reported that platelet dressing could not enhance healing of burn wounds (25). Horn et al. reported that although PRP gel could effectively decrease the width and depth of chronic wounds, changes were not statistically significant compared with a control group (26). Although PRP has been used effectively in several conditions such as dental and oral surgeries (27) and in the treatment of musculoskeletal injuries (19), few human studies are available on its usage in DFUs. Despite controversies, the present study confirmed the effectiveness of PRP in the treatment of DFUs.

Despite many advances in the treatment of DFUs, many diabetic patients are yet living with this destructive complication (12). Lack and malfunction of some growth factors disrupt the natural healing process in diabetic patients, which leads to DFUs. It seems that PRP provides the growth factor needed for healing (28). It is reported that seven fundamental protein growth factors that are actively secreted by platelet initiate wound healing process. PRP also includes three proteins in blood known to act as cell adhesion molecules: fibrin, fibronectin and vitronectin (29). Platelets also secrete transforming growth factor-Beta (TGF-Beta) and Monocyte Chemoattractant Protein-1 (MCP-1) that would attract monocytes and neutrophils to the wound site (28).

Our findings supported that PRP can facilitate healing of DFUs and therefore can reduce the risk of amputation (2). Autologous PRP can be prepared easily and does not need a large volume of patient's blood. Therefore, its usage is economical and affordable, and as it is autologous, the risk of transmission of blood borne diseases is diminished.

PRP preparation in the present study did not change

Table 2. Ulcer Characteristics

Variable	Time			Mauchly's Test	Time	Time × Group
	First Week	Second Week	End			
Surface Area, mm²				< 0.001	0.001	0.08
PRP dressing Group	6.98 ± 9.82	5.10 ± 8.48	2.68 ± 5.94			
Simple dressing Group	13.22 ± 9.35	12.32 ± 11.01	11.88 ± 13.65			
Depth, mm				< 0.001	< 0.001	0.01
PRP dressing Group	9.91 ± 7.39	5.40 ± 6.05	4.56 ± 5.76			
Simple dressing Group	16.86 ± 10.19	16.51 ± 11.56	13.03 ± 14.10			

biochemical composition of plasma, which is considered as its advantage. Moreover, PRP dressing used in the present study is a noninvasive method and seems to be more safe and affordable than methods used in previous studies (i.e. PRP gel or injection method). PRP gel is usually prepared from bovine plasma and therefore may cause allergic reactions. PRP injection method may also put patients at risk of injection related complications (19, 30).

Lack of control over some confounding factors such as patients' nutrition, activities and their level of adherence to their medical treatments can be considered as some limitations of the present study. Moreover, the study was conducted on a small sample. Therefore, replication of study with larger sample sizes is recommended.

This study revealed that PRP dressing could significantly decrease the depth and surface area of DFUs in a three-week period. None of the patients receiving PRP developed any side effects. Therefore, using PRP in the treatment of DFUs is recommended. In this study we used a PRP with concentration of 100000 platelets per mL. More studies with different concentrations of PRP, in periods longer than this study and using PRP after amputation are suggested.

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Footnotes

Authors' Contribution: Rouhangiz Karimi, data gathering; Mohammad Afshar, supervision; Morteza Salimian, PRP preparation; Alireza Sharif, diagnosis of ulcer; Milad Hidariyan, co-edithor.

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