

Study of PRP assisted wound repair and regeneration in chronic non healing wounds

Gupta A, Shrivastava S.

Investigation performed at Department of Orthopedics, Datta Meghe Institute of Medical Sciences, Jawaharlal Nehru Medical College, Sawangi, Wardha, Maharashtra, India

Abstract

Background: Chronic non healing wounds are a cause of severe pain and disability in a large number of patients. Usually these patients are suffering from conditions which has lowered their capacity to heal the wound because of variety of systemic, metabolic or local disturbances in milieu interior. Some of the commonest types of these non-healing wounds are vascular ulcers, diabetic ulcers and pressure ulcers. The usual strategy to treat these ulcers like TIME strategy is not always sufficient and some wounds remain unresponsive to current therapies. Platelet rich plasma (PRP) is a rich source of complex group of growth factors essential for natural wound repair. PRP therapy was used in current study to assess its role in healing of chronic wounds.

Methods: The present study was carried out in 35 patients of various age and sex groups. All these patients had chronic non healing wounds which had not responded to conventional therapy. Wound size was measured at the initiation of protocol by graph method. PRP was prepared using standard two spin technique. The PRP so prepared was injected in the periphery of the wound as per STARS protocol every fourth day. Saline dressings were done regularly and no antiseptics or antibiotics were used. No analgesics were given. Wound size was determined at every fourth day at the time of injection using graph method. **Results:** Out of 35 wounds, 30 wounds healed completely within 40 days of starting therapy. In none of the cases antibiotics or wound debridement was required. Of the remaining 05 wounds there was a significant reduction in size, pain and infection.

Conclusion: PRP injection for treatment of chronic non-healing wounds is an effective method of treatment irrespective of their etiology

Keywords: Non healing ulcer, wound care, PRP injections, STARS protocol

Address for Correspondence: Dr. Anuraag Gupta,
Department of Orthopaedics, Datta Meghe Institute of Medical
Sciences, Jawaharlal Nehru Medical College, Sawangi,
Wardha, MH, India

Email: anuraaggupta@gmail.com

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Introduction

Right from the days of Sushruta in Ancient India, due to frequent battles and wars, healing of wounds was a matter of concern [1] Sushruta Samhita has two separate chapters dealing with healing of these wounds, and describes more than 100 plants for treatment of wounds both singly and in combination [2] Sushruta has mentioned not only procedures and drugs to obtain a clean wound (VranaShodan)

followed by healing (VranaRopan) but also medicines to help treatment of keloids [3].

Chronic wounds can be classified into vascular ulcers (eg, venous and arterial ulcers), diabetic ulcers, and pressure ulcers. Some common features shared by each of these include a prolonged or excessive inflammatory phase [4,5]. Persistent infections, formation of drug-resistant microbial biofilms, and the inability of dermal

and/or epidermal cells to respond to reparative stimuli [6].

Pressure ulcers develop as a result of prolonged unrelieved pressure and shearing force applied to skin and the underlying muscle tissue leading to a decrease in oxygen tension, ischemic injury, and tissue necrosis. Pressure ulcers are common in patients with compromised mobility and decreased sensory perception (neuropathies) [7,8].

The phenotypic abnormalities of epidermis- and dermis-derived cells residing in chronic wounds include lower density of growth factor receptors and lower mitogenic potential preventing them from responding properly to environmental cues. For instance, fibroblasts, isolated from patients with chronic diabetic, chronic nondiabetic wounds, or patients with venous insufficiency, have lower mitogenic response to PDGF-AB, IGF, bFGF, and epidermal growth factor applied separately or in combination. These findings are likely due to a decrease in receptor density [9, 10, 11].

Successful treatment of a particular chronic wound requires a detailed understanding of the molecular and cellular components present within each wound bed. Currently, chronic (and acute) wounds of different etiologies are treated using a multistep approach based on contemporary knowledge of wound healing and known by the acronym TIME. First, nonviable tissues (T) from within and around a wound are removed using surgical debridement or debriding agents, such as bacterial Collagenase, Papain, Bromelin etc. Second, infection and inflammation (I) are minimized with antibiotics and anti-inflammatory preparations. Next, moisture (M) imbalance is corrected, generally with carefully selected dressings. Finally, epithelialization (E) and granulation tissue formation are

promoted by the application of specific therapies, such as growth factors [12].

The use of TIME strategy is not always sufficient, however, and some wounds remain nonresponsive to current therapies.

Platelet and platelet rich plasma

A rich source of the complex group of growth factors (GF's) essential to natural wound repair is the platelet [13]. The platelets act in the hemostasis; wound healing and re-epithelialization liberating diverse growth factors that stimulate the angiogenesis, promoting growth and vascular fibroblast proliferation that in turn provide an increase in the collagen synthesis [14,15].

Platelet rich plasma (PRP) therapy has accumulated considerable attention over the two last decades, mainly due to its potential ability in regenerative medicine, including oral and maxillofacial surgery, sports and veterinary medicine. Platelets as a main components of the PRP, contain more than 1100 different proteins, with numerous post-translational modifications, resulting in over 1500 protein-based bioactive factors [16, 17, 18]. These factors include immune system messengers, growth factors, enzymes and their inhibitors and other factors which can participate in tissue repair and wound healing. Another important characteristic of PRP is that represents an autologous product, which is prepared from the patient's own blood. Therefore, the use of autologous PRP eliminates any concerns about the risk of crossed contamination, disease transmission or immune reactions [19, 20, 21].

Materials and Methods

Study Design: Prospective Interventional Randomized Control Study.

Sample Size: 34 consecutive patients with chronic (more than 90 days duration) wounds of varying etiology and depth were taken up for the study.

Method: These patients were thoroughly evaluated. The study group patients were subjected to wound infiltration of autologous inactivated PRP prepared by double spin technique as per the standard protocol, using 20-40 ml of patient's blood as per the size of the wound, using a 22 gauge needle under strict asepsis. This will be done on the day of recruitment and subsequently every 4th day till the wound has reached the size of less than 100 sq. mm. These patients were dressed with moist saline dressing only. No antibiotics were used as far as possible. Similarly, analgesics were avoided. No surgical intervention was done once the patient was enrolled.

Method of preparing PRP

It was done with a double spin method, using 20ml of venous autologous blood, freshly drawn from the patient. 5ml blood was then transferred to 4 EDTA test tubes each and centrifuged at 2000 RPM for 15 minutes. RBCs got settled in lower portion of test tube and plasma in the upper part. Plasma was extracted and collected in a separate test tube and re-centrifuged at 1200 RPM for 10 minutes. The plasma further separated into upper buffy coat with platelet poor plasma and lower 3-4 ml layer containing platelet rich plasma.

STARS THERAPY PROTOCOL [22]

The autologous PRP thus obtained is transferred to a 10 ml syringe and locally infiltrated into wound margins through a 22 gauge needle at a distance of approximately 1cm. The process is repeated every 4th day. Local moist saline dressing is performed on alternate days. Diluted cetrimide and chlorhexidine gluconate solution are used for cleaning dirty wounds. No further surgical interventions were undertaken.

Inclusion Criteria

1. All age and genders
2. Wound of the size at least 100 square mm.

3. All chronic wounds (more than 90 days) including trophic ulcers, decubitus ulcers, diabetic and venous ulcers.

Exclusion criteria

1. Wound size less than 100 square mm in area.
2. Wounds less than 90 days duration.
3. Patient on I/V antibiotics.
4. patient with serious systemic illness and moribund patients

Results

The results of the study were as follows

Table: 01

Distribution of patients according to size of wound at start of treatment

Size (cm)	Number	Percentage
1 – 10	9	25.71
11 – 100	22	62.86
> 100	4	11.43
Total	35	100%

Table: 02

Distribution of patients according to rate of

Rate of Healing	20 days	40 days	60 days
50±5% reduction	25	8	2
Complete healing	2	12	16

healing of wound

5 patients did not show complete healing even at the end of 60 days. However, 4 of them had significant reduction in size of wound while 1 did not show significant healing.

Table: 03

Distribution of patients according to Pain scoring and initial assessment for pain

VAS score	At start of therapy		4th day of therapy		20th day of therapy		40th day of therapy	
	No.	%	No.	%	No.	%	No.	%
1-2	19	54.29	31	88.57	29	93.55	9	90
3-4	7	20	2	5.71	0	0	0	0
5-6	3	8.57	2	5.71	0	0	0	0
7-8	6	17.14	0	0	0	0	0	0
9-10	0	0	0	0	2	6.45	1	10
Total	35	100	35	100	31	100	10	100

Table 4

Distribution of patients according to infection

Infection	Number	Percentage
Start	12	34.29
4 th Day	4	11.43
20 th day	0	0
40 th day	0	0
Total	16	

Discussion

Non healing wounds has been a cause of concern to mankind since time immemorial. Not only they cause significant morbidity and suffering, but the treatment is prolonged and expensive. Further in spite of all modern modalities of treatment, a large number of these wounds fail to heal. A variety of growth factors and cytokines have been discovered in last 4 decades which promote rapid wound healing.

Shrivastava et al in their protocol STAR therapy have advocated direct injection of platelet rich plasma into the wound instead of making gel preparation with good results. The STAR protocol in our study was found to be simple, rapid, early reproducible, with

use of minimal equipment and inexpensive [22].

We had used autologous blood for PRP preparation, and therefore none of our patient had any adverse reaction.

There are large number of studies in the published literature are on chronic non healing wounds like trophic ulcers of leprosy, diabetic foot ulcers, chronic lower limb ulcers etc. but with mix results.

In the present study, we have studied chronic wounds sustained because of variety of reasons being treated with PRP alone without use of analgesics and antibiotics, no debridement and moist saline dressings only. Chronic wounds were classified as per center for medical services definition [23].

Graph paper tracing was used to record the size of the wound. More than 71.43% of patients showed 50% + 5% reduction in the size of the wound with in 5 infiltration and 90% wounds reached 50% + 5% size with in 10 infiltrations i.e. in 40 days. However complete healing took much long time. The larger was the surface area of the wound, the longer it took to heal. More than 90% of cases healed with in 40 days (20 infiltrations).

One of the patient had a non healing ulcer over the sole of the foot which was of about 7 years duration. Using the present protocol with only 10 infiltrations the patient showed complete healing. Not only the STARS protocol of PRP therapy brought about the complete healing but this healing left behind minimal scar which was of good quality and unlikely to breakdown in near future. Further, as the amount of scarring was minimal there were no secondary deformities because of scar contraction. Similarly a patients with non healing ulcers over both ankles of over 10 year duration showed complete healing.

One of the most remarkable things was the quality of wound healing in these cases. The healed area shows much less scarring. Whatever scar was left behind was of good quality and unlikely to breakdown in future. Even skin showed remarkable regeneration.

In a study by Manish Suthal et al, the potential safety and efficacy of autologous platelet rich plasma for treatment of non-healing ulcers was demonstrated [24]. These wounds were initially treated by standard methods of wound care including skin grafting and other method of skin cover and reconstructive surgery and pain management with management of comorbidities, but satisfactory healing could not be achieved. In the trial, they gave a single subcutaneous injection of PRP along with one PRP gel dressing for each ulcer. They observed ulcer healing as early as 4

weeks PRP treatment and the mean healing time was on to be almost 8.2 weeks. They also noted reduction of discharge and pain reduction was noted within first week post treatment.

In present series, the pain was assessed using VAS scoring. At the start of therapy the VAS score was between 7-8 in 17.14% cases, 8.6% between 5 and 6, 20% had a VAS score between 3 and 4 and 54.29% showed score of 1-2.

This score decreased rapidly after infiltrations and on 4th day of infiltrations there was a significant shift of patients with 88.57% showing VAS score of 1-2 and 5.71% showing VAS score of 3-4. The number of patients showing VAS score of 5 -6 was 5.71%. As almost all of these patients had not received analgesics, this signifies a very positive effect of PRP infiltration.

In only one patient with chronic wounds over ankles of more than 10 years duration, the VAS score was 8 at the start of the treatment and this further increased to 9. Though the wound healed with infiltrations, the pain persisted and the patient had to undertake separate intervention for control of pain. The cause of this pain was ill understood and may be related to nerve entrapment in the scar and altered perception because of chronic problem.

Pain relief had dramatic effect on patient outcome, they became more cooperative, happier, and demand for analgesics were almost absent.

Kathelene M. Lacci have postulated that PRP may suppress cytokine release and thus inflammation leading to decrease in pain [25].

Marissa J Cartier et al found that PRP wound therapy may positively impact the patient by reducing pain [26].

In none of the patient, oral or systemic antibiotic were given. However, most of

these patients were on antibiotics before the start of protocol, which were stopped. At the start of study, 34.29% of patients had positive culture of wound, which decrease to 11.43% by 3rd infiltration and complete eradication of infection by 5th infiltration. None of the patient showed increased clinical signs of infection and there were no complication related to infection. The wound cultures showed a growth of variety of bacteria including MRSA, pseudomonas and E. coli. Usually, these were present as individual infecting organisms.

Marrisa J Cartier et al in their meta-analysis found that superficial and deep infections and postoperative complications were significantly lower in PRP treated subjects than in control group. Further, infection was significantly increase in control group than in PRP group [26].

In this series during the whole course of study, there was no significant complication.

Some patients experienced pain of injection which was not severe and subsided immediately after the procedure.

One patient had severe pain to start with (VAS score 8). This patient had long standing ulcers over both ankles. The pain increased after infiltration and persisted throughout the course of treatment.

Overall, it was found that PRP infiltrations not only helped in wound healing but also significantly promoted skin regeneration leading to minimization of scar formation and its resulting problems.

Conclusion

Autologous PRP could be easily prepared and used, utilizing minimal instrumentation even at centers with limited resources. The STARS protocol of preparing and infiltrating PRP in the wound margin was easy and effective irrespective of their etiology with minimal complications.

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