e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2022; 14(10); 573-579

Original Research Article

Autologous Platelet Rich Fibrin Matrix versus Zinc Oxide and Phenytoin Paste in Non-Healing Ulcers: A Comparative Assessment

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Received: 12-08-2022 / Revised: 08-09-2022 / Accepted: 11-10-2022
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Conflict of interest: Nil

Abstract

Aim: The aim of the present study was to compare the therapeutic efficacy of autologous PRFM versus triple combination paste (zinc oxide, phenytoin, and mupirocin ointment) in non-healing ulcers.

Methods: The present clinical trial was conducted in the Department of Dermatology, Patna Medical College and Hospital, Patna, Bihar, India for one year. A total of 20 patients were included in the study with the age range 20–70 years. Patients with non-healing ulcers were selected from the outpatient department of Skin and VD.

Results: A total of 20 cases of non-healing ulcers of varying etiologies were treated using autologous platelet rich fibrin (PRF) at weekly intervals for maximum frequency of 6 sittings respectively. The mean age of the patients was 39.7 ± 13.02 years. Out of 20 ulcers, there were 7 (35%) venous ulcers, 3 (15%) traumatic ulcers, 6 (30%) diabetic ulcers and 4 (20%) trophic ulcers. Group A showed a mean reduction in the ulcer area by 8.26 mm2 (75.90%) which is highly significant (P-value = 0.0002). Group B showed a mean reduction in the ulcer area by 4.500 mm2 (45%) which is also significant (P-value = 0.015)

Conclusion: This procedure is simple, patient-friendly, cost-effective, painless and can be performed as an outpatient procedure. We concluded that autologous platelet-rich fibrin matrix is much more effective than the triple combination paste (zinc oxide, phenytoin, and mupirocin ointment) in the treatment of non-healing ulcers.

Keywords: Non-Healing Ulcer, phenytoin, PRF, zinc

Introduction

Ulcer is defined as wounds with a “full thickness depth” and a “slow healing tendency” and may result in complete loss of the epidermis and often portions of the dermis and even subcutaneous fat. [1] Chronic non-healing ulcer is defined as a persistent defect in the skin for the period of more than six weeks and does not exhibit any tendency to heal following three or more months. The symptomatologies of non-healing ulcers include increase in pain, friable granulation tissue, foul odor, and breakdown of wound rather progressing for healing. These results in social outcast

and face more challenges in providing healthcare. [2,3]

Various modalities of treatment for venous ulcers include compression stockings, good wound care and sometimes surgical therapies. The treatment is often difficult and is generally associated with high recurrence rate. [4-6] Dressings play a major role in healing of these ulcers. Moist occlusive dressings with saline are known to improve wound healing. Platelet concentrates have been widely used in regenerative medicine to promote wound healing as they contain transforming growth factor (GF) F, platelet‐derived GF, vascular‐endothelial GF, platelet derived epidermal GF, insulin‐like GF‐I and basic fibroblast GF.

Tissue engineering has demonstrated regeneration potentiality without any permanent sequelae. Many researchers have proven that human skin has the ability to regenerate through available stem cell populations. Stem cell has the ability of self-renewal and ability to differentiate into function specific daughter cells. Regenerative medicine has a definite role in regulating these stem cell populations for promoting skin regeneration. [7] The contents of platelet-rich fibrin (PRF) include platelets, leukocytes, cytokines and adhesive proteins including fibrinogen, fibronectin, vitronectin, and thrombospondin-1 respectively. [8,9] It enhances wound healing through induction of locally accessible growth factors which favors recruitment and proliferation of mesenchymal stem cells (MSCs) and synthesis of extracellular matrix.

The recent literature shows autologous platelet-rich fibrin matrix (PRFM) being rich in growth factors. It is effective in the treatment of chronic non-healing ulcers. [10,11] Phenytoin increases fibroblast activity, decreases collagenase activity with the formation of granulation tissue. Mupirocin ointment possesses antibacterial action. Topical zinc oxide promotes re-epithelization, and thus, has a role in wound healing. It is clinically established that the healing of leg ulcers is delayed in patients with a low level of zinc and thus, zinc can be given either orally or topically to improve healing in such patients. [12,13]

The aim of the present study was to compare the therapeutic efficacy of autologous PRFM versus triple combination paste (zinc oxide, phenytoin, and mupirocin ointment) in non-healing ulcers.

Methods

The present clinical trial was conducted in the Department of Dermatology, Patna medical College and hospital, Patna, Bihar, India for one year. A total of 20 patients were included in the study with the age range 20–70 years. Patients with non-healing ulcers were selected from the outpatient department of Skin and VD.

Inclusion Criteria

- Patients with trophic ulcers due to Hansen disease or diabetes mellitus
- Patients with stasis dermatitis
- Pyoderma gangrenosum
- Venous ulcer
- Traumatic ulcer
- Age group 18–85 years

Exclusion criteria

- Patients with age group below 18 years
- Patients with a history of bleeding disorders
- Anemia and other hematological disorders
- Platelet count <1.5 lakhs/cu mm
- Patients on anticoagulant medications (aspirin, warfarin, heparin)
- Patients with malignant ulcers, pregnant and lactating females

The study population was randomized into two groups, i.e.,

Group A consisted of 10 patients on autologous PRFM.

Group B consisted of 10 patients on triple combination paste (zinc oxide, phenytoin, and mupirocin ointment).

Preparation of PRFM

After taking informed consent, the length and breadth of the ulcer were measured. Under aseptic conditions, 10 mL of venous blood was collected from the median cubital vein and added to a sterile centrifugation tube without any anticoagulant. The tube was rotated at 3,000 rpm for 10 min. Three layers were obtained following this topmost acellular layer which is the platelet-poor plasma (PPP), the lowermost layer containing red blood cells (RBCs), and the middle layer containing the PRFM [Figure 1]. The upper layer (PPP) was discarded. The PRFM was separated from the RBCs at the base with the help of sterile forceps and scissors in a clean and sterile petri-dish. The matrix was then placed onto a sterile gauze and applied over the ulcer followed by the application of a secondary non-absorbable dressing. The patient was advised to take adequate rest. The dressing was removed after a period of minimum of 5 days. The procedure was repeated every week for up to five sittings. The healing of the ulcer was assessed, the area was calculated, and photographs were taken at the beginning and end of every week. The wound area was calculated using the formula for an ellipse: Length × width × 0.7854 (an ellipse is closer to a wound shape than a square or rectangle). The use of an ellipse for calculating the wound measurement has been used in randomized controlled trials in wound healing literature. [14,15]

Preparation of triple combination paste (zinc oxide, phenytoin, and mupirocin ointment)

Under aseptic conditions, 10 phenytoin tablets (100 mg) were crushed finely in mortar and pestle. These were mixed with 10 g of zinc oxide powder and mupirocin ointment until a smooth paste was obtained. The patient was asked to apply it twice daily. At the beginning and every week, the healing of the ulcer was assessed, the area was calculated and photographs were taken The wound area was calculated using the formula for an ellipse: Length × width × 0.7854 as done in the preparation of the PRF matrix.

Results

Table 1: Demographic details of patients

Age group (in years)	Number of patients	Percentage (%)
20-30	3	15
30-40	2	10
40-50	7	35
50-60	6	30
60-70	2	10
Total	20	100

A total of 20 cases of non-healing ulcers of varying etiologies were treated using autologous platelet rich fibrin (PRF) at weekly intervals for maximum frequency of 6 sittings respectively. The mean age of the patients was 39.7 ± 13.02 years. Out of 20 ulcers, there were 7 (35%) venous ulcers, 3 (15%) traumatic ulcers, 6 (30%) diabetic ulcers and 4 (20%) trophic ulcers. The length of non-healing ulcer ranged from 3 months to 14 months with a mean of 6.48 ± 1.72 months. The mean duration of ulcer healing was 4.75 ± 3.45 weeks. The mean VAS score for ulcer showed a declining trend from 9.04 ± 0.09 to 2.29 ± 1.36. The reduction of pain in the ulcer was due to the anti-inflammatory property of platelet rich fibrin matrix. We observed

no significant complications in the entire duration of the study.

Table 2: Improvement in both groups

Groups	Initial Size (mean ±SD)	Final Size (mean± SD)	P
Group A	10.850±5.150	2.600±1.630	0.0002
Group B	10.050±4.850	5.250±3.170	0.015

Group A showed a mean reduction in the ulcer area by 8.26 mm2 (75.90%) which is highly significant (P-value = 0.0002). Group B showed a mean reduction in the ulcer area by 4.500 mm2 (45%) which is also significant (P-value = 0.015)

Table 3: Improvement Percentage

Groups	Group A	Group B	P
Improvement percentage (%)	75.90%	45%	0.0045

The statistical difference between group A and group B was significant P=0.0042 (P< 0.05).

Discussion

Chronic non-healing ulcers due to various etiologies have become cumbersome and add morbidity for patients and society. These wounds pose a greater challenge for healthcare personnel to treat. These usually present with exposed underlying tissues and further complicates the process of granulation tissue whilst simple dressing. Additionally, at the same time it presents with an equivocal challenge to maintain the viability of surrounding tissue. These non-healing ulcers need a multimodal approach for treating by ascertaining the underlying pathology and the systemic condition of the individual. Conventional approach of treatments were not able to provide growth factors to induce neovascularization at the ulcer site to initiate the healing process. [16]

PRF is an autologous platelet and leucocyte‑rich fibrin material and is an important advancement in regenerative medicine. It forms an organised network where the platelets and leucocytes are concentrated leading to the sustained release of various GFs, resulting in wound healing. Hence, it can also be used for the treatment of venous ulcers. [17] A study conducted by Margolis et al. which included 26,599 patients, concluded that patients who were treated with products derived from platelets, tend to heal faster than patients who were treated without the products derived from platelets. He also concluded that even though the ulcers that were treated with these derivatives were bigger and deeper than the other groups these showed better improvement at the end of 12 weeks. [18]

In our study, we determined the efficacy of autologous PRFM in non-healing ulcers. According to the present study, the improvement is 75.90% in the area of the ulcer. In a similar study by Anirudh Somani and Reena Rai, [19] the improvement was 80% which is much higher than the present study. According to a study by G. Yuvasri, [20] the mean reduction in the area of the ulcer size observed was 86.03%. In the present study, the improvement in the ulcer size is 45% using triple combination paste (zinc oxide, phenytoin, and mupirocin ointment). In a study by Sehgal et al. [21] zinc oxide and phenytoin paste were used in the treatment of trophic ulcers of leprosy. Complete resolution was seen in 55% of the patients. In a study by Shafer et al., [22] granulation tissue was formed in 50–90% of his patients.

PRF contains enormous platelets with fibrin. Once alpha granules in the platelets start degranulation, it releases various growth factors (transforming growth factor β, platelet derived growth factor,

epidermal growth factor, nerve growth factor) along with vitronectin, fibronectin and sphingosine 1‑phosphate which helps in enhancement of wound healing and microenvironment homeostasis. This unique organization in the form of the 3-dimentional fibrin matrix provides a binding site for platelets as well as growth factors. This flexible mesh serves as scaffold to promote cellular migration in micro-environment and perquisite in repairing and regenerating tissue. Overall, in PRF preparation, leukocyte and fibrin acts as mutual stimulatory actors by imitating the physiological process of wound healing and boosting angiogenic, osteogenic and antimicrobial activities. [23,24]

Suryanarayan S., et al. showed the mean duration of ulcer healing with PRP was 5.6 weeks. [25] Kim SA., et al. showed 90 - 100% epithelization after 15.18 days of treatment with PRP for non-healing ulcers. [26] Frykberg., et al. showed that 63 of 65 ulcers responded with a reduction in area, volume and undermining of the ulcers in a mean duration of 2.8 weeks with PRP treatments. [27]

The treatment of non-healing ulcer with this platelet concentrate is a breakthrough in terms of challenges faced during the treatment and morbidity in the quality of life of the patients. This method is comparatively fast, cost-effective and applicable without any requirement of hospitalization with benefit of reduction in hours lost for work. [28]

Conclusion

This procedure is simple, patient‑friendly, cost‑effective, painless and can be performed as an outpatient procedure. We concluded that autologous platelet-rich fibrin matrix is much more effective than the triple combination paste (zinc oxide, phenytoin, and mupirocin ointment) in the treatment of non-healing ulcers. The results of our series should encourage future prospective studies to assess the efficacy of PRF dressing in ulcers. Its distinctive advantages include use of autologous blood and low cost and limited resource requirement in its preparation. However, it's wide spread application is still limited due to poor accessibility of health care facilities by leprosy patients, disease stigmatisation and lack of manpower resources.

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