

Effectiveness of Intralesional Injection of Autologous Platelet-Rich Plasma vs. Normal Saline Dressing for Chronic Non-Healing Ulcers

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Abstract:

Background and Aim: Non-healing ulcers pose a significant global health challenge, affecting individuals on personal, professional, and social levels. The impact is substantial, both in terms of human well-being and the allocation of resources. In this study, we conducted a randomized prospective study to assess the impact of using autologous PRP versus normal saline on chronic non-healing ulcers. Our main focus was to evaluate the effectiveness of these treatments in terms of reducing ulcer area and volume, shortening the healing duration, and achieving complete or partial healing. Additionally, we also monitored for any potential side effects that may arise from these interventions.

Material and Methods: In this randomized study, 80 eligible male and female patients attending the surgical outpatient department were enrolled. These patients had chronic non-healing ulcers and met the inclusion criteria. A total of 80 patients diagnosed with chronic non-healing ulcers were randomly assigned to two equal groups: the PRP group, which received treatment with PRP, and the NS group. Throughout the course of four weeks, various aspects of the condition were closely monitored. These included pain levels, presence of slough and discharge, the development of granulation tissue, as well as any changes in the size and volume of the ulcer. These observations were conducted on a weekly basis, providing valuable insights into the progression of the condition.

Results: The average age in the PRP group was 49.54±11.20 years, while in the NS group it was 50.25±10.15 years. The typical duration of ulcers in both groups ranged from 3 to 6 months. Most of the ulcers in the PRP group lasted between 3 to 6 months, while a smaller percentage lasted between 6 to 12 months. No statistically significant difference was found between the PRP group and NS group in terms of age, gender, occupation of patients, pre-treatment duration, size of ulcer, and etiology of ulcer. The p-value is greater than 0.05.

Conclusion: PRP has proven to be more effective than NS in treating chronic non-healing ulcers. It promotes faster healing, provides quicker pain relief, and leads to an early decrease in discharge and sloughing. These benefits are observed across all age groups and genders, regardless of the type, size, site, duration, and cause of the ulcer.

Keywords: Granulation Non-Healing Ulcers, Normal Saline, Platelet-Rich Plasma.

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Introduction

Chronic ulcers, also known as non-healing ulcers, are lesions that occur spontaneously or as a result of trauma. These ulcers typically appear on the lower extremities and do not respond to initial treatment or heal within a specific timeframe. They may be caused by systemic diseases or local disorders. [1,2] Chronic ulcers are wounds that exhibit a prolonged healing process. In the realm of chronic ulcers, Falanga proposed a new term, 'impaired or weak healing process', to better capture the non-healing mechanism, moving away

from the previous notion of a 'healing failure'. [3] Chronic ulcers can be treated through either conservative or surgical methods. The goal of conservative management is to either fully heal the ulcer or prepare the wound bed for a successful surgical intervention. There are various types of ulcers that do not heal, such as venous, arterial, diabetic, pressure, and traumatic ulcers. The objective of ulcer treatment is to achieve wound closure as quickly as possible. For non-healing ulcers, the usual course of action involves cleansing

the wound, removing dead tissue, addressing any infections, relieving pressure on the affected area, controlling blood sugar levels, and applying appropriate dressings for local ulcer care. [4-6]

Certain factors can increase the risk of poor wound healing. These include local causes like the presence of necrotic tissue, tissue hypoxia, and repeated trauma in the ulcer, as well as systemic diseases like diabetes mellitus and medications such as steroids. There are several advanced treatments available for non-healing ulcers, such as hyperbaric oxygen therapy, skin grafting, VAC (vacuum assisted closure), and surgical management options like angioplasty and reconstructive surgery as needed. [7,8]

PRP is a concentrated form of the patient's blood that is rich in platelets. There are different terms used in the literature to refer to it, including 'concentration of platelets', 'platelet gel', or 'releasate of platelets'. [9,10] When it comes to the PRP, it's worth noting that not only does it contain a high concentration of platelets, but it also shows an increase in all coagulation factors. Activated platelets release various growth factors that play a crucial role in cell migration, proliferation, differentiation, angiogenesis, tissue debris removal, and tissue regeneration. [11,12] Platelets contain a variety of proteins known as secretion proteins, which play a crucial role in the process of wound healing. These factors are part of a group of growth factors, specifically cytokines and chemokines. Some examples include platelet-derived growth factors, transforming growth factors- β , interleukin1, platelet-derived angiogenesis factor, vascular endothelial growth factor, epidermal growth factor, platelet-derived endothelial growth factor, epithelial cell growth factor, insulin-like growth factor, fibronectin, and others. [13,14]

The accelerated soft tissue wound healing is believed to be at least 2-3 times faster than normal, possibly due to higher concentrations of growth factors. Using an autologous PRP has several advantages, such as eliminating the risk of cross reactivity, immune reaction, or disease transmission. For this study, we conducted a randomized prospective analysis to assess the impact of applying autologous PRP versus normal saline on chronic non-healing ulcers. Our focus was on measuring wound healing based on factors such as reduction in ulcer area and volume, time taken for healing, and the occurrence of complete or partial healing. We also monitored for any potential side effects.

Material and Methods

This randomized study was conducted in a department of General Surgery at a Tertiary Care Teaching Institute in India over a period of 1 year. After obtaining approval from the institutional

ethics committee and written informed consent, a total of 80 eligible male and female patients with chronic non-healing ulcers were enrolled from the surgical outpatient department. These patients met the inclusion criteria for the study.

Inclusion Criteria

Individuals suffering from chronic non-healing ulcers with an ulcer area smaller than 20 cm.² Ulcers that are considered chronic typically have been present for over three months, exhibit pale granulation tissue at the floor of the ulcer, and have indurated base and edges.

Exclusion Criteria

Individuals with a history of bleeding disorders or who are taking anticoagulant medications, those with blood transmissible diseases, uncontrolled diabetes mellitus, pregnant and lactating mothers, individuals with immunosuppressive disorders or who are taking immunosuppressive medication, those with psychiatric illness, and individuals with ulcers accompanied by underlying osteomyelitis and/or exposed bones.

As part of the pre-treatment process, a comprehensive history was gathered, including information about age, sex, occupation, potential causes, and any related medical conditions. Additionally, a thorough examination was conducted, both locally and systemically, along with routine blood tests. During a local examination of an ulcer, the size was measured by directly pressing the ulcer against a gauze piece or butter paper, and then transferring the tracing onto graph paper. The length, width, and depth of the ulcer were measured using cotton tipped applicators and disposable scales. Observations were made regarding the ulcer site, discharge, margin, surrounding skin, granulation tissue, tenderness, temperature, and regional lymph nodes.

Exploring the Method of PRP Preparation Using aseptic technique, approximately 8-8.5 ml of blood is drawn from the antecubital fossa using an 18 G needle. The blood is then collected in a sterilized vacuutainer tube, which contains 1.5 ml of anticoagulant acid citrate dextrose solution. After collecting the sample, it was placed in a centrifugation machine. The sample underwent a soft spin at 1200 rpm for 8 minutes at 20°C.

This process resulted in the separation of the whole blood into three distinct layers: the lower red blood cell region, the middle buffy coat layer, and the upper straw-colored plasma region. The separated buffy coat and platelet poor plasma (PPP) are carefully aspirated using a pipette and collected into a sterilized collecting tube without any anticoagulant, with a capacity of 10 ml. After a second centrifugation at 2400 rpm for 4 minutes, the tube was able to separate its contents. The

upper portion of the tube contained a clear yellow supernatant serum, which had a low concentration of platelets and fibrinogen. On the other hand, the lower bottom layer of the tube was highly concentrated with PRP and had a red tinge. The upper layer containing PPP was removed using a pipette, while the lower layer of PRP was used for platelet count. By utilizing the potential of collagen to activate PRP in the body, it becomes possible to administer unactivated PRP through intralesional injections using a small gauge needle.

A total of 80 patients were randomly assigned to two groups, with each group consisting of 40 patients. Group A: platelet rich plasma (PRP). Group B received normal saline. Patients were given antibiotics when signs of infection appeared, along with supportive treatment such as analgesics, proteins, iron, and multivitamins. Various observations were made regarding the subjective complaints of pain, fever, discomfort, discharge, granulation, and other related symptoms. Upon admission, any necessary debridement was performed on the ulcer.

Group A received PRP injections every 7th day, while group B received normal saline dressing every alternate day. Ulcer size reduction was noted on a weekly basis, with measurements taken before treatment and then again on the 7th, 14th, 21st, and 28th day or the day the wound healed. Photographs were taken at regular intervals of 7th, 14th, 21st, and 28th day to document visual findings. The photographs were captured from a fixed distance, angle, focal length, and illumination.

Statistical Analysis

The data was compiled and entered into a spreadsheet computer program (Microsoft Excel 2019) before being exported to the data editor page of SPSS version 15 (SPSS Inc., Chicago, Illinois, USA). Quantitative variables were reported using measures such as means and standard deviations or median and interquartile range, depending on their distribution. The presentation of qualitative variables was in the form of counts and percentages. Confidence level and level of significance were set at 95% and 5% respectively for all tests.

Results

In the PRP group, the average age was 49.54 ± 11.20 years, while in the NS group it was 50.25 ± 10.15 years (Table 1). Out of a group of 40 patients, 30 (75%) were male and 10 (25%) were female. Out of the 40 patients in the NS group, 32 (80%) were male and 8 (20%) were female, as shown in Table 1.

The most prevalent type of ulcer in the PRP group is venous, accounting for 29.62% of cases. The most common ulcers in the NS group were post

cellulitis (29.62%) and traumatic (29.62%) (Table 2). The most common duration of ulcers in both groups was 3-6 months. Most of the ulcers in the PRP group lasted between 3 to 6 months, while a smaller percentage lasted between 6 to 12 months. According to Table 3, a significant majority of ulcers in the NS group were between 3-6 months old. A total of 40 patients were included in the study. Among them, 55% (22 patients) had an ulcer surface area ranging from 10 to 15 cm², while 32.5% (13 patients) had an area less than 10 cm². The remaining 12.5% (5 patients) had ulcer areas between 15 to 20 cm². Out of the 40 patients in the NS group, 60% (24 out of 40) had ulcer surface areas ranging from 10 to 15 cm². Additionally, 37.5% (15 out of 40) had ulcer areas less than 10 cm², while only 2.5% (1 out of 40) had ulcer areas between 15 to 20 cm². The p-value is greater than 0.05.

No statistically significant difference was found between the PRP group and NS group for age, gender, occupation of patients, pre-treatment duration, size of ulcer, and etiology of ulcer. The p-value is greater than 0.05. In the PRP group, the baseline mean area and volume of the ulcer measured 12.20 ± 4.56 cm² and 5.10 ± 2.32 cm³, respectively. The average area and volume of the ulcer at the end of the study were 1.20 ± 0.86 cm² and 1.05 ± 2.30 cm³, respectively. The baseline mean area and volume in the NS group measured 12.69 ± 3.24 cm² and 5.47 ± 2.70 cm³, respectively. The NS group had a final mean area of 5.25 ± 1.50 cm² and a volume of 2.40 ± 1.36 cm³. In the PRP group, there was a mean reduction in ulcer area and volume of 12.50 ± 4.22 and 6.70 ± 4.89 , respectively. On the other hand, the NS group showed a mean reduction in area and volume of 9.10 ± 1.80 and 4.58 ± 1.22 . The statistical significance level was found to be less than or equal to 0.05.

At the end of the treatment, a remarkable 75% of patients in the PRP group showed no signs of slough from ulcers. In contrast, only 15% of patients in the NS group experienced the same outcome. A majority of patients (62%) in the PRP group reported being pain-free by the end of their treatment. Among the participants in the NS group, a significant majority of 74% reported experiencing minimal pain, while only a small number of 3 patients reported having no pain. Most patients in the PRP group (60%) experienced no discharge, while 40% of patients had minimal discharge by the end of their treatment. Among the participants in the NS group, a majority of 77% experienced minimal discharge, while 11% reported mild discharge and 12% did not experience any discharge. The statistical significance level was found to be less than or equal to 0.05.

In the PRP group, moderate granulation tissue was observed in 85% of cases, while mild granulation tissue was observed in 15% of cases. The majority of cases in the NS group exhibited mild granulation tissue, while a smaller percentage showed only mild granulation tissue. A significant difference was observed in both groups ($p < 0.01$). In the PRP

group, 23% of the ulcers showed complete healing, while 77% of the ulcers showed partial healing. All ulcers in the NS groups showed partial healing. A significant difference was found between both groups in the healing of ulcers. The statistical significance level was found to be less than or equal to 0.05.

Table 1: Demographic data of cases

Variable	PRP group (n=40)	NS group (n=40)	P value
Male	30 (75%)	32 (80%)	0.12
Female	10 (25%)	8 (20%)	
Mean age \pm SD (years)	49.54 \pm 11.20	50.25 \pm 10.15	0.09

Statistically significance at $p \leq 0.05$

Table 2: Etiology in both groups

Type	PRP group (n=40)	NS group (n=40)	P value
Diabetic	9 (22.5%)	8 (20%)	0.58
Postcellulitis	10 (25%)	12 (30%)	
Traumatic	9 (22.5%)	12 (30%)	
Venous	12 (30%)	8 (20%)	
Total	40	40	

Statistically significance at $p \leq 0.05$

Table 3: Duration of ulcer persisted before treatment in both groups.

Duration (in months)	PRP group (n=40)	NS group (n=40)	P value
3-6 months	32 (80%)	36 (90%)	0.23
6-12 months	8 (20%)	4 (10%)	
Total	40	40	

Statistically significance at $p \leq 0.05$

Discussion

Throughout the process of wound healing, various stages occur simultaneously and in sequence. These stages include haemostasis and inflammation, proliferation, and remodelling. Chronic ulcers are characterized by a disrupted healing process that results in a cycle of inflammation and proliferation, leading to persistent chronic inflammation. [17,18]

Various factors, such as the patient's overall health, the specific conditions of the ulcer, and the planned therapeutic interventions, can have a significant impact on the different stages of healing. Chronic ulcers, also known as non-healing ulcers, are lesions that occur spontaneously or as a result of trauma, usually in the lower extremities. Despite receiving initial treatment and appropriate care, these ulcers do not respond and fail to heal within a specific timeframe. The underlying cause of these ulcers may be associated with systemic disease or local disorders. [19,20]

PRP is an autologous preparation, which means it is a safe treatment option when compared to allogenic preparations. It is also free from any worries about transmissible diseases. [21,22] All patients in the PRP group experienced a decrease in pain, discharge, and slough from their ulcers after treatment. Additionally, they had more granulation

tissue on the surface of their ulcers. It is worth noting that there were no reported side effects. Autologous PRP has been shown to be both safe and effective in the treatment of chronic nonhealing ulcers. In a recent study by Frykberg et al., researchers examined the effects of treatment on non-healing ulcers in 49 patients. The results were quite promising, as 63 out of the 65 ulcers showed significant improvement in terms of reduced area, volume, and undermining. On average, it took about 2.8 weeks and 3.2 treatments for these positive changes to occur. [23] In a recent study conducted by Kakudo et al., they examined the effectiveness of autologous PRP in treating intractable skin ulcers. The results were quite promising, as three out of the five cases treated saw complete healing within just four weeks. On average, the wounds showed signs of epithelialization within 6.6 weeks. These findings highlight the potential of autologous PRP as a treatment option for stubborn skin ulcers. [24]

PRP has shown promising results in the treatment of chronic ulcers, as it can speed up the healing process, reduce local pain, and lower the risk of infection. Experimental studies often yield positive results when it comes to the application of PRP. In a study conducted by Carter et al [25] PRP was applied to ulcers in equine lower limb wounds,

showing promising outcomes. The histological results indicate a positive outcome in the wound healing process, as evidenced by the presence of well-organized fibroblasts and improved alignment of collagen fibers. [26] In a study conducted by Yazawa, a combination of PRP and fibre glue was used to treat rabbit ear ulcers. The findings revealed that starting from the seventh day after treatment, the ulcers treated with PRP showed enhanced epithelialisation and reduced presence of granulation tissue, in comparison to the ulcers treated solely with fibre glue. [27] In a recent study, Suthar et al conducted a case series to assess the impact of autologous platelet rich plasma on the treatment of chronic non-healing ulcers. [28]

A total of 36 patients with nonhealing ulcers of various causes were included in the study. These patients received a single dose of subcutaneous PRP injections and also had PRP gel applied topically. The treatment was administered under compassionate use and the patients were followed up for duration of 24 weeks. All the patients demonstrated positive progress in wound healing, with a noticeable decrease in wound size. On average, it took 8.2 weeks for the ulcers to fully heal.

According to recent research, PRP has shown promise in effectively treating chronic non-healing ulcers. Nevertheless, additional large-scale controlled randomized prospective clinical trials are needed to conclusively establish its effectiveness. When plasma is injected into the wound, it offers several advantages over the traditional method of simply placing PRP on the ulcer. PRP intralesional injection enables the ongoing application of dressings without compromising the wound's growth factors. Additionally, PRP can be used in conjunction with reconstructive procedures, even in a single-stage operation.

There have been numerous studies exploring the use of PRP for non-healing ulcers, and the findings have shown promise. However, there is currently a lack of comprehensive scientific data on the specific benefits of PRP in clinical procedures. PRP is an autologous preparation, which means it is a safe treatment option when compared to allogenic preparations. It is also free from any worries about transmissible diseases. [29] In addition, PRP does not require any specific precautions regarding antibody formation. This effectively reduces the risk of graft vs. host disease and makes it more readily accepted by patients.

There are some limitations to this study. Certain patients are excluded from this study, such as those with large size ulcers, a history of bleeding disorders or on anticoagulant medications, hemodynamic instability, or an immunosuppressive

disorder. A study was conducted on a small group of patients, so in order to apply the findings to the general population, a larger study with a larger group is necessary.

Conclusion

PRP is more effective than NS on chronic non-healing ulcers as it causes more rapid healing, rapid relief from pain and early decrease in discharge and slough in all age groups and sex; irrespective of type, size, site, duration and etiology of ulcer. Further research and controlled, randomized prospective clinical trials on larger patient population are necessary to validate the results.

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