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Evaluation of wound healing in diabetic foot ulcer using platelet-rich plasma gel: A single-arm clinical trial

Mohammad Hossein Mohammadi [a], Behnam Molavi [b], Saeed Mohammadi [c,*], Mohsen Nikbakht [c], Ashraf Malek Mohammadi [c], Shayan Mostafaei [c], Amir Hossein Norooznezhad [c], Ali Ghorbani Abdegah [b], Ardeshir Ghavamzadeh [c]

[a] BMT and Cell Therapy Center of Taleghani Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[b] Surgery Department, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran
[c] Hematology, Oncology and Stem Cell Transplantation Research Center, Tehran University of Medical Sciences, Tehran, Iran

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ABSTRACT

The aim of the present study was to evaluate the effectiveness of using autologous platelet-rich plasma (PRP) gel for treatment of diabetic foot ulcer (DFU) during the first 4 weeks of the treatment. In this longitudinal and single-arm trial, 100 patients were randomly selected after meeting certain inclusion and exclusion criteria; of these 100 patients, 70 (70%) were enrolled in the trial. After the primary care actions such as wound debridement, the area of each wound was calculated and recorded. The PRP therapy (2 mL/cm^2 of ulcers) was performed weekly until the healing time for each patient. We used one sample T-test for healing wounds and Bootstrap resampling approach for reporting confidence interval with 1000 Bootstrap samples. The p-value < 0.05 were considered statistically significant. The mean (SD) of DFU duration was 19.71 weeks (4.94) for units sampling. The ratio of subjects who withdrew from the study was calculated to be 2 (2.8%). Average area of 71 ulcers in the mentioned number of cases was calculated to be 6.11 cm^2 (SD: 4.37). Also, the mean, median (SD) of healing time was 8.7, 8 weeks (SD: 3.93) except for 2 mentioned cases. According to one sample T-test, wound area (cm^2), on average, significantly decreased to 51.9% (CI: 46.7–57.1) through the first four weeks of therapy. Furthermore, significant correlation (0.22) was not found between area of ulcers and healing duration (p-value > 0.5). According to the results, PRP could be considered as a candidate treatment for non-healing DFUs as it may prevent future complications such as amputation or death in this pathological phenomenon.

1. Introduction

Diabetic foot ulcer (DFU) is a common accompanying complication and the most important cause of hospitalization among diabetic patients. This phenomena, with an incidence of 15% in diabetic population, is an important issue for health and care services [1–3]. During the lifetime of a diabetic patient, the risk of any lower extremity involvement with DFU is estimated to be about 25%, which is affected by several risk factors including arterial disorders, peripheral neuropathy and infection. Among diabetic patients, 20% are diagnosed with inadequate blood flow and 50% with peripheral neuropathy. These incidences are highly significant in DFU population because 80% of them are suffering from both conditions [4]. Moreover, the vascular problems in these cases not only postpone the wound healing process but also hinder the reaction of the immune system to the accompanying infections. The vascular complications in diabetic patients mostly develop as 3 major disorders of thrombosis or arteritis of arterioles, peripheral neuropathy (mostly due to the ischemic situations) and atherosclerosis of arteries. Besides the mentioned risk factors, physical and

* Corresponding author. Hematology, Oncology and Stem Cell Transplantation Research Center, Tehran University of Medical Sciences, Tehran, Iran. Fax: +982188004140.
E-mail address: saeedm_58@yahoo.com (S. Mohammadi).

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mechanical traumas causing neuropathy in lower extremity may also lead to DFUs [5–7]. Chronic DFU is defined as an ulcer not decreased by 50% of the primary size during a month [4]. Proper treatments suggested for DFU mostly include local actions such as ulcer debridement, antibiotic therapy and bedside surgery [8,9]. Although ulcer debridement is suggested as the primary step, it could only be helpful when the patient does not suffer from arterial insufficiency. So far, different surgical methods such as percutaneous transluminal angioplasty [10], luminal stenting and arterial reconstruction surgery have been practiced in order to improve blood supply in patients with ischemic DFUs [11,12]. Moreover, new strategies such as hyperbaric oxygen therapy [13], bioengineered tissues [14], electrical stimulation, phototherapy [15] and platelet derived growth factors [16] are also recommended and applied. Platelet derived growth factors, which have been available clinically since 1985, are biologic active compounds acting in different mechanisms and pathways including activation or induction of chemotaxis, cellular proliferation and angiogenesis to induce and accelerate wound healing [17].

The aim of this longitudinal and single-arm clinical trial was to evaluate the effectiveness of using autologous PRP gel for treatment of DFU during the first 4 weeks of treatment.

2. Materials and methods

2.1. Ethics

This clinical trial was approved by the Medical Ethics Committee of Research Institute for Hematology, Oncology and Stem Cell Transplantation of Shariati Hospital, Tehran University of Medical Sciences (approval code: 1394.103.3). Also all the team members considered the 1975 Declaration of Helsinki and its following revisions during the trial. The aim of this study was clearly explained to each patient. After the purpose of the study was explained (according to the patients' level of understanding), eventually, all patients were requested to sign a written consent form to declare that they were joining this study freely. This clinical trial was registered in the Iranian Registry of Clinical Trials (under supervision of the Ministry of Health and Medical Education) with IRCT2015123018842N10 number. Current study originally was a part of a bigger trial which unfortunately was canceled and removed from IRCT due to financial problems. Thus we had to submit for another IRCT code. During the study, however, this trial was under the supervision of the Ethics Committee.

2.2. Study design and patients

This longitudinal and single-arm clinical trial was conducted between May 2014 and December 2015 in Shariati Hospital (Tehran University of Medical Sciences, Tehran, Iran). In the mentioned period of time, 100 patients with chronic DFUs were selected in the study by simple random sampling. Then the inclusion and exclusion criteria were applied to all patients. Chronic DFU, as defined before, is a wound found in the lower extremity (usually a foot) of a known diabetic patient, which has not decreased by 50% of the primary size in one month (inclusion criteria). No other limitations such as age, sex, fasting blood sugar (FBS) or hemoglobin A1C (Hb A1C) levels were applied. Exclusion criteria included osteomyelitis, malignant arterial insufficiency, exposed bone in an ulcer, antibiotic resistance DFU, wounds with Charcot deformity, history of anti-proliferative medication or radiation in past 3 months, serum Hb < 10 mg/dL, platelet count < 10^3/μL and history of growth factor therapy within last 2 weeks. Finally, according to exclusion criteria, 70 (70%) patients as units sampling were enrolled in the trial (Fig. 1).

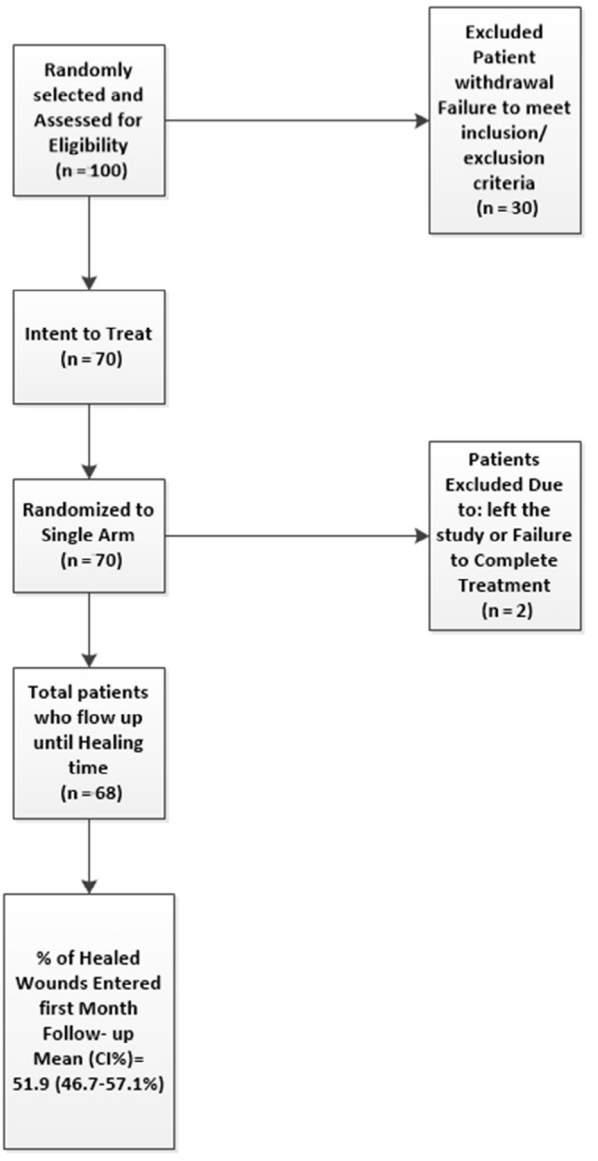

Fig. 1. A follow-up diagram of study design and methodology.

2.3. Primary care

First of all, demographic data and full medical history including the present illness and drug history were collected

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from all patients. Then primary laboratory tests such as FBS and HbA1C were re-checked. For those patients with imbalanced glucose level (abnormal FBS and/or Hb A1C), new insulin prescriptions were ordered by consulting with an expert endocrinologist while consequent laboratory results were evaluated again. For the infection complications, a specialist was consulted with to prescribe proper antibiotics for prophylaxis in all cases (intravenous or oral). Also debridement was performed wherever required by an expert surgeon under sedation (in some cases) in an operating room in order to remove all necrotic tissues, foreign bodies and sinuses. Afterward, the area (length and wide) of the ulcer was measured and recorded separately for each patient by a trained physician in order to find the ulcer area. For each ulcer, the most likely geometric shape was estimated and then the area was calculated according to the appropriate shape formula (such as triangle, square, rectangular or elliptic).

2.4. Platelet rich plasma preparation

PRP preparation was performed by Rooyagen PRP Gel kit (Arya Mabna Tashkhis Co, Iran). According to kit instructions, first, 27 mL of peripheral blood was drawn from donor using a 30 mL syringe containing 3 mL anticoagulant: sodium citrate. Then, the blood was shaken gently 4 times. Afterward, it was transferred into three 10 mL tubes using the transfusion kit adaptor connected to the syringe, and centrifuged in $2000 \times g$ for 10 minutes in 24 °C. After first centrifugation, the 2 fold rich platelet in the supernatant plasma was achieved. This PRP was then transferred to a second tube containing 2 mL 25 mM $CaCl_2$ leading to gel formation after 20 minutes. Finally, the platelet gel was applied topically on the wound which would be explained in detail in the next step.

2.5. Ulcer dressing and evaluation

After debridement and washing of the DFU, 2 mL/cm^2 of PRP was applied on the ulcers as a covering layer. Then a non-absorbing wet dressing was layered on the lesion as the first contact surface. Every week until the healing was achieved, this procedure was repeated and wound area was re-measured and recorded by the physician over times. All photos were taken using a 10 mega pixel camera (Sony). At the end of the treatment, all patients were asked to follow discharge orders to prevent possible episodes of diabetic foot ulcer remission and also keep a scheduled visit with an endocrinologist. After the first four weeks, the changes between measured ulcer areas before and after treatment were calculated by dividing the pre-treatment and post-healing (or measured ulcer area at the end of the first four weeks) areas to be considered as the effect size of PRP therapy in this study.

2.6. Statistical methods

From the first visit to the end of the trial, all collected information was added to patient's file in Excel 2007 (Microsoft Office, Microsoft, Washington D.C., USA). According to normality assumption of wound changes, we used one sample T-test for evaluating the wound changes from pre-treatment to 4th week of treatment and for reporting confidence interval of the effect size, Bootstrap resampling approach with 1000 Bootstrap samples was applied. The p-value <0.05 was considered statistically significant. Data were analyzed using Statistical Package for Social Sciences version 18.0 (SPSS, Inc., Chicago, IL, USA).

3. Results

During the mentioned time period, 100 patients were evaluated from which 30 were excluded according to the already defined exclusion criteria. Among the remaining 70 cases with 71 ulcers, 58 (83%) individuals were male and 12 (17%) were female. The average age of participants (in the range of 30–79 years old) was calculated to be 53.8 years (SD: 10.59). As already explained, a full medical history of patients was collected and according to these histories, duration of diabetes was recorded between 2 and 32 years with the mean of 16.2 years (SD: 7.9). Also the average ulcer duration time was found to be 136.9 days (SD: 32.7 in the range of 74–207 days). Moreover, Hb A1C mean was 6.2% (SD: 0.65) with minimum of 4.6% and maximum of 8.0%. For those cases with abnormal Hb A1C levels, new insulin orders were prescribed by an expert endocrinologist and continued to reach a proper serum glucose levels.

After measuring and calculating the ulcer area in the pre-treatment step, the median value obtained was 6.11 cm^2 (SD: 4.37), holding a range between 0.2 and 15.12 cm^2. Also the average ulcer at the end of 4th week was calculated to be 2.82 cm^2 (SD: 2.25 in the range of 0.07–9.42). At weeks 11 and 12, two of the patients left the study due to personal problems not related to the process of the study. At the end of the study, 69 remaining ulcers were healed in an average of 8.7 weeks (SD: 3.93 in the range of 4–17) (Fig. 2). Although wounds were re-measured every week as the ulcer dressing was changed, inferable evaluation was documented at the 4th week.

According to the results of Kolmogorov–Smirnov test for normality of effect size, one sample T-test was used. Result of this test showed that the wound area (cm^2), on the average, significantly decreased up to 51.9% (CI: 46.7–57.1) from the beginning of treatment to the end of the first four weeks by PRP therapy. The correlation between ulcers area in pre-treatment and at the end of 4th week (after treatment by PRP gel) was 0.78 (p value = 0.008); then the correlation between the primary wound area and the healed time (by week) was calculated to be 0.22, which was not significant (p value > 0.5). Also no significant relation was detected between effect size and other variables such as age, HbA1C, gender and duration of diabetes (p-values > 0.05).

4. Discussion

Diabetes has long been known as a serious issue in health care system due to its growing prevalence. According to the literature, patients with diabetes are estimated to be 422 million (2014) [18], which could reach to 552 million people in 2030 [19]. As any other chronic disease, diabetes is associated with various complications in different organs [20,21]. Among these complications, DFUs, which almost

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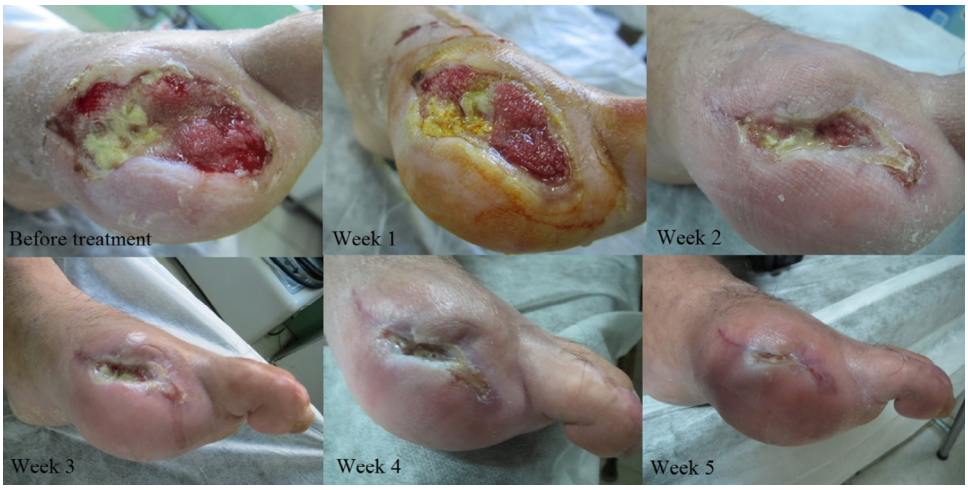

Fig. 2. The treatment process of PRP therapy in one case (selected randomly) in different weeks from beginning of the treatment until the healing time.

always are observed in lower extremity, occur in 15% of diabetic population [22]. This phenomenon may lead to infection, gangrene, amputation [23] or even death [24]. This fact could reach a new level of importance when data reveal that 50–70% of lower extremity amputations are caused by DFUs [3]. In the United States, 66,000 lower extremity amputations ensued after untreated DFUs were recorded in 2006 [25]. In this issue any non-invasive therapeutic process is preferred to invasive ones especially when the results are predicted to be the same. So, in the cases of non-healing DFUs, amputation is considered as the last choice in the treatment process [26]. In this clinical trial, the effect of autologous PRP gel application as a non-invasive method was evaluated on the treatment of chronic DFUs. The results showed a significant decrease in the ulcer area in the pre-treatment step compared to the 4th week (after beginning of treatment). Also it was shown that there is no significant correlation between primary ulcer area and the healed time which means that there is no limitation for any case with any wound area to undergo PRP therapy while considering the exclusion criteria. Thus a wide range of patients with varying levels (areas of wound) of DFUs are able to use this treatment. Also, most of the HbA1Cs did not increase dramatically before the treatments. As data showed, age and gender as confounding variables have no significant association with the ulcers area. Fortunately, at the end of the current clinical trial, amputation was not necessary in any of the cases; however, no further information was collected from two patients who discontinued from the study. So far, several studies including case studies and clinical trials have reported using PRP for treatment of DFUs with different success ratios. Scimeca et al. successfully treated a 49-year-old man with a 15 cm^2 DFU (3 months wound) using PRP in 7 weeks [27]. Also, according to what Suresh et al. [28] declared, a 57-year-old man suffering from a 15 cm^2 non-healing DFU for 4 years was treated by PRP and healed after 7 weeks or six settings of PRP. In a clinical trial held in the United States in 2006 by Driver et al., efficacy of PRP treatment on non-healing chronic DFUs was investigated on two groups of patients: controls and standard care (PRP gel) with the wound area of 3.2 cm^2 (SD: 3.5) and 4 cm^2 (SD: 5.3), respectively. In PRP treated group (72 cases) during 12 weeks, 68.4% of ulcers were healed while in controls 42% was cured [29]. However, after adjacent outliers for PRP treated group, this percentage increased to 81.3% versus 42% in controls. In another study carried out in Japan in 2012 by Sakata et al. on 39 cases with 40 DFUs in lower extremity with a wound mean of 16.8 cm^2 (SD 26.67), it was shown that 83% of DFUs were healed completely in 145.2 days with a significant p-value [16].

Platelet rich plasma is known as an autologous fraction from the blood containing high amounts of platelet and growth factors [30]. PRP renowned as a chemotaxis and mitogenic agent is commonly and successfully used in clinic for treatment of wound in different fields of periodontal and oral, maxillofacial, orthopedic, cosmetic and plastic surgeries [17]. In the general aspect, the mechanism by which PRP associates with wound healing begins by α-granules degradation [31]. This phenomenon in turn activates other related growth factors including platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), platelet derived endothelial growth factor (PDEGF), transforming growth factor β (TGF-β), epidermal growth factor (EGF), insulin like growth factor (IGF), platelet growth factor 4 (PGF-4) and interleukin 1 (IL-1). All these mentioned growth factors have been proved to play roles in wound healing process [17,30]. PRP is also capable of recruiting macrophages due to its chemotaxis properties and acts as an anti-infection agent as well [32].

Angiogenesis, the formation of new blood vessels from pre-existing ones, mostly occurs in pathological conditions such as tumor growth, corneal neovascularization and psoriasis [33,34]. On the other hand, in physiological conditions, this process is mainly responsible for two main events: wound healing and female reproductive cycle [35]. Although PRP consists of different growth factors affecting different cells, it seems that one of the possible pathways of its action is through the induction of angiogenesis. In

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angiogenesis process VEGF is a strong inducer of angiogenesis that affects proliferation and migration of endothelial cells (ECs), which are two main steps of angiogenesis. Also VEGF is responsible for induction of other angiogeneic factors [36–38]. Also PDGF, TGF-ß and IGF in PRP are noted as strong angiogenic factors [39]. In this condition the new vascular branches could provide enough blood supply (both nutritional and oxygenation support) and also are able to remove dusts of wound healing process [40].

This study affirmed a highly efficient application of PRP gel in treatment and healing of chronic non-healing DFUs such that in all 70 evaluated cases, a relative improvement and healing of wound were observed.

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Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.transci.2016.10.020.

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