



Research Paper

STUDY TO EVALUATE THE EFFICACY OF PLATELET-RICH PLASMA INJECTION IN THE MANAGEMENT OF CHRONIC NONHEALING WOUNDS

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ABSTRACT

OBJECTIVE: The aim was to evaluate the efficacy of platelet-rich plasma (PRP) in treating chronic nonhealing wounds. **BACKGROUND:** Nonhealing ulcers are a significant health problem with a high costs in terms of human and material resources. The application of autologous PRP has been a significant breakthrough for treating nonhealing ulcers, as it is an easy and cost-effective method and provides the necessary growth factors that enhance wound healing. **METHODOLOGY:** A prospective study was conducted from September 2018 to March 2020 at Narayana general hospital attached to Narayana Medical college after ethical clearance from the ethical clearance committee. A total of 30 cases with chronic nonhealing ulcers of various causes (traumatic, diabetic, pressure, venous ulcers), at least six weeks old with wound area measurement between 2 cm² and 10 cm² are injected with autologous platelet-rich plasma injection twice weekly. Haemoglobin level <10 g/dL, platelet count <105000/ uL and osteomyelitis cases are excluded from the study. Wound contraction was measured every 4th day and represented as a percentage of healing wound area and followed up for 6 weeks. **RESULTS:** The mean age \pm SD of the patients in the present study was 49.36 \pm 14.05. According to the etiology, nonhealing ulcers are again classified as traumatic, diabetic, decubitus ulcers, venous, and others (postoperative wounds, Hansen's disease), which constitutes 40%, 27%, 17%, 3%, 13% respectively. The healing rates of the various ulcers were monitored in weekly intervals till the sixth week. Among 30 patients, 17 patients (57%) showed complete healing, 3 patients (10%) showed 99-90% healing, 5 patients (17%) showed 80-89% healing, 5 patients (17%) showed 80-89% healing, 4 patients (13%) showed 50-79% healing, 1 patients (3%) showed <50% healing. Area reduction was statistically significant (p-value is <0.001). **CONCLUSION:** Conventional therapies do not provide good healing for chronic nonhealing ulcers as they are not able to provide the necessary growth factors essential for the healing process. PRP is a safe, affordable, biocompatible, and simple OPD based procedure for treating nonhealing ulcers.

KEYWORDS

autologous platelet-rich plasma, chronic wound, growth factors, nonhealing ulcers

INTRODUCTION

The chronic nonhealing wounds are recalcitrant to primary wound care will deteriorate unless a clinical intervention or alternative care is provided. Chronic wounds have been shown to halt in the inflammatory phase of wound healing and either do not heal or deteriorate. These stalled wounds have consistently high levels of matrix metalloproteinases and proinflammatory cytokines (i.e., tumor necrosis factor- α) and consistently low levels of tissue inhibitors of matrix metalloproteinases and growth factors (i.e., platelet-derived growth factor). Under the treatment of conventional debridement and dressing, the curative effect is low. With the aging of the population, chronic diseases such as diabetes and hypertension have increased. These chronic diseases aggravate arteriosclerosis and excessive blood sugar that are also not conducive for wound healing⁽¹⁾.

Platelets contain these growth factors, cytokines, and chemokines, which are crucial in the early stages of wound healing. Growth factors are a collection of soluble and diffusible polypeptide substances that control the growth, differentiation, proliferation, and cellular metabolism of numerous cell types. They promote epithelial and endothelial regeneration, stimulate angiogenesis, collagen synthesis, soft tissue healing, and hemostasis. Harnessing factors from platelets and applying them to a nonhealing wound could restart the healing process, moving the wound out of the inflammatory cycle into the proliferative phase of healing⁽²⁾.

Platelet-rich plasma (PRP) is an endogenous therapeutic substance that is attaining interest in regenerative medicine due to its capacity to stimulate and accelerate tissue healing. Platelet-rich plasma is defined as an autologous biological product derived from the patient's blood. A plasma fraction is procured with a platelet concentration higher than that in circulating blood by the centrifuge process⁽³⁾. Platelets play a vital role in the wound healing process due to their hemostatic function. There are numerous growth factors which are known to be involved in the wound healing process like insulin-like growth factor (IGF1, IGF2), vascular endothelial growth factor (VEGF), platelet-

derived growth factor (PDGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), transforming growth factor (TGF- β), and keratinocyte growth factor (KGF)⁽³⁾.

For all the reasons mentioned above, this study evaluates the efficacy of platelet-rich plasma injection in managing chronic nonhealing wounds.

METHODOLOGY

A prospective study was conducted from September 2018 to March 2020 at Narayana general hospital attached to Narayana Medical college on 30 patients with nonhealing ulcers.

Ethical clearance was received before the beginning of the study from the Ethical Clearance Committee. Detailed history, including the name, sex, age, address, occupation, contact number, and medication history, was noted.

Types of outcome measures

1. Total area epithelialized at the end of the intervention (measured in cm²).
2. Wound complications: infection, necrosis.

INCLUSION CRITERIA:

- Age between 18 to 70 years of age.
- Chronic nonhealing ulcer of etiology (traumatic, diabetic, pressure, venous ulcers) is at least six weeks old.
- Wound area (width x length) measurement between 2 cm² and 10 cm²
- Nonhealing ulcer without exposure to the bone.

EXCLUSION CRITERIA:

- Haemoglobin level < 10 g/dL
- Platelet count < 105000/ uL
- Any history of allergic sensitivity to components of the PRP kit (calcium chloride, acid citrate dextrose solution A (ACD-A),

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- thrombin)
- Chronic kidney disease patients, Chronic liver disease patients
 - Patient with known or suspected osteomyelitis

Treatment Procedure

Described the treatment procedure to each patient and consent taken in the local language. Before PRP application, the wound bed was debrided (mechanical method), cleansing with normal saline. Platelet-rich plasma is typically applied two times per week. The activated PRP was injected over the ulcer edges. The remaining PRP, if any, was applied onto the wound and dressed with a non-absorbent dressing (paraffin gauze).

Preparation of PRP

27 ml of blood was first collected from the patient's upper limb cubital vein using an 18 G needle. Acid citrate dextrose A solution was added to the sample as an anticoagulant. The first spin is light-spin centrifugation. It is slow to prevent spinning down platelets and to separate plasma at 1500 rotations for 15 min. Platelets are concentrated right on top of the buffy coat, followed by Red blood cells being the heaviest, followed by White blood cells, whereas platelets are the lightest. Heavy spin centrifugation is a faster type of centrifugation at around 3500 rotations for 7 min, and thereby the platelets are spun down and get separated at the bottom of the tube. The left behind is platelet-poor plasma (PPP) resulting suspension is used as platelet-rich plasma. The mean platelet count was 3.7 Lakhs/cumm, and the mean final concentration of platelets in PRP was 6.05 Lakhs/cumm⁽⁴⁾.

Wound Measurements

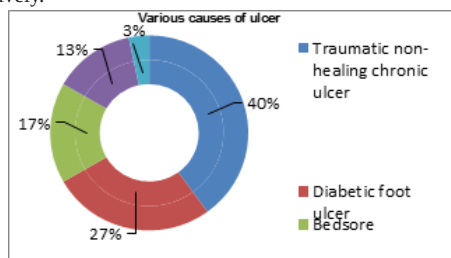
Measurements were taken and recorded before each application, before each dressing change. The length and width of the wound are measured using the standard "clock face" method detailed by Sussman⁽⁵⁾. The length was from 12:00 to 6:00 with 12:00 towards cephalad, and width was from 9:00 to 3:00. The wound area is calculated using the formula for an ellipse: width × length × 0.7854 (an ellipse is nearer to a wound shape than a rectangle or square that would be described by simple width × length. Wound contraction was measured every 4th day and represented as percentage of healing wound area. Percentage of wound contraction was calculated taking the initial size of the wound as 100% using the following formula:

$$\% \text{ wound contraction} = \frac{(\text{Initial wound area} - \text{Specific day wound area})}{\text{Initial wound area}} \times 100.$$

FIGURE 1:Percentage of wound contraction formula

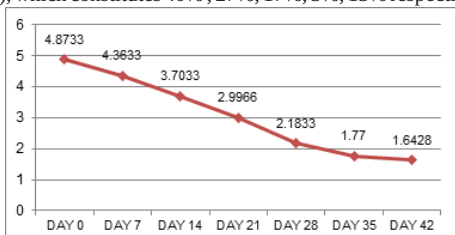
RESULTS:

The mean age of the patients in the present study was 49.36±14.05. Percentage of males and females in the current study was 73% and 27% respectively. Based on the duration of the ulcers, they are again classified into the < 3 months, 3 to 6, 6 to 12 months, and > one-year category, which showed 5 (17%), 15 (50%), 4 (13%), 6 (20%) respectively.



Graph 1: Distribution according to various causes of ulcer

According to the etiology, ulcers are classified as traumatic, diabetic, decubitus ulcers, venous, and others (postoperative wounds, Hansen's disease), which constitutes 40%, 27%, 17%, 3%, 13% respectively.

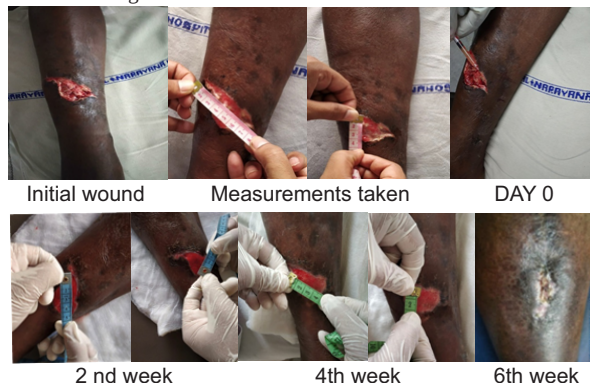


Graph 2: Distribution according to Sum of the mean area of ulcers from baseline to the last visit

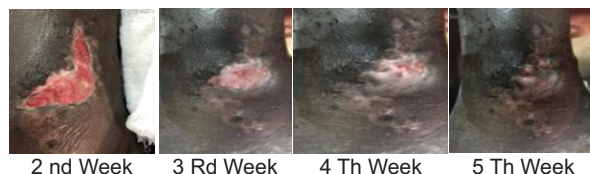
The healing rates of the various ulcers were monitored in weekly intervals till the sixth week. Among 30 patients, 17 patients (57%) showed complete healing, 3 patients (10%) showed 99-90% healing, 5 patients (17%) showed 80-89% healing, 5 patients (17%) showed 80-89% healing, 4 patients (13%) showed 50-79% healing, 1 patients (3%) showed <50% healing.

There is a significant difference in change of area from pre PRP injection to post 6 weeks follow up (p-value is <0.001).

Case :1 Post traumatic nonhealing wound over right side lower end of tibia measuring 4x3 cm



Serial measurements shows decrease in wound area and wound closure seen on 6th week **Case 2:** Post surgical nonhealing wound over Rt ankle measuring 6x3 cm treated with 6 sittings of PRP injection



Serial measurements shows decrease in wound area and wound closure seen on 5th week

DISCUSSION

Chronic non healing ulcers come with significant cost and morbidity for the patients and society altogether. The main aim of any treatment modality is to obtain wound closure efficiently. The conventional treatment compromise adequate debridement, control of infection, avoidance of undue pressure on the wound and revascularization of ischemic tissue.

The wound healing process is typically divided into three/four overlapping phases: hemostasis/inflammation, cell proliferation and remodeling, which are managed by various cells, cytokines, and growth factors which can act directly over the responsible cells for their release, nearby cells or even distant cells

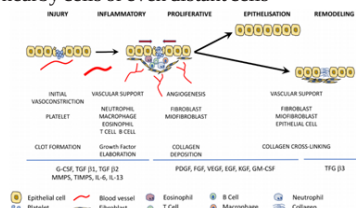


Figure 3:Stages of wound healing^[6]

The normal platelet count is 1,50,000– 4,00,000 per microliter of blood. Platelets usually remain alive for 5-11days.Platelets begin secreting these growth factors within 10 minutes of activation. PRP release supraphysiologic amounts of essential growth factors and cytokines from their alpha granules to provide a regenerative stimulus that augments healing and promotes repair in tissues with low healing potential.

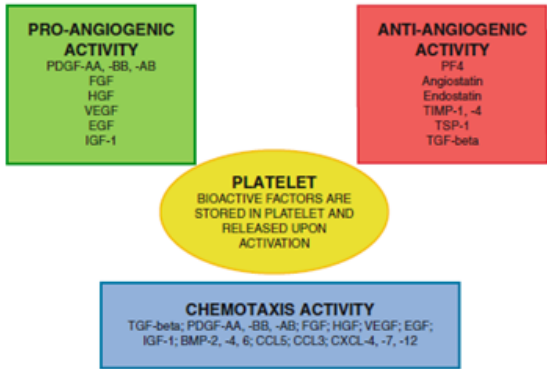


FIGURE 4: Principal PRP bioactive factors involved in angiogenesis and chemotaxis.

GROWTH FACTORS	FUNCTIONS
PDGF	Stimulates cell proliferation Chemotaxis Stimulates angiogenesis
TGF-β	Stimulates production of collagen type I and type III Angiogenesis Re-epithelialization Prevents collagen break down
BMP2	TGF-β signaling pathway Hedge hog pathway Cytokine and cytokine receptor interactions
BMP7	Phosphorylation of SMAD1 and SMAD5- induce transcription of numerous osteogenic genes.
IL-1RA	Pain relief

FIGURE 5:Growth factors present in mesenchymal stem cells

The mean age of patients in this study was 49.36±14.05 comparable to Atef et al.^[7], who had an average age was 48±7.38 years. In this study, the younger patients and older patients showed the same rate of healing.

The number of wounds taken into this study was 30 of different etiologies. Studies conducted by Weed et al. 2004^[8], Anitua et al. 2008^[9] are also done on different etiologies.

In this study,89% of wounds showed a positive response within four treatments over two weeks. Frykberg et al.^[10] showed 97% of different etiologies' wounds improved over three weeks of reduction in wound surface area with the application of 3 times. de Leon et al.^[11] showed that a good response was seen in 86.5% of ulcers of various etiologies within 2.2 weeks and 2.8 applications.

In this study, PRP injected on 30wounds. The number of wounds in studies conducted by Suthar et al.^[12], Croveti et al.^[13], Bernuzzi et al.^[14] was around 20 Wounds.

In this study, the ulcer's duration on which PRP was injected was around 3 -15 months. The duration of ulcer in studies conducted by Suthar et al.^[12], Mohammadi et al.^[15], Bernuzzi et al.^[14], Yilmaz et al.^[16], Kontopodis et al.^[17] was around four months.

Regarding the effect of duration of ulcers on the healing rate, this study showed a positive correlation between healing rate percent and duration of ulcers. That may be due to the short duration of all ulcers (<12 months).

The studies conducted by Patel et al.^[18], and Margolis et al.^[19] showed that ulcer healing rate was higher in cases whose duration of the ulcer was within one year compared to ulcers longer than one year. It was explained by time-dependent changes occurring in the ulcer microenvironment, making the healing process difficult to be achieved.

In this present study, the mean size of the wounds was 4.87 cm2, which is comparable to other studies like Kontopodis et al.^[17], Yilmaz et al.^[16], Mohammadi et al.^[15].

There was a significant and firm proportional correlation between the size of the wounds and treatment duration—the larger the surface of wounds, the longer the duration to heal. Although the percentage healing rate decreased with increased wound size, absolute healing rates increased enormously with initial wound size.

In this study, chronic nonhealing wounds of larger size showed a better response to a greater number of injections. There was a significant and firm proportional correlation between the size of the wounds and the number of PRP injections. Many studies concluded that the larger the ulcer, the longer the duration required for treatment and the greater the number of injections.

Name	Wounds	Duration In Months	Mean Area Cm2	Appication duration	Wound Improvement
Croveti et al ^[13]	24 patients	1–360	67.4 (0.5–56)	Weekly applications for ten applications	Topical hemotherapy with platelet gel may be considered as an adjuvant treatment
Frykberg et al ^[10]	65 wounds	12 (0.75–65)	19	Mean 2.8 weeks with 3.2 applications	Volume reduction, mean 51%.PRP gel can reverse nonhealing trends
Bernuzzi et al ^[14]	17	Minimum 1 month (mean duration unspecified)	63 (36–90)	Mean weekly treatments: 14.5 (4–25)	Considerable variability in clinical outcomes exists, due to differences in biological properties of platelet concentrates from individual patients
de Leon et al ^[11]	285 wounds	12	26	2.2 weeks with 2.8 treatments	PRP gel can restart the healing process.
Sakata et al ^[20]	40wounds	3.3±3.6	13.4 (0.01–140)	One to two weekly applications until healing	Good healing outcomes and a low amputation rate
Salazar-Alvarez et al ^[21]	11 wounds	17 (6–108)	0.79–63.59	Maximum of four local applications of PRP, 1-week intervals, unspecified method for PRP obtainment	PRP could serve as a useful adjunct therapy
Yilmaz et al ^[16]	19 wounds	1.75±6.6	8.61±3.44	weekly until complete wound healing (mean 3.57±1.83 sessions)	Effective in promoting healing, especially in deep venous ulcers
Kontopodis et al ^[17]	72 wounds	Minimum 1 month	4.1±3.9	PRP for 16 weeks	PRP could serve as a useful adjunct therapy
Suthar et al ^[12]	24 wounds	4 (2.25–6)	NIL	PRP system, one single application, gel and intralesional	PRP is safe and efficient in chronic wounds

Moham madi et al ⁽¹⁵⁾	70 woun ds	4.6 ±1	6.11 (0.2–1 5.12)	weekly topical applications performed until healing	PRP may be considered a suitable treatment for recalcitrant diabetic foot ulcers
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Table: Similar studies showing efficacy of PRP**Strength of the study**

As it is autologous, it presents no risk of immunogenic reactions or human to human disease transmissions like HIV or Hepatitis B, thus making it a safe modality of treatment.

Limitations of the study

- No control group was used
- Single centre study

Recommendations for further work

- Despite the encouraging results of several animal studies, well-controlled human studies are lacking. The research is still in its infancy. However, more extensive comparative studies are needed to verify these results.
- There is no consensus or protocol for the use of PRP. There is shortage of comparative studies to standardize the platelet-rich plasma procedural parameters. The heterogeneity of PRP preparations, both presently and historically, has made interpreting the existing literature difficult and limits our ability to make definitive treatment recommendations
- PRP's role in addressing infection is being widely investigated and holds promise, as reports of the antibacterial nature of platelet-rich plasma are pouring in the literature.

CONCLUSION

With the use of autologous PRP dressings for the treatment of chronic nonhealing ulcers the following conclusions were derived

- Area reduction was statistically significant (p-value is <0.001).
- There were no adverse effects or reactions seen
- It is a cost-effective procedure and easy to use
- Showed faster and better healing rates

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