

Original Research Article

Potential therapeutic effects of autologous platelet rich plasma on impaired wound healing: a prospective clinical study

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ABSTRACT

Background: The evolving state of technology in field of medicine has led to a plethora of new options in the realm of wound healing. The primary objective of present study was to assess the potential therapeutic effects of autologous platelet rich plasma in the healing of wound.

Methods: A prospective clinical nonrandomized cohort study was performed in 50 patients (40 males, 10 females) with recalcitrant, chronic; non-healing wounds of different etiologies. Age ranged from 25 to 70 years (mean 39.14 ± 13.38). Eligible patients were treated with autologous platelet rich plasma (PRP) at 4-days intervals for a maximum of 14 sessions. Patients were evaluated (reduction in size of the wound (area)) every week and continued until final healing has achieved. The primary outcome was positive response to therapy, defined as complete healing of the wound and absence of any raw area. Significance was set at $p < 0.05$.

Results: The mean time duration to wound healing was 6.33 weeks (S.D ± 2.16). A statistically significant difference ($p < 0.05$) was observed between the baseline parameters and mean area after PRP injection. After 7 sessions (28 days of therapy) the wounds showed the significant change in wound surface area ($p < 0.05$). None of the patients developed deep or superficial wound infections.

Conclusions: There is considerable improvement in wound characteristics like healthy granulation tissue, healing edge, pain, slough, bleeding on touch and discharge after 7 days of PRP therapy and considerable improvement on follow up.

Keywords: Autologous platelet rich plasma, Wound healing, Non-healing wound

INTRODUCTION

Wound healing is an intricate multifactorial and dynamic process that involves the coordination of a sequence of many biological events; varying with changing wellbeing of the individual.¹ Various local contributing factors as well as systemic and psychosomatic factors considerably influence the extent and effectiveness of the healing process.²⁻⁵ Abnormalities in any of these domains may lead to impaired healing of wounds resulting in chronic non healing ulcers owing to deficiency of growth factors

and cytokines.⁶ Impaired wound healing cause's significant socio-economic impact to the healthcare sector and society at large. There is a plethora of wound care techniques advocated for impaired healing.⁶⁻¹⁰ However, none of these are universally reliable, and impaired healing or non-healing wounds remains a challenging problem in current practice.⁶ Currently, there is a focus on cellular therapy for the treatment of non-healing ulcer.^{11,12} The introduction of autologous PRP has been one of the major breakthroughs in the management of non healing wounds owing to its property of releasing growth factors and chemokines that augments the healing

cascade.¹³ The primary objective of present study was to assess the potential therapeutic effects of autologous platelet rich plasma (PRP) in the healing of wound.

METHODS

The study was designed as a single centre prospective clinical research. The present research was approved by institutional review board, and informed consent was obtained from each subject. The current study recruited patients with chronic or non-healing ulcers of various etiologies (Lacerated wounds associated with open fractures, amputation wounds, diabetic foot ulcer, venous ulcer and bed sores.) reporting to the Department of Orthopaedic, Traumatology and rehabilitation NSCB Medical College Jabalpur MP India from April 2016 to March 2017. A medical and demographic history was taken, and patients were thoroughly examined (Table 1). Routine pre-procedure laboratory investigations data were obtained. Inclusion and exclusion criteria were defined. To be included, all participants aged 25-70 years of either sex had to chronic ulcers or non-healing wounds; subject must understand the risk and benefit of the protocol and be able to give informed consent; availability for the duration of entire study period.^{8,14} Exclusion criteria included hematological disorders or any history of coagulopathies; medically unfit patient, hypersensitivity to NSAIDs; skin disorders; severe infection; uncontrolled sugar levels; pregnant, breast feeding or planning to become pregnant. Among ninety subjects, 50 were satisfied the inclusion/eligibility criteria. Eligible patients were treated with autologous platelet rich plasma (PRP) injection at 4-days intervals for a maximum of 14 sessions.

Device description

The present study utilized a REMI centrifuge C-854/6 System; a dedicated Mini Centrifuge system (Capacity: 6x15; Type of Head: Swing Out; Max. Speed: 3500 rpm; Max. RCF: 1600g; W x D x M (mm): 310 x 310 x 295; Supply: 220-240 Volts 50 Hz Single Phase).



Figure 1: Following density gradient centrifugation, the blood sample is separated in different blood fractions (from bottom to top of tube) red blood cells; buffy coat; plasma rich in platelet.



Figure 2: PRP layer is collected and is ready for infiltration into the subcutaneous margins.

Platelet rich plasma (PRP) was prepared using density gradient centrifugation. A dual-spin preparation method was used. The preparation (PRP) can be performed in the operating theater during the actual procedure and takes about 25 minutes. Under aseptic precautions 20 ml blood was withdrawn from antecubital vein in sterile EDTA-coated disposable test tube. Blood was properly mixed before transferring to the processing device, to avoid formation of fibrin clots. For the first spin the sterile disposable test tube was, centrifuged at 22-24 degree room temperature at 5000 rpm/min for 15 minutes in a REMI centrifuge C-854/ 6 System (Medico/ Doctor Centrifuge). The blood sample after first spin was separated into different blood fractions (from bottom to top of tube): lowest or red cell and granulocytes; middle or whitish opaque layer of buffy coat which contains osteoprogenitor cells, mononuclear cells and some platelets and the top one is yellowish transparent layer and contains plasma and platelets. This top layer is divided in two zones; upper platelet poor plasma (PPP) and lower platelet rich plasma (PRP) (Figure 1). Following first centrifugation, entire plasma portion above the buffy coat was transferred to an empty tube and was subjected for second spin at 2000 rpm for 5 min.^{15,16} The second spin produces two major components: platelet poor plasma (PPP) as supernatant and platelet rich plasma (PRP: fall onto the bottom). The PPP layer was discarded with the help of a long bore sterile micropipette and around 2 ml of PRP was collected. Just before infiltrating into the wound, a few drops of 10% Calcium Chloride were added to PRP. The surgical procedure included: debridement of soft tissue at the wound site and infiltration of activated PRP into the wound in subcutaneous margins and a non-absorbent dressing (Figure 2). The procedure was repeated every fourth day. After every 4 days, the dressing was removed with normal saline and assessed for improvement. The healing response was critically assessed every week. The amount of blood needed for this protocol decreases each session as wound showed the improved healing response. Formula for the area of an ellipse (Length×width×0.7854) was used to calculate the wound area (Figure 3).^{17,18} The outcome will be defined as a percentage difference in area calculated as difference between the measurements (original measurement and assessment day measurement) divided by the original measurement.

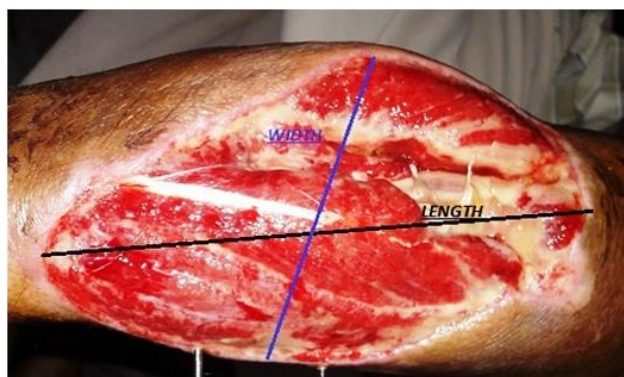


Figure 3: The use of an ellipse for calculating wound measurement.

RESULTS

Among the included patients 40 (80%) were males and 10 (20%) were females with a mean age of 39.14±13.38 years (Table 1). Ten patients (20%) were presented with diabetic ulcer, three patients (06%) with venous ulcer, five patients (10%) with bed sores, twelve patients (24%) with open stump wounds after amputation and twenty patients (40%) with lacerated wound (Table 2). A total of 4 (8%) patients were lost to follow up, 75% of which occurred within first two weeks. A complete Follow-up data were available for 46 (92%) patients. Wound area was significantly reduced at follow-up compared with baseline parameters (p<0.05) (reduction from 49.98cm² ±83.29 to 2.48 cm² ±S.D.-8.71) (Table 3). Wound/ulcer healing (reduction in wound size, appearance of granulations Tissue) was observed as early as 28 days (Four weeks) of therapy or after seventh session of PRP therapy. The mean healing time was found to be 6.33 ±2.16 weeks. Wound showed significant improvement in mean percentage of reduction in area from 7th day of therapy to 56th day of therapy (from 19.75±15.76 to 96.81±9.96) (p<.05) (Table 4) (Figures 4-6). Smaller wound area healed significantly faster than

larger wounds (p<0.05) (Table 5). Complete healing was seen in 4(8.69%) wound at 21st day, 36 (78.2%) wounds at 56th day. Smoking delayed healing. Six (13.04%; smokers) out of forty six patients wound still require PRP injections. There were no signs of infection or any adverse reactions observed during the therapy (Table 5).

Table 1: Demographic variables.

	Frequency	Percentage (%)
Age (years)		
Mean age 39.14±13.38 years		
<20	4	8
20-29	10	20
30-39	10	20
40-49	14	28
50-59	8	16
Sex		
Male	40	80
Female	10	20
Co-morbidity		
Diabetes mellitus	10	20
Hypertension	30	60
Smoking	6	12

Table 2: Etiology (Table showing case distribution according to underlying causes or causation).

Type of wound	Frequency	Percentage (%)
Diabetic ulcer	10	20
Venous ulcer	3	6
Bed sore	5	10
Wounds following amputation (traumatic and elective)	12	24
Lacerated wound	20	40
Total	50	100

Table 3: Wound areas on follow up days (wound area was significantly reduced at follow-up compared with baseline parameters).

Day	Mean (cm ²)	Std. dev.	Min.	Max.	t test	P value
Starting day	49.98	83.29	2.35	451.60		
7th	42.45	75.50	0.78	431.97	4.73	<0.0001
14th	31.85	63.57	0	379.34	5.16	<0.0001
21st	22.68	52.50	0	314.16	5.02	<0.0001
28th	12.51	25.31	0	127.23	4.6	0.0001
56th	2.48	8.71	0	37.69	4.04	0.0005

Table 4: Improvement in mean percentage of reduction in area on follow up days.

Day	Mean (cm ²)	Std. dev.	Min.	Max.	t test	P value
7th	19.75	15.76	0	100		
14th	47.41	21.00	16	100	14.23	<0.0001
21st	64.86	23.52	23.43	100	19.71	<0.0001
28th	76.21	21.02	34.37	100	23.58	<0.0001
56th	96.81	9.96	53.12	100	29.26	<0.0001

Table 5: Size of wound and healing.

Area of wound (cm ²)	No. of wounds	Complete healing by (days)	P value
0-10	04	28 th	<0.05
11-20	14	35 th	
21-30	07	40 th	
31-40	06	46 th	
41-50	09	56 th	

*40 wounds showed complete healing; 06 patients, with associated history of smoking showed significant reduction in wound area but didn't attain complete wound healing after 56th day.



Figure 4: Non healing diabetic foot ulcer showing healing after PRP injection.

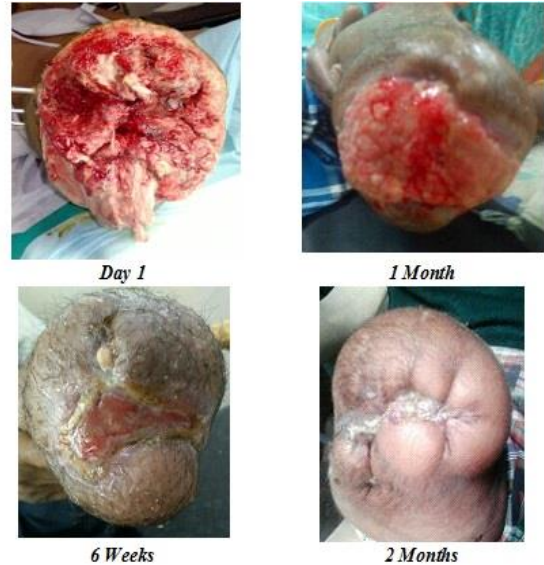


Figure 6: Guillotine amputation showing healing after repeated PRP infiltration.



Figure 5: Road side accident; post op case of distal tibia locking plate for closed fracture distal IIIrd tibia fibula and after 1 month PRP infiltration started. PRP induces healthy granulation tissue even on exposed implant.

DISCUSSION

The rationale behind PRP therapy is to accelerate or to assure the healing of a chronic wound, which is likely not able to heal without intervention. A variety of invasive or noninvasive treatment interventions are used to enhance healing of chronic wounds. One method that is well documented in the literature to facilitate wound healing is to involve the use of PRP to deliver growth factors into the chronic wound. Clinical demonstration by Knighton et al were among the first contemporary study to show that the healing can be enhanced with autologous PRP therapy that delivers locally acting growth factors into wound tissue to stimulate a physiologic response.¹⁹ An ever-increasing amount of clinical evidence exists regarding potential positive effects of autologous PRP therapy on the healing process of chronic wounds that are not responding to conventional treatment.²⁰⁻²³ Although there is mounting evidence the clinical data have been hindered by the number of PRP injection and adequate dosage.²⁰⁻²³ The authors suggested that the number of injections varies based on each patient's individualized condition that can be categorized into risk factors and associated co-morbidities.²⁴ Furthermore, the authors noted that the dosage and frequency of PRP injections were directly proportional to the size of wound, with

more frequent injection necessitated in patients with larger wounds and vice versa. Ulcers that were less than 10 cm² at initial presentation healed significantly faster (8.69% at 21st day) than ulcers measuring 10 to 50 cm² (78.2% wounds at 56th day; p<0.05). In present study authors have found that besides escalating the healing process PRP-prevented the occurrence of large area hematomas thus reducing a potential source for infection.^{25,26} The association between deleterious effects of smoking and delayed wound healing is well recognized in clinical practice. In the present study the authors have pointed out that compared with non-smokers, smokers showed delayed healing response even with PRP injections and were found to have more number of injections. Smoking impaired healing through the effects of tissue ischemia, impaired epithelialisation, and impaired proliferation of cells, vasoconstriction and enzymatic system toxicity.²⁷

In present study, we present the results of the autologous PRP therapy of patients with chronic wounds that were associated with impaired healing. The potential benefits of PRP therapy include their wide applicability, safer, cost effective and less time consuming manner with no side effects. However, PRP therapy requires multiple sitting depending on size of wound and patient compliance. Authors advocated the need for the development of a standards and guidelines for the preparation of PRP, as no standardized methodology is currently available in the literature.

CONCLUSION

In conclusion, autologous PRP therapy remains a reliable, cost-effective intervention for patients with chronic wounds associated with impaired healing, particularly those with smaller wounds and without co-morbidities. Larger wound areas and smokers often experience delayed healing and may require multiple PRP therapy.

Limitations

This study has some limitations. Small cohort included. Another limitation of this study is the absence of a control group. A controlled randomized in a large-scale cohort study with a longer follow-up would be needed to better demonstrate the clinical outcome of autologous PRP therapy. Further studies are required to optimize the number and spacing of injections for obtaining maximum desired functional outcome.

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Conflict of interest: None declared

Ethical approval: The study was approved by the institutional ethics committee

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