Original Research Article

A comparative study between platelet rich plasma and conventional dressing in chronic non healing leg ulcers

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ABSTRACT

Background: Chronic non-healing leg ulcers are a major health problem worldwide and have great impact on personal, professional and social levels, with high cost in terms of human and material resources. The present study was conducted with an aim to demonstrate the efficacy of autologous platelet rich plasma (PRP) in chronic non-healing leg ulcers in comparison to conventional dressings.
Methods: A total of 50 patients with leg ulcers were randomized into two groups (A and B) with each group comprising of 25 patients each. Group A were treated with autologous platelet rich plasma (PRP) dressings and group B were treated with conventional dressings using normal saline. Ulcer measurements were taken on day 1, day 7, days 15 and after 30 days. The end point of study was complete wound epithelialization or appearance of granulation tissue, which ultimately lead to spilt skin grafting or secondary healing; whichever is earlier.
Results: There was statistically significant difference in the average time taken for complete healing of ulcer in PRP dressings, 3.68 weeks against 6.2 weeks in conventional dressing group (p value <0.0001). PRP dressing group showed a 43.96% reduction in ulcer size as compared to 13.81% in conventional dressing group (p <0.0001). It was observed that PRP dressing group has faster wound healing and contraction of wound.
Conclusions: PRP dressing of leg ulcers was better than conventional normal saline dressing as it leads to early reduction of ulcer size and enhances rate of wound healing.

Keywords: Chronic non healing leg ulcer, Platelet rich plasma dressing, Platelet derived growth factor

INTRODUCTION

Chronic nonhealing leg ulcer is defined as the "loss of skin and subcutaneous tissue on the leg or foot, which takes more than 6 weeks to heal. Chronic ulceration of the lower leg, including the foot, is a frequent condition, causing pain, social discomfort, and generating considerable costs.[1] The prevalence of leg ulcers is well documented to be vary between 0.18% and 1%.[2] The major causes of lower extremity ulcers are diabetic, venous, arterial, and neuropathic.

Chronic wounds are characterized by a long inflammatory phase that hinders the regenerative wound healing. Chronic wounds, especially in patients with diabetes mellitus (DM), are a major health challenge. The goal of wound care in chronic ulcers is to facilitate healing and prevent lower extremity amputations using Standardized protocols of wound care. The standard treatment algorithm includes a complete patient and wound assessment, history, physical examination, and a variety of diagnostic tests that determine the need for infection control intervention, revascularization, excision and debridement, skin graft/flap, wound protection, and

education. Apart from these conventional methods to facilitate wound healing various new methods are emerging such as cellular therapies which include platelet-rich plasma (PRP). This can have an adjunctive role in a standardized, quality treatment plan. The main aim of this study was to evaluate the safety and efficacy of autologous PRP in treating chronic non healing leg ulcer when compared to conventional dressing. The therapeutic effect of PRP is attributed to the abundance of various growth factors such as platelet derived growth factor, transforming growth factor-β, fibroblast growth factor, insulin-like growth factor-1, insulin-like growth factor-2, vascular endothelial growth factor, epidermal growth factor, and also some cytokines primarily stored in alpha granules.[4,5]

Currently, there is a paucity of critical scientific data regarding the beneficial effects of PRP in clinical procedures. In the current study, PRP was found to be useful in treating chronic leg ulcers. However, further controlled, randomized prospective clinical trials are necessary to definitively demonstrate its efficacy. There is also a need for the development of a standard protocol for the preparation of PRP, as literature currently there is no standardization of the procedure.

METHODS

This is a prospective randomized controlled study, to test the efficacy of autologous platelet rich plasma in epithelialization and wound reduction in chronic wounds. The study was conducted in the department of surgery, Mamata Medical College, Khammam from October 2016 to September 2018. Study was approved by the Institutional ethics committee. A series of 50 cases was compiled for this study during this period, the source of data were patients attending the outpatient on a regular basis or those admitted as inpatients for the management of chronic wounds. 50 patients were studied. 25 cases were randomly chosen for study with autologous PRP and 25 cases received conventional dressing for the chronic wounds.

Inclusion criteria

Age group of 18 to 80 years with long standing non healing leg ulcers (pressure sores, diabetic ulcers, venous ulcers, other non-specific ulcers), non-diabetic patients and patients with type 1 or type 2 diabetes mellitus, ulcer ≥4 weeks duration, ulcer ≤15 cm^2 in size, Hb ≥10 gm%, patients willing to take part in the study.

Exclusion criteria

Screening platelet count <1 lac/mm^3, patients with known or suspected osteomyelitis, patients with immuno-deficiency, patients with serum creatinine above 1.5 mg/dl, severe infection (presence of visible pus or copious wound exudates), presence of cellulitis,

inadequate perfusion, ischemia, gangrene, Patients not willing to take part in the study.

Statistical analysis

Paired T test for quantitative data and Pearson chi square test for qualitative data were used to evaluate the p value. Differences were considered statistically significant, if p <0.05. IBM SPSS Statistics for Windows, version 24 (IBM Corp., Armonk, N.Y., USA) software program was used for statistical calculations.

Preparation of the material

Under aseptic precautions 20 ml of venous blood was drawn and added to a test tube containing acid citrate dextrose in a ratio of 9:1 (blood: acid citrate dextrose), In the first spin the test tube is centrifuged at 5000 rpm for 15 min to separate the red blood cells from the platelets and plasma. After the first spin, 3 layers appeared. This is due to differences in the density of the blood components: the deep layer consists of red blood cells, the middle layer contains platelets and leukocytes, and the top layer is made up of platelet-poor plasma (Figure 1). The middle layer and top layer were collected directly by gentle aspiration with a pipette and transferred to a new, sterile centrifuge tube. And it centrifuged again at 2000 rpm for 5-10 min.[6] Studies have shown that these frequent but small blood draws do not have an effect on haemoglobin, hematocrit, or platelet count.
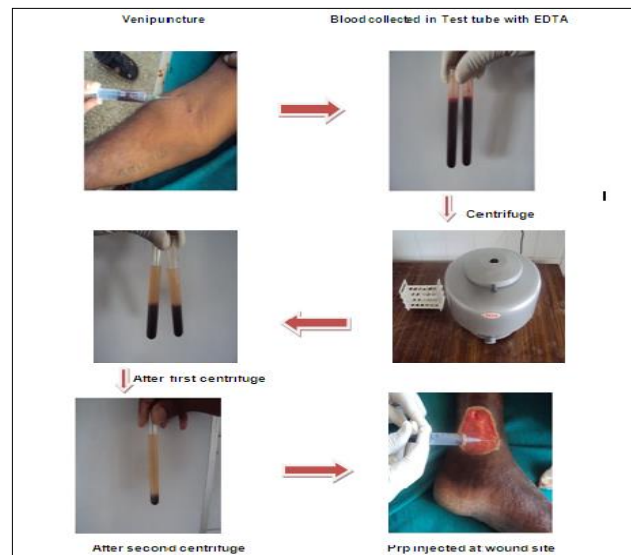

Figure 1: Preparation of material.

Dressing technique

For conventional dressing

The ulcer was cleaned with normal saline and saline soaked gauze piece was kept over the ulcer which was covered with pad and roller bandage.

For platelet rich plasma dressing

The ulcer was cleaned with normal saline. Platelet rich plasma was prepared from patient's blood and injected into the surrounding wound area twice weekly. The end point of study was complete wound epithelialization or appearance of granulation tissue, which ultimately lead to spilt skin grafting or secondary healing; whichever is earlier. Rate of wound healing was calculated as the difference between the primary wound on the day 1 and at end of 7 days, 15 days and 30 days. Ulcer measurements were taken using ruler (in cm) as two largest perpendicular diameters. The ulcer area was measured in cm^2 by multiplying these two diameters.

RESULTS

The present study was conducted in Mamata Medical College, Khammam. During the study from October 2016 to September 2018, 50 patients with chronic ulcers were randomized into study (PRP) and control (conventional dressing) group. These groups were studied for the effect of conventional dressing versus PRP on epithelialization and wound reduction.

Age distribution

In this study, the age of the patients ranged from 20 years to 80 years and the maximum numbers of cases were in age group between 51 to 65 years (42%) Mean age of subjects in present study in study group (PRP group) was 60.56 years±15.446 and in control group (conventional dressing group) was 47.80±13.85years with a p-value of <0.0035. This is statistically significant. The details of age group are depicted in (Table 1).

Table 1: Age distribution.

Age (yrs)	Study group (n=25)		Control group (n=25)	
	N	%	N	%
20-35	1	4	6	24
36-50	6	24	7	28
51-65	9	36	12	48
66-80	8	32	0	0
>80	1	4	0	0
Total	25	100	25	100
Mean age±SD	60.56±15.446		47.80±13.85	

Table 2: Aetiology of ulcer.

Aetiology of ulcer	Study group (n=25)	%	Control group (n=25)	%
Diabetic	13	52	12	48
Traumatic	5	20	8	32
Venous	3	12	3	12
Trophic	3	12	2	8
Arterial	1	4	0	0
Total	25	100	25	100

Aetiology of ulcer

In this study, 50% (25 cases) of the wounds were of diabetic ulcers. The next most common wounds were traumatic 13 (26%), venous 6 (12%), trophic 5 (10%) arterial 1 (2%). The details of aetiology of ulcer were depicted in (Table 2).

In the present study, the contraction area in study group was 580.68 mm^2±63.55 mm^2, whereas in control group, it was 209.85 mm^2±143.23 mm^2 with a p value was <0.0001, which was statistically significant. There was a statistically significant difference between the area before the treatment and after the treatment among the cases (p<0.001). The mean ulcer size at day 1, day 7, day 15 and day 30 is shown in (Figure 2). Size of ulcer was comparable though statistically not significant. The percentage reduction in mean ulcer size in study group was 43.96 % compared to 13.81% in control group at the end point of the study, with a p value <0.0001 which was statistically significant.
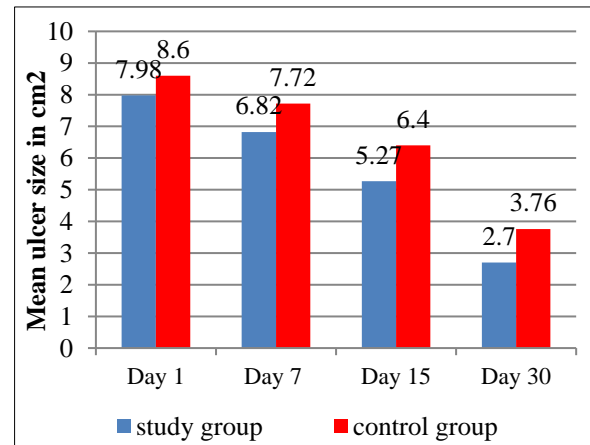

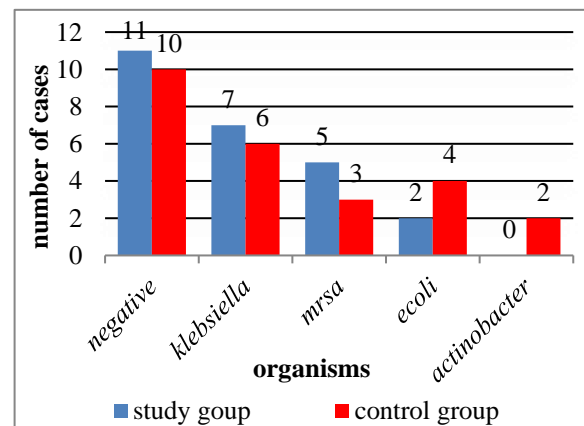**Figure 2: Surgical management.**

Figure 3: Organisms isolated during the study.

Bacteriological profile

Figure 3 shows bacteriological profile of ulcer. In the present study, bacteriological results prior to treatment

with PRP at the wounds are mostly sterile wounds followed by *Klebsiella* and MRSA.

Commonest site for metastasis was regional lymph node. 8 patients had secondary deposits in liver, 2 were having deposit in anterior abdominal wall and two females were having secondary deposits in both ovaries.

The mean time taken for complete healing of the ulcers were 3.68±0.47 weeks in study group as compared to 6.2±0.5 weeks in the control group.

DISCUSSION

Chronic non-healing leg ulcers are a major health problem worldwide and have great impact at personal, professional and social levels, with high cost in terms of human and material resources. Recalcitrant non-healing ulcers are inevitable and detrimental to the lower limb and are a major cause of non-traumatic lower limb amputations. These ulcers pose a therapeutic challenge, particularly in developing countries with resource constraints. Response to conventional therapies such as dressings, surgical debridement and skin grafting may be unsatisfactory.

The use of intra-lesional injection of PRP for treatment chronic wounds was first described by Dionyssiou et al.[7] In their study the technique was tried experimentally on rabbits and showed excellent results, then it was applied clinically in 26 patients who have non-healed chronic wounds. None of these patients had diabetic ulcers (most of the ulcers were traumatic or post-operative) the results of our study are in accordance with study done by Dionyssiou et al and we were able to achieve similar outcome.

Mechanism of action of platelet-rich plasma

PRP functions as a tissue sealant and drug delivery system, and platelets initiate wound repair by releasing locally acting growth factors via α-granules degranulation.[8-10]

α-granules of platelets contains

Platelet-derived growth factor (PDGF-AA, BB, and AB isomers), transforming growth factor-β (TGF-β), platelet factor 4 (PF4), interleukin-1 (IL-1), platelet-derived angiogenesis factor (PDAF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), platelet-derived endothelial growth factor (PDEGF), epithelial cell growth factor (ECGF), insulin-like growth factor (IGF), osteocalcin (Oc), osteonectin (On), fibrinogen (Ff), vitronectin (Vn), fibronectin (Fn), and thrombospondin-1 (TSP-1).

These growth factors help in healing by attracting un-differentiated cells in the newly formed matrix and triggering cell division. PRP may suppress cytokine release and limit inflammation, interacting with macrophages to improve tissue healing and regeneration Promote new capillary growth. And accelerate epithelialization in chronic wounds.[11]

The recent Cochrane review lists 9 eligible RCTS comparing autologous platelet rich plasma with placebo or alternative treatments for chronic wounds in adults, results of our study compared to other studies was depicted in (Table 3).

In a study done by Suryanarayan et al on chronic non healing leg ulcers, in 24 patients with 33 ulcers were treated with PRP and the mean duration of healing of the ulcers was 5.6 weeks (SD 3.23).[6] They also noticed a decrease in pain where as in the present study mean duration of healing of the ulcers 3.68±0.47 weeks. In a study conducted by Frykberg et al, on 49 patients with 65 non-healing leg ulcers showed that 63 (96%) of 65 ulcers responded with a reduction in area, volume and undermining of the ulcers in a mean duration of 2.8 weeks.[12] This is comparable with the present study. study done by Kakudo et al, in five cases of intractable skin ulcer with autologous PRP, among which three ulcers healed completely within 4 weeks and epithelialization of wound occurred within 6.6 weeks on average.[13]

Table 3: Comparisons with 9 other RCTS.

Study	No of patients	Type of wounds	Duration of treatment	Demonstrated efficacy of PRP
Weed 2004	26	Mixed wounds	24 weeks	No
Driver 2006	72	Diabetic foot ulcers	24 weeks	Yes
Kakagia 2007	51	Diabetic foot ulcers	8 weeks	Yes
Planinsek 2007	10	Venous ulcers	-	Yes
Anitua 2008	15	Mixed wounds	8 weeks	Yes
Present study	50	Mixed wounds	7 weeks	Yes

Sachidanand et al demonstrated that mean percentage of reduction in volume and area of chronic ulcers was 95% respectively with a mean PRP treatment duration of 5.1 weeks. Whereas in the present study it was around 43.96

% in 3.68±0.47 weeks. According to study done by Driver et al, in diabetic foot ulcers in 72 patients they carried out the first prospective, randomized, controlled multicenter trial in the United States.[14] Patients were

randomized into two groups; standard of care with PRP or control (saline dressing) and were evaluated biweekly for 12 weeks. the authors found that 68.4 percent (13/19) of patients in the PRP group and 42.9 percent (9/21) in the control group had wounds that healed. Wounds in the PRP group healed after a mean of 42.9 days (SD 18.3) vs. 47.4 days (SD 22.0) in the control group.

In the present study, bacteriological results prior to treatment with PRP at the wounds are mostly sterile wounds followed by klebsiella and MRSA. Sterile wounds was found in 11 (44%) cases in PRP group and 10 (40%) cases in control group, klebsiella was found in 7 (28%) cases in PRP group and 6 (24%) cases in control group. MRSA was found in 5 (20%) cases in PRP group and 3 (12%) cases in control group. Findings of our study are comparable to study done by Bielecki et al.[21]

Comparison of % reduction in mean ulcer size with other studies

In the present study, the % reduction in mean wound area in study group was 43.96% compared to 13.8% in control group, with a p value <0.01 which is statistically significant. In a study done by Kumar, the mean difference in decrease in wound size was 46.95%±15.16% in experimental group, whereas in control group, it was 2.28%±2.54%.[15]
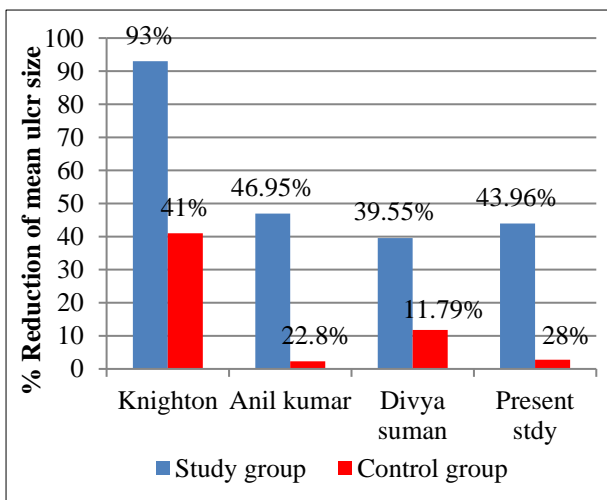
Figure 4: Comparison of % reduction in mean ulcer size.

Knighton, in his study the mean difference in decrease in wound size was 93%±17% in experimental group, whereas in control group, it was 41%±39%.[16] The mean difference in decrease in wound size in a study done by Anitua was 72.94%±22.25% in experimental group, whereas in control group, it was 21.48%±33.56%.[17] In a study done by Suman, the mean difference in decrease in wound size was 39.55±2.52 in experimental group, whereas in control group, it was 11.79%±2.35.[18] The detailed comparative figures are depicted in (Figure 4).

Figure 5: Comparative image showing before and after application of PRP (A) before PRP application, (B) after PRP application.

Comparison of time taken for complete healing with other studies

The mean time taken for complete healing of the ulcers was 3.68±0.47 weeks in study group as compared to 6.02±0.5 weeks in the control group. In a similar study done by Yousof et al, the mean time taken for complete healing of ulcers was 4 weeks in study group when compared to control group it was 12 weeks.[19] The mean time taken for complete healing of ulcers in the study done by Hardikar was 10 weeks in study group when compared to control group it was 20 weeks.[20]

Table 4: Comparison of time taken for ulcer healing.

Study	Study group	Control group
Yousef et al[19]	4 weeks	12 weeks
Hardikar et al[19]	10 weeks	20 weeks
Present study	3.68 weeks	6.02 weeks

Comparison of contraction area with other studies

In the present study, the contraction area in study group is 580.68 mm^2±63.55 mm^2 compared to control group is 209.85mm^2±143.23 mm^2 where as in the study done by Anil Kumar the contraction area in study group is 237.67 mm^2 compared to the control group is 17.04 mm^2 as depicted in (Table 5).[15]

Table 5: Comparison of contraction area.

Study	Study group	Control group
Present study	580.68	209.85
Kumar et al[15]	237.67	17.04

The major limitation of our study was the small number of cases, single institutional study, and short follow-up period. A longer follow-up period is required to assess whether the wound healing progresses at a similar rate. Other parameters like the number of debridements required in each week, quality of life, and cost of the total treatment were not studied.

CONCLUSION

In conclusion, the results from our case series showed that PRP is a safe and effective treatment modality for chronic non-healing leg ulcers. Using PRP to treat chronic wounds/ulcers may not only enhance healing, but also prevent lower extremity amputations caused by nonhealing wounds. Therefore, further research and controlled, randomized prospective clinical trials on larger patient population are necessary to validate the results.

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