RESEARCH ARTICLE

Proficiency of topical platelet-rich plasma with vacuum-assisted closure over platelet-rich plasma alone in diabetic foot ulcers - A clinical prospective comparative study

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ABSTRACT

Background: Type 2 Diabetes Mellitus is usually associated with peripheral neuropathy, peripheral vascular disease with consequential limb ischemia, and eventually diabetic foot ulcers (DFU). The healing process is slow due to microangiopathy and wound is easily infected with microbials leading to superficial infection, progressing to deep infection and eventually landing in amputation most of the times. Plate rich plasma (PRP) is very cost-effective, readily available blood derivative and has the capability to stimulate cell proliferation and differentiation. It improves tissue healing and regeneration and exhibits potent activities against a number of pathogens. Vacuum assisted closure (VAC), on the other hand, is a new novel way to treat DFU by having negative pressure wound healing. The present study focused on the advantage of (PRP+VAC) dressing over (topical PRP application with its peripheral injection) alone for aiding and enhancing the process of wound healing in DFU. Aims and Objectives: To appraise the advantage of (PRP+VAC) dressing over (topical PRP application with its peripheral injection) alone for aiding and enhancing the process of wound healing in DFU. Materials and Methods: This was a prospective comparative study of 100 cases to compare the outcomes of wound healing by (topical PRP application with its peripheral injection) alone and (PRP+VAC) dressing. Results: Mean time taken for appearance of granulation tissue, 100% granulation tissue, average reduction in wound surface area, showed significant (P => 0.005) differences between the (PRP+VAC) and the (topical PRP application with its peripheral injection) dressing groups. Conclusion: (PRP+VAC) dressings are more effective than conventional (topical PRP application with its peripheral injection) dressings in wound healing of DFU.

KEY WORDS: Type 2 Diabetes Mellitus; Plate Rich Plasma; Vacuum Assisted Closure

INTRODUCTION

Type 2 Diabetes Mellitus is usually associated with peripheral neuropathy, peripheral vascular disease with consequential limb ischemia, and eventually diabetic foot ulcers (DFU). The healing process is slow due to microangiopathy and wound is easily infected with microbials leading to superficial infection, progressing to deep infection, and eventually landing in amputation most of the times. Previously and even now the DFU are treated with antibiotics, antidiabetic drugs, peripheral vasodilators, antiplatelet aggregating drugs but the outcome of these drugs is not up to mark due to underlying diabetes with comorbidities and lack of compliance on the part of the patient and lack of knowledge about the effectual treatment for these ulcers.

Activated platelet-rich plasma (PRP) and negative pressure wound therapy (NPWT), are effectual novel ways to treat DFU, recently introduced.

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With activated PRP, the healing process of DFU is a complex mechanism involving the integrated interface between molecular signals and different cells together with platelets. These platelets in the presence of a diabetic wound get activated with thrombin and this aPRP releases growth factors like platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), epidermal growth factor, insulin-like growth factor, and transforming growth factor-beta (TGF-β). These growth factors trigger angiogenesis, extracellular matrix production, and cytokine release, and help in wound healing.[1]

Activated PRP, aPRP with 4× of normal concentration platelets (10,000,000 platelets/microlitre) is very cost-effective, readily available blood derivative, with a capacity to stimulate cell proliferation and differentiation. Cellular mitogenesis and angiogenesis are both upregulated by activated platelets.[2] Its high leukocyte concentration, autologous property helps in local debridement and antibacterial activity without an immune reaction.[3] Autologous aPRP not only enhances wound healing but also helps to regenerate skin tissue.[4] It is much advantageous in fracture wound healing, is remedy for skin defects or dental mucosal wounds.[5,6]

Proper preparation and centrifuge technique is decisive to obtaining high-quality active PRP.[7,8] Lack of biological effect may be due to poor PRP processing or inadequate standard laboratory centrifuges that cannot properly prepare PRP rather than the specialized FDA cleared equipment with validated processes.[9]

As activated aPRP is autologous, immune rejections are a non-issue.[3] It contains the same materials present in the blood that induce clotting, except in higher concentration.[10] It secretes growth factors that function by activating a cytoplasmic signal that further promotes normal gene expression.

Platelets are composed of a cytoskeleton and intracellular structures such as glycogen, lysosomes, and two granules, the dense granule and the alpha granule.

The alpha granule contains clotting factors, growth factors, proteins and works through degranulation process.[9] (PRP is collected in an anticoagulated form in a tube containing sodium citrate for the these growth factors to remain inactive).[11,12] These alpha granules bind to the transmembrane receptor of target cells such as mesenchymal stem cells, fibroblasts, endothelial cells, and epidermal cells, activating these.[13] This further activates intracellular signal proteins that express a gene sequence directing cellular proliferation, collagen synthesis, extracellular matrix formation, and numerous other pathways to promote healing and repair processes.[11]

Damaged platelets with degraded/non-viable cellular components are incapable of inducing this response.[14]

NPWT, on the other hand, is a novel way to treat DFU by creating an intermittent negative pressure across these ulcers. NPWT is effective in managing wound infections, soft tissue loss, vascular insufficiency, and traumatic wounds.[15,16] It is a non-invasive method based on well-defined, controlled negative pressure application via medical grade reticulated polyurethane ether or polyvinyl foam dressing to wound surfaces. The technique characteristically removes exudates from wounds and hence reduces extravascular, interstitial fluid; subsequently leading to enhanced microcirculation.[17] Taking away of wound fluid removes factors that suppress fibroblasts, vascular endothelial cells, and keratinocytes, all of which promote wound healing.

Experimental studies have revealed a positive influence on both local microcirculation and granulation tissue formation. Local mechanical physical factors, yet not completely understood, similar to tissue expansion, and seem to promote cell growth. These studies have revealed that cells, allowed to stretch, tend to divide and proliferate in the presence of soluble mitogens.[18]

One explanation for the high acceptance on part of the therapists and the widespread use of the vacuum assisted closure (VAC) method in these are the excellent clinical results.[19]

The present was a clinical, prospective comparative study to appraise the proficiency of topical PRP with vacuum-assisted closure over only topical PRP application in DFU.

**MATERIALS AND METHODS**

This was a clinical prospective comparative study, done on 100 patients of either sex, in their middle years of life, from the demographic profile, having type 2 diabetes mellitus and on oral hypoglycemic drugs (Biguanide/Thiazolidinediones/Alpha-glucosidase inhibitors) with DFU admitted in the Department of Orthopedics at a tertiary care hospital in Punjab, India, to compare the outcomes of wound healing with topical aPRP application, to topical aPRP combined with VAC from January 2019 to December 2020. An informed consent was taken from each participant. Thereafter ethical clearance was taken from the institutional ethical committee of the institution to conduct the study.

**Inclusion Criteria**

- Patients within the age group of 30–60 years
- Patients with a DFU of >4-week duration, not healed with conventional conservative methods of treatment.

**Exclusion Criteria**

- Patient with systemic disorder (COPD, tuberculosis) etc
- Psychological disorders, unable to maintain personal hygiene
• Patient with coagulopathies
• Advanced peripheral vascular disease secondary to type 2 diabetes mellitus
• Other confounding factors (old age, compliance, arteriosclerosis, varicose ulcers, alcoholism, and smoking).

Patients were allocated to one of the treatment arms through sampling without replacement method for their random allocation. Patients included in the study underwent routine investigations and initial debridement. Wound size (cm²) measured on day “0” (zero) with two largest perpendicular diameters and calibrated on a graph paper, depth of the wounds was not a consideration in the present series as the included patients had sloughing of epidermis and dermis only. On the same day, deep tissue swab was taken, and appropriate antibiotics initiated as per culture and sensitivity report. After achieving adequate hemostasis after debridement, a foam band dressing applied over the wounds under aseptic condition with application of intermittent negative pressure after 4 h of application of dressing; removed and replaced with a new one every 4 days. All the patients had punch biopsies taken as a part of protocol during follow-up at 3, 6, and 12 weeks and at regular intervals thereafter. This was done to confirm the appearance, presence, and progression of the granulation tissue, revascularization and to crosscheck any adverse change/malignant transformation of wound, if any. Decrease in size of wound documented at each visit until permanent healing of the wound was there.

The following steps were followed in the study.

Preparation

Autologous PRP (1,000,000 platelets per microlitre of blood) was obtained from freshly drawn blood (30 ml of venous blood) of the patient with an added anticoagulant (sodium citrate). It drawn blood experienced two centrifugation (spin) steps.[7]

The first spin, known as HARD SPIN, (more than 3000 rpm for 15 min) separated the red blood cells (RBC) from the plasma containing the platelets, white blood cells (WBC), and clotting factors. Three layers resulted from the hard spin: an upper layer containing platelets and WBC, a middle layer known as the Buffy coat containing maximum number of platelets, and a bottom layer containing RBC. The RBC layer was removed and discarded.

The second spin (also called SOFT SPIN [more than 2000 rpm for 5 min]), separated the PRP in the bottom of the tube of the platelet-poor plasma in the top of the tube by removal of more RBC. This created a bottom layer rich in platelets and leucocytes used for aPRP dressing.[8]

In (aPRP+VAC), the dressing sealed at periphery of the wound and connected it to VAC unit through a drainage tube to produce a desired pressure of 125 mm of hg, intermittently for 2 min every 5 min for half an hour, 6 times a day, after 4 h of PRP dressing to allow the maximum imbibition of topically placed aPRP over the ulcer bed. Once the vacuum was on, it sucked air out of the dressing causing its collapse and drawing the edges of the wound inwards. It also took away exudates from the wound in tandem, through evacuation tube embedded in the dressing on one side and connected to a fluid collection canister contained within the vacuum/suction machine on other side, to collect these.

Method of Application of Dressings

1. In the first group, the sterile foam dressing cut to the approximate size of the wound placed gently into position after the application of freshly prepared aPRP.

2. In the second group, as per VAC application guidelines described by Lee et al.,[12] a drainage tube was placed on top of the foam and a second piece of foam was placed over it. (For shallower wounds, a single piece of foam used and the drainage tube inserted inside it). The foam, together with first few inches of the drainage tube and the surrounding area of healthy skin, checked to ensure that the dressing formed an airtight seal both with the skin and drainage tube [Figure 3]. The distal end of the drain connected to a vacuum unit, programmed to produce the desired negative pressure of 125 mm of hg, intermittently for 2 min every 5 min for half an hour, 6 times a day, after 4 h of PRP dressing. It sucked air out of the dressing causing its collapse and drawing the edges of the wound inwards. It also took away exudates from the wound in tandem through evacuation tube connected to a fluid collection canister contained within the vacuum/suction machine.

Change of Dressing

Performed every 4 days in both the groups with wound inspection at that time. The resident doctor conducted manual measurement of size and granulated area in cm². Findings calibrated on a graph paper.

Parameters for Evaluation

Patients were evaluated clinically for appearance of granulation tissue, 100% appearance of granulation tissue, full coverage of the ulcer, reduction in wound surface area, and duration of hospital stay.

Statistical Analysis

The results of observations of individual patients were pooled for each intervention group. Data analysis was performed using SPSS version 20 (SPSS Inc., Chicago, Illinois, USA). Numerical data expressed as mean, ± standard deviation (SD) or percent as proportionate to the sample size. The significance of difference between two groups was determined using “P” value.

A “P < 0.05” considered significant.
Period of Follow Up

The follow-up done at 3, 6 and 12 weeks [Figures 1 and 2] and thereafter at regular intervals until definitive wound coverage was there.

RESULTS

All the patients were having type 2 diabetes mellitus (80% were on oral hypoglycemic agents and rest 20% were on insulin therapy) with an ulcer of ≥4 weeks duration ulcer on sole/dorsum of the foot. The size of the wound ranged from 5.1 to 8.7 cm (mean of 7.6 ± 0.8 cm). Foot angiopathy (assessed by color Doppler) was the most associated comorbidity. The mean time taken for the appearance of granulation tissue, 100% granulation tissue, permanent wound coverage, reduction in wound surface area, and hospital stay, was lesser in the (aPRP+VAC) dressing group, with a significant \( P \) value \( (P < 0.005) \), than in the (topical aPRP application) dressing group. Successive punch biopsies at regular follow-up visits showed no adverse cellular change/malignant change in the present series. Complications such as infection, presence of exudates, and persistent pain were there in both modalities of treatment but were less in the (aPRP+VAC) dressing group. Only 86% of the patients needed the split skin grafting in (aPRP+VAC) dressing group when compared to topical aPRP application dressing group [Table 1 and Figures 1-3].

DISCUSSION

Despite many advances in the treatment of DFU, patients are yet living with amputation as a destructive complication.\(^{[20]}\) Topical application of activated PRP and NPWT are two familiar modalities in the management of chronic DFU. Although the former could significantly increase the rate of healing of DFUs, latter leads to a faster granulation tissue formation and reduction in wound size in comparison to the former.\(^{[21,22]}\)

Autologous aPRP is economical and affordable as it is prepared with a small volume (7 cc of aPRP prepared from 30 cc of freshly drawn venous blood) of patient’s blood and the risk of transmission of blood-borne diseases or immunological reaction is not an apprehension.

Autologous aPRP provides the growth factor needed for natural healing process in diabetic patients.\(^{[23]}\) Seven fundamental protein growth factors, actively secreted by platelet initiate wound healing process. aPRP also has three proteins known to act as cell adhesion molecules, that is., fibrin, fibronectin, and vitronectin.\(^{[7]}\) Platelets also secrete TGF-β and Monocyte Chemo attractant Protein-1 that would attract monocytes and neutrophils to the wound site.

PRP can facilitate healing of DFUs and therefore can reduce the risk of amputation\(^{[24]}\) but more so when it is used in alliance with NPWT. In the present study, the granulation tissue appeared on the 9th day after application of aPRP alone, 100% granulation tissue appeared on the 23rd day with 1.6% decrease in wound size and total wound coverage on the 25th day as compared to its combination with VAC dressing where the granulation tissue appeared on 4th day, 100% granulation tissue appeared on 13th day with total wound coverage in 15 days decreasing the hospital stay by 14 days and more so there was considerable decrease in size of the wound by 3.9%.

The present study is in accordance with previous studies in support of “PRP” dressing as documented in previous studies also. Driver et al.\(^{[21]}\) conducted a study on 72 patients type 2 diabetes, aged between 18 and 95 years suffering from an ulcer of at least 4 weeks duration and compared the effectiveness of autologous aPRP gel to that of normal saline gel for 12 weeks. The primary objective of the study was to
evaluate the safety of aPRP and 100% re-epithelialization when compared to the control treatment, and a secondary objective was rate of re-epithelialization. Patients were randomized into two groups: standard of care with aPRP gel or control (saline gel) and were evaluated biweekly for 12 weeks. They found that 68.4% (13/19) of patients in the aPRP group and 42.9% (9/21) in the control group had wounds that healed. Wounds in the aPRP group healed after a mean of 42.9 days (SD 18.3) versus 47.4 days (SD 22.0) in the control group. They thus confirmed the positive effect of autologous aPRP gel in the treatment of DFUs. The present study confirms their findings. Lacci and Dardik\[25\] used aPRP as an adjuvant therapy and showed the effectiveness of it on healing of chronic wounds. The most important steps in their study were debridement, offloading and frequent dressing changes, procedures that transformed a chronic wound into an acute wound. They eliminated other factors that impeded healing like pressure, friction, and shear. After preparing the wound bed, aPRP applied as a gel or injected in the wound. The healing time in their study was shorter as compared to saline dressings. The present study confirms their findings. Mehrannia et al\[26\] reported a single case of non-healing DFU, successfully treated by injection of aPRP inside and around the peripheral skin. The present study confirms their findings. Tran et al\[27\] conducted a study aimed to evaluate the effects of activated PRP on DFU healing on 6 patients. Activated PRP was isolated from peripheral blood and activated with calcium chloride. Patients injected with activated PRP 2 times with 14-day interval. All patients monitored during 12 weeks. The results showed that 100% (6/6) ulcers completely closed after about 7 weeks. This result initially suggested that aPRP injection was efficient method to treat the non-healing foot ulcers. The present study confirms their findings. Sutharet et al\[28\] demonstrated the potential safety and efficacy of autologous activated PRP for the treatment of chronic non-healing ulcers. The mean age of the treated patients was 62.5 ± 13.53 years and they were followed-up for a period of 24 weeks. All the patients showed reduction in wound size, and the mean time duration to ulcer healing was 8.2 weeks. The present study confirms their findings. Horn et al\[29\] reported that aPRP gel could effectively decrease the width and depth of chronic wounds. The present study confirms their findings. Hence, most of the researchers stated above used PRP effectively in non-healing DFU. Several of the researchers used it effectively in dental and oral surgeries\[30\] and also in the treatment of musculoskeletal injuries\[31\] and the present study confirms their findings.

There are previous studies supporting the usage of “VAC” also, Lone et al\[32\] conducted a prospective casecontrol study on the usage of Vacuumassisted closure (VAC) versus conventional dressings in the management of DFU. He reported that granulation tissue appeared in 26 (92.85%)
patients by the end of week 2 in group A (vacuum-assisted closure), while it appeared in 15 (53.57%) patients by that time in-group B (conventional dressing). They achieved 100% granulation in 21 (77.78%) patients by the end of week 5 in Group A compared to only 10 (40%) patients in Group B. Patients in Group A had fewer number of positive blood cultures, secondary amputations. They concluded that VAC was more effective, safe, and patient satisfactory compared to conventional dressings for the treatment of DFUs. The present study affirms that. Lehman[33] combined the benefits of Collagen and NPWT to heal a chronic DFU in a case and suggested combining the advanced wound healing properties of collagen with NPWT has a beneficial effect on wound healing. Swaminathan et al.[34] conducted a study on 30 patients with DFU to evaluate the efficacy of vacuum-assisted wound closure VAC therapy for the treatment of DFU. They showed that mean initial wound surface area before VAC therapy was 103.07 cms and after VAC therapy there was a significant reduction in wound size to 94.53 cms over a mean duration of 31.9 days. That significantly increased the wound bed granulation tissue and good percentage of graft and flap take up. The daily requirement of antibiotic and analgesic was reduced. Duration of hospital stay was reduced due to faster wound healing. There was overall reduction in pain and further complications like amputations were avoided thereby increasing the patients compliance. They Concluded that VAC therapy is newer and a safe method of treatment for DFU. In present study, we combined both these methods to appraise the synergistic effect of these if any since Ahmed et al.[35] documented that PRP and NPWT were effective in neovascularization and stimulation of healing process but the latter was more effective. They showed expression of VEGF in PRP patients after the 3 weeks was mild 10%, moderate 20%, strong 70% and was mild 10%, moderate 20%, strong 70% in peripheral and central tissue biopsy respectively. While in NPWT patient was mild 20%, moderate 30%, strong 50% in both peripheral and central tissue biopsy. The average of blood vessels formation in CD-31 was 9.95 ± 3.64 after 3 weeks in peripheral tissue biopsy and was 8.58 ± 3.51 in central tissue biopsy in PRP patients. While in NPWT patients was 8.35 ± 3.25 in peripheral tissue biopsy and was 8.38 ± 3.12 in central tissue biopsy. These facts got confirmed in the present study. Leon[36] reported synergism in using NPWT with alternated applications of autologous PDGFs. He concluded that there was a significant change in wound volume (20–50%), 2–6 weeks after beginning one or both treatment modalities. They further demonstrated that using NPWT and PRP treatments alternately resulted in successful wound healing. The present study focused to have a synergistic effect of both methods proved that mean time taken for the appearance of granulation tissue, 100% granulation tissue and average reduction in wound surface area, showed significant (P <= 0.005) differences between two above stated dressing groups. This study showed that (aPRP+VAC) dressing, which was an attempt to amalgamate the advantages of both methods, is a superior alternative for achieving a better healing, in shorter duration, with lesser complications, thus establishing it, a better method of management of DFU.

**CONCLUSION**

We conclude that (aPRP+VAC) dressings are superior to topical aPRP application dressings in wound healing of DFU. At the same time, lack of control over some confounding factors such as patients’ nutrition, activities, and their level of adherence to their medical treatments are some of the limitations of the present study. Moreover, a very small sample is another limitation of the study. Therefore, replication of study with larger sample sizes is recommended.

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