

**Assessment of the Therapeutic Efficacy of Autologous PRFM versus Triple Combination Paste in Non-Healing Ulcers**Pratap Chandra Das<sup>1</sup>, Aswini Kumar Das<sup>2</sup>, Pallavi Kashyap<sup>3</sup><sup>1</sup>Assistant Professor, Department of Skin & V.D, Lord Buddha Koshi Medical College and Hospital, Saharsa, Bihar, India<sup>2</sup>Associate Professor, Department of Skin & V.D, Lord Buddha Koshi Medical College and Hospital, Saharsa, Bihar, India<sup>3</sup>Assistant Professor, Department of Skin & V.D, Lord Buddha Koshi Medical College and Hospital, Saharsa, Bihar, India

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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 Skin and V.D for two years. A total of 50 patients were included in the study with the age range 20–70 years. Patients with non-healing ulcers were selected from the outpatient department.**Results:** A total of 50 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. Most of the patients belonged to the age group 40-50 years. The mean age of the patients was  $36.4 \pm 12.08$  years. Out of 25 ulcers in group A, there were 8 (32%) venous ulcers, 3 (12%) traumatic ulcers, 7 (28%) diabetic ulcers and 6 (24%) trophic ulcers. Out of 25 ulcers in group B, there were 8 (32%) venous ulcers, 5 (20%) traumatic ulcers, 8 (32%) diabetic ulcers and 4 (16%) trophic ulcers. The length of non-healing ulcer ranged from 3 months to 14 months with a mean of  $6.54 \pm 1.78$  months. Group A showed a mean reduction in the ulcer area by  $8.23 \text{ mm}^2$  which was highly significant (P-value = 0.0002). Group B showed a mean reduction in the ulcer area by  $4.79 \text{ mm}^2$  which was 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

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**Introduction**

A non-healing ulcer is defined as an ulcer that fails to undergo healing while undergoing continuous therapy for a duration of at least 3 months. It is prevalent among adult men and causes significant health issues, greatly affecting their quality of life. This condition is characterised by uncomfortable symptoms such as discomfort, difficulties in walking, and disrupted sleep. They may occur as a consequence of arterial disease, venous disease, diabetic neuropathy, metabolic disorders, haematological disorders, and infective illnesses. Autologous platelet-rich fibrin (PRF) is a straightforward and cost-effective approach for treating ulcers that do not heal. It supplies the essential polypeptide growth factors that improve tissue repair. One example is the transforming growth factor beta (TGF)-beta, which has strong

profibrotic properties and great potency. These elements promote fibrosis and the deposition of collagen under the skin, enhancing biomechanical strength, as well as facilitating epithelial resurfacing and differentiation. Recent studies indicate that autologous platelet-rich fibrin matrix (PRFM) has a high concentration of growth factors. It is efficacious in the management of persistent non-closing ulcers. [1-3]

The continuous use of phenytoin sodium leads to gingival hyperplasia, which is caused by inflammation and/or fibrosis. This discovery has led to the investigation of its function in the process of wound healing. [4-6] Phenytoin in the concentrations enhances the functioning of fibroblasts, reduces the activity of collagenase, and promotes the development of granulation tissue.

Mupirocin ointment has antimicrobial properties. Treatment options for venous ulcers include a range of methods, such as the use of compression stockings, meticulous wound care, and even surgical interventions. The therapy is often challenging and typically linked to a high likelihood of recurrence. [7-9] Dressings are crucial in facilitating the healing process of these ulcers. Saline-soaked occlusive dressings are recognized for their ability to enhance wound healing. Platelet concentrates have been extensively used in regenerative medicine for their inclusion of several growth factors such as transforming growth factor (GF) F, platelet-derived GF, vascular-endothelial GF, platelet derived epidermal GF, insulin-like GF-I, and basic fibroblast GF, which contribute to the promotion of wound healing.

Tissue engineering has shown the ability to regenerate tissue without causing any long-term complications. Multiple studies have shown that the human skin has the capacity to regenerate by using existing populations of stem cells. Stem cells possess the capacity for self-renewal and the ability to differentiate into specialized daughter cells with specified functions. Regenerative medicine plays a crucial role in controlling these populations of stem cells to enhance skin regeneration. [10]

The objective of this research was to evaluate and compare the effectiveness of autologous PRFM (Platelet-Rich Fibrin Matrix) with a triple combination paste in the treatment of non-healing ulcers.

### Methods

The present clinical trial was conducted in the Department of Skin & V.D, Lord Buddha Koshi Medical College and Hospital, Saharsa, Bihar, India for two years. A total of 50 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 25 patients on autologous PRFM.

Group B consisted of 25 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. 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 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  $\times$  width  $\times$  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. [11,12]

### 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  $\times$  width  $\times$  0.7854 as done in the preparation of the PRF matrix.

## Result

**Table 1: Demographic details of patients**

Age group (in years)	Group A N (%)	Group B N (%)
20-30	4 (16)	5 (20)
30-40	3 (12)	3 (12)
40-50	10 (40)	12 (48)
50-60	5 (20)	3 (12)
60-70	3 (12)	2 (8)
Total	25	25
<b>Ulcers</b>		
Venous ulcers	8 (32)	8 (32)
Traumatic ulcers	3 (12)	5 (20)
Diabetic ulcers	7 (28)	8 (32)
Trophic ulcers	6 (24)	4 (16)

A total of 50 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. Most of the patients belonged to the age group 40-50 years. The mean age of the patients was  $36.4 \pm 12.08$  years. Out of 25 ulcers in group A, there were 8 (32%)

venous ulcers, 3 (12%) traumatic ulcers, 7 (28%) diabetic ulcers and 6 (24%) trophic ulcers. Out of 25 ulcers in group B, there were 8 (32%) venous ulcers, 5 (20%) traumatic ulcers, 8 (32%) diabetic ulcers and 4 (16%) trophic ulcers. The length of non-healing ulcer ranged from 3 months to 14 months with a mean of  $6.54 \pm 1.78$  months.

**Table 2: Improvement in both groups**

Groups	Initial Size (mean $\pm$ SD)	Final Size (mean $\pm$ SD)	P
Group A	10.845 $\pm$ 5.150	2.615 $\pm$ 1.630	0.0002
Group B	10.055 $\pm$ 4.850	5.265 $\pm$ 3.170	0.015

Group A showed a mean reduction in the ulcer area by  $8.23 \text{ mm}^2$  which was highly significant (P-value = 0.0002). Group B showed a mean reduction in the ulcer area by  $4.79 \text{ mm}^2$  which was also significant (P-value = 0.015).

## Discussion

Chronic non-healing ulcers caused by several factors have grown burdensome and contribute to illness for individuals and society. These injuries provide a more formidable obstacle for healthcare staff to address. These often manifest as exposed underlying tissues, which further complicates the formation of granulation tissue with the application of a simple dressing. Furthermore, it simultaneously poses a difficult task in preserving the vitality of the surrounding tissue. To effectively treat these ulcers that do not heal, a comprehensive strategy is required. This involves determining the root cause of the ulcers and evaluating the overall health state of the person. The traditional methods of therapy were unable to provide the necessary growth factors to stimulate the formation of new blood vessels at the ulcer location and commence the process of healing. [13]

PRF is a substance made from the patient's own platelets and white blood cells, and it is a significant development in the field of regenerative medicine. An ordered network is formed, in which platelets and leukocytes are concentrated, leading

to the continuous release of different growth factors (GFs) that promote wound healing. Therefore, it may also be used for the management of venous ulcers. [14] A research done by Margolis et al. including 26,599 individuals found that patients treated with platelet-derived products had quicker healing compared to those treated without such products. In addition, he determined that despite the ulcers treated with these compounds being larger and more profound than those in the other groups, they exhibited superior recovery after 12 weeks. [15]

Fifty instances of non-healing ulcers with different causes were treated with autologous platelet rich fibrin (PRF) at weekly intervals, with a maximum of six sessions per patient. The majority of the patients were between the age range of 40-50 years. The average age of the patients was  $36.4 \pm 12.08$  years. Among the 25 ulcers in group A, 8 (32%) were venous ulcers, 3 (12%) were traumatic ulcers, 7 (28%) were diabetic ulcers, and 6 (24%) were trophic ulcers. Among the 25 ulcers in group B, there were 8 (32%) venous ulcers, 5 (20%) traumatic ulcers, 8 (32%) diabetic ulcers, and 4 (16%) trophic ulcers. The duration of non-healing ulcer varied between 3 months and 14 months, with an average of  $6.54 \pm 1.78$  months. Group A exhibited a statistically significant reduction in ulcer area, with a mean decrease of  $8.23 \text{ mm}^2$  (P-value = 0.0002). Group B had a statistically

significant mean decrease in ulcer area of 4.79 mm<sup>2</sup> (P-value = 0.015). In comparable research conducted by Anirudh Somani and Reena Rai, the observed enhancement was 80%, surpassing the results of the current investigation. [16] G. Yuvasri's research found that the average decrease in the size of the ulcer area was 86.03%. [17] The current research demonstrates a 45% reduction in ulcer size when using a triple combination paste consisting of zinc oxide, phenytoin, and mupirocin ointment. Sehgal et al. [18] conducted research where they used zinc oxide and phenytoin paste to treat trophic ulcers caused by leprosy. They observed complete remission in 55% of the patients. Shafer et al. conducted research where granulation tissue developed in 50-90% of their subjects.

Platelet-rich fibrin (PRF) consists of large platelets together with fibrin. Upon degranulation, alpha granules in platelets release a variety of growth factors (including transforming growth factor  $\beta$ , platelet derived growth factor, epidermal growth factor, and nerve growth factor) as well as vitronectin, fibronectin, and sphingosine 1-phosphate. These substances play a crucial role in promoting wound healing and maintaining a stable microenvironment. The 3-dimensional fibrin matrix serves as a distinctive structure that both binds platelets and facilitates the presence of growth factors. This versatile mesh functions as a scaffold to facilitate the movement of cells in a small-scale environment, which is essential for the process of tissue repair and regeneration. In PRF preparation, leukocytes and fibrin mutually stimulate each other by mimicking the natural wound healing process and enhancing angiogenic, osteogenic, and antibacterial properties. [19,20,21] In a study conducted by Suryanarayan S. et al., it was shown that the average time required for ulcer healing with platelet-rich plasma (PRP) was 5.6 weeks. [22] Kim SA., et al. shown that the application of platelet-rich plasma (PRP) resulted in complete epithelization in 90 - 100% of non-healing wounds during a treatment period of 15.18 days. [23] Frykberg et al. demonstrated that out of 65 ulcers, 63 exhibited a decrease in size, volume, and undermining after an average treatment period of 2.8 weeks using PRP therapy. [24]

### Conclusion

This technique is uncomplicated, user-friendly, economical, painless, and may be conducted on an outpatient basis. Our findings indicate that autologous platelet-rich fibrin matrix is significantly superior than the triple combination paste (consisting of zinc oxide, phenytoin, and mupirocin ointment) for treating non-healing wounds. The outcomes of our series should motivate future prospective studies to evaluate the effectiveness of PRF dressing in ulcers. The

notable benefits of this method include the use of the patient's own blood and its cost-effectiveness, as well as its little need for resources throughout the preparation process. Nevertheless, the extensive use of leprosy treatment is now constrained by the inadequate availability of healthcare facilities for leprosy patients, the social stigma associated with the illness, and the scarcity of human resources.

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