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Treatment Of Chronic Foot Ulcers In Patients With Diabetes Using Platelet-Rich Plasma

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ABSTRACT

Introduction: Diabetic foot ulcer is a major complication of diabetes mellitus, and is the major component of the diabetic foot. The major problem with diabetic foot ulcers (DFUs) is the length of time they take to heal. It has been reported that the levels of metalloproteinases (MMPs) and tissue inhibitor of metalloproteinase (TIMPs) can significantly contribute to a delay in healing. **Objective**: This study examined the application of PRP in the treatment of diabetic foot ulcers (DFUs) in patients. **Methods**: This is a prospective study was conducted at the dept. of Transfusion Medicine, Diabetic Foot Care & PRP Center (Diagnostic & Hospital), Sirajganj & Desh PRP Centre, Bogura, Bangladesh from January 2018 to December 2021. One hundred one (101) patients included to our study according to the following inclusion and exclusion criteria an application of PRP in the treatment of foot ulcers in patients with diabetes. Informed consent was obtained from patients, which include the use of photographs in this paper. Patients with the following criteria were excluded: platelet dysfunction syndrome, critical thrombocytopenia, unstable haemodynamic and pregnancy. The significance of changes in time for wound healing was statistically assessed. **Results**: Total 101 foot ulcers were assessed. Wound size reduction was detected in patients after four weeks of treatment. In DFUs with a 2–5.5 cm^2 surface area, complete closure happened after 7.2 weeks, 5.5–8.5cm^2 DFUs completely closed after 7.5 weeks, and 8.5–12.5cm^2 DFUs healed completely after 8.8 weeks. None of the wounds reopened after eight months of monitoring. **Conclusion**: This study will provide more evidence for the use of autologous PRP for DFUs in patients. Our results substantiate the fact that the procedure is cost beneficial and can be easily adapted to most clinical settings for many patients, at low cost. However, for large, non-healing ulcers in patients with diabetes, it is imperative to use skin graft as the definitive procedure for wound healing.

Keywords: *Ulcer, Platelet, Plasma, Diabetic.*

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INTRODUCTION

Diabetic foot ulcer is a major complication of diabetes mellitus, and is the major component of the diabetic foot [1]. The major problem with diabetic foot ulcers (DFUs) is the length of time they take to heal. It has been reported that the levels of metalloproteinases (MMPs) and tissue inhibitor of metalloproteinase (TIMPs) can significantly contribute to a delay in healing [2]. Diabetes is a major health problem that is currently showing an alarming rise in its prevalence [3]. This can be serious when combined with ischaemia and vascular disease, which reduces oxygen and nutrients to the wound, preventing the wound healing mechanism from performing as it would in patients without diabetes. Alvarsson *et al.* [4] 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 [5]. Accepted therapeutic objectives and standardsof 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 [6]. 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 [7]. Platelets are known to start the wound healing process through the release of locally active growth factors [8910]. Due to

the lack of oxygen and nutrients, epithelial cells at the wound site are unable to express essential factors for healing, such as vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF); almost all of the cells at the wound will change metabolism and activity [11]. These changes in the cells, and other factors affecting DFUs, such as the presence of infection, will delay the normal healing process. Platelet-rich plasma (PRP) has been proposed as an adjunct for the treatment of DFUs, as well as a diabetic foot ulcer treatment for other chronic and acute wounds [12,13]. PRP has also been demonstrated to be of some antimicrobial properties against microorganisms, such as *Escherichia coli*, MRSA, *Candida albicans*, and *Cryptococcus neoformans* [14]. 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 [15]. For more than 20 years, PRP gel has been used to stimulate wound healing [16]. Autologous PRP gel consists of cytokines, growth factors, chemokines, and a fibrin scaffold derived from a patient's blood [17,18]. It is thought to stimulate the molecular and cellular induction of normal wound healing responses. The alpha granules of platelets contain growth factors that include molecules such as PDGF, VEGF and transforming growth factor (TGF-beta-3), which stimulate cell proliferation and differentiation resulting in new tissue formation [19,20].

Materials and Methods

This is a prospective study was conducted at the dept. of Transfusion Medicine, Diabetic Foot Care & PRP Center (Diagnostic & Hospital), Sirajganj&Desh PRP Centre, Bogura, Bangladesh from January 2018 to December 2021. One hundred one (101) patients included to our study according to the following inclusion and exclusion criteria an application of PRP in the treatment of foot ulcers in patients with diabetes. Informed consent was obtained from patients, which include the use of photographs in this paper. Patients with diabetes and with a chronic foot ulcer, with average wound duration of six months, were included in the study. Patients with the following criteria were excluded: platelet dysfunction syndrome, critical thrombocytopenia, unstable haemodynamic and pregnancy.

Patients who were unable to avoid taking non-steroidal anti-inflammatory drugs (NSAIDs) for one week before and one week post-study were also excluded. Patients' were categorised into three groups, according to the size of ulcer at the start of the study:Group 1: wound size diameter of 2–5.5cm^2. Group 2: wound size diameter of 5.5–8.5cm^{2}and Group 3: wound size diameter of 8.5–12.5cm^2.Traditional dressing treatments (Alpha and Comfeel cream, mupirocin for skin infections, and nitrogen gas bandages) used by patients had failed, with a lack of reduction in wound surface area at six months. In all patients, blood sugar was under control with Insulin, in the range of 150–200mg/dl. HbA1c range was 6–7.5mg/dl. Patients avoided NSAIDs one week before treatment and for one week post-treatment, as these drugs decrease platelet activity. In this study, we defined 'complete wound closure' as being complete epithelialisation over the ulcer area.

Physical examination and treatment:

All patients would be done physical examination, Doppler ultrasound scan of the leg with DFUs, X-ray of the foot. Then, patients were classified to grades according to Classification of Texas University. The size of DFUs also recorded before treatment. Only patients satisfied with criteria would be collected 20 mL of peripheral blood to make PRP.

PRP preparation:

20 mL of peripheral blood was used to prepare PRP and PPP according to the guideline of New-PRP Pro Kit (Gene world Ltd., HCM, VN). Briefly, blood was centrifuged at 1.500 rpm in 5 min to obtain plasma. Then, this plasma was centrifuged at 3.500 rpm in 5 min to collect platelets as a pellet at the bottom of the centrifuge tube. Pellet was diluted in 3 mL plasma and was considered as PRP, and remaining plasma was considered as PPP. Both PRP and PPP were activated to release growth factor by Calcium chloride. When calcium chloride was added into PRP, fibrin gel was formed, and this gel was used to dress on the wound, and activated PPP was stored in -20C for using in the next days.

Wound treatment and monitoring:

The PRP was applied, in a single application, to the surface of the wound and covered by a protective bandage. Wounds were photographed before treatment and every week after treatment initiation, using a digital camera. The ulcer area was determined using computer software (Silhouette Connect wound assessment software).

Platelet recovery:

PRP produced by this method was 2–4ml platelet gel with an average of 1.2 million platelets per ml of platelet gel. The initial platelet level was around 100,000 cells per µl of blood. We recovered 86.9–92.5% of total platelets. In general, for clinical applications, 1ml gel is used for approximately 100–150cm2 burn wounds and for 10cm2 chronic wounds.

Statistical analysis:

The significance of the changes in time for wound healing in the three groups were statistically assessed using one-way analysis of variance, followed by Bonferroni post-hoc multiple comparison by SPSS 21.0 (SPSS Inc., Chicago, IL, US). This CI, being narrow in width, shows more precise estimates, whereas CIs from small sample sizes tend to be wide, producing less precise results.

Results

Total 101 patients completed the study were57 men and 44 women. The median age of women was 30 years and men, 35 years. No patient had more than one ulcer. Traditional treatment of the ulcer had failed, with average wound duration of six months before the start of the study. Application of PRP induced the formation of healthy granulation tissue and allowed successful closure of all wounds. There were no complications associated with treatment by PRP. Re-epithelialisation of the wound took, on average, four weeks. There was wound size reduction in patients after four weeks, following treatment with PRP (Table-1). In ulcers with a 2–5.5cm^2 surface area (Group 1) complete closure happened after 7.2 weeks, 5.5–8.5cm^2 ulcers (Group 2) completely closed after 7.5 weeks, and 8.5– 12.5cm^2 ulcers (Group 3) healed completely after 8.8 weeks (Fig-1 & 2).A 40-year-old man with wound size of 8.5–12cm^2 at starts of study (a), after four weeks (b) and complete healing at nine weeks (c). A 38-year-old man with wound size of 5.5-8.5cm^2 at starts of study (d). Wound after four weeks (e). After complete healing at seven weeks (f). Our study showed that complete closure time was significantly shorter for those patients with a wound surface area of 2–5.5cm^2 (Group 1) in comparison with those patients with a wound surface area of 8.5–12.5cm^2 (Group 3) ($p< 0.05$). Average wound area closure rate per day, in the three groups, after treatment by PRP, was 0.055cm^2 per day (Fig-3).

Table-1: Frequency of patients with different wound size in cm^2 (N=101)

Initial wound size(cm^2)	Number	Percentage
2-5.5	65	64.4
5.5-8.5	10	9.9
8.5-12.5	26	25.7

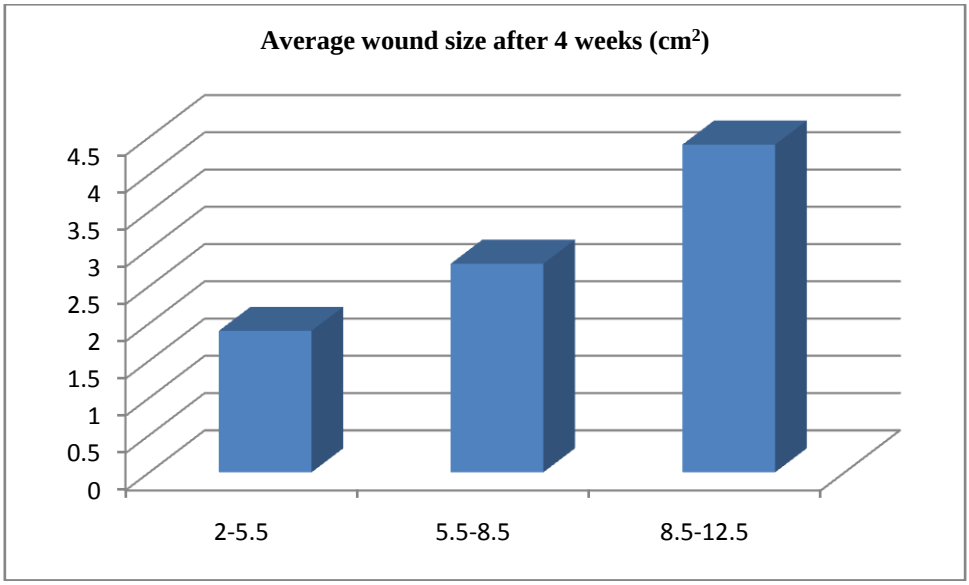

Fig-1: Reduction of wound size after four weeks treatment with platelet rich plasma (PRP).

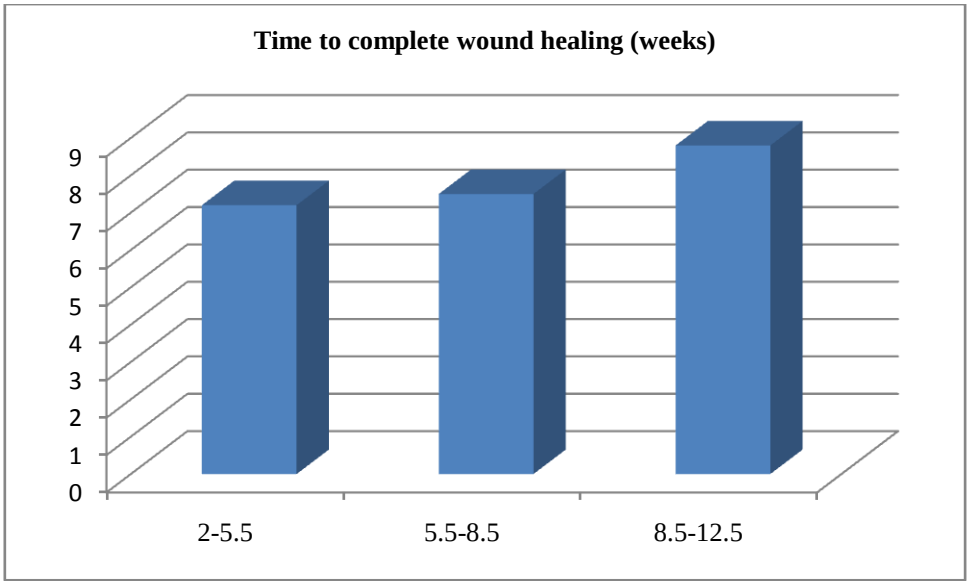

Fig-2: Complete wound healing in weeks after treatment with platelet rich plasma (PRP).

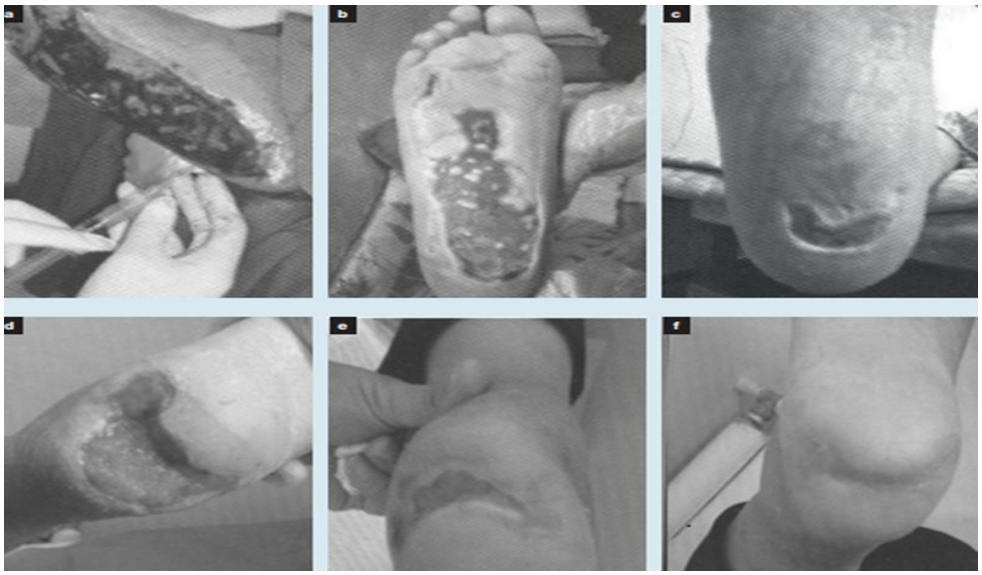

Fig-3: Representative images of wound healing by platelet-rich plasma (PRP).

DISCUSSION

Over the last decades, the use of emerging cellular therapies, such as platelet-rich plasma (PRP), has more attention in a variety of diseases and settings for its potential use in the regenerative medicine as a therapeutic agent and can have an adjunctive role in a standardized, quality treatment plan.PRP is defined as plasma containing above-baseline concentrations of platelets, which is from 140 000-400 000/µl. PRP is isolated through the centrifugation of whole blood. Simply, its actions are based on the infusion of elevated platelets, thereby theoretically enhancing the biological healing capacity and tissue generation in the wound bed. Enzyme-linked immunosorbent assay studies of PRP have quantified the presence of increases in GFs such as transforming GF β, epidermal GF, and platelet-derived GF. Through degranulation of the alpha granules in platelets, PRP can secrete various GFs, which have been documented to initiate the wound healing process.The gold standard for DFU treatment consists of debridement of the wound, management of any infection, revascularisation strategy when indicated, and off-loading of the ulcer [12]. Other methods have also been proposed as beneficial adjunct therapies, such as hyperbaric oxygen therapy (HBOT), use of advanced wound care products, and negative-pressure wound therapy (NPWT) [13].Growth factors (GFs) play an essential role in the process of wound healing and tissue regeneration. Each GF has more than one effect on the healing process and acts by binding to specific cell membrane receptors on the target cells. Growth factors effects include promoting chemotaxis, inducing cell migration and proliferation, and stimulate cells to up regulate protein production. These growth factors not only regulate cell migration and proliferation but also promote angiogenesis and remodel the extracellular matrix, creating an ideal environment that favors the cutaneous wound healing process.However, data so far have not provided enough evidence of the efficacy and cost-effectiveness of these adjunct treatment methods. PRP provides a promising alternative

to traditional methods by promoting safe and natural healing. Upon activation by CaCl [14], platelets change shape and develop branches, called pseudo-pods that spread over injured tissue. The granules contained within platelets release growth factors which stimulate the inflammatory cascade and healing [15].Platelet materials also exert antimicrobial activity against some types of skin flora bacteria [16]. Clinical data shows that the presence of infection is reduced in PRP-treated wounds [17]. However, there are few controlled trials and only case reports are available, such as McAleer et al. who found that the use of autologous PRP was successful in healing a chronic lower extremity wound, in a case study of a 57-year-old man with diabetes and a wound of six months' duration [18]. A recent review of PRP for the treatment of DFUs, explains the efficiency of PRP is still rather controversial, and shows lack of evidence [19,20]. In addition, sample sizes are frequently small, limiting the generalisation of the findings. Limitations of this study included the measurement of wound healing as the position of the DFU may vary in different patients making access to take an accurate measurement more difficult. The study was not conducted blind as patients were informed about the test at all stages of the study. There was also no negative control within the study.

CONCLUSION

A significant difference in wound healing time was observed, related to the size of the ulcer, with a quicker wound closure time for patients with the smallest ulcer size (2–5.5cm^2) compared to those in the largest group size (8.5–12.5cm^2). Our results substantiate the fact that the procedure is cost beneficial and can be easily adapted to most clinical settings for many patients, at low cost. However, for large, non-healing ulcers in patients with diabetes, it is imperative to use skin graft as the definitive procedure for wound healing.

Conflict of Interest: None.
Source of Fund: Nil.

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