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THE AUTOLOGOUS PLATELET RICH FIBRIN MATRIX(PRFM) AS CELL THERAPY IN THE TREATMENT OF CHRONIC NON- HEALING ULCERS- A PROSPECTIVE STUDY

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ABSTRACT

Background: Non-healing ulcers are a major cause of disability and impairment, interfering with physical and mental well-being of patients chronic non- healing ulcers. They are difficult to manage having longer duration of treatment and increased financial burden. Minimizing the duration of healing can be a major step in rehabilitation of such patients. Platelet rich fibrin (PRF) is a newer modality to hasten wound healing. It is a concentration of platelets suspended in plasma extracted after centrifuging patient's own blood.
Aim: To study the efficacy of platelet-rich fibrin (PRF) in management of non-healing ulcers.
Methodology- Prospective Study was conducted in the Department of Dermatology, Venereology, and Leprology, Nalanda Medical College and Hospital, Patna.with Sample size of 60 patients over a period of 18 months.
Results: The mean age of cases in our study was 48.41±11.52 years. The average percentage improvement in area and volume was 97.12% and 98.72% respectively. The mean duration of healing was found to be 2.41 weeks. The procedure was safe with no noted adverse events.
Conclusion-PRF is an inexpensive, safe, feasible and effective procedure in the management of non-healing ulcers.

Keywords- Platelet rich fibrin, PRF , Non-healing ulcer.

INTRODUCTION

Chronic ulcers, also known as non-healing ulcers, are characterised as spontaneous or traumatic lesions, usually found in the lower extremities, that do not respond to initial treatment or persist despite appropriate care and fail to heal within a specific timeframe. These ulcers may be caused by systemic diseases or local disorders[1,2]. There are several categories of ulcers that do not heal well, including venous, arterial, diabetic, pressure, and traumatic ulcers. The typical wound healing process is characterised by its dynamic and intricate nature, consisting of three distinct phases: inflammation, tissue creation, and tissue remodelling. If the normal healing process is disrupted, the absence of growth hormones and cytokines, which impede the healing process, might lead to the chronic character of an ulcer [3]. Chronic ulcers, specifically those associated with venous illness, diabetes, or arterial disease, frequently impact the lower extremities [2]. Estimates indicate that there are between 2 to 6 million individuals in the United States who experience chronic non-healing ulcers. The global prevalence of this condition varies between 1.9% and 13.1% [5, 6]. With the increasing ageing population and the growing frequency of risk factors such as smoking, obesity, and diabetes, it is expected that the incidence of chronic ulcers will also increase. Based on a 2.5% mortality rate associated with wounds, it is estimated that around 10% of the population will suffer from a persistent wound at some stage in their lifetime [6]. These ulcers impose a substantial economic burden on both the patient and the healthcare system [7], while also negatively impacting the patient's quality of life and productivity.

According to research, foot or ankle ulcers are present before more than 85% of lower limb amputations occur. Diabetes is a major contributor to non-traumatic amputations globally. Between 15% and 25% of individuals diagnosed with diabetes experience the development of foot ulcers, and out of this group, approximately 12% require amputation of the lower extremities [2]. Individuals with diabetic foot ulcers are susceptible to infection, and the persistent non-healing ulcers resulting from diabetic neuropathy impede the healing process. The main cause of chronic lower extremity ulcers is venous illness, as the increased pressure in the veins destroys the walls of the blood vessels, leading to the breakdown of the skin [2]. Venous non-healing ulcers account for approximately 75-80% of all vascular ulcers, and they are seen in 1-2% of the general population [1, 9, 10].

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The primary goal of ulcer treatment is to expedite the process of wound healing. The standard approach to treating non-healing ulcers involves cleaning the wound, removing dead tissue, preventing and diagnosing infection, relieving pressure on the affected area, controlling blood sugar levels, and using dressings for local ulcer care [2,11,12]. Nevertheless, there exist specific risk factors that frequently impact and contribute to inadequate wound healing. These encompass: 1) Local factors, such as the presence of debris or dead tissue, infection in the ulcer, reduced oxygen supply to the tissues, and repetitive injury; 2) Systemic conditions, such as diabetes mellitus, weakened immune system, or inadequate nutrition; and 3) Medications, such as corticosteroids [3]. The conventional treatment options for non-healing ulcers effectively target these issues and provide optimal local therapy for ulcers by eliminating dead tissue, creating a moist environment for wound healing, reducing local pressure, managing infection through the use of antibiotics, antiseptics, and topical antibacterial agents, addressing ischemia, and medically managing any accompanying health conditions.Hyperbaric oxygen therapy, skin grafting, vacuum aided closure (VAC), and surgical interventions such as angioplasty and reconstructive surgery are among the sophisticated treatments available for non-healing ulcers [3, 13, 14].

Many chronic ulcers reoccur or persist for extended periods of time, even after receiving therapy. This requires the use of more advanced wound care therapies. Cellular therapy has emerged as a notable advancement in vascular therapeutics for the treatment of non-healing ulcers. The blood and platelet concentrate contains a diverse range of cytokines and growth factors, which are crucial for utilising the patient's own body cells in the healing of wounds and ulcers. These modular treatment solutions are devoid of any adverse effects and are characterised by their safety and efficiency. Platelet-rich fibrin (PRFM) therapy, a recently developed cellular therapy, has gained considerable interest for its potential application in regenerative medicine as a therapeutic agent for various chronic conditions. It can serve as an adjunctive component in a well-structured and effective treatment plan [13,15]. Autologous platelet-rich fibrin matrix (PRFM), a suspension of platelets in plasma derived from the patient's own blood, is being increasingly employed in clinical praxis for the treatment of chronic ulcers.Compared to whole blood, PRFM has a much increased concentration of platelets, ranging from 2 to 6 times higher [1, 16]. Platelets function as a natural storage for various growth factors that have healing characteristics and play an active role in tissue regeneration. This is the basis for the therapeutic effects of PRFM [15].

PRFM is being employed as a novel alternative technique in various medical disciplines, including dentistry, traumatology, cosmetic surgery, ophthalmology, and dermatology. Growth factors initiate the vital processes of chemotaxis, angiogenesis, cell proliferation, and differentiation, which are crucial for tissue repair and regeneration.

Platelets contain several factors. Furthermore, several studies have been published [17] that examine the efficacy of platelet-rich plasma in treating non-healing ulcers. Prior to administration, PRFM is commonly mixed with thrombin to generate a fibrin gel and an exudate that is abundant in platelet growth factors[18].The -granules of thrombin activated platelets release a variety of growth factors that can regulate cell proliferation, differentiation, and accelerate soft tissue healing in vivo [19] [20].

Based on a recent meta-analysis examining the application of PRFM therapy in cutaneous wounds, it was found that PRFM had a substantial positive impact on ulcers in small, challenging-to-heal acute and chronic wounds. Additionally, PRFM was found to enhance the process of wound healing when compared to standard wound care practises [21,22]. Moreover, platelets exhibit antimicrobial properties against certain skin bacteria, and clinical evidence suggests that wounds treated with PRFM experience reduced infection rates [22]. Hence, PRFM therapy provides several advantages that can serve as a valuable and effective treatment approach for tiny, recalcitrant ulcers [23].

A significant advancement has been made in the promotion and speeding up of the healing process of soft tissues by utilising autologous platelet rich plasma to treat non-healing wounds. PRFM therapy delivers a diverse range of signalling cytokines and growth factors that play a vital role in tissue healing through several processes, such as regulating inflammation, promoting the formation of new blood vessels, and facilitating the synthesis and restructuring of new tissue [15]. This facilitates the establishment of a biological milieu within the body that is very conducive to the restoration of tissue homeostasis. PRFM is a safe therapy option with excellent clinical outcomes due to its simplicity, affordability, and superior effectiveness compared to other traditional treatments. Additionally, it is derived from the patient's own cells and does not contain any disease-causing bacteria. There has been a significant resurgence of interest in the field of autologous platelet or fibrin biologics.

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The role of platelets in wound healing is clearly elucidated. In order to gain a deeper comprehension of their function in the process of healing, there have been limited investigations conducted on the molecular level to examine the impact of platelets on wounded skin, specifically in terms of gene expression, cell survival, and effectiveness.

Recent research has shown that autologous Platelet Rich Fibrin Matrix (PRFM), which contains a high concentration of growth factors, is a successful treatment for persistent ulcers that do not heal.Thus, employing blood extract as a means to promote the development of a persistent ulcer that is not healing is an intriguing, secure, and costly approach that poses no risk of an allergic response.We examined a collection of cases that demonstrated the use of autologous platelet-rich fibrin matrix (PRFM) in the treatment of non-healing chronic ulcers. The wound area was determined by applying the formula for an ellipse, which is derived by multiplying the length and breadth of the wound and then multiplying the result by 0.7854. This calculation assumes that the shape of the wound more closely resembles an ellipse rather than a square or rectangle. The volume was determined by multiplying the area by the depth, according to the formula.

MATERIALS&METHODS

Prospective Study was conducted in the Department of Dermatology, Venereology, and Leprology, Nalanda Medical College and Hospital, Patna.with Sample size of 60 patients over a period of 18 months.

Criteria for inclusion

• Patients who are willing to participate in the study. • Patients between the ages of 18 and 80. • Inpatients with nonhealing ulcers lasting more than four weeks, who have received therapy, are eligible for inclusion in the study.

The study included chronic non-healing ulcers caused by Hansen's disease, traumatic injuries, arterial issues, venous problems, and other factors. Exclusion criteria for the study included patients who were not willing to participate, patients already undergoing treatment for an active infection, and patients with...

→Age group below 18 years → Having a history of bleeding disorders

→Anaemia with a haemoglobin level below 10g% and other haematological disorders. →Platelet count below 1.5 lakhs per cubic millimeter. →Currently taking anticoagulant medicine such as Aspirin, Warfarin, or Heparin. →Uncontrolled diabetic mellitus with malignant ulcers. →

Study Design • The study was conducted under the approval of the institutional ethics committee.

• Every patient was provided with a detailed explanation of the study's nature in their respective language, and their consent was obtained.

• Participants were included in the study for a period of three months and were monitored on a weekly basis for up to five weeks, or until complete healing occurred, whichever came first.

• The case record performer would document a comprehensive record of demographic and clinical characteristics. A thorough investigation was conducted to trace the accurate historical account. Photographs of the patients were captured digitally and stored during every visit.

• Initial assessments were conducted: A comprehensive blood analysis including complete blood count, fasting and postprandial blood sugar levels, bleeding time, clotting time, liver function tests, and kidney function tests were performed to exclude any pre-existing medical issues.

Elisa tests for HIV1&2, HBsAg, and AntiHCV were performed in all instances.

• Specific studies include: - Colour Doppler to assess vascular causes - HbA1C and Slit skin smear for Acid-fast bacilli to evaluate neuropathic causes.

If the ulcers exhibit signs of secondary infection, it is advisable to do surgical debridement and administer both topical and systemic antibiotics as needed prior to initiating treatment. Subsequently, over a period of 7-10 days, if the infection was successfully managed, the PRFM surgery was performed.

RESULTS

Table1 Age and Gender distribution of subjects

Age	Number	%	Pvalue
22-30	5	8.3%	
31-40	11	18.3%	

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41-50	18	30%	0.001
51-60	17	28.3%	
>60	9	15%	
Total	60	100%	
Mean±SD	48.41±11.52		
Male	38	63.3%	
Female	22	36.7%	

Table 2 Residence and Occupation distribution of subjects

RESIDENCE	Number	%	Pvalue
Rural	38	63.3%	0.166
Urban	22	36.7%	
Total	60	100%	
Mean±SD	30.00±11.31		
OCCUPATION			
Businessman	1	1.7%	0.024
Businesswomen	1	1.7%	
Clerk	1	1.7%	
Driver	4	6.7%	
Farmer	16	26.7%	
Housewife	6	10%	
Labour	21	35%	
Shopkeeper	3	5%	
Student	1	1.7%	
Teacher	1	1.7%	
Technician	1	1.7%	
Other	4	6.7%	
Mean±SD	5.00±6.60		

Table 3 ETIOLOGY of patients

ETIOLOGY	Number	%	Pvalue
Arterial	2	3.3%	0.146
Diabetic	5	8.3%	
Hansen	39	65%	
Traumatic	4	6.67%	
Venous	5	8.3%	
Others	5	8.3%	
Total	60	100%	
Mean±SD	10.00±14.25		

Table 4 SITE distribution of patients

SITE	Number	%	Pvalue
DLH	1	1.7%	0.001
LH	9	15%	
LLM	1	1.7%	
LMM	2	3.3%	
LPBT	1	1.7%	
LRF	1	1.7%	
LS	1	1.7%	
LSM1	4	6.7%	
LSM2	1	1.7%	
LSM23	1	1.7%	
LSM3	2	3.3%	
PLBT	1	1.7%	
PRBT	1	1.7%	
RE	1	1.7%	
RH	7	11.7%	
RK	1	1.7%	

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RLM	4	6.7%	
RMM	3	5%	
RPBT	4	6.7%	
RSM1	9	15%	
RSM2	3	5%	
RSM3	2	3.3%	
Total	60	100%	
Mean±SD	2.72±2.54		

Table 5 Duration (in month) distribution of subjects

Duration	Number	%	Pvalue
1.7-3	11	18.33%	0.001
3.1-6	27	45%	
6.1-9	15	25%	
9.1-12	4	6.67%	
>12	3	5%	
Total	60	100%	
Mean±SD	6.03±2.99		

Table 6 Mean value of Area (cm2) and Volume (cm3) distribution

	Mean±SD	't'test	Pvalue
INITIAL AREA	6.76±3.85	13.581	0.001
AFTER 1ST SITTING	5.15±3.03	13.146	0.001
AFTER 2 ND SITTING	3.42±2.43	10.692	0.001
AFTER 3 RD SITTING	2.01±1.87	8.040	0.001
AFTER 4 TH SITTING	1.03±1.58	4.883	0.001
FINAL AREA	0.94±1.97	3.669	0.001
INITIALVOLUME	6.65±4.64	11.103	0.001
AFTER 1 ST SITTING	4.92±3.43	11.105	0.001
AFTER 2 ND SITTING	3.32±2.38	10.634	0.001
AFTER 3 RD SITTING	1.95±1.70	8.591	0.001
AFTER 4 TH SITTING	0.77±1.14	5.078	0.001
FINAL VOLUME	0.55±1.14	3.733	0.001

Table 7 Sessions distribution of patients

Sessions	Number	%	Pvalue
2	5	8.33%	0.001
3	29	48.33%	
>4	26	43.33%	
Total	60	100%	
Mean±SD	4.11±1.02		

DISCUSSION

Doctors face challenges in treating chronic non-healing ulcers. Several treatment modalities for non-healing wounds include routine dressings, vacuum-assisted closure, hyperbaric oxygen therapy, reconstructive surgery, and surgical debridement. The use of topical platelet derived growth factors for treating chronic non-healing ulcers is permitted by the FDA. However, it is expensive and not affordable in underdeveloped countries. PRFM serves as a cost-effective, secure, and uncomplicated alternative to the aforementioned process.

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The initial investigation that showcased the favourable impact of growth factors with localised action was conducted in 1986 by Knighton et al. [24]. Platelets contain a significant quantity of growth factors, cytokines, and chemokines. Macrophages play a crucial role in the initial phases of wound healing by actively participating in the processes of inflammation and tissue repair. This attribute of platelets gives rise to the concept of utilising platelets as a therapeutic tool for non-healing wounds [25,26].The process of isolating platelets from blood and putting them to a nonhealing ulcer can shift the ulcer from an inflammatory phase to a proliferative phase due to the platelets' anti-inflammatory properties.

PRFM was initially developed by Choukroun et al.[27] for application in oral and maxillofacial surgery. The production of PRFM is a straightforward process that does not necessitate the use of any anticoagulant. The Choukroun PRF, sometimes referred to as blood centrifugation, is a method for obtaining it. Since anticoagulants are absent, there is no need to activate platelets, unlike in the case of PRP. Platelets get activated upon contact with the walls of the tube, initiating the coagulation cascade. At the end of centrifugation, three distinct layers are formed: the base layer consisting of red blood cells, the top layer of acellular plasma, and a clot of platelet-rich fibrin in the centre.The PRF does not, unlike PRP.

The dissolution process occurs rapidly, while the formation of a robust fibrin matrix takes place gradually, resembling the remodelling of a normal blood clot (Dohan et al., 2006).

A study conducted by Yazawa et al. (2003) demonstrated that Platelet-Rich Fibrin (PRF) yields better outcomes compared to Platelet-Rich Plasma (PRP). The reason for this is because when included in drug delivery systems like fibrin, the average concentration of growth factors in the platelet concentrates was three times or greater than what was reported with traditional platelet-rich plasma. The release of growth factors from the fibrin matrix occurs gradually over a period of one week. It has been discovered that this leads to more effective healing of ulcers compared to platelet-rich plasma (PRP) (Dohan et al., 2006).

A study conducted by Suchetha et al. demonstrated that platelet-rich plasma (PRP) has a greater platelet concentration in comparison to platelet-rich fibrin (PRF). Nevertheless, the enhanced efficacy of PRF in comparison to PRP can be elucidated by the research conducted by Yazawa et al. This study demonstrated that when PRF is integrated into drug delivery systems like fibrin, the average concentration of growth factors in the platelet concentrates is three times or more than what is observed with conventional platelet-rich plasma. In addition, the growth factors were systematically released over a period of approximately one week (Suchetha et al).

Dohan et al. also demonstrated that PRFM exhibited a slower release of growth factors compared to PRP, and they observed superior healing qualities with PRF.

Steenvoorde et al. conducted a study on the application of autologous platelet-rich fibrin (PRF) utilising Vivostat PRF on a variety of challenging wounds that are difficult to cure. They successfully achieved complete wound closure in eight wounds and reduced the diameter by up to 66% in three wounds. The other two wounds only experienced a reduction in depth, requiring an average of 2.2 applications. The average duration of treatment was 4.2 weeks, and there were no negative side effects observed.

CONCLUSION

The use of PRFM for the treatment of trophic ulcers is a viable, secure, uncomplicated, and cost-effective approach. The use of the treatment was problem-free and no complications were recorded, resulting in high patient compliance and satisfaction.

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