"STUDY OF EFFICACY OF AUTOLOGOUS PLATELET RICH FIBRIN VERSUS PLATELET RICH PLASMA AS A REGENERATIVE MEDICINE STRATEGY FOR CHRONIC CUTANEOUS ULCERS"

Submitted by

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Under the guidance of

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I hereby declare that this dissertation entitled **"STUDY OF EFFICACY OF AUTOLOGOUS PLATELET RICH FIBRIN VERSUS PLATELET RICH PLASMA AS A REGENERATIVE MEDICINE STRATEGY FOR CHRONIC CUTANEOUS ULCERS"** is a bonafide and genuine research work carried out by me under the guidance of **DR. ARUN C. INAMADAR** M.D, D.V.D, F.R.C.P, Professor and HOD, Department of Dermatology, Venereology and Leprosy at BLDE (Deemed to be University) Shri B. M. Patil Medical College Hospital and Research Centre, Vijayapura.

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LIST OF ABBREVIATIONS

PRF - Platelet rich fibrin

PRP – Platelet rich plasma

IGF - Insulin-like growth factor

EGF - Epidermal growth factor

VEGF - Vascular endothelial growth factor

TGF - b - Transforming growth factor - beta

PDGF - Platelet derived growth factor

VLU - Venous leg ulcer

MLU - Mixed leg ulcer

ALU - Arterial leg ulcer

HYTILU - Hypertensive ischaemic leg ulcers

DFU - Diabetic foot ulcer

CVI - Chronic venous insufficiency

CEAP - Clinical, Etiological, Anatomical, and Pathophysiological classification

NPUAP - National Pressure Ulcer Advisory Panel

DUSS - Diabetic ulcer severity score

SINBAD - Site, Ischaemia, Neuropathy, Bacterial infection And Depth

ABPI - Ankle/ brachial pressure index

RCT - Randomized control trials

SSB - Short - stretch bandages

GM-CSF - Granulocyte-macrophage colony-stimulating factor

TNF - a - Tumour necrosis factor - a

ACD-A - Anticoagulant Citrate Dextrose Solution, Solution A

L-PRF - leukocyte - platelet rich fibrin

L-PRP - Leukocyte - platelet rich plasma

P-PRP - Pure platelet rich plasma

P-PRF - Pure platelet rich fibrin

VAS - Visual Analogue Scale

ABSTRACT

Introduction

Management of non-healing ulcers is a major challenge clinically. Current therapies include debridement, offloading etc. which show poor response. Newer modalities include stem cells, platelet derived growth factors and fibrin glues which reduce the healing time of chronic wounds. Platelets have a major role in wound healing through the secretion of growth factors, chemokines etc.

Aims & Objective

To study the comparative efficacy of autologous Platelet Rich Fibrin (PRF) versus Platelet Rich Plasma (PRP) as a regenerative medicine strategy for chronic cutaneous ulcers.

Materials & Methods

Patients with ulcers of duration >6 weeks were enrolled for a comparative study comprising of two groups. A total of 42 cases of chronic cutaneous ulcers were evaluated in the study. The 42 ulcer cases were divided into group A receiving weekly PRF dressings and group B receiving weekly PRP dressing for a maximum of 6 weeks. Ulcer evaluation was performed at baseline, each weekly dressing and at 2 week follow-up post final procedure.

Results

Primary efficacy was assessed by percentage reduction of ulcer size and patients with 100% re-epithelization at 8 weeks. 95.2% of ulcers in Group A and 90.4% ulcers in Group B showed complete re-epithelization. One ulcer in group A and two ulcers in group B showed development of infections during procedure, hence procedure had to be abandoned. The ulcers developing infection were also associated with pain and sero-purulent discharge. Recurrence of ulcer was seen in 4 ulcers in PRF group and 3 ulcers in PRP group, however these did not occur during the duration of follow-up.

Conclusion

Dressings done with platelet rich fibrin (PRF) and platelet rich plasma (PRP) showed similar efficacy in percentage reduction in volume of ulcer and re-epithelization of chronic cutaneous ulcers. Both forms of dressings were associated with similar complications of infection and recurrence. PRF and PRP dressings provide a safe, efficacious and inexpensive regenerative medicine strategy in the healing of chronic cutaneous ulcers.

Keywords: Chronic cutaneous ulcers, platelet rich fibrin, platelet rich plasma

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INTRODUCTION

INTRODUCTION

An ulcer is the breach of the continuity of skin, epithelium or mucous membrane caused by sloughing out of inflamed necrotic tissue.[1]

Chronic ulcers(wounds)are defined as ulcers that have failed to proceed through the orderly process that produces satisfactory anatomic and functional integrity or that have proceeded through the repair process without producing an adequate anatomic and functional result.[2]

The wounds which have not healed in 6 weeks are generally considered chronic.[2] Skin ulcers which occur in traumatized or vascular compromised soft tissue are also a major component of chronic non healing ulcers.[2] Repeated trauma, poor perfusion or oxygenation, and/ or excessive inflammation contribute to the causation and the perpetuation of the chronicity of ulcers.[1]

The management of non healing ulcers is a major challenge clinically. Current therapies include debridement, offloading and supplementary treatments.[1] However, the response to treatment is often poor and the outcome disappointing. These wounds place a limb at risk of infection and amputation and also puts the patients at risk of mortality.[1] Newer modalities of treatment include the use of stem cells, platelet derived growth factors, fibrin glues etc which increase the response time of healing chronic wounds.[2]

Blood platelets have a major role in the initiation of cutaneous wound healing.[2] Due to their essential role in homeostasis, platelets are deployed to sites of injury or infection to modulate inflammatory processes through the secretion of growth factors, chemokine and other inflammatory mediators.[2] The majority of secreted substances found in platelets are

localised within granules.[3] Numerous growth factors with healing roles are released by activated platelets including Insulin-like growth factor (IGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), transforming growth factor-b1 (TGF-b1) and platelet derived growth factors (PDGF).[3]

Platelet rich preparations are a safe, reliable and cost-effective means to accelerate the healing and improve the probability of efficient repair following injury.[4] Autologous PRP is a platelet suspension in plasma derived from whole blood that is increasingly being used in clinical practice for the treatment of chronic ulcers. The concentration of platelets in PRP is 2–6 folds higher than that of whole blood.[4]

Platelet rich Fibrin (PRF) is a new member of platelet concentrates developed by Choukroun et al.[5] It is classified as one of the four families of platelet concentrates as PRF is a cross between fibrin glue and classic platelet concentrates. Platelet and growth factors are theoretically trapped in the fibrin clot as platelets are not measured and growth factors in the exudate are well below the other PRP preparations.[6]

Currently, platelet rich plasma (PRP) and platelet rich fibrin (PRF) are being used widely for many purposes without side effects. Due to a lack of sufficient literature there is a need to evaluate the efficacy of platelet rich fibrin versus platelet rich plasma as a regenerative strategy on chronic cutaneous ulcers.

OBJECTIVE OF THE STUDY

OBJECTIVE OF THE STUDY:

To compare the efficacy of autologous platelet rich fibrin versus platelet rich plasma as a regenerative medicine strategy for chronic cutaneous ulcers

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The process of cutaneous wound healing is a complex, intricate interplay between a number of highly regulated factors working together to restore barrier function. In majority of superficial wounds the sequence of events occurs normally; however, it can go awry at numerous steps along the pathway, especially in case of underlying disease states such as diabetes or existing skin conditions. A chronic wound may result when wound healing does not progress normally and this is at significant burden to both the patient and the medical system.[7]

Chronic ulcers (wounds) are defined as ulcers that have failed to proceed through the orderly process that produces satisfactory anatomic and functional integrity or that have proceeded through the repair process without producing an adequate anatomic and functional result. The majority of wounds that have not healed in more than 6 weeks are considered chronic. Skin ulcers, which usually occur in traumatised or vascular compromised soft tissue, are also considered chronic in nature, and proportionately are the major component of chronic non healing ulcers. Repeated trauma, poor perfusion or oxygenation, and/ or excessive inflammation contribute to the causation and the perpetuation of the chronicity of ulcers.[1]

Physiologic wound healing:

Once a superficial wound occurs, a myriad of systems are activated at the site of injury in order to clear out the foreign material, as the primary barrier function of the skin is lost, and to restore the normal structure of the skin. Physiological wound healing is successful only to a limited degree - a wound will never reach the maximum tensile strength of normal skin, about 70% - however, most of the essential functions of the skin will return to normal.[7]

Healing of ulcer involves multiple cell populations, the extracellular matrix and the action of soluble mediators such as growth factors and cytokines. Although the process of healing is continuous, it may be arbitrarily divided into four phases:[8]

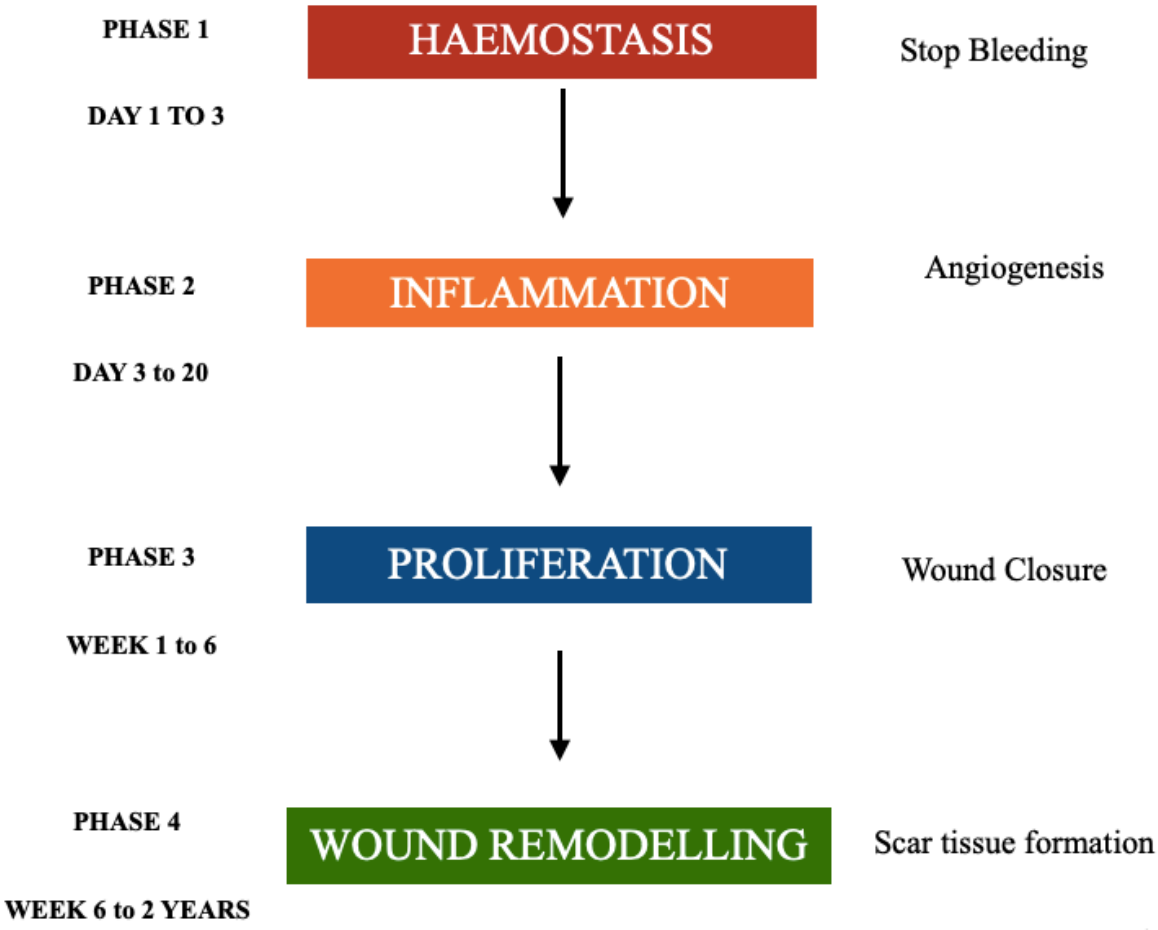
A. Coagulation and haemostasis

B. Inflammation

C. Proliferation

D. Wound remodelling with scar tissue formation

Figure 1: Phases of wound healing



The inflammatory phase of wound healing starts shortly after achieving hemostasis, and the primary goal of this phase is to clear pathogens and foreign material from the wound and also to contain the damage to a localized area. With increase in vascular permeability due to vasodilation neutrophils and monocytes localize to the wound site.[1,7]

Regulation of this phase is by a complex interplay of cytokines, which culminates in conversion of monocyte to macrophages, which is thought of as the master regulator of the inflammatory phase of wound healing. The macrophages phagocytose and digest the tissue debris and remaining neutrophils, along with secretion of growth factors and cytokines that promote tissue proliferation and cell migration.[1,7]

About 3 days after the initial wound, the proliferative phase centres around fibroblasts with production of both collagen and ground substance that forms the basis for the tissue scaffold of the previous wound area. At this time, the endothelial cells enter a rapid growth phase and angiogenesis occurs within the granulation tissue, creating a rich vascular network supplying this very active area of healing.[1,7]

After about 2–3 weeks, the wound transitions to a remodelling or maturation, phase where the collagen type is restored to usual (type I, rather than type III seen in a new wound) and the wound tissue matures which results in full cross-linking and restoration of a near normal tissue structure. The vascular network rapidly regresses as well. However, as previously mentioned, the wound strength does not reach its normal, pre-injury state.[1,7]

Etiopathogenesis of chronic ulcer:

In chronic ulcers the series of biological events that close any defect in the skin may be impaired by factors interfering with inflammation, angiogenesis, re-epithelization and wound remodelling.[9] Most wounds have a tendency to heal, however longstanding, but this process may be very slow. Recurrent injury over a previous scar, as in a leg ulcer or pressure sore, and recurrent breakdown following healing, can give rise to chronic skin wounds or ulcers that appear to have lost the capacity to heal.[9]

The chronic wound environment may also be deficient in the stimulatory growth factors, growth factor receptors or proteolytic enzymes required for growth factor activation, or may be overproducing any of these factors.[9]

Resolution of inflammation is important for wound closure. Chronic ulcer fluid has been shown to decrease the proliferation of fibroblasts, endothelial cells and keratinocytes. Fibrin accumulates in chronic ulcers (unlike acute ulcers), and forms complexes that may bind or inactivate other molecules such as growth factors.[9]

- There are four major categories of cutaneous leg ulcers: [9]

A. Venous leg ulcers (VLUs)

B. Mixed venous and arterial leg ulcers (MLUs),

C. Arterial leg ulcers (ALUs) and hypertensive ischaemic leg ulcers (HYTILUs)

D. Neuropathic ulcers (includes Diabetic Ulcers and Trophic ulcers seen in Leprosy)

Between 2% and 10% of all people with diabetes mellitus suffer from foot ulcers. The incidence rate is 2.2 to 5.9% annually. In poorly controlled diabetics, ulceration predominantly around the feet can develop. Often amputation is the last resort and risk of reamputation is high.[1]

Approximately 20% of venous ulcers do not heal after 1 year and 8% do not heal after 5 years. Annual recurrence is 6 - 15 % and most ulcers recur more than once. Thus, ulceration of lower limbs is a common complication of a wide spectrum of pathologies that cause a negative impact on the quality of life of patients.[1]

Venous leg ulcers

They are chronic skin ulcers at the gaiter area that result from chronic peripheral venous hypertension.[10]They represent the most advanced grade of chronic venous insufficiency (CVI).[10] CVI results from chronic peripheral venous hypertension caused by venous reflux and/or obstruction, or by neuro musculoskeletal dysfunction of the leg.[9]

The lifetime incidence of leg ulcers is around 1%, with a point prevalence of 0.1%.[9] Since approximately half of all leg ulcers are VLUs, the lifetime incidence of VLUs can be calculated at 0.5%, and the point prevalence at 0.05%.[10] Venous leg ulcers primarily affect individuals aged over 65 years.[9]

The CEAP classification system is used to assess venous pathology, pathophysiology and chronic venous insufficiency including venous ulcers.[10] Seven clinical categories are recognised & it classifies active VLU as C6 and healed VLU as C5.[5]

Complications and comorbidities include chronic pain and impairment of quality of life, local wound infection, systemic infection and sepsis, infestation with maggots (fly larvae), secondary squamous cell carcinoma and secondary lymphoedema (periulcer lymphoedema and/or foot and toe lymphoedema).[9]

Mixed leg ulcers

Mixed leg ulcers are venous leg ulcers in a leg with peripheral arterial disease.[9] They are more resistant to treatment than VLUs and cannot be distinguished from VLUs by clinical appearance alone. Diagnosis can only be made after vascular assessment.[9]

Arterial leg ulcers

Arterial leg ulcers are chronic skin ulceration primarily caused by skin ischaemia due to advanced peripheral artery disease. They may occur spontaneously or after minor trauma to an area of ischaemic skin that is at risk of ulceration.[9]

Clinically, there is a well-delineated zone of skin necrosis covered with eschar or remnants of necrotic wound border.[10] Steep ulcer margins are usual, with a white or black wound base, exhibiting virtually no granulation tissue[10]. Smaller ALUs are round in shape and have a ‘punched-out’ appearance. Larger ALUs are polycyclic and figurated[9].

Arterial leg ulcers generally affect the elderly, that is patients aged 60 years and above.[9] They are generally associated with obesity, smoking, diabetes, hyperlipidaemia, hypertension, coronary heart disease and stroke. Classification of severity is done by Fontaine Classification ranging from Grade I to Grade IV.[9]

Hypertensive ischaemic leg ulcers

Hypertensive ischaemic leg ulcers represent a skin infarction due to ischaemic, subcutaneous arteriolosclerosis occurring in a patient with hypertension.[9]

In more than 90% of patients the eschar or wound is located at the latero-dorsal aspect of the leg and/ or over the Achilles tendon.[9] The wound border maybe livid or violaceus to black. The ulcer usually occurs within an area of livedo.[9]

Neuropathic ulcer

A neuropathic ulcer is a form of chronic ulceration which develops in anaesthetic skin. Characteristically, neuropathic ulcers are painless, persistent and uninflamed.[9]

The underlying neuropathology is commonly a distal polyneuropathy encompassing motor, sensory and autonomic components: the vast majority of neuropathic ulcers occur in patients with type II diabetes.[10]Other causes include peripheral nerve injury, peripheral neuropathy (can be secondary to leprosy), renal failure, alcoholism, vitamin deficiencies, leprosy, pernicious anaemia, syringomyelia, tabes dorsalis, spinal dysraphism, spinal cord injury, and hereditary sensory and autonomic neuropathies.[9]

Of people with diabetes, 25% develop foot ulcers at some point in their lives.[10] In diabetic patients, hyperglycaemia leads to a complex of abnormal enzyme activity which results in a decrease in normal neuron conduction as well as nerve dysfunction and ischaemia, causing further injury to, and eventual death of, nerve cells.[9]

As patients experience sensory loss, trauma to the affected site often goes unnoticed, and progressively worsens as the area is continuously subjected to repetitive pressure and shear forces.[9]

Neuropathic ulcers typically occur on the foot, under the metatarsal heads or on the heel.[10] They tend to be surrounded by thick hyperkeratosis, and have a pink punched-out base that bleeds easily and is painless.[9] The foot tends to be anaesthetic, warm with palpable pulses and dilated veins. The skin is dry and hyperkeratotic under the forefoot and heel.[9]

According to University of Texas Wound Classification System & Wagner Grading system neuropathic ulcers can be graded from Stage A upto Stage D and from Grade 0 to Grade 3 depending upon depth of ulcers and presence or absence of infection and ischaemia.[1]

Neuropathic(Trophic) Ulcers in Leprosy

Leprosy is a chronic granulomatous infection caused by Mycobacterium leprae affecting mainly peripheral nerves and skin. The clinical spectrum of leprosy varies from a single skin patch to widespread damage to nerves, bones, and the eyes.[3]

Neural damage due to disease per se, as well as resulting from reactions, are the foremost causes of deformities and crippling in leprosy. Hence trophic ulcers developing in leprosy are neuropathic in nature.[3]

A primary impairment is a direct consequence of leprosy (e.g.madarosis, collapse of nasal bridge) and secondary impairment is not directly related to the disease (e.g. trophic ulcers).[3]

Causes of chronic ulcers seen in dermatology:

Ulcers associated with or due to systemic inflammatory conditions are often a major diagnostic and therapeutic challenge. These chronic ulcerations maybe called as "inflammatory ulcers" (i.e., pyoderma gangrenosum, vasculitic ulcers, cryoglobulinemic ulcers, etc.) because a major and primary component of their pathophysiology indeed rests on inflammation and immunologic phenomena.[11]

However, this group of ulcers also includes conditions due to microcirculatory occlusion where a primary localized inflammatory component is less obvious. Therefore, inflammatory ulcers include the two aspects of chronic ulcers that are not due to classical vascular diseases or neuropathy. [11]

Some of common dermatological causes of chronic ulcers include:[10,12]

Table 1: Common dermatological causes of chronic ulcers	
Infections	Hansen's disease
	Cutaneous Tuberculosis
	Atypical mycobacteria
	Deep fungal infections
Eczematous disorders	Chronic Eczema
Neutrophilic dermatosis	Pyoderma gangrenosum
Vasculitis	Medium & small vessel vasculitis
	Cryoglobulinemic vasculitis
Malignant disorders	Basal cell carcinoma
	Squamous cell carcinoma
Metabolic disorders	Necrobiosis lipoidica diabeticorum
Drug induced	Warfarin induced skin necrosis

Management of chronic ulcers:

Workup for treatment of chronic non healing ulcer includes clinical assessment of the ulcer and discerning underlying cause, assessment of tissue and vasculature of the ulcer and skin surrounding the ulcer. Biopsy, pus culture & sensitivity, patch testing may be done if required, depending upon the possible etiology of the ulcer.

A. **Clinical assessment:** It includes full clinical history and physical examination of the patient. History includes, duration and/ or number of recurrences of an ulcer, pain, trauma, comorbid factors, and associated medical causes. The presence of comorbid factors such as malnutrition, poor hygiene, older age group, intravenous drug abuse, obesity, varicose veins, deep vein thrombosis, and coexisting medical causes such as diabetes mellitus, peripheral arterial diseases, rheumatoid arthritis, connective tissue disorders, infections systemic vasculitis etc. adversely affect prognosis and outcome of the treatment.[13]

B. **Assessment of ulcer:** It includes the assessment of site, size, depth, edge, margins, floor, base, and condition of the surrounding skin. The site of the ulcer may give a clue to the underlying etiology of the ulcer. The size and surface area of the ulcer is determined along with clinical photography. [13] Grading of an ulcer can be done using various assessment tools such as:[1]

    a. NPUAP Guidelines

    b. The Wagner Classification System (Merritt-Wagner)

    c. University of Texas Diabetic Foot Ulcer Classification System

d. Diabetic Ulcer Severity Score (DUSS)

e. Site, Ischemia, Neuropathy, Bacterial Infection, and Depth (SINBAD) Wound Classification System

The lesser-known classification systems also include MAID, the CHS system, Margolis, Saint Elian Wound Score System, Van Acker/Peters, and Foster and Edmonds.

a. NPAUP Guidelines: According to the National Pressure Ulcer Advisory Panel an ulcer can be graded from Grade 1 to Grade 4 depending on the loss of layers and the involved tissue necrosis.[9] Only pressure injuries should be staged with the NPUAP Pressure Injury Staging System.[1]

b. The Wagner Classification System (Merritt-Wagner): Developed in the 1970s, it comprises of six ulcer grades, ranging from 0 to 5. This system can used to assess the ulcer depth and also the presence of osteomyelitis or gangrene.[1]

c. University of Texas Diabetic Foot Ulcer Classification System: The University of Texas Diabetic Foot Ulcer Classification System is frequently used in clinical practice especially for diabetic foot ulcers (DFUs) and has proved effective at predicting lower extremity amputation. It uses four grades (0–3) and four stages (A–D) to classify DFUs. The grades correspond to depth, whereas the stages account for the severity of the wound by marking the presence of infection, ischemia, or both.[1]

d. Diabetic Ulcer Severity Score (DUSS): The Diabetic Ulcer Severity Score (DUSS) is based on the categorization of wounds into specific severity subgroups for a comparison of outcomes. Assessment using the DUSS system includes the presence of

pedal pulses, the ability to probe to bone within the ulcer, and ulcer quantity and location. The sum of points determines severity, with the score ranging from 0 to 4.5[1]

e. Site, Ischemia, Neuropathy, Bacterial Infection, and Depth (SINBAD) Wound Classification System: Also used in evaluation of diabetic foot ulcers, this system uses five clinical features (site area, ischaemia, neuropathy, bacterial infection, and depth), which are graded as either present (0) or absent (1). The maximum score is 6.6.

C. **Vascular assessment:** In case of patients with lower extremity ulcers, the accurate assessment of the arterial and venous systems is necessary to establish the diagnosis and essential for adequate treatment selection. It may be done by the following methods -[13]

a. Doppler measurement of ankle/brachial pressure index (ABPI)

b. Colour flow Doppler imaging - for arterial and venous flow

D. **Biopsy:** Biopsy should be considered if the appearance of the ulcer is atypical, if etiology of underlying disease is unclear or if there is deterioration or failure to progress after 12 weeks of active treatment.[13]

E. **Culture & sensitivity:** Bacteriological swabs for culture and sensitivity should be done in case of evidence of clinical infections such as inflammation, redness, cellulitis, increased pain, purulent exudates, rapid deterioration of the ulcer, pyrexia, and foul odour. Limitations of swab cultures include the presence of numerous organisms, which may have little to no clinical relevance, no standard technique for obtaining a swab culture that may give reproducible results, inappropriate technique for taking swabs like from necrotic or nonviable tissue. They also lack the ability to differentiate between

bacteria resting on the wound surface versus infecting organisms. There are no reports in the literature that validate the use of swab cultures in chronic wounds. Gold standard for the treatment of infection is $>10^5$ colony-forming units of bacteria per gram of tissue on quantitative biopsy with the exception of β-hemolytic streptococcus, which is harmful at any level in the wound tissue.[13]

F. **Patch Testing:** Ulcers following dermatitis/eczema should be considered for patch-testing. The incidence of contact allergy may increase with the duration of ulceration. The principal sensitizers demonstrated in various studies are ingredients of applications, dressings, and bandages, with common sensitizers being lanolin, antibiotics, antiseptics, preservatives, emulsifiers, resins, and latex.[13]

Wound Care & Treatment:

The treatment of chronic ulcers is a therapeutic challenge. Treatment based on cause of ulcer should be a priority as per multiple studies. The basic principles of treatment are removal or treatment of precipitating cause such as surgical intervention; promoting circulation and improve venous return, with aids such as compression therapy; promoting healing with wound care, lifestyle changes, symptom management, and preventative care.[14]

Basic steps included in wound care are:

a. Cleaning of ulcer

b. Ulcer debridement

c. Dressing of ulcer

Cleaning of ulcer: Recommendations for cleaning of an ulcer is using simple irrigation with either normal saline compresses or plain tap water. Wounds and skin are colonized by bacteria. However, there is a lack of evidence that the presence of colonizing bacteria impedes wound healing. The dressing technique should be aimed at prevention of cross-infection and should be clean.[13,16]

A systematic review of the effects of antimicrobials including topical antiseptics on chronic wounds identified no randomized controlled trials (RCT's) to support the cleansing by antiseptic solutions.[13,15] Another systematic review that looked for effects of using tap water in comparison to distilled water or boiled water or normal saline for cleansing of wound found no difference in infection or healing rates while using any of them.[13,16]

Ulcer debridement: Debridement is required if slough and wound debris obscure the base of the ulcer. Necrotic and devitalized tissue can be removed by mechanical, autolytic, chemical, or enzymatic debridement. Persistent necrotic tissue in the ulcer contributes to reduced host resistance to infection because it acts like a foreign body. There may be a high concentration of harmful proteases and bacteria in the necrotic tissue that can inhibit wound healing.[13]

Skin debridement comprises of removal of nonviable, non bleeding skin. The chronic wound should be converted to an acute wound by debridement so that it can proceed through the normal healing phases. Debridement is contraindicated in ulcers when healing is complicated by severe arterial insufficiency.[13,17]

Wound debridement can be done by various techniques. These include autolytic, chemical, mechanical, surgical and biological modalities. Autolytic debridement which causes breakdown and removal of dead tissues by body's own cells and enzymes, is

recommended for wounds with minimal debris and without clinical signs of infection. This is facilitated through maintenance of the moist environment in the ulcer by simple nonadherent wound dressing. Surgical debridement is appropriate in wounds with large amounts of necrotic tissue and eschar.[13]

Dressing of ulcer: Management of chronic ulcer should be aimed at using wound dressings that provide the optimal "moist" environment. Dressing should be simple, minimally adherent or non-adherent, low cost and acceptable to the patient. No single dressing material is favoured. The different types of ulcer dressings available are occlusive plastic films, hydrocolloid dressing, absorbent dressings, calcium alginate, hydrogels, and biological dressings. The choice of dressing should be made on the basis of personal experience, availability, type, and state and site of the wound. Patient preference and tolerance should also be considered.[18] For example, biological wound dressings are effective when used along with compression therapy in venous ulcers as compared with compression therapy alone.[13]

1. **Topical antibiotics and antiseptics:** Antibiotics are indicated when there are classical signs of infection. In chronic wounds, reduction of certain microbial species, such as anaerobic bacteria in order to limit undesirable odours or perhaps mixed communities of four or more bacterial species that impede healing use of topical antibiotics may be justified.[13,19,20] The most commonly used topical antimicrobials in wound care are chlorhexidine, mupriocin, fucidic acid, iodine, silver containing products etc. Acetic acid, honey, hydrogen peroxide, sodium hypochlorite, potassium permanganate, and proflavine have also been used.[13]

a. Chlorhexidine impregnated dressings - They are efficacious in reduction of bacterial colonization and increasing overall rate of healing of ulcer. However, there is lack of sufficient data to assess efficacy and safety for long term use in ulcers.[13,21]

b. Mupirocin - Mupirocin is a bactericidal agent which inhibits protein synthesis in susceptible bacteria by reversibly binding to bacterial isoleucine - tRNA ligase. It is one of the most commonly used topical antibiotic preparations used for dressing in acute and chronic ulcers and for treatment of superficial bacterial infections. However, due to increased cases of resistance, mupirocin is now sparingly used.[22]

c. Silver - Wounds treated with ionized nanocrystalline silver dressing showed improved clinical parameters along with decreased bioburden on the wound surface, in an uncontrolled, prospective study of a series of chronic wounds. However, the deep tissue loads were unchanged. This showed that, the surface flora contributed more significantly to delayed healing than deeper.[13,22]

2. **Compression therapy:** High compression, graduated, multi-layered system with adequate padding are recommended as the first line of treatment for uncomplicated venous leg ulcers with ABPI ≥0.8 in all settings. Compression systems may be classified into 3 groups:[13]

   a. Short-stretch bandages (SSB),

   b. long-stretch bandages, and

   c. stockings

All compression bandage systems must create a pressure gradient from ankle to knee. In case of oedema around the ulcer and over its limb, it is recommended to use an SSB system. Compression pressures of a minimum of 30-40 mm Hg at the ankle should be given in the management of venous leg ulcers.[13,23-26]

3. **Negative Pressure wound therapy:** Negative pressure wound therapy, or vacuum-assisted closure, was first used in the USA in 1997. It functions by maintaining a moist environment, optimisation of blood flow, removal of exudates, and application of pressure to promote wound closure. Hence, these devices may be able to diminish numerous factors that could be deficient in a chronic wound.[7] Various studies have shown that these devices are also associated with reduction in rates of infection in chronic wounds.[7,27] Negative pressure wound therapy may thus be used as an adjuvant or temporary measure to reduce the volume of the wound, thereby simplifying the subsequent surgical repair of the wound.[7,27,28]

4. **Hyperbaric Oxygen:** Although controversial, hyperbaric oxygen therapy has been used thus far in wound healing on the basis of the principle that it can advance fibroblast proliferation, enhance immune function, and promote angiogenesis, along with other functions. As localized delivery of oxygen has not shown to be effective in the treatment of wounds, this therapy is applied to the patient in a hyperbaric oxygen chamber. This could lead to significant adverse effects including myopia, oxygen toxicity in the brain leading to seizures, pneumothorax etc.[7,29] Owing to these factors, hyperbaric oxygen should only be considered in wounds hypoxia has been demonstrated as in an ischemic diabetic ulcer. However, this modality may be used after other more beneficial modalities have been tried.[7,30]

5. **Skin substitutes:** Skin substitutes such as grafts (mostly autologous) have been previously used either for large surgical defects or burns, requiring replacement of a significant surface area of tissue. However, novel bioprosthetic skin substitutes have been developed due to recent advances in this field. Skin substitutes usually consist of a biologically derived substance that is combined with a material to allow for its placement on a wound.[7,31]

The cost effectiveness of these dressings are a concern and hence, there is a significant barrier to widespread adoption. However, several studies that have been conducted in this regard have shown that they have an advantage with using the skin substitutes, considering that reduction in even a single day of hospital admission represents a large chunk in cost saving along with being beneficial to the patient.[1,31]

The multiple options of skin substitutes developed include mesh material coated with porcine collagen or polypeptides or a porcine xenograft. One study showed that these were associated with cost reduction in care and improved quality of outcome in a limited scope of facial burns.[1,31]

The substitutes developed using fibroblasts derived from newborn foreskin tissue, extracellular matrix, and a bioabsorbable polyglactin mesh are able to generate growth factors, collagen, cytokines, and glycosaminoglycans to promote the wound healing environment. Numerous studies have shown its efficacy,[7,32,33] especially in case of burn wounds and venous/pressure ulcers. However, a few adverse effects seen are that of a theoretical risk of rejection (thus far unreported) and hypersensitivity (seen with bovine serum that may be present in trace amounts in some preparations).[7]

6. **Surgery:** Surgical approach is considered generally in patients with chronic venous leg ulcer and superficial venous reflux, in order to promote ulcer healing and to prevent recurrence of the ulcer.[13,17,34] Surgical ablation of incompetent superficial veins is considered in case of a non healing ulcer (no sign of healing after 6 weeks of best management of wound). Newer surgical techniques can be used in case of perforator incompetence and disease of the superficial venous system, as these are associated with minimal morbidity.[13,17] Surgical ablation/ligation should be considered after ulcer healing, incase of significant superficial and/or perforator vein incompetence, as a preventive measure to avoid recurrence of ulcer.[13,17,34]

7. **Other therapies:** Multiple other modalities are being used conventionally and experimentally, targeting specific ulcer types and underlying conditions. These include endovascular laser therapy, sclerotherapy, drugs like pentoxifylline, zinc and phenytoin, GM-CSF, compression therapy, hydrocolloids, hydrogels, hydrofibers, extracorporeal shock wave therapy etc.

8. **Growth Factors:** In recent times growth factors have received significant attention in the field of wound healing.[7,35] Since a chronic wound contains so many disruptions in growth factors and cytokines, inclusion of an environment that contains and promotes the growth of these factors may be helpful. For example, a chronic wound tends to express low levels of fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), transforming growth factor-b (TGF- b), and platelet derived growth factor (PDGF). It also shows low levels of interleukins (IL) 1 and 6, and tumour necrosis factor-a (TNF-a).[7,36]

Simple replacement of a certain growth factor is unable to rescue the chronic wound phenotype as a chronic wound is an environment where many factors are deficient and dysregulated. Alternatively, it is also possible that the ‘‘master regulator’’ of wound healing has yet to be seen.[7,37]

PLATELET RICH GROWTH FACTORS:

The use of blood derivatives (fibrin glues, or platelet concentrates) for surgical purpose has been suggested since the last 50 years for the management of skin wounds.[38-41] Platelet-rich plasma (PRP) was used as an umbrella term for platelet concentrates, and the objectives of this modality of treatment was first to concentrate platelets and to force them to release their growth factor contents on a wounded site.[42,43,44] This is one of the oldest approaches of regenerative medicine in modern medicine.[38] Fibrin matrix and platelet components (especially growth factors) offer a complex of healing properties as surgical adjuvants.[38,45] In the recent years, platelet concentrates are being used as surgical adjuvants in the form of plasma, fibrin, mesh, foams, suspensions etc. for regenerative medicine.[42,46,47]

Blood Components:[42,45,46]

Blood is mainly composed of red blood cells (RBCs), white blood cells (WBCs), platelets and plasma.

a. Red Blood Cells (RBCs)**:** They are large, microscopic cells that are anucleate and make up 40- 50% of the total blood volume. Their main function is oxygenation of tissues.

b. White Blood Cells (WBCs)**:** They are a component of the body's defence mechanism and constitute 1% of the total blood volume. They are comprised of neutrophils, eosinophils, basophils, monocytes, lymphocyte.

c. Platelets**:** They are produced in the bone marrow and are cytoplasmic fragments of megakaryocytes (a type of white blood cell). Structurally, they are round or oval in shape, approximately 2 mm in diameter. Platelets are anucleate, however, they contain organelles and granules (α, δ, λ). The α granules hold around 30 bioactive proteins which play a role in hemostasis and tissue healing.

d. Plasma**:** Plasma is yellow tinted and relatively clear and constitutes 55% of the blood volume. It carries RBCs, WBCs, platelets and contains various hormones, enzymes, proteins and antibodies.

The basic objective in PRP/PRF therapy is applying supra - pharmacological dose of platelets directly at the site where tissue regeneration is required. It helps to rejuvenate & regenerate injured tissues along with modulation of wound healing. However, various other factors maybe present in the concentrate, sometimes more important than platelet growth factors. This may especially be seen in the form of the fibrin matrix and leukocytes.[42,45,46]

History of Platelet rich growth factors: Platelet rich plasma to platelet rich fibrin:

Autologous platelet rich plasma (PRP) was first developed in the early 1970s and was made popular in the 1980s.[8,48,49] The first generation of PRP was developed by mixing collected blood with excess calcium and thrombin, which resulted in activated platelets trapped within a fibrin network. Since then, various protocols for platelet preparation have been formulated and are traditionally prepared by a dual-speed centrifugation process.[8]

The first spin segregates red blood cells from the plasma and buffy coat. Subsequently, in the second spin cycle, the platelet plug is separated from the platelet-poor plasma thus generating PRP, with a platelet concentrate that contains 6-8 times the concentration of growth factors when compared with whole blood.[8,43,49]

The platelets obtained through the 2 stage centrifugation process have been shown to secrete high levels of bioactive substances that slowly diffuse to the surrounding micro-environment facilitating tissue regeneration. It was thus demonstrated that PRP could further enhance surgical wound healing of either soft or hard tissues.[8,43,49] Despite its widespread use, one of the reported drawbacks was the use of anticoagulation factors delaying normal wound healing events. Due to the above mentioned limitations, a second-generation platelet concentrate without utilising anti-coagulation factors was further developed. This was later termed as platelet rich fibrin (PRF)[8]

Platelet rich fibrin (PRF), also termed as leukocyte-PRF (L-PRF), in addition to the platelet concentrate, contains more white blood cells (WBCs), that are necessary during the wound-healing process.[5,8,50,51] Moreover, since WBCs, including neutrophils and macrophages, are the initial cell types found in wounded sites, they also play an important role in phagocytizing debris, microbes, and necrotic tissue, consequently preventing infection.[8,52,53]

Macrophages are one of the key cells implicated in growth factor secretion during wound healing which includes transforming growth factor beta (TGF- beta), PDGF, and vascular endothelial growth factor (VEGF). These cells, together with neutrophils and platelets, are key players in wound healing and in combination with their secreted growth

factors/cytokines are capable of facilitating tissue regeneration, new blood vessel formation (angiogenesis), and prevention of infection.Till date, numerous studies have investigated the regenerative potential of PRF in various medical situations.[8,52,53]

- The various growth factors present in PRP/ PRF can be described as follows – [42,45,46]

Table 2: Growth factors present in PRP/ PRF	
Platelet derived growth factor (PDGF) αα	It is chemotactic for fibroblasts and macrophages. PDGF ββ and αβ – They are mitogenic for fibroblasts, smooth muscle cells and endothelial cells.
Transforming growth factor beta (TGF)	
a. **β1**	Mediates angiogenesis
b. **TGFβ 2**	Acts as chemotactic for fibroblasts, keratinocytes and macrophages. It is also mitogenic for smooth muscle cells and fibroblasts. It also has potential to regulate matrix proteins, collagen and proteoglycans.
Vascular Endothelial Growth Factor (VEGF)	Chemotactic and mitogenic for endothelial cells.
Epidermal growth factor	It is mitogenic for fibroblasts, keratinocytes and endothelial cells.
Fibroblast growth factor-2 (FGF-2)	It has a role in tissue organization and regeneration.
Fibroblast growth factor 9	Helps in regeneration of hair follicle.
Hepatocyte growth factor	Helps in regeneration.

It was Ehrenfest *et al.* who first proposed a classification for the platelet concentrate.[46] It was classified into four types depending upon the leucocyte and fibrin content:[46]

Table 3: Classification of platelet concentrate

Platelet Derivative	Feature
P-PRP (Pure Platelet Rich Plasma)	This can be prepared by collecting only the buffy coat alone after the first soft spin. Very few leucocytes will be present.
L-PRP(Leucocyte and Platelet Rich Plasma)	This type contains mostly platelets with few but appreciable amount of leucocytes. There is difference in collection of PRP. After soft spin, plasma, buffy coat and topmost layer of RBC is harvested. Later, after hard spin, lowest fraction of product is harvested which contains all platelets and few leucocytes.
P-PRF (Pure Platelet Rich Fibrin)	This is obtained by mixing PRP with activator and incubating it for some time so that a stable platelet rich fibrin clot is formed.
L-PRF (Leucocyte and Platelet Rich Fibrin)	In this no anticoagulant added and no activator required. First blood is collected without any anticoagulant and centrifuged without delay. The process results in three layers. L-PRF layer is formed in middle and harvested.

Platelet Rich Plasma (PRP):

Platelet-rich plasma (PRP), also termed as autologous platelet gel or plasma-rich growth factors and, platelet-concentrated plasma means "abundant platelets that are concentrated into a small volume of plasma."[54]

The unearthing of platelet-derived growth factor (PDGF) in encouraging wound restoration, angiogenesis and skin remodelling brought to notice this fresh autologous therapeutic modality. This mixture of growth factors plays pivotal role in modulation of tissue repair and regeneration.[55]

The pre-packaged growth factors in platelets get "activated" after coming in contact with coagulation triggers, leading to its degranulation and release of these growth factors. The secreted growth factors attach themselves to their individual trans-membrane receptors that are present over various cell types. This causes an internal signal-transduction pathway, unraveling the expression of a normal gene sequence of a cell, like growth and cell division, formation of collagen, osteoid tissue and matrix etc., thus enhancing the ordinary wound-healing progression.[55]

History of PRP:

PRP has been in use as a therapeutic modality in dentistry since 1998, and the clinical application of PRP was subsequently expanded to other fields, including aesthetic medicine.[56] Multiple preparation techniques have been developed such as: orthodox blood centrifugation, commercial systems that can be applied as platelet suspension or as a gel. Further improvement in these techniques is an on-going process.

PRP is a biological by-product that includes platelet-derived factor along with other growth factors that when introduced into a tissue acts to generate the wound healing process without producing an actual trauma. It promotes angiogenesis along with local tissue remodelling by activation of tissue-resident and marrow-derived progenitor/stem cells.[57]

Preparation of PRP can be done by two methods depending upon the number of steps used in centrifugation: the first one is the single spin method, second being the double spin method. The thus formed platelet-rich plasma x in the anti- coagulated state is stable for eight hours and requires activation for the release of their content.[57]

PRP can be prepared on an out-patient basis just before the procedure. “The process must be carried out under aseptic conditions along with optimum temperature regulations i.e. 20-22°C. In order to inhibit platelet aggregation. PRP is prepared with an anticoagulant, the most commonly used anticoagulant being anticoagulant citrate dextrose solution formula A (ACD-A) or sodium citrate. The platelets need to be isolated in high concentrations, in order to achieve therapeutic benefit and to be in a viable state simultaneously, so that they can actively secrete their growth factors.”[58]

The current technique permits us to focus platelets and white blood cells from blood and allows the prompt discharge of growth factors by injection directly into injured tissue, encouraging the identical healing process in a more directed fashion.

PRP preparation:[60,61]

Materials required include:

a. Table centrifuge

b. 5ml ACD-A or sodium citrate tubes

c. Syringes for segregation of contents

d. Blood collection kit

The basic principle behind the PRP separation procedure is as follows: Different blood components have different specific gravities. So on centrifugation they get separated into different layers. First the 'platelet-rich plasma' is divided from whole blood by 'soft or light-spin' centrifugation following which the platelets are concentrated by 'hard or heavy-spin' centrifugation. This is in accordance to The American Association of Blood Banks technical manual.[60,61]

The red blood cells settle at the bottom as they are heaviest, following which come the white blood cells. The topmost layer is that of the platelets which are lightest. The aim of the first step is to separate the plasma from the rest of the components. This is done by a slow centrifugation of the tub during which platelets get concentrated just above the buffy coat. In the subsequent step, the test tube is rapidly spined in order to concentrate the platelets and let them settle down at the lowermost portion of the tube.[60,61]

The platelet-rich pellet is re-suspended in residual plasma by discarding almost three fourth of the supernatant. Platelets may be activated by addition of calcium chloride ($CaCl_2$) or thrombin as an "activator".[60,61]

The activated PRP must be used as early as possible, as rapid secretion of growth factors occurs initially with maximum secretion occurring within ten minutes. Depending upon the methods used, rate and time of spin, anticoagulant profile and container used, there may be variability in the yield of platelets obtained. These yields may vary from 4 to 7 times the baseline. Temperature should be maintained between 20- 22^{o} C in order to assure the viability of platelets. This can be confirmed by Trypan blue staining.[60,61]

Although various protocols have been used for preparation of PRP, the double spin method is preferred over the single spin method, as studies have shown that the single spin method is disadvantageous in achieving therapeutic levels of platelets. Different platelet types and concentrates may be generated using different protocols, devices and centrifuge speeds for preparing PRP.[60,61]

Concentration of platelets in PRP:

The average range for concentration of platelets in blood is 200,000 ± 75,000/μL. For a preparation to be labeled as "PLATELET RICH", the concentration of platelets should rise to level of five to ten times the base line.[45,50]

Table 4: Indications of PRP in dermatology	
Alopecias	PRP has been used in androgenetic alopecia in the form of mesotherapy, as incubation medium in Follicular Unit Transplant and has also shown success in cases of alopecia areata and telogen effluvium.[54]
Skin rejuvenation	PRP has shown to remove photo damaged extracellular matrix and induce synthesis of new collagen and has thus gained importance in the world of aesthetics. PRP can be applied topically under occlusion or given as intradermal injections.[56,57]
Acne scars and contour defects	PRP can be used as mesotherapy alone or in combination with other techniques such as subcision, derma roller or laser resurfacing.[57,58]
Wound ulcers and connective tissue disease associated ulcers	Stasis ulcers, trophic ulcers, diabetic ulcers, venous ulcer, lipodermatosclerosis and traumatic ulcers have shown good healing after treatment with PRP.[51,53,55]
Striae distensae	Some studies have shown use of intradermal radio- frequency device along with injectable PRP as tissue augmentation through its needle electrode.[54,59]
Lichen Sclerosus	PRP and autologous fat transfer in combination can be used as a novel technique in management of lichen sclerosis of vulva.[56,57]

Safety of PRP:

a. The mitogenic effect of autologous PRP is limited to the standard healing procedure. PRP remains non-mutagenic as whatever growth factors it delivers, act through signal transduction only. These growth factors fail to move into the cells or nucleus.[55]

b. Secondary infection is rare when the procedure is carried out under strict aseptic precautions.

c. Since PRP is autologous, there is no risk of transmission of hepatitis B, C or HIV.

Limitations of PRP:[57,59]

a. There is a lack in uniformity of preparation protocol for PRP protocol, as different platelet concentrations have different storage time.

b. Short duration of release of growth factors.

c. Chance of producing coagulopathies and rare bleeding episodes due to cross reaction between antibodies to bovine factor Va with human factor Va.

d. There may be limited injection site reactions like transient erythema or pain

e. Nowadays in the market, various automated devices are available for preparing PRP. However, these devices are expensive compared to manual methods and the commercial interest of the manufacturers can deteriorate the quality of platelet concentrates.

Platelet Rich Fibrin (PRF):

History & Evolution of PRF: Platelet rich fibrin is a second-generation platelet derivative that was developed by Choukroun *et al.* in France in 2001. Unlike other platelet concentrates like PRP, preparation technique for PRF does not require anticoagulants or bovine thrombin or any gelifying agent. It is strictly an autologous fibrin matrix that contains a large quantity of platelet and leukocyte cytokines. In the absence of artificial biochemical modification like

the use of bovine thrombin, PRF is a novel measure in the therapeutic concept with elementary processing.[52,59]

The essence of PRF synthesis lies in the ability to accumulate platelets and release growth factors in a fibrin clot. The PRF clot is produced by natural polymerization during centrifugation, and the natural fibrin architecture is considered to be responsible for the slow release of growth factors and matrix glycoproteins during ≥7 days.[62] This slow release is not seen in most PRP techniques due to their rapid platelet activation and release of growth factors along with very light fibrin network that is generated to sustain the concentrate injection.[59,63]

Biologic Characteristics of PRF:

Platelet rich fibrin is a new generation of platelet concentrate that kick-starts the wound healing process and maximises predictability. Platelets, growth factors including cytokines, and the fibrin matrix constitute PRF.[59,64] Degranulation of platelets causes release of cytokines which in turn stimulates cell migration and proliferation in the fibrin matrix. This launches the first stage of healing. Fibrin matrix supporting them constitutes the determining element responsible for the real therapeutic potential of PRF. The biologic activity of the fibrin molecule highlights its significant cicatricial capacity.[59,65]

Fibrin Matrix:

Fibrin is the activated form of fibrinogen molecule that is present in plasma and α-granules of platelets and has a significant role in platelet aggregation and hemostasis. Transformation of soluble fibrinogen into insoluble fibrin occurs and it further polymerizes to

a cicatricial matrix. The centrifugation process causes slow, natural polymerization of fibrin resulting in a homogenous 3-D arrangement. This further leads to intrinsic incorporation of platelet, growth factors, cytokines and glycan chains in the fibrin meshes.[59,63]

The fibrin matrix is flexible, elastic, and has good tensile strength and consists of weak thrombin concentrations which are composed of equilateral junctions. These connected junctions facilitate the establishment of a fine and flexible fibrin network that is capable of supporting growth factors, cytokines and the occurrence of cellular migration. This further increases the lifespan of the cytokines and cause their release at the time of initial cicatricial matrix remodelling. Thus, the cytokines are available in the crucial period required by the cells to initiate wound healing.[59,66]

There are significant differences between the fibrin meshwork in PRF and PRP. Due to the increased thrombin requirement for rapid setting of the PRP it forms a rigid polymerized material with bilateral junctions, that does not allow for cytokine enmeshment and cellular migration.[59,66]

Role of Fibrin in Angiogenesis:

Sustained release of growth factors and cytokines is seen due to entrapment of cytokines in the 3-dimensional architecture of fibrin matrix. This is monumental in initiation of angiogenesis.[59,67] The growth factors seen actively in the fibrin gel include the FGF, VEGF, angiopoietin, and PDGF. The rigidity of the fibrin matrix is instrumental in the process of angiogenesis in response to stimulation by FGF and VEGF.[59,68] The binding of endothelial cells to fibronectin, and vitronectin occurs due to increased expression of $\alpha v\beta 3$ integrin in response to the fibrin.[59,69]

Role of Fibrin in immune response:

Fibrin and fibronectin modulate the wound colonization by the macrophages. Fibrin matrix also aids in the increased expression of CD11c/CD18 receptor on endothelial cells thereby enhancing adhesion to endothelial cells and fibrinogen, hence causing transmigration of neutrophils.[59,70]

Effect of Fibrin on Mesenchymal Stem Cells:

Fibrin matrix works as a frame work for the undifferentiated mesenchymal cells in order to facilitate the differentiation of these cells hence aiding in tissue regeneration.[59,63]

Role of Fibrin in Osseous Tissue:

Bone morphogenic proteins (BMPs) that are enmeshed in fibrin matrix have the ability to be consistently released and show angiogenic, haemostatic, and osteoconducive properties. Fibrin acts as a support matrix for BMP, which are progressively released and may induce bone formation. The fibrin clots helps in achieving haemostasis by trapping circulating stem cells, allowing vascular and tissue restoration.[59,71]

PRF Preparation:

As with PRP, PRF can be prepared on an out-patient basis, however it has to be prepared just prior to its application. The protocol for preparation is very simple and was developed by Choukroun *et al.* in Nice, France.[59,63,72]

Materials required include:

a. Table centrifuge

b. 10-mL dry glass test tube or plain blood collection tube (without anticoagulant)

c. Blood collection kit

PRF preparation has some advantages over PRP preparation, that being, single-stage centrifugation and absence of bovine thrombin. Blood obtained from the patient is placed into the test tube and centrifuged immediately for 10 minutes at 3000rpm.[59,63] Some other studies have used 2700 rpm for 12 minutes with similar findings.[59,73]

Steps in preparation of PRF include:

A. Collection of blood from the patient in sterile plain tubes

B. Placement of the collected blood specimen in the centrifuge and immediate spin for the stipulated time

C. The blood sample is seen to settle into various separate layers following centrifugation

The layers that are formed are as follows:

a. Upper fraction containing the straw-coloured acellular plasma.

b. Middle fraction containing the fibrin clot (buffy coat)

c. Lower fraction containing the RBCs

Due to the absence of any anticoagulant a coagulation cascade is set off due to activation of platelets and the blood coagulates almost immediately. Initially, fibrinogen occupies the upper part of the tube, until the circulating thrombin transforms it into a fibrin network.[59,74]

D. Further, the upper portion of the test tube with the acellular plasma is discarded and the middle portion containing the fibrin clot is carefully removed so as not to disturb the clot and is scraped off from the lower part containing the red blood cells.[46,59]

Fibrin clot is formed due to the natural and progressive polymerization with embedding of platelets and leukocyte growth factors into the fibrin matrix in substantial quantity.[46,59]

PRF Membrane:

The PRF clot can be converted into an inexpensive autologous fibrin membrane by pressing the clot between two gauge pieces.[59,74] The serum exudate thus expressed from the clot is rich in proteins such as vitronectin and fibronectin and can be used to hydrate graft materials, rinse the surgical site, and store autologous graft.[52,59,74] Equipments to prepare PRF membrane are also available. The membrane is prepared here by placing the clot on the grid in the PRF box and covering it with a compressor lid which squeezes out the fluid from the clot. The membranes formed using this method have constant thickness and stay hydrated for several hours. Serum exudates expressed from these clots have also been recovered.[59]

Table 5: Indications for PRF in dermatology and aesthetics	
Dermal augmentation & skin rejuvenation	Can be applied given as intradermal injections for augmentation of areas with volume deficit.[59]
Acne scars & depressed scars	PRF may be used as alone or in combination with other techniques such as subcision, derma roller or laser resurfacing. It is ideal to be used in depressed and atrophic scars.[59]
Chronic non healing ulcers	Stasis ulcers, trophic ulcers, diabetic ulcers, venous ulcer, lipodermatosclerosis and traumatic ulcers have shown good healing response to PRF.[59]
Augmentation purpose in plastic and reconstructive surgery	Long term diminution of deep naso-labial folds, facial volumization, rhinoplasty, auricular cartilage defects etc.[59]

Use of PRF in periodontology and oral and maxillofacial surgery has also been largely described.[59]

PRF membrane functionalized by incorporation alkaline phosphatase induces the mineralization of PRF. Thus, PRF can also be a suitable material for bone replacement.[59]

Advantages of PRF:[59]

A. PRF acts as an adjunct to the natural healing process and has the following effects:

   a. The fibrin clot acts as a scaffold due to its mechanical properties which gives protection to the graft materials and also acts as a biological bridge between the bone particles

b. The fibrin network is engaged in cellular migration, mainly for the endothelial cells which is essential for angiogenesis, vascularization, and survival of graft

c. Persistent release of various growth factors that include PDGF, TGF-β and IGF-1 aiding the healing process

d. Self-regulation of infectious and inflammatory processes due to presence of leukocytes and various cytokines

B. There is no use of anticoagulants

C. Occurrence of slow natural polymerization

D. Presence of 3D fibrin network forming a matrix that helps in retention of growth factors for extended periods

E. East formulation of a PRF membrane that possesses elasticity and flexibility

F. Economical viability and simple to prepare

Shortcomings & Limitations of PRF:[59,70]

A. As less quantity of PRF is obtained per procedure and owing to the fact that PRF is an autologous product, its availability in larger amounts especially for usage in larger surgical procedures is a concern.

B. It cannot be used as an allogenic material as PRF possesses circulating immune cells and antigenic molecules.

C. Increased risk of transmitting infectious agents if not processed with caution.

STUDIES SHOWING EFFICACY OF PRF/ PRP IN HEALING OF CHRONIC ULCERS:

In the study conducted by Shreyas NS *et al.* comparing PRF dressings with conventional therapy, it was found that in comparison to conventional dressing, platelet rich fibrin dressings had shown faster healing of chronic cutaneous ulcer, thus being a safe non surgical adjuvant therapy for chronic cutaneous ulcer.[2]

The study also observed that, unlike PRPs, PRF does not dissolve quickly after application, instead, the strong fibrin matrix is slowly remodelled in a similar way to a natural blood clot.[2]

In the study it was also seen that the PRF was especially helpful in volume deficient wounds where the PRF applied transformed into corresponding adjacent tissue muscle, subcutis and skin, although they could not quantify its benefit as the volume of ulcer was not measured in each visit.[2]

In a systematic review conducted by Miron R J *et al.* on all currently available in vitro, in vivo and clinical literature utilising PRF for soft tissue regeneration, augmentation and/or wound healing. It was found that 18 studies (58% clinical studies) reported positive wound healing events associated with the use of PRF, despite using controls. Furthermore, 27 out of 31 clinical studies (87%) supported the use of PRF for soft tissue regeneration and wound healing for a variety of procedures in medicine and dentistry.[8]

In conclusion, the results for the study highlighted the positive effects of PRF on wound healing after regenerative therapy for the management of various soft tissue defects found in medicine and dentistry.[8]

In a prospective study conducted by Nagaraju U *et al.* where seven treated patients of Hansen's disease were included with healthy ulcers. These were treated with Platelet Rich Fibrin Matrix (PRFM) at weekly intervals, repeated once a week for a maximum of five sittings as per requirement. It was concluded that PRFM for the treatment of trophic ulcers in treated patients with Hasnsen's disease is a feasible, safe, simple and inexpensive method.[3]

In a study conducted by Crovetti *et al.,* they used PRP in 24 patients with single or multiple cutaneous ulcers with different etiologies such as diabetes, venous insufficiency, infection and post-traumatic, neuropathic or vasculitis origin.[75,76]

After 10 sessions of L-PRP application, complete wound closure was seen in 9 patients, 2 were required cutaneous graft, 4 were lost to follow up, 9 showed partial response and continued receiving treatment. However, after the first session, there was increase in granulation tissue formation in all cases, however, complete re-epithelization needed different times due to different size and duration of the ulcers. Pain relief was seen in all patients. They concluded that topical hemotherapy with L-PRP may be considered as an useful adjuvant treatment for cutaneous ulcers in a multidisciplinary process.[75,76]

Bielecka *et al*. studied the effect of application of L-PRP in 12 patients with venous leg ulcers, post debridement. They noted a total wound closure in all cases using injectable PRP in impermeable dressings.[75,77]

The ulcer healing processes were evaluated using clinical observations, but also with histopathological, immunohistochemical and molecular tests of tissue samples (obtained with the ethic committee agreement) taken before and at the 10^{th} day after treatment. The positive effects of L-PRP were obvious.[75,77]

In the prospective, randomized controlled multicenter trial conducted by Driver *et al*., regarding the use of L-PRP for the treatment of diabetic foot ulcers. Evaluation of the wounds was done biweekly for 12 weeks or until healing. Healing was confirmed 1 week following closure and followed up for another 11 weeks.[75,78]

The authors noted that 68.4% of cases in L-PRP group and 42.9% in the control group showed healing of ulcers. The authors suggested that the majority of non-healing diabetic foot ulcers treated with autologous platelet-rich plasma gel can be expected to heal.[75,78]

Among the 4 families of platelet concentrates widely available, 2 families i.e. PRP (Platelet-rich Plasma) and PRF (Platelet-Rich Fibrin) are particularly used and are continuously being tested for efficacy. These two platelet concentrates may have some common qualities like the presence of significant concentrations of platelets and leukocytes that are important in immune regulation, growth factor release and the overall wound healing process. However, the main difference between them, that is the fibrin architecture considerably influences the healing potential and the therapeutical protocol associated to each platelet concentrate technology.[75] As there is a paucity in studies that truly compares the healing potential of these two families of platelet concentrates, our study aims to establish a comparison in efficacy of PRF and PRP.

METHODOLOGY

METHODOLOGY

MATERIALS AND METHODS

SOURCE OF DATA

Patients with cutaneous ulcers more than 6 weeks duration, attending outpatient department of Dermatology, Venereology and Leprosy of B.L.D.E. (Deemed to be University) Shri. B.M. Patil Medical College Hospital and Research Centre, Bijapur, were enrolled for the study.

Period of study:

The study was conducted during the period of October 2018 to July 2020.

Study design:

A hospital based randomised comparative study.

METHOD OF COLLECTION OF DATA:

21 patients above the age of 18 years with a total of 44 chronic non healing ulcers were enrolled for the study. As each ulcer showed individual variation in morphology and response to treatment, each ulcer was treated as an individual case. Of the 44 ulcer cases, 2 cases were lost to follow-up at the start of treatment. Hence, the effective sample size evaluated was that of 42 cases of chronic ulcers.

Inclusion criteria:

1. Ulcers more than 6 weeks old.
2. Ulcer size (0.5 cms upto 10 cms)

3. Patients with normal platelet count

Exclusion criteria:

1. Ulcers less than 6 weeks old
2. Infected ulcers
3. Patients with Haemoglobin < 10gm%

Methods:

Detailed history with respect to the onset and duration of ulcer, any treatment for ulcers received within past 6 weeks and pre-existing medical conditions was recorded (ANNEXURE - II).

Initial clinical examination of the patient was done by one of the investigators to determine the size, volume and condition of ulcers. These findings were recorded in the proforma (first visit record). Informed consent for the study was undertaken from all the patients (ANNEXURE - III).

Forty-four cases of chronic cutaneous ulcers were allocated alternately into two groups. Patients in GROUP A were treated with platelet rich fibrin (PRF) dressing. Patients in GROUP B were treated with platelet rich plasma (PRP) dressing.

The ulcer site was examined, cleaned with antiseptic solution and if required was paired and then prepared for the designated dressing.

The PRF or PRP was prepared following the standard protocol as discussed below. Then under aseptic precautions the PRF was used to cover the floor of the ulcer, whereas PRP

was injected along the margins of the ulcer and the ulcer will be dressed with non-adherent dressing.

After one week, the dressing was removed completely and the ulcer was assessed for improvement and reduction in volume. A new PRF/ PRP dressing was then applied. This procedure was repeated weekly for a maximum of 6 weeks or until complete re-epithelization depending on the healing response.

Methodology:

Evaluation of Ulcer volume:

All ulcers were measured using callipers and cotton-tipped applicators to measure length, width, and depth of the ulcer. The largest vertical measurement of the ulcer was taken as length and the largest horizontal measurement (side to side) as width. Depth was taken from the deepest point of the ulcer base to the level of normal skin surface. Ulcer volume was calculated using the formula for an ellipse:[3,78,79]

$$\text{Volume of ulcer} = (\text{Length} \times \text{Width} \times 0.7854) \times \text{Depth}$$

(As an ellipse is closer to a wound shape than a square or rectangle that would be described by simple length × width)[3,78]

Equipments:

i. *Method of Platelet-rich Fibrin preparation:* The PRF was prepared following the protocol developed by Choukroun *et al.* For PRF preparation we used a table centrifuge (Remi R-8C) and collection kit (20 ml syringe, 5 ml blood collection tubes). A few minutes before wound care, 20 ml blood was drawn by venipuncture

under aseptic precautions in four sterile collection tubes of 5-ml capacity without anticoagulant for every 4cm^3 of ulcer. The tubes were placed at opposite sites in a centrifugal machine at 3000 rpm (704.34g / radius 7 cm) for 10 minutes and immediately centrifuged. Blood centrifugation immediately after collection creates a well-structured and resistant fibrin clot in the middle of the tube, just between the red corpuscles at the bottom and straw coloured acellular plasma at the top. The upper straw coloured layer was then be removed and middle fraction collected and cut at the erythrocyte zone as close as possible to the fibrin clot (2 mm below lower dividing line) as PRF where, platelets trap massively in the fibrin meshes.[2]

Figure 2: REMI R-8C centrifuge used for preparation of PRF/ PRP

Fig. 2

Figure 3: PRF prepared in plain tubes after single spin

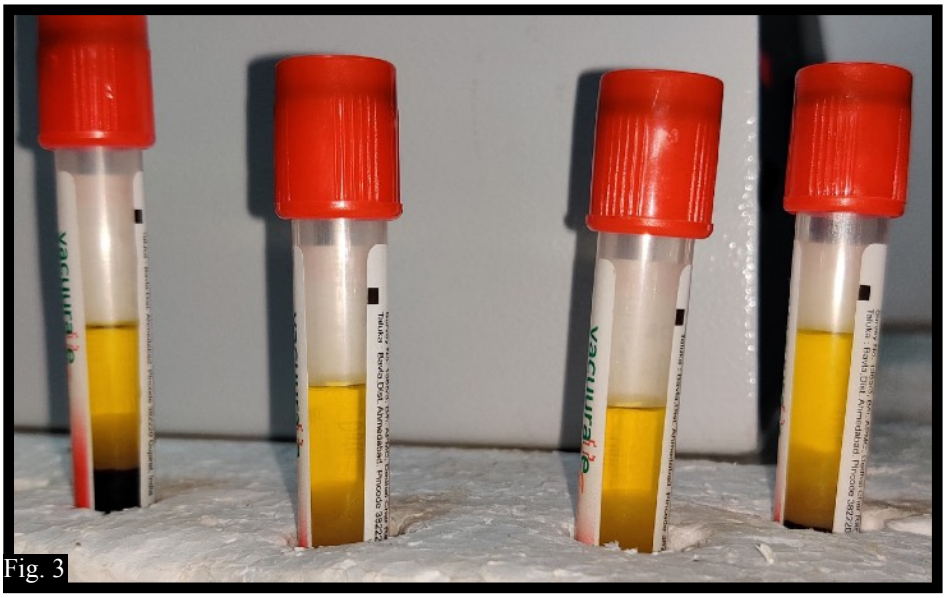
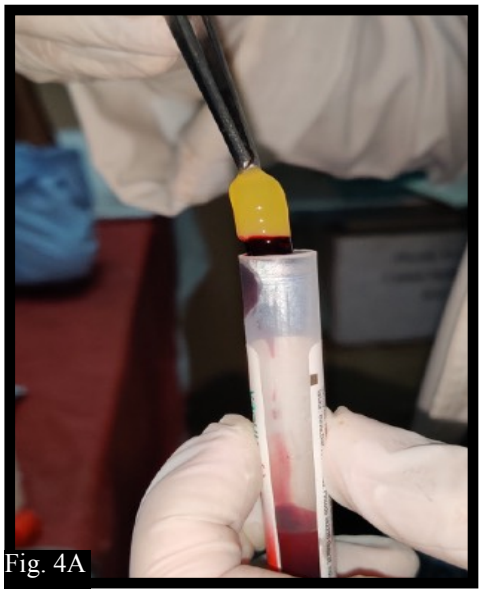
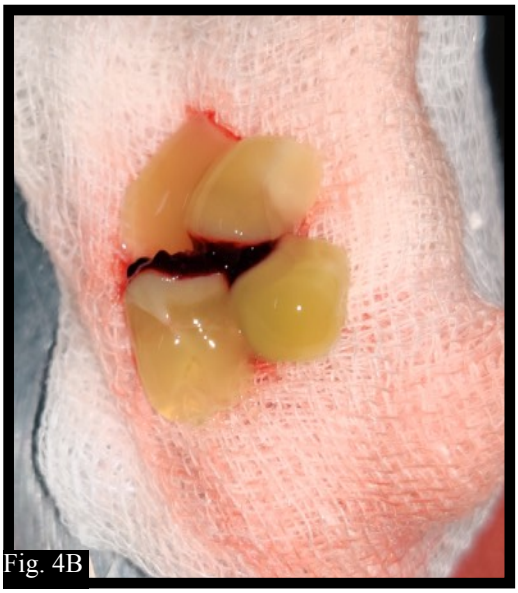


Figure 4: (A) & (B) - Fibrin clot of PRF





ii. *Method of Platelet-rich Plasma Preparation:* Two-stage centrifugation process (double-spin method) was employed in the preparation of PRP. Whole blood samples (20ml) were drawn from the patient and transferred into a tube pre-filled with citrate anticoagulant solution. The mixture was centrifuged at 3000rpm for 7 minutes (first spin). After the first spin, the lower red blood cell portion was discarded and the supernatant containing platelet-poor plasma and buffy coat was centrifuged again at 4000rpm for 5 minutes (second spin). The lower 1/3rd of this solution provided approximately 2ml of autologous platelet-rich plasma for dressing.[4]

Figure 5: PRP preparation after 1st spin

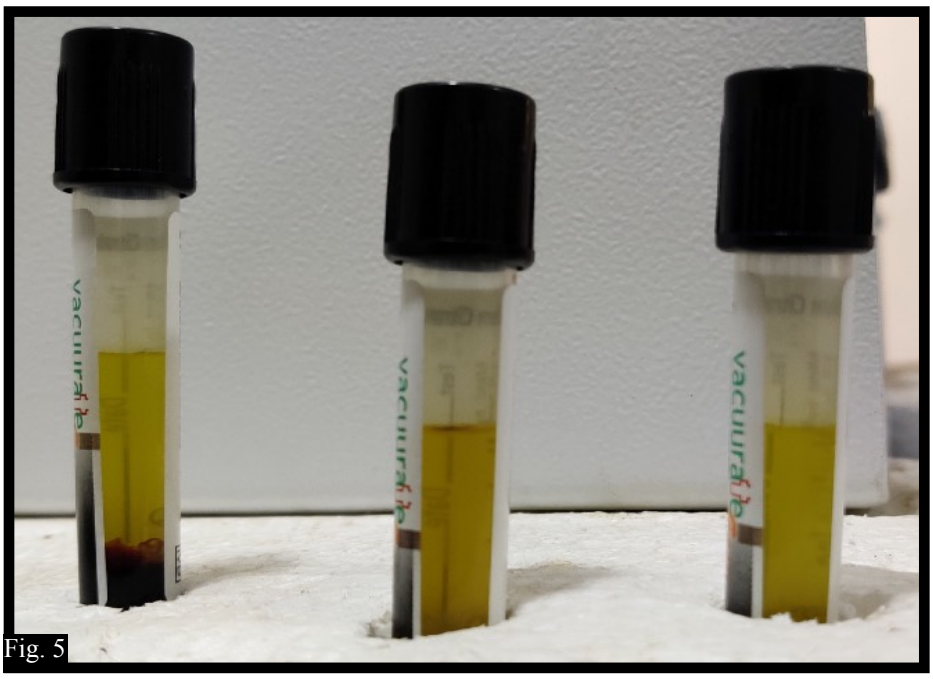


Fig. 5

Figure 6: Presence of Fibrin clot in PRF tube (Red) versus straw coloured plasma in PRP tube (Black)

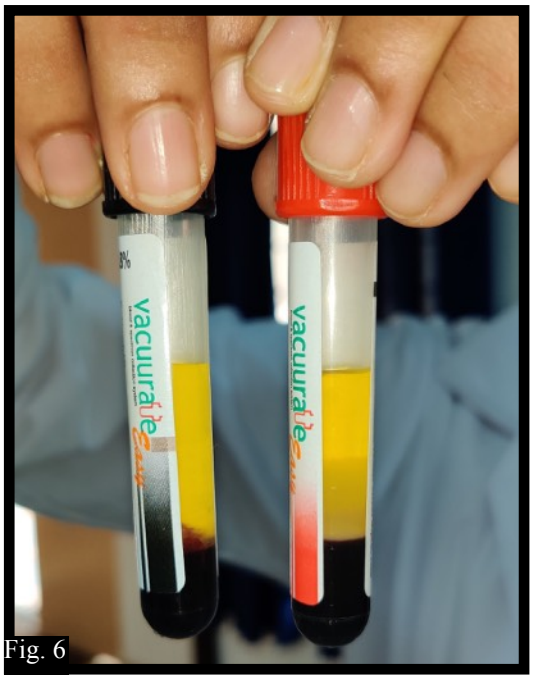


Fig. 6

Procedure:

The ulcer site was examined, assessed for volume and condition, then cleaned with antiseptic solution and if required paired and then prepared for PRF/ PRP dressing.

In group A, the PRF was used to cover the floor of the ulcer and in group B, PRP was injected into the margin of the ulcer. The ulcer was further dressed with non-adherent dressing. After one week, the dressing was removed completely and a new PRF/ PRP dressing was applied. This procedure was repeated weekly for the maximum of 6 weeks or until complete re-epithelization depending on the healing response.

Follow -up:

Ulcer evaluation was performed at baseline and then every week until 8 weeks after enrolment by investigator for ulcer area, volume, characteristics of ulcer exudates (presence, colour, amount and odour), necrotic tissue & granulation tissue, pain infection and complete healing. Patient satisfaction and general perception regarding the treatment was also noted. Clinical photographs were taken in identical settings and lighting at every follow up before successive dressing. Any adverse effects related to therapy were recorded in the proforma immediately at each sitting. At the end of 8 weeks the final response was evaluated according to the above mentioned procedure.

Efficacy evaluation:

The 2 primary efficacy parameters were assessed:

i) Objective assessment- Percentage reduction in volume of ulcer and number of patients with completely healed ulcer (100% re-epithelization) at 8 weeks.

ii) Subjective assessment- Patient assessment of improvement of ulcers after at the end of 8 weeks; patients in both the groups were asked to mark their response on a 10 inch long 'visual analog scale (VAS).

Investigations:

Following investigations were done:

1.Pus Culture & Sensitivity

2. Random blood sugar (RBS)

3. HIV, HbSAg

4. Haemoglobin / Total Count with Differential Count/ Platelet Count

5. Serum Protein/ Serum Albumin levels

Sample size calculation

If there is truly no difference between the PRF and PRP treatment, then 40 ulcers (20 per group) were required to be 80% sure that the limits of a two-sided 80% confidence interval will exclude a difference between the PRF and PRP group of more than 11.5%.[2]

Calculation based on the formula:

$$n = 2 \times f(\alpha, \beta/2) \times \pi \times (100 - \pi) / d2$$

where π is the true percent 'success' in both the control and experimental treatment groups

Statistical analysis

All characteristics were summarized descriptively. For continuous variables, the summary statistics of mean± standard deviation (SD) were used. For categorical data, the number and percentage were used in the data summaries and diagrammatic presentation. Chi-square (χ^2) test was used for association between two categorical variables.

The formula for the chi-square statistic used in the chi square test is:

$$\chi_c^2 = \sum \frac{(O_i - E_i)^2}{E_i}$$

The subscript “c” are the degrees of freedom. “O” is observed value and E is expected value. C= (number of rows-1)* (number of columns-1)

The difference of the means of analysis variables between two independent groups was tested by Kolmogorov-Smirnov test. The Kolmogorov-Smirnov Z test and the Wald-Wolfowitz runs test are more general tests that detect differences in both the locations and shapes of the distributions. The Kolmogorov-Smirnov test is based on the maximum absolute difference between the observed cumulative distribution functions for both samples. When this difference is significantly large, the two distributions are considered different. The Wald-Wolfowitz runs test combines and ranks the observations from both groups. If the two samples are from the same population, the two groups should be randomly scattered throughout the ranking.

If the p-value was < 0.05, then the results were considered to be statistically significant otherwise it was considered as not statistically significant. Data were analyzed using SPSS software v.23 (IBM Statistics, Chicago, USA) and Microsoft office 2007.

Ethical clearance:

Institutional ethical committee clearance was undertaken for the study.

RESULTS

RESULTS

A hospital based prospective, double blinded, randomized, comparative study was conducted from October 2018 to June 2020. A total of 42 cases of chronic non healing ulcers were evaluated in the study. The enrolled ulcers were allotted into 2 groups:

- Group A (PRF)

- Group B (PRP)

Age and Gender distribution

Among the 42 ulcer cases evaluated, 15 ulcers (35.7%) were in males and 27 ulcers (64.2%) were seen in females. There was no significant difference in the gender distribution between the two groups (Table 6, Table 7, Figure 7, Figure 8)

Table 6: Sex Distribution of cases		
Sex	**N**	**%**
Male	15	35.7
Female	27	64.3
Total	42	100.0

Figure 7: Sex Distribution

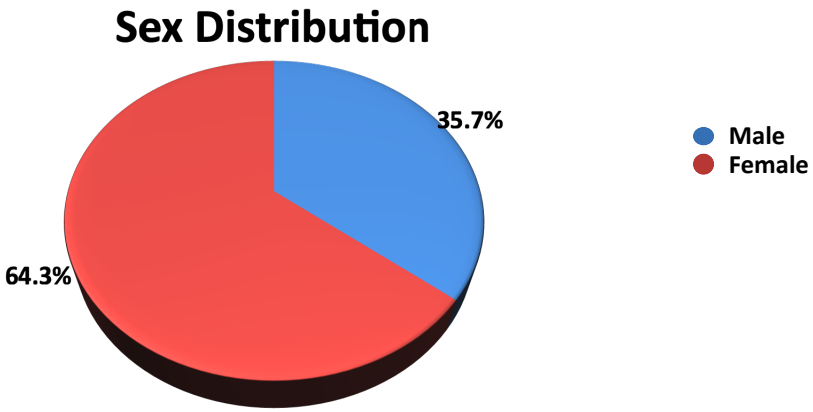
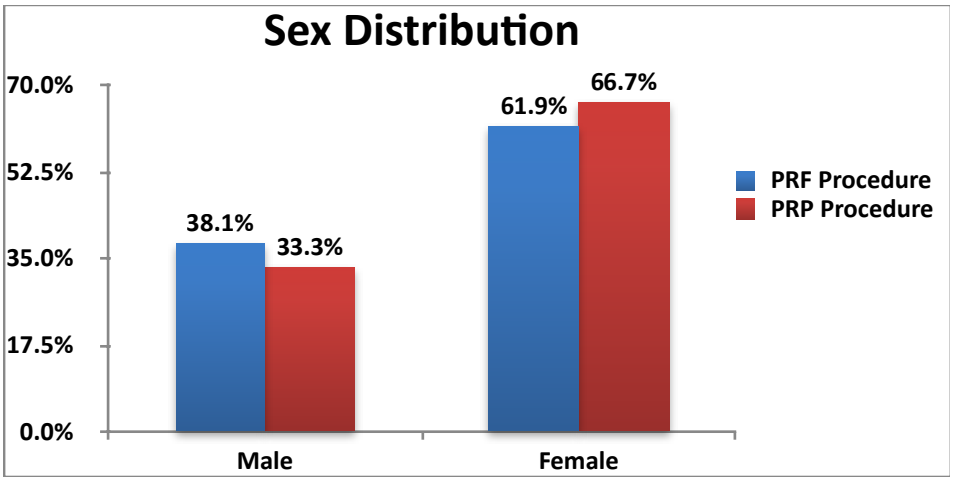


Table 7: Distribution of Sex between Study Procedures					
Sex	PRF Procedure		PRP Procedure		p value
	N	%	N	%	
Male	8	38.1%	7	33.3%	0.747
Female	13	61.9%	14	66.7%	
Total	21	100.0%	21	100.0%	

Figure 8: Distribution of Sex between Study Procedures



Mean Age, Distribution of age between procedures:

Mean age in the PRF group was 46.2 ± 20.8yrs and in PRP group was 48.7 ± 19.1 years. There was no significant difference in the age distribution between the two groups (Table 8)

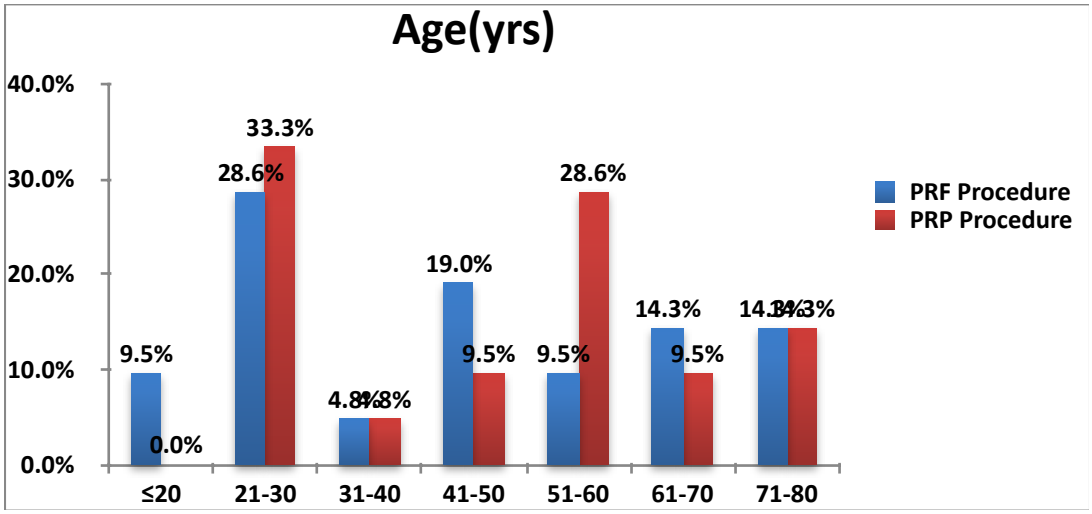
Table 8: Mean age between procedures						
	PRF Procedure		PRP Procedure		Kolmogorov-Smirnov Z	p value
	Mean	SD	Mean	SD		
Age(yrs)	46.2	20.8	48.7	19.1	0.463	0.983

The most common age group showing chronic non healing ulcers in both groups was 21-30 years of age followed by 41-50 years in PRF group and 51-60 years in PRP group. The comparative distribution in the two groups was not significant (Table 9, Figure 9)

Table 9: Distribution of Age between Study Procedures

Age(yrs)	PRF Procedure		PRP Procedure		p value
	N	%	N	%	
≤20	2	9.5%	0	0.0%	0.551
21-30	6	28.6%	7	33.3%	
31-40	1	4.8%	1	4.8%	
41-50	4	19.0%	2	9.5%	
51-60	2	9.5%	6	28.6%	
61-70	3	14.3%	2	9.5%	
71-80	3	14.3%	3	14.3%	
Total	21	100.0%	21	100.0%	

Figure 9: Distribution of Age between Study Procedures



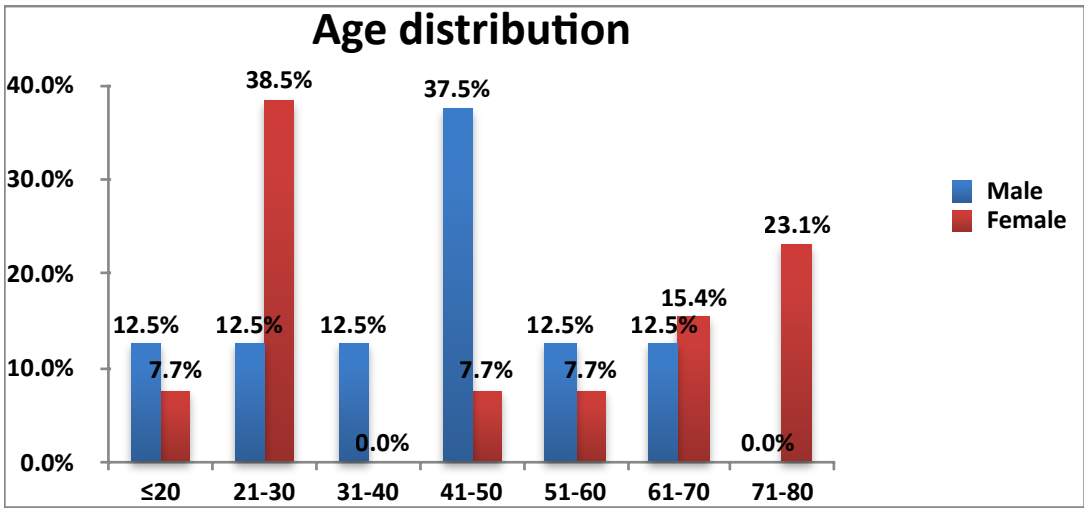
Association of Age between sex in the procedures:

In Group A i.e. ulcers receiving PRF dressing the maximum male patients were between the 41-50 years age group. Whereas, females were amongst the 21-30 years age group. (Table 10, Figure 10)

Table 10: Association of Age between Sex in PRF Procedures cases

Age(yrs)	Male		Female		p value
	N	%	N	%	
≤20	1	12.5%	1	7.7%	0.301
21-30	1	12.5%	5	38.5%	
31-40	1	12.5%	0	0.0%	
41-50	3	37.5%	1	7.7%	
51-60	1	12.5%	1	7.7%	
61-70	1	12.5%	2	15.4%	
71-80	0	0.0%	3	23.1%	
Total	8	100.0%	13	100.0%	

Figure 10: Association of Age between Sex in PRF Procedures cases

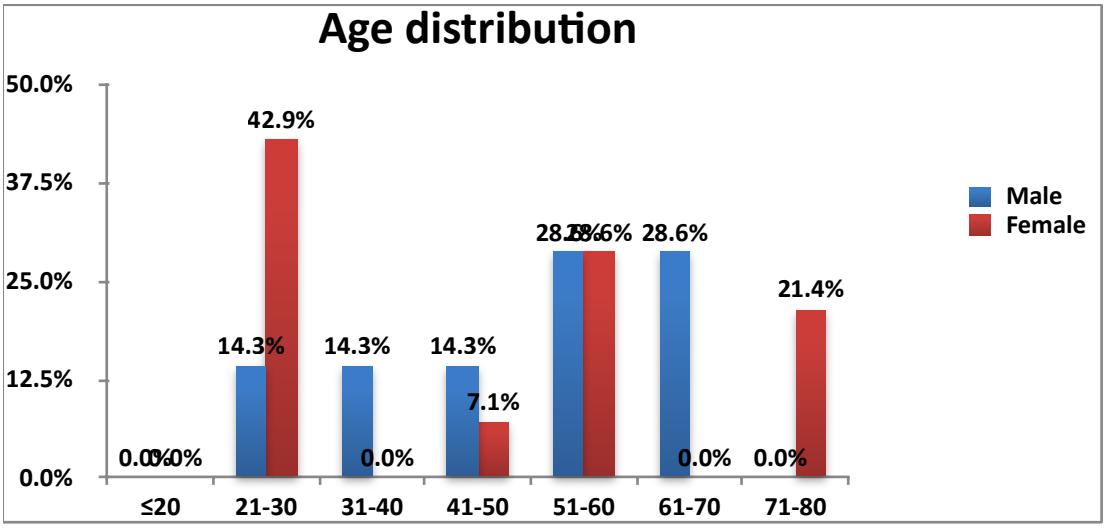


Patients in Group B i.e. ulcers receiving PRP dressing, showed that male patients were between 51-70 years of age and females were between 21-30 years of age. (Table 11, Figure 11)

Table 11: Association of Age between Sex in PRP Procedures cases

Age(yrs)	Male		Female		p value
	N	%	N	%	
≤20	0	0.0%	0	0.0%	0.113
21-30	1	14.3%	6	42.9%	
31-40	1	14.3%	0	0.0%	
41-50	1	14.3%	1	7.1%	
51-60	2	28.6%	4	28.6%	
61-70	2	28.6%	0	0.0%	
71-80	0	0.0%	3	21.4%	
Total	7	100.0%	14	100.0%	

Figure 11: Association of Age between Sex in PRP Procedures cases



Distribution of diagnosis between procedures:

Between the two procedures studied i.e. PRF and PRP the most common diagnosis seen in patients attending the dermatology OPD with chronic non healing ulcers was that of Hansen's disease (73.8%) i.e. 71.4% in PRF and 76.2% in PRP group respectively. This number was significantly higher than ulcers occurring due to other dermatological conditions. (Table 12 (A) & (B), Figure 12)

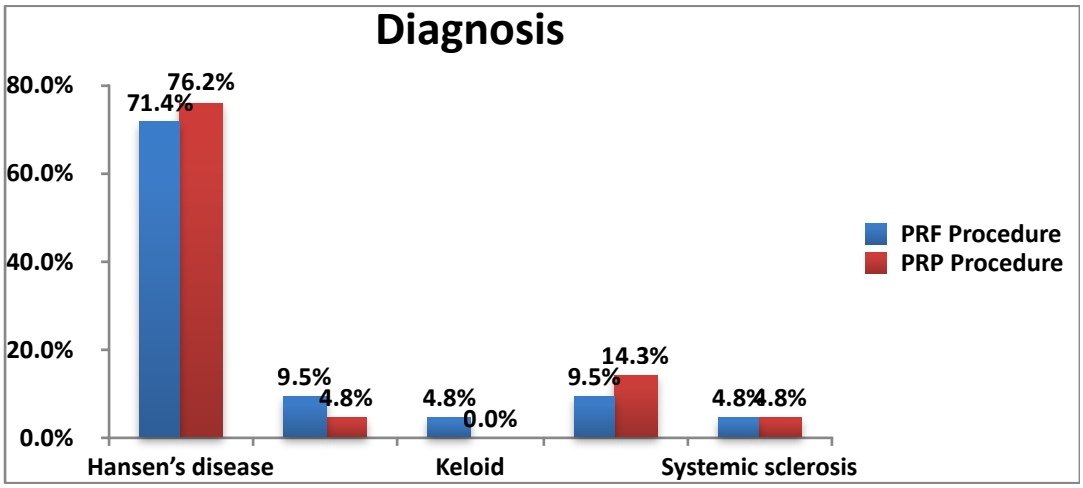
Table 12: (A) Distribution of Diagnosis between Study Procedures

Diagnosis	PRF Procedure		PRP Procedure		p value
	N	%	N	%	
Hansen's disease	15	71.4%	16	76.2%	0.726
Non Hansen's disease	6	28.6%	5	23.8%	
Total	21	100.0%	21	100.0%	

Table 12: (B) Distribution of Diagnosis between Study Procedures

Diagnosis	PRF Procedure		PRP Procedure		p value
	N	%	N	%	
Hansen's disease	15	71.4%	16	76.2%	0.815
Infected eczema	2	9.5%	1	4.8%	
Keloid	1	4.8%	0	0.0%	
Nec. acral erythema	2	9.5%	3	14.3%	
Systemic sclerosis	1	4.8%	1	4.8%	
Total	21	100.0%	21	100.0%	

Figure 12: Distribution of Diagnosis between Study Procedures



Distribution of Site between Study Procedures:

In our study, lower limb ulcers were most commonly seen in both groups of the study with right foot being predominantly involved in both groups (57.14% and 42.8% in Group A and Group B respectively). (Table 13)

Table 13: Distribution of Site between Study Procedures

Site	PRF Procedure		PRP Procedure	
	N	%	N	%
Right Hand	1	4.8%	0	0.0%
Left Hand	1	4.8%	2	9.5%
Right Leg	2	9.5%	1	4.8%
Left Leg	0	0.0%	0	0.0%
Right Foot	12	57.14%	9	42.8%
Left Foot	2	9.5%	5	23.8%
Right Ankle	2	9.5%	3	14.3%
Left Ankle	1	4.8%	1	4.8%
Total	21	100%	21	100%

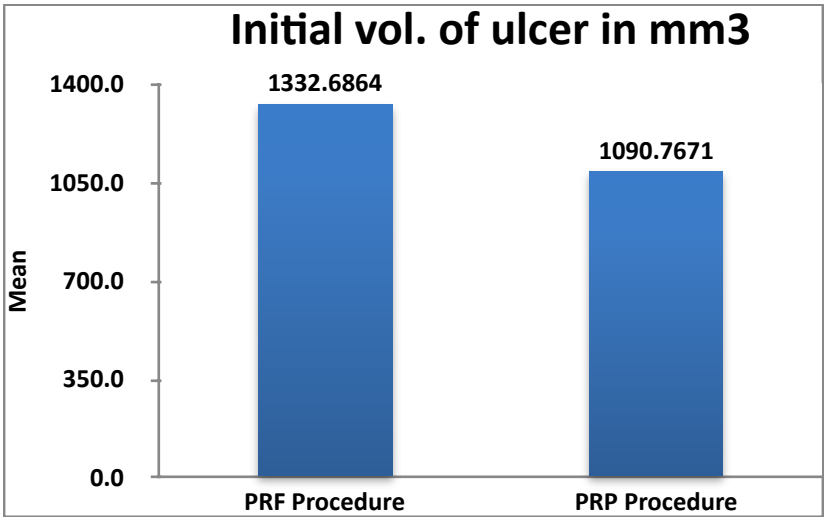
Mean Initial vol. of ulcer:

The mean initial volumes of ulcer was also comparable, being 1332.7mm3 ± 1266.6mm3 in Group A i.e. PRF and 1090.8mm3 ± 1655.4mm3 in Group B i.e. PRP. (Table 14, Figure 13)

Table 14: Mean Initial vol. of ulcer between Study Procedures

Parameters	PRF Procedure		PRP Procedure		Kolmogorov-Smirnov Z	p value
	Mean	SD	Mean	SD		
Initial vol. of ulcer in mm3	1332.7	1266.5	1090.8	1655.4	0.926	0.358

Figure 13: Mean Initial volume of ulcer between Study Procedures

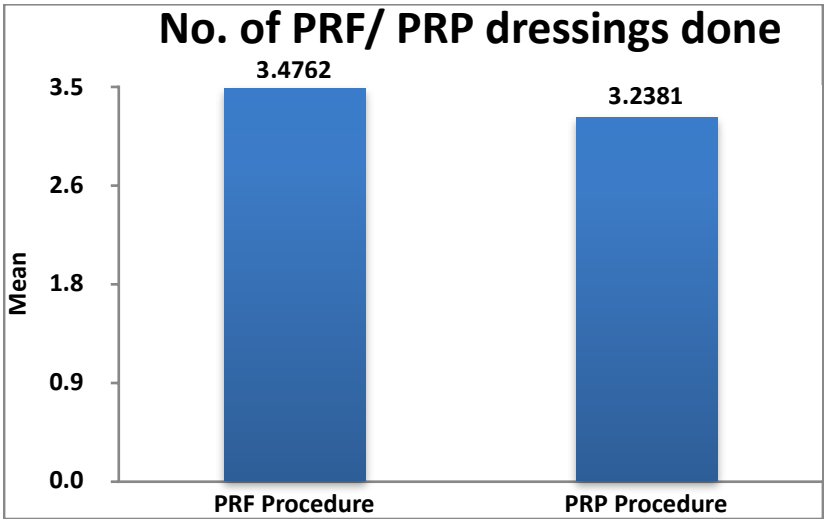


Mean No. of PRF/ PRP dressings done between Study Procedures:

There was no statistically significant difference between the mean number of PRF/ PRP dressings required in patients. These were comparable being 3.5 with SD of 1.5 in the PRF group and 3.2 with SD of 1.2 in PRP group of patients. (Table 15, Figure 14)

Table 15: Mean No. of PRF/ PRP dressings done between Study Procedures						
Parameters	**PRF Procedure**		**PRP Procedure**		**Kolmogorov-Smirnov Z**	**p value**
	Mean	**SD**	**Mean**	**SD**		
No. of PRF/ PRP dressings done	3.5	1.5	3.2	1.2	0.463	0.983

Figure 14: Mean No. of PRF/ PRP dressings done between Study Procedures



No. of PRF/ PRP dressings done between Diagnosis:

It was seen that patients with non Hansen's disease required fewer number of PRF/ PRP dressings than those required in patients with Hansen's disease. However, cumulative number of dressings showed that this difference was not statistically significant. (Table 16)

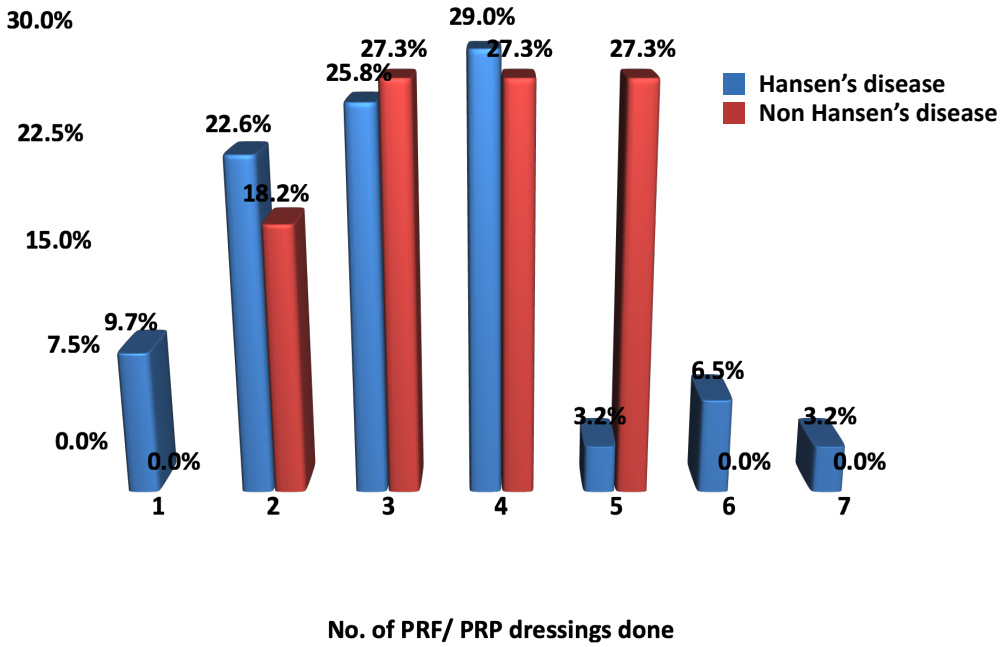
Table 16: No. of PRF/ PRP dressings done by Diagnosis					
No. of PRF/ PRP dressings done	**Hansen's disease**		**Non Hansen's disease**		**p value**
	Mean	**SD**	**Mean**	**SD**	
1	3	9.7%	0	0.0%	0.307
2	7	22.6%	2	18.2%	
3	8	25.8%	3	27.3%	
4	9	29.0%	3	27.3%	
5	1	3.2%	3	27.3%	
6	2	6.5%	0	0.0%	
7	1	3.2%	0	0.0%	
Total	31	100.0%	11	100.0%	

Mean No. of PRF/ PRP dressings done between Study Procedures:

There was no statistically significant difference between the mean number of PRF/ PRP dressings required in patients with Hansen's disease and non Hansen's diseases. These were comparable being 3.3 with SD of 1.5 in the Hansen's disease group and 3.6 with SD of 1.2 in non Hansen's disease group of patients. (Table 17, Figure 15)

Table 17: Mean No. of PRF/ PRP dressings done by Diagnosis					
Parameters	**Hansen's disease**		**Non Hansen's disease**		**p value**
	Mean	**SD**	**Mean**	**SD**	
No. of PRF/ PRP dressings done	3.3	1.5	3.6	1.1	0.44

Figure 15: No. of PRF/ PRP dressings done by Diagnosis

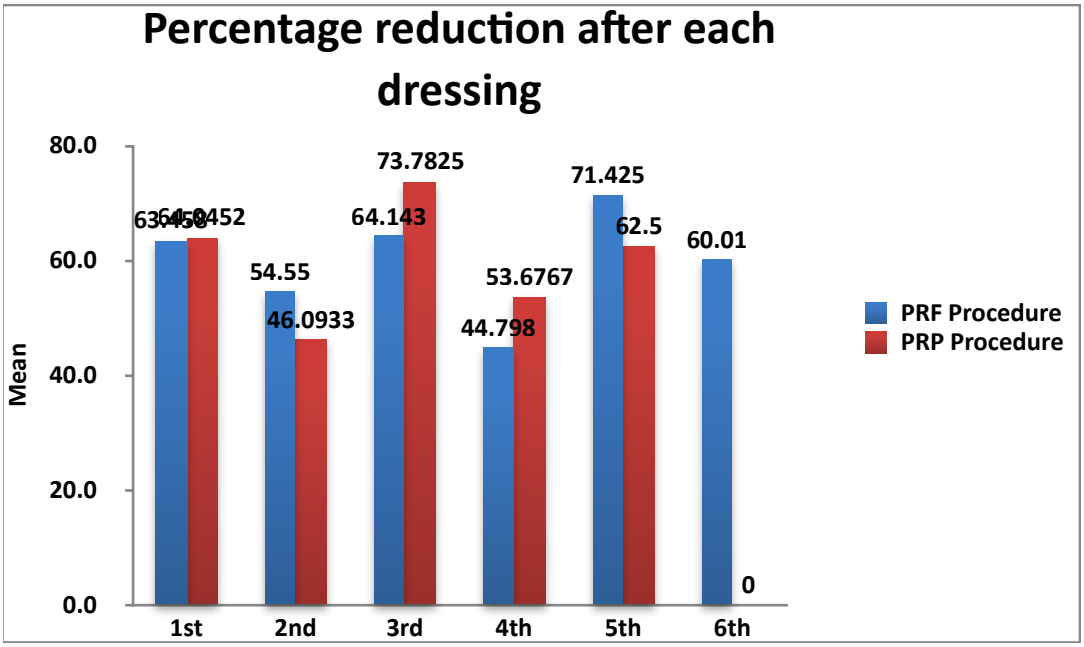


Percentage reduction after each dressing between study procedures:

Uniform reduction in volume of ulcer was seen per dressing in both procedures. The mean percentage reduction seen between each dressing during the PRF procedure ranged from 44.8% to 71.4%. During PRP procedures range of mean percentage reduction was from 46.1% to 73.8%. There was no statistically significant difference in this parameter between the two procedures. (Table 18, Figure 16)

Table 18: Mean Percentage reduction after each dressing between Study Procedures						
Percentage reduction after each dressing	PRF Procedure		PRP Procedure		Kolmogorov-Smirnov Z	p value
	Mean	SD	Mean	SD		
1st	63.5	23.1	64.0	45.7	1.156	0.138
2nd	54.6	27.1	46.1	31.1	0.548	0.925
3rd	64.1	22.6	73.8	18.3	0.831	0.494
4th	44.8	39.4	53.7	31.3	0.655	0.785
5th	71.4	2.5	62.5	0.0	-	-
6th	60.0	0.0	-	-	-	-

Figure 16: Mean Percentage reduction after each dressing between Study Procedures



Between ulcers due to Hansen's disease and that due to other dermatological conditions (non Hansen's) there was no significant difference between mean percentage reduction per PRF dressing done. (Table 19)

Table 19: Mean Percentage reduction after each PRF dressing between Diagnosis

Percentage reduction after each PRF dressing	Hansen's disease		Non Hansen's disease		p value
	Mean	SD	Mean	SD	
1st	67.2	25.7	55.2	14.7	0.306
2nd	53.7	28.2	56.3	27.8	0.87
3rd	62.6	23.2	66.8	24.7	0.786
4th	10.8	4.5	78.8	5.4	0.423
5th	71.4	2.5	-	-	
6th	60.0	0.0	-	-	

Similarly after each PRP dressing done, there was no statistically significant difference in mean percentage reduction in volume of ulcer seen in ulcers due to Hansen's disease and other dermatological conditions. (Table 20)

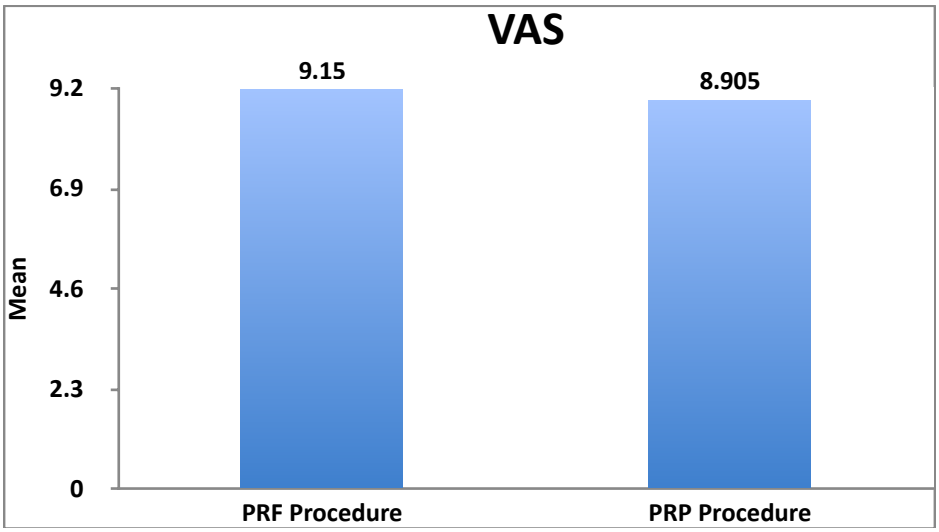
Table 20: Mean Percentage reduction after each PRP dressing between Diagnosis					
Percentage reduction after each PRP dressing	**Hansen's disease**		**Non Hansen's disease**		**p value**
	Mean	**SD**	**Mean**	**SD**	
1st	63.0	49.5	67.4	35.1	0.857
2nd	54.9	23.3	22.0	40.6	0.068
3rd	69.4	19.0	87.1	8.6	0.265
4th	50.9	43.7	59.2	0.0	0.902
5th	62.5	0.0	-	-	
6th	-	-	-	-	

Distribution of VAS between Study Procedures

Mean VAS for patient's assessment of improvement was higher in subjects in Group A (9.2±1.6) when compared to those in Group B (8.9±0.9). This was not significant (Table 21, Figure 17).

Table 21: Distribution of VAS between Study Procedures					
Parameters	**PRF Procedure**		**PRP Procedure**		**p value**
	Mean	**SD**	**Mean**	**SD**	
VAS	9.2	1.6	8.9	0.9	0.551

Figure 17: Distribution of VAS between Study Procedures



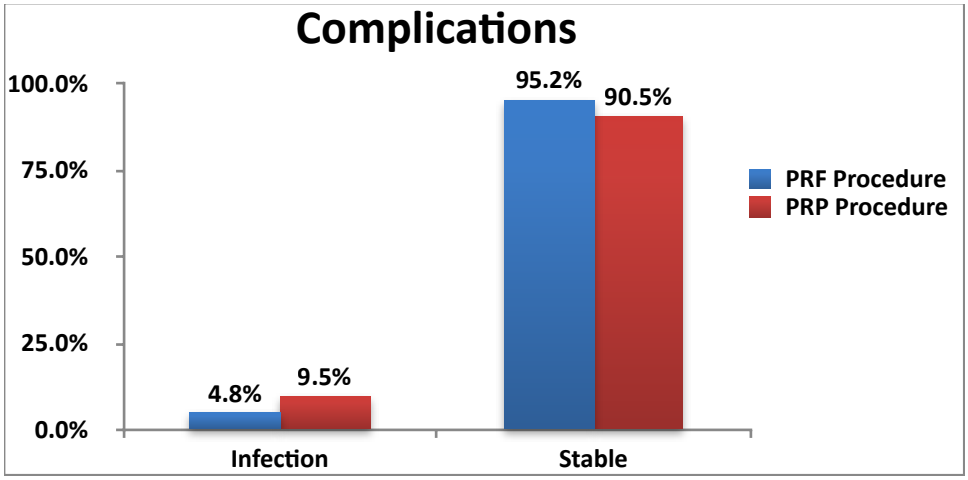
Post procedure complications between study procedures:

Infections were seen in one ulcer in PRF (Group A) i.e. 4.8% and two ulcers in PRP (Group B) i.e. 9.5%. Procedures had to be abandoned for two ulcers, one ulcer each from Group A and Group B due to development of infection in the ulcers. One ulcer patient from Group B presented with infection at the two week follow-up, after complete re-epithelization. (Table 22, Figure 18)

Table 22: Distribution of complications between Study Procedures

Complications	PRF Procedure		PRP Procedure		p value
	N	%	N	%	
Infection	1	4.8%	2	9.5%	0.549
Stable	20	95.2%	19	90.5%	
Total	21	100.0%	21	100.0%	

Figure 18: Distribution of Complications between Study Procedures



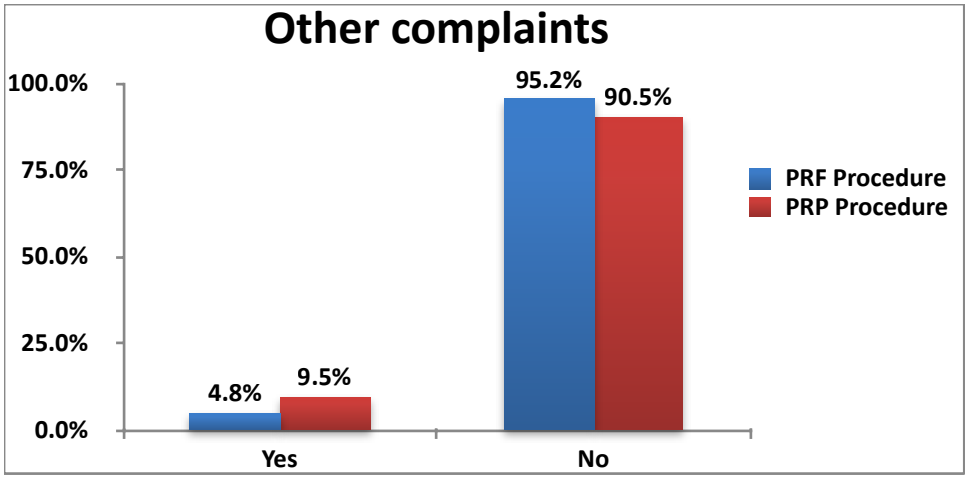
Other complaints:

Patients with ulcers that developed infection gave complaints of pain and discharge from ulcer. This was seen in 1 ulcer in PRF group (4.8%) and 2 ulcers in PRP group (9.5%). There was no difference in complaints caused during with of the procedures. (Table 23, Figure 19)

Table 23: Distribution of Other complaints between Study Procedures

Other complaints	PRF Procedure		PRP Procedure		p value
	N	%	N	%	
Yes (Pain, discharge)	1	4.8%	2	9.5%	0.549
No	20	95.2%	19	90.5%	
Total	21	100.0%	21	100.0%	

Figure 19: Distribution of Other complaints between Study Procedures



Recurrences:

Recurrences were seen in 4 ulcers in the PRF group (19%) and 3 ulcers in the PRP group (14.3%). However, these recurrences was not seen during the duration of the study. Other ulcers showed no evidence of recurrence in either of the group during the duration of the study. (Table 24, Figure 20)

Table 24: Distribution of Recurrence between Study Procedures

Recurrence	PRF Procedure		PRP Procedure		p value
	N	%	N	%	
Yes	4	19.0%	3	14.3%	0.788
No	16	76.2%	16	76.2%	

Figure 20: Distribution of Recurrence between Study Procedures

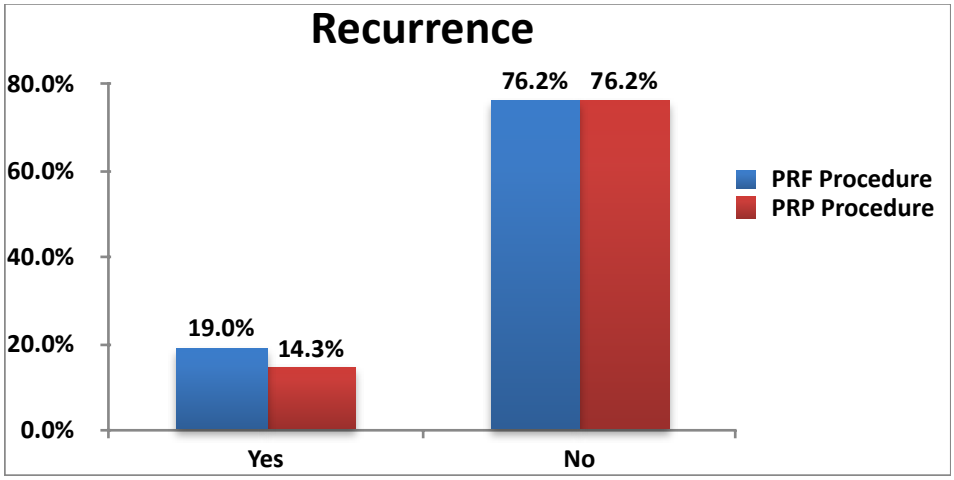


Figure 21: Clinical picture of PRF dressings in ulcer due to Hansen's disease: (A) at baseline (B) after 1st PRF dressing (C) after 3rd PRF dressing (re-epithelized)

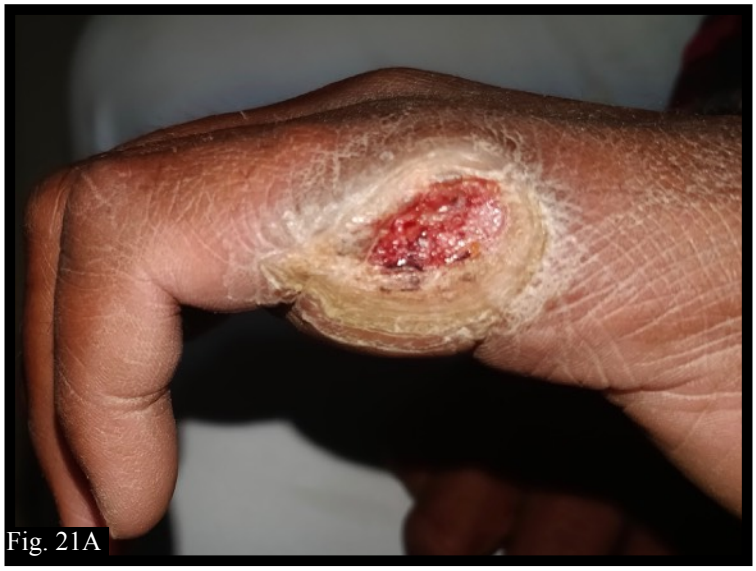


Fig. 21A

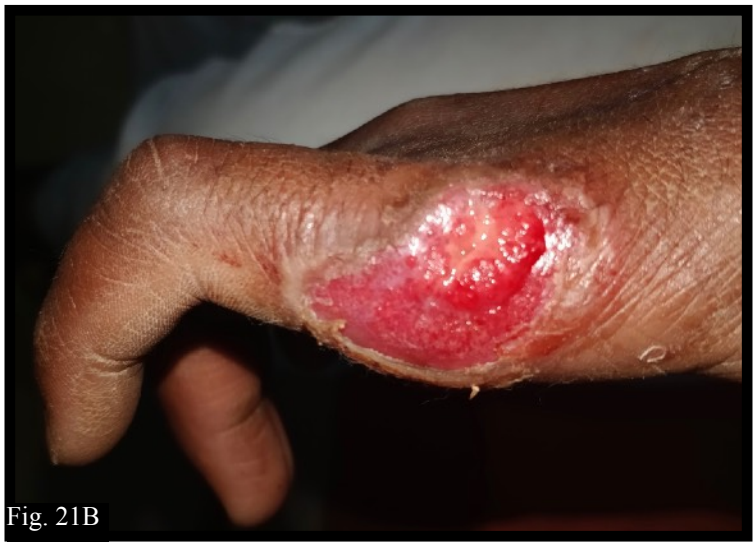


Fig. 21B

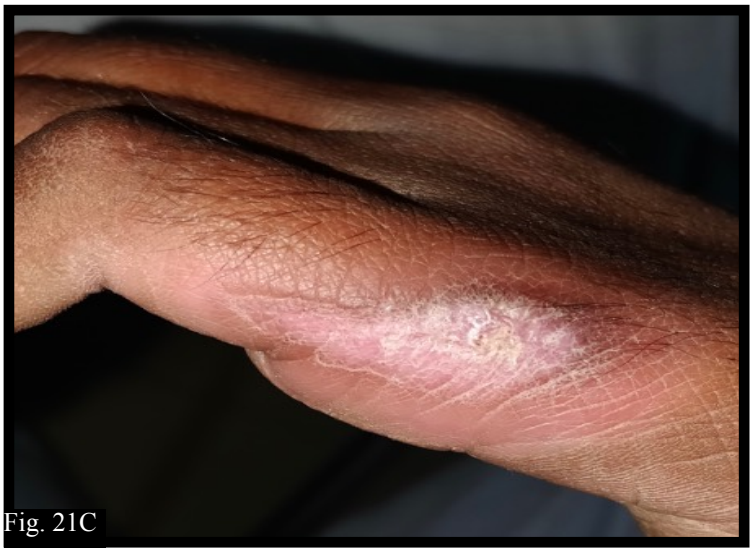


Fig. 21C

Figure 22: Clinical picture of PRP dressing in ulcer due to Hansen's disease: (A) at baseline (B) after 1st PRP dressing (C) after 3rd PRP dressing (re-epithelized)

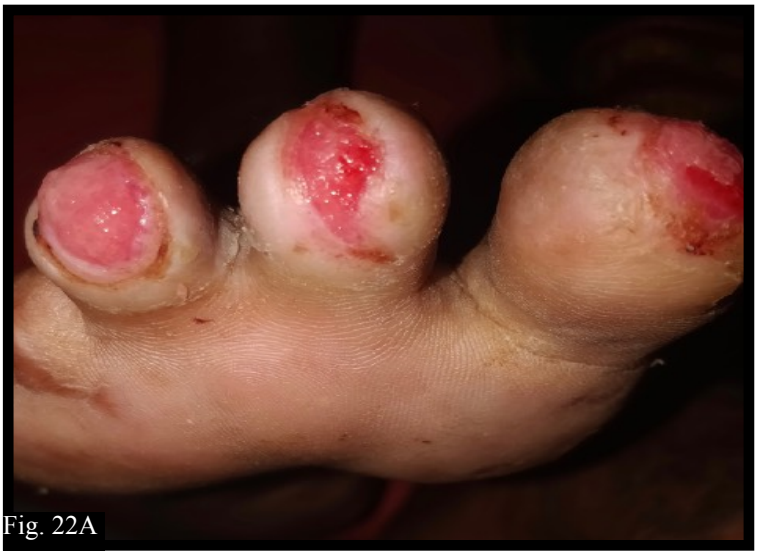


Fig. 22A

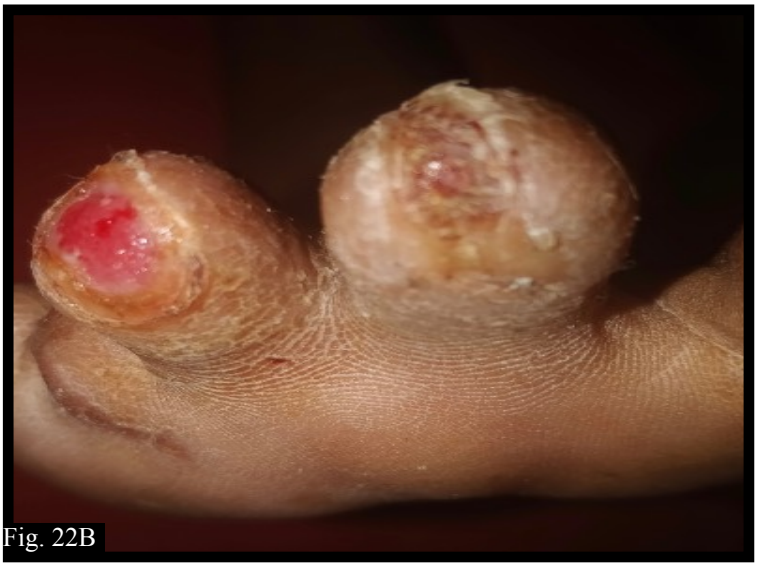


Fig. 22B

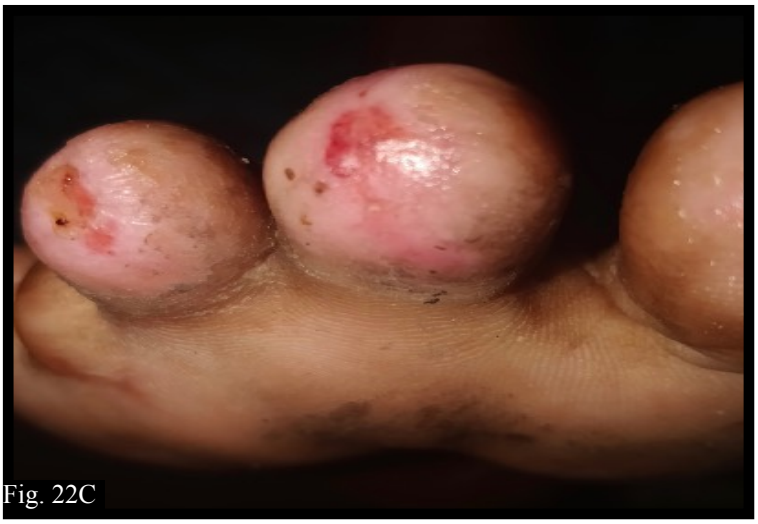


Fig. 22C

Figure 23: Clinical picture of PRF dressing in ulcer due to Hansen's disease: (A) at baseline (B) after 1st PRF dressing (C) after 6th PRF dressing

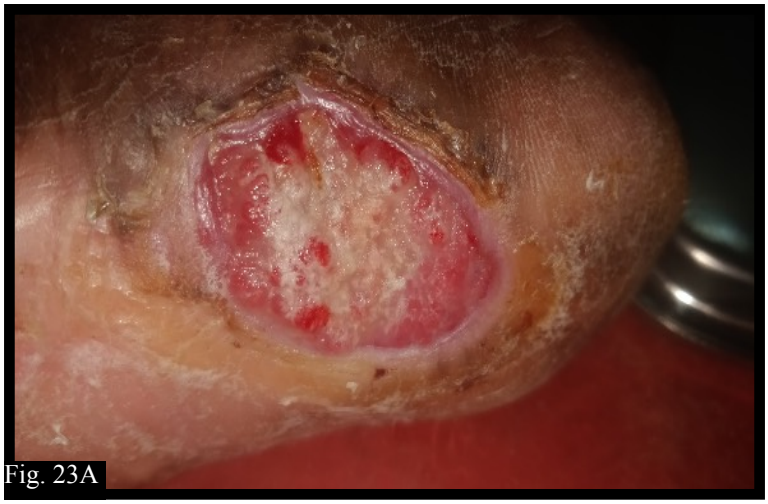


Fig. 23A

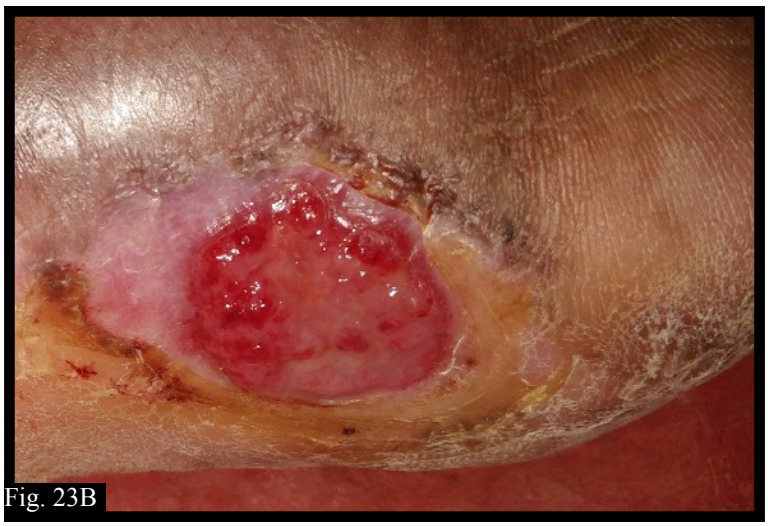


Fig. 23B

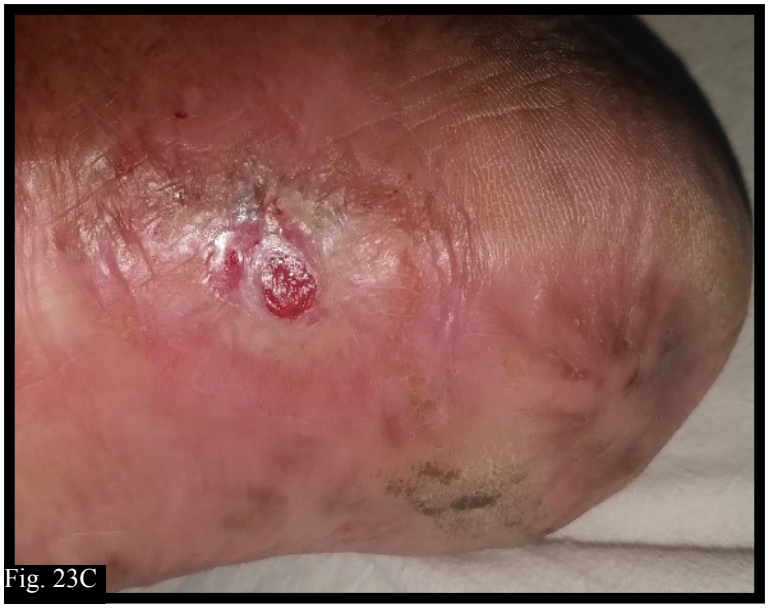


Fig. 23C

Figure 24: Clinical picture of PRP dressing in ulcer due to Hansen's disease: (A) at baseline (B) after 1st PRP dressing (C) after 4th PRP dressing

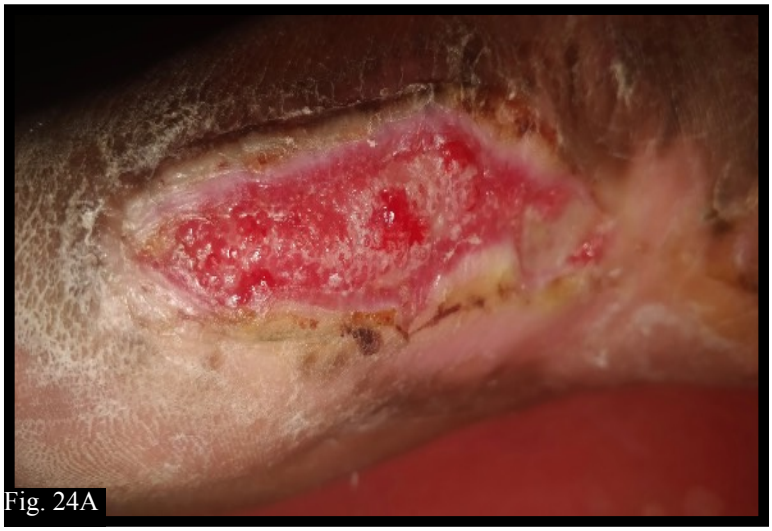


Fig. 24A

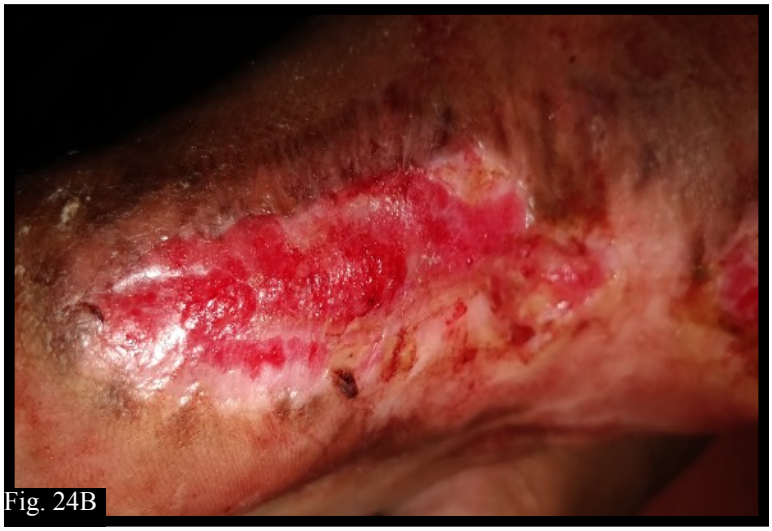


Fig. 24B

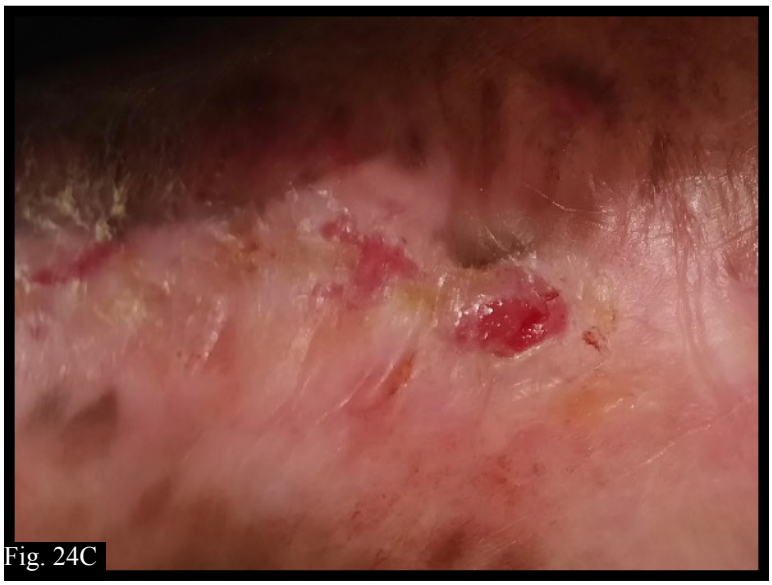


Fig. 24C

Figure 25: Clinical picture of completely re-epithelized ulcers secondary to Hansen's disease 1 month after completion of PRP and PRF dressings.

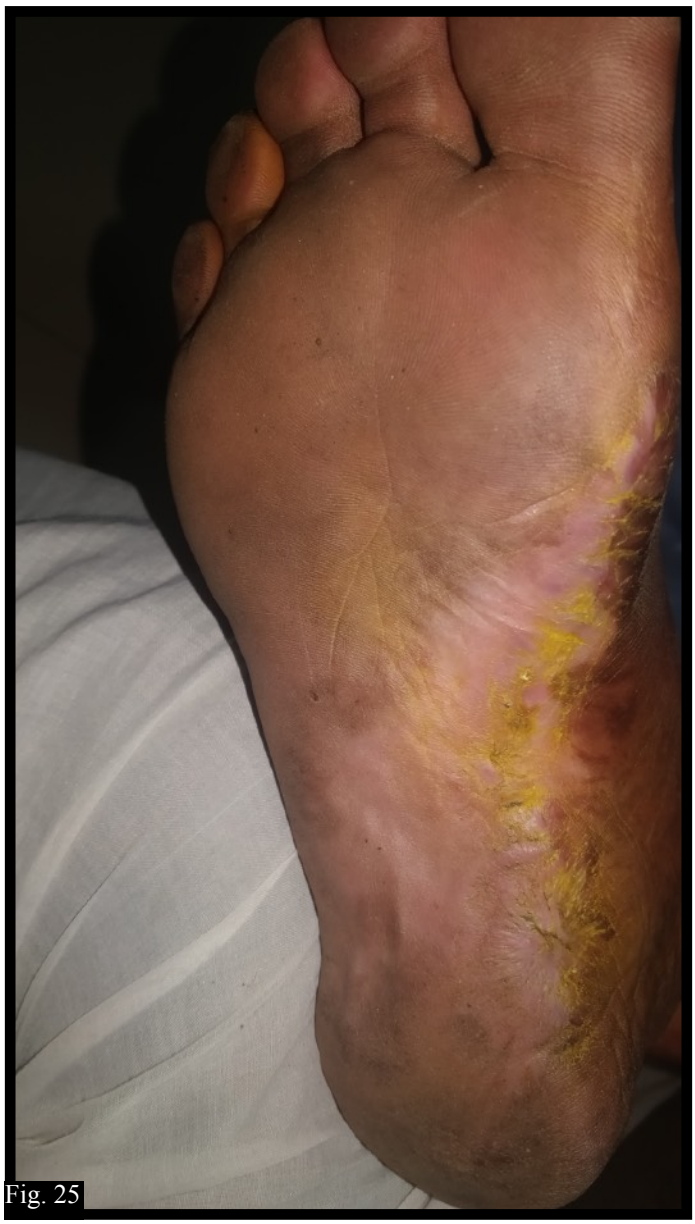


Figure 26: Clinical picture of PRF dressing in ulcer due to Keloid with chronic ulcer:

(A) at baseline (B) after 1st PRF dressing (C) after 4th PRF dressing

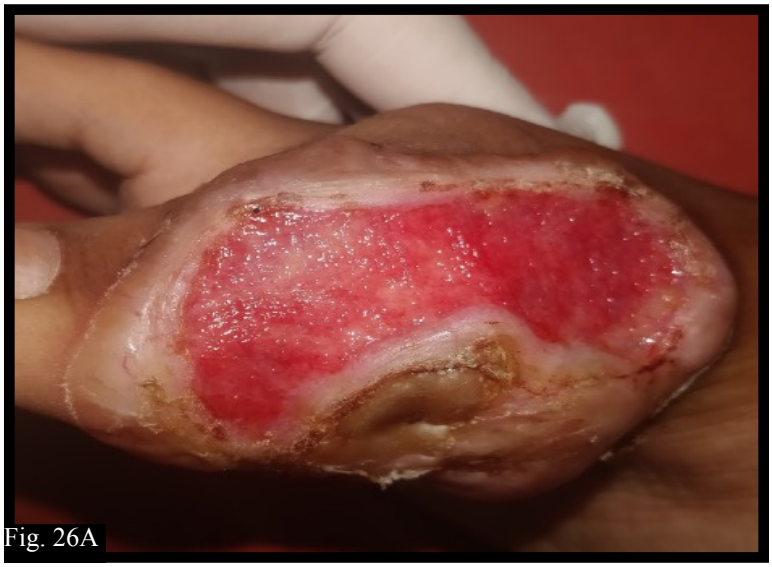


Fig. 26A

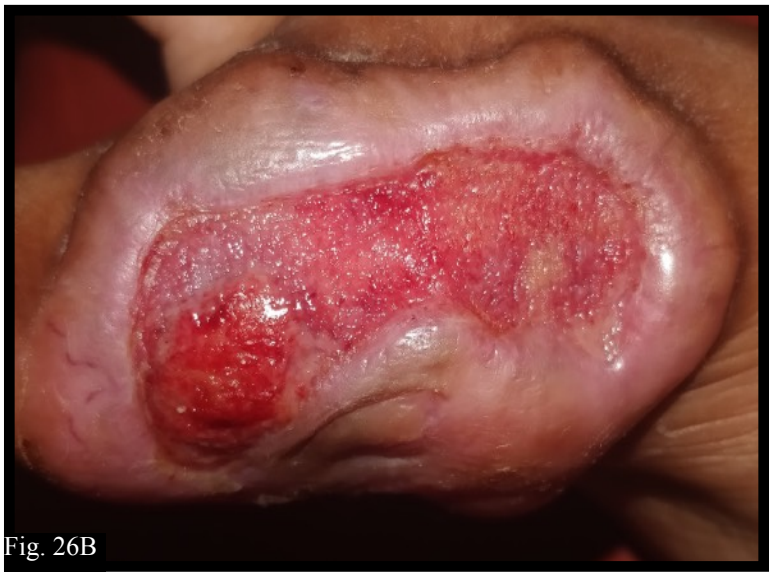


Fig. 26B

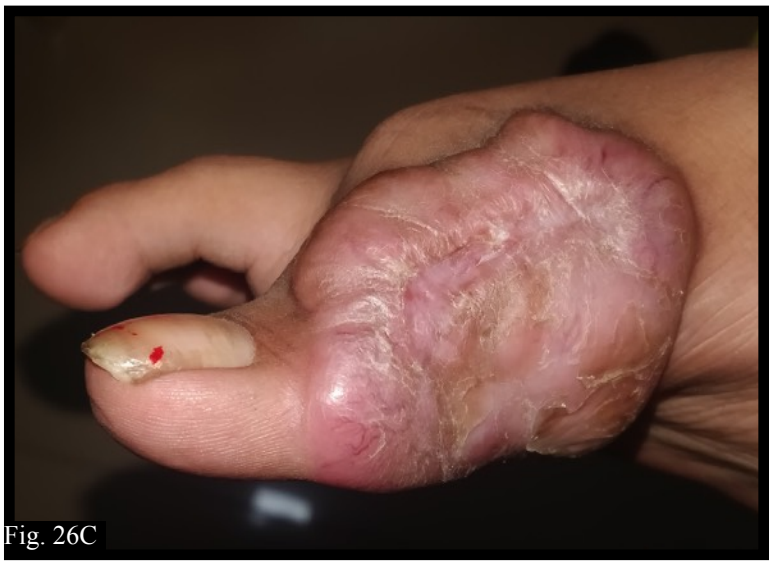


Fig. 26C

Figure 27: Clinical picture of PRP dressing in ulcer due to Necrolytic acral erythema:

(A) at baseline (B) after 1st PRP dressing (C) after 3rd PRP dressing (re-epithelized)

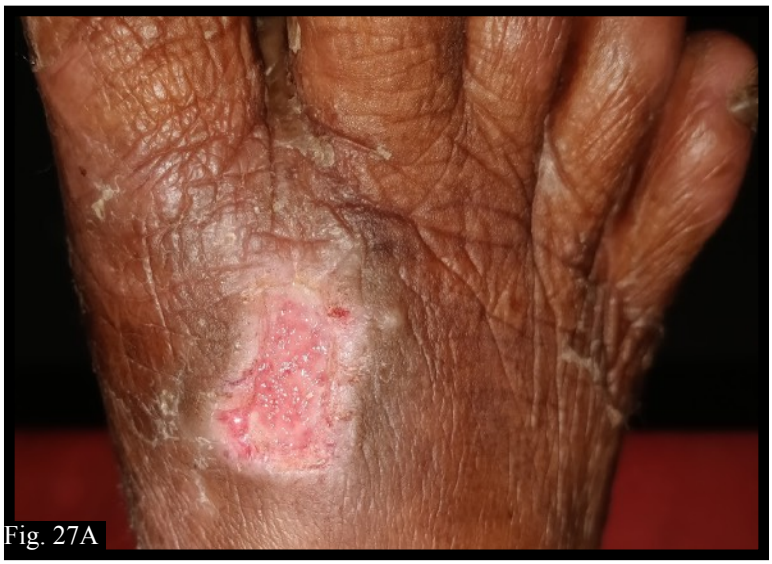


Fig. 27A

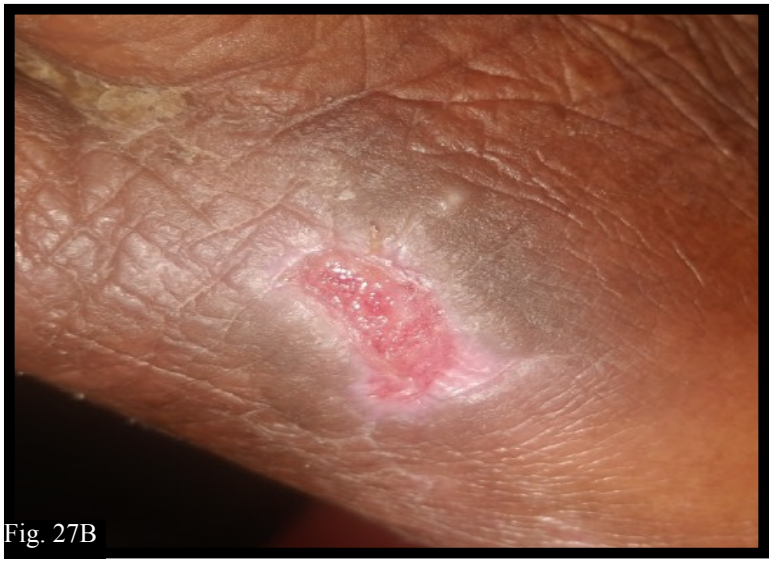


Fig. 27B

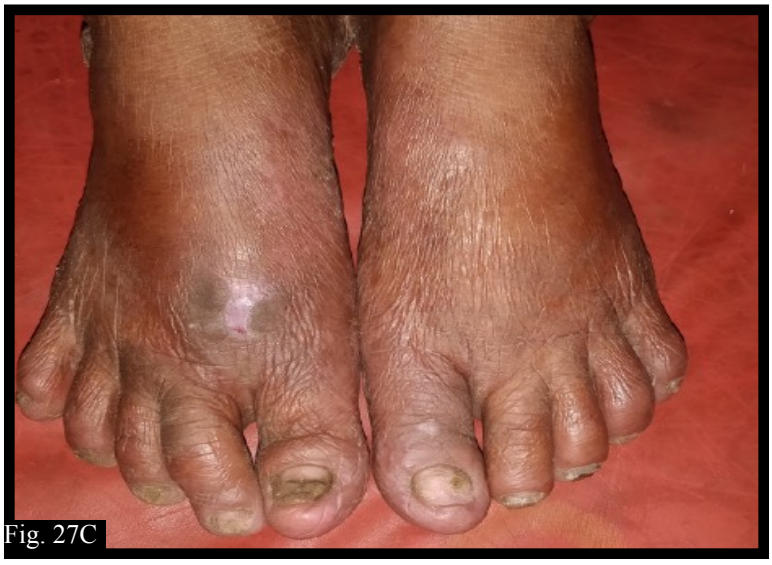


Fig. 27C

Figure 28: Clinical picture of PRF dressing in ulcer due to Necrolytic Acral Erythema: (A) at baseline (B) after 1st PRF dressing (C) after 3rd PRF dressing (re-epithelized)

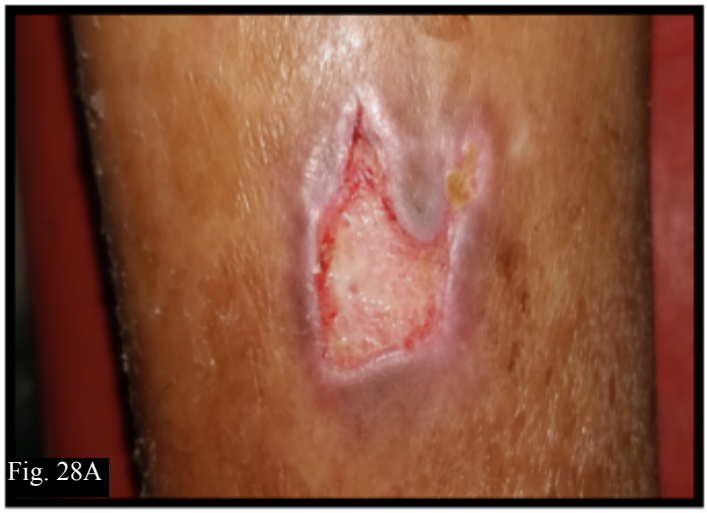


Fig. 28A

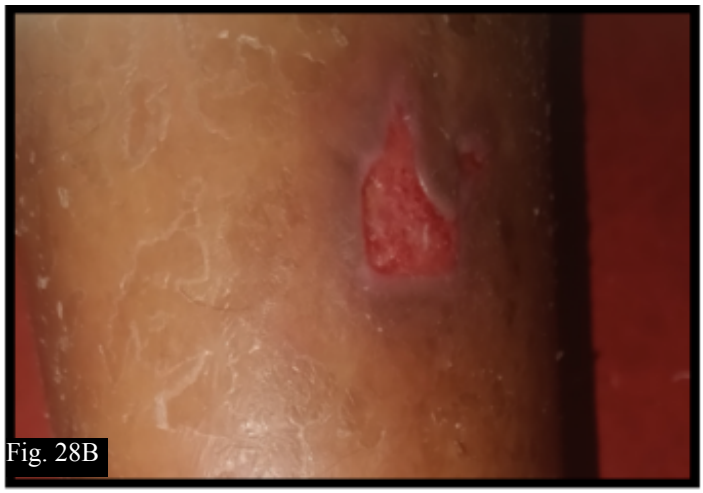


Fig. 28B

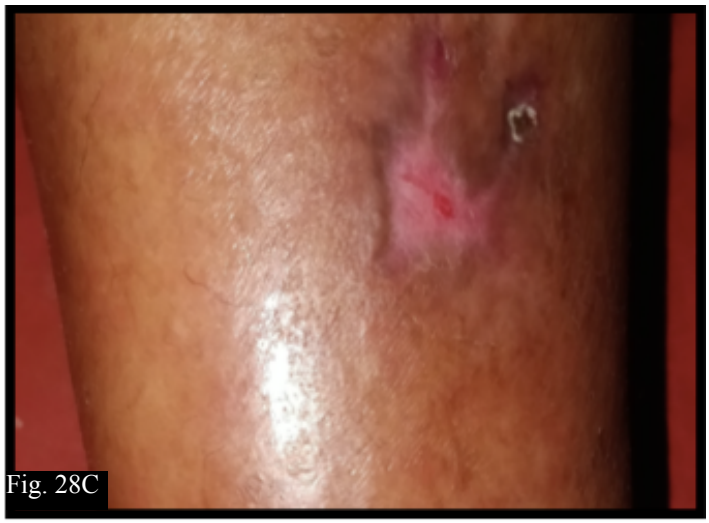


Fig. 28C

Figure 29: Clinical picture of PRP dressing in ulcer due to Necrolytic acral erythema:

(A) at baseline (B) after 1st PRP dressing (re-epithelized)

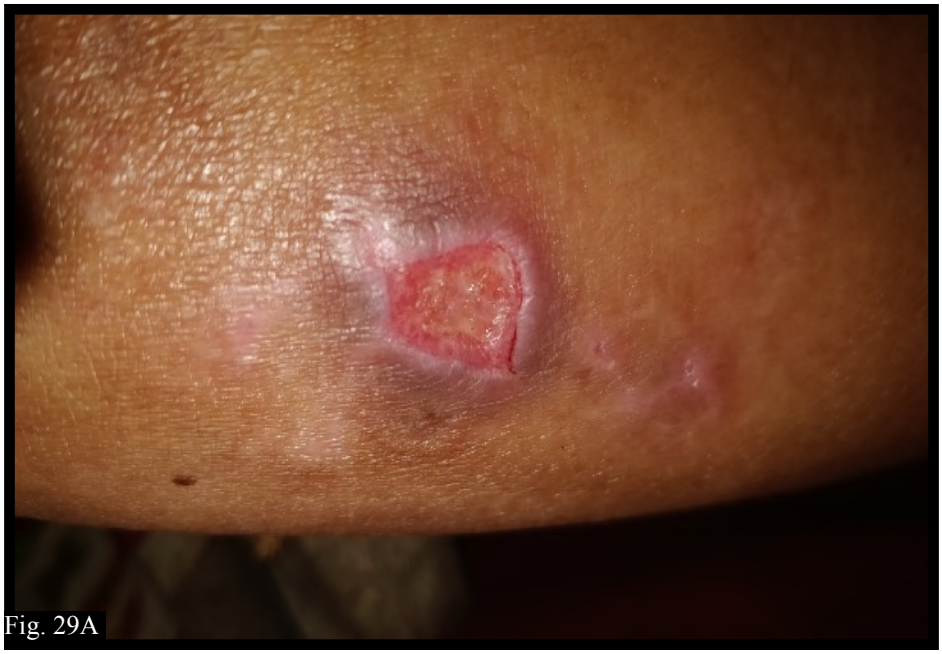


Fig. 29A

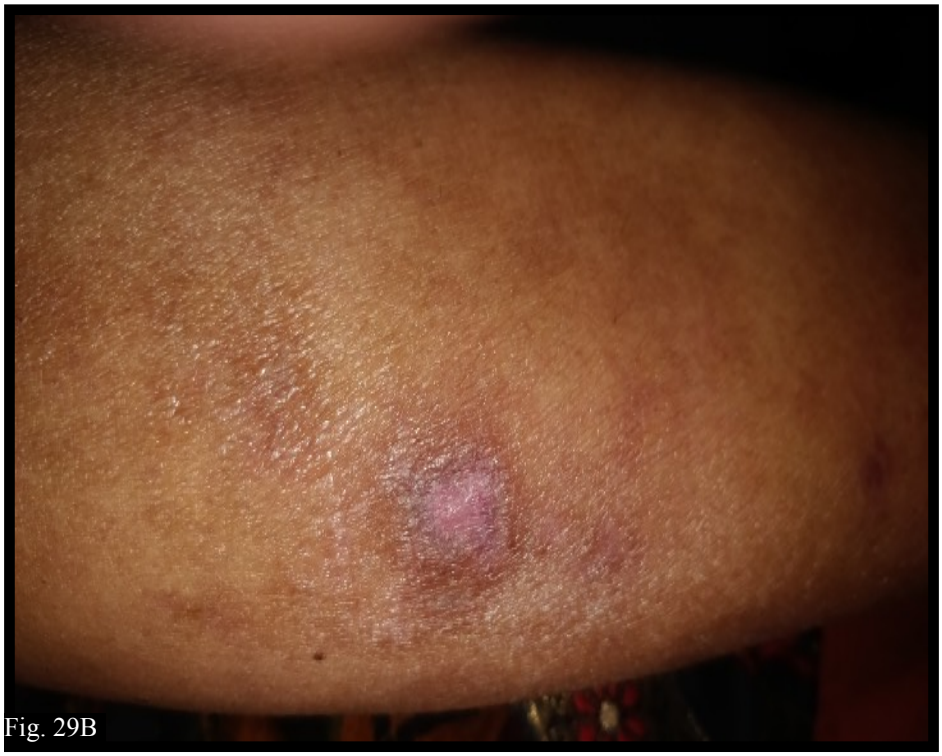


Fig. 29B

Figure 30: Clinical picture of PRF dressing in ulcer due to Hansen's disease: (A) at baseline (B) after 1st PRF dressing (C) after 7th PRF dressing (re-epithelized) (D) Recurrence after 3 months

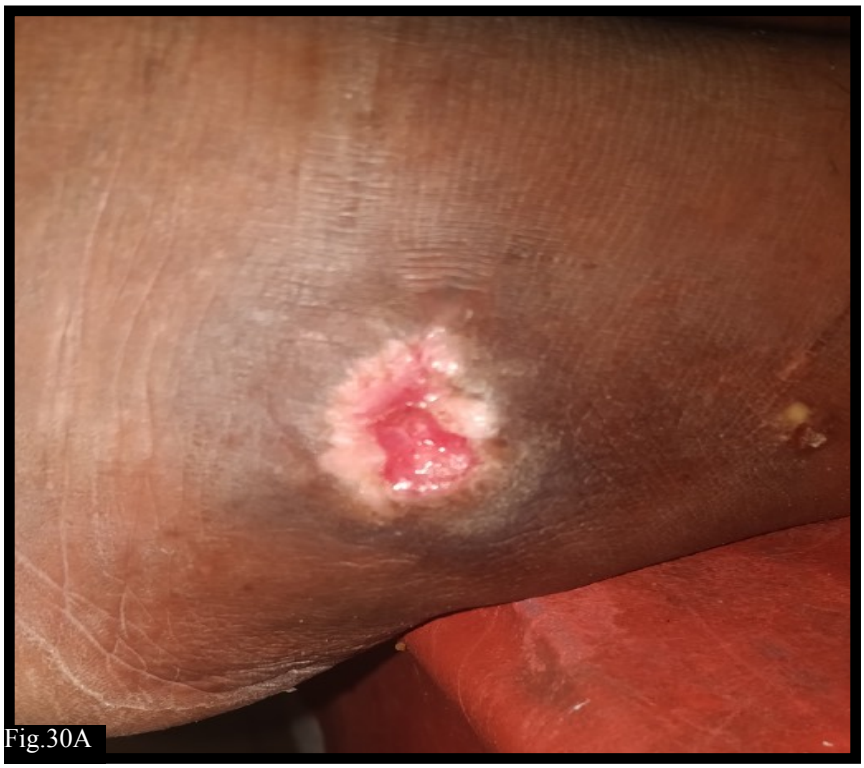


Fig.30A

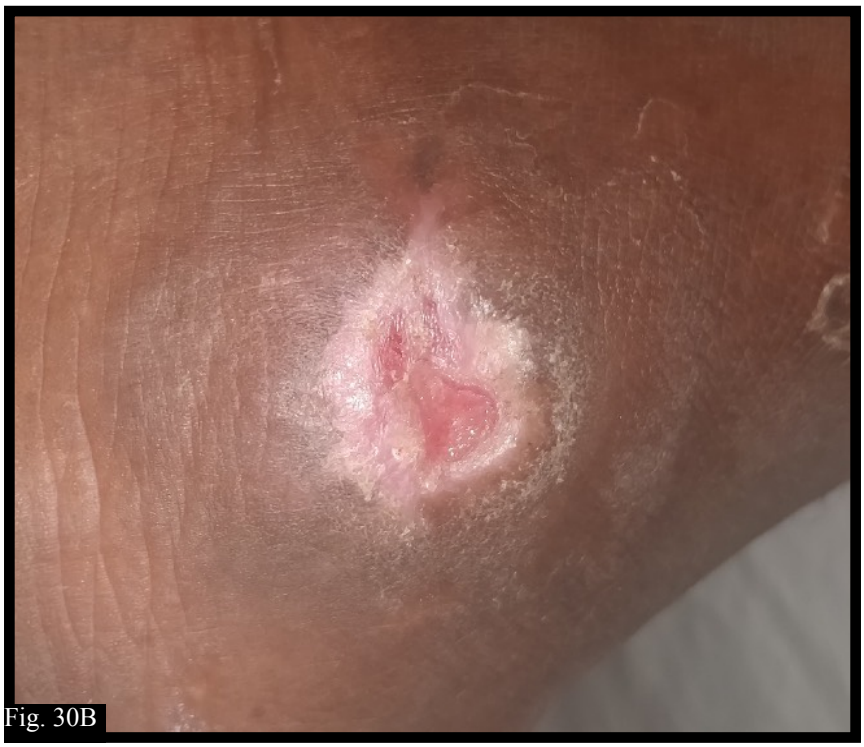


Fig. 30B

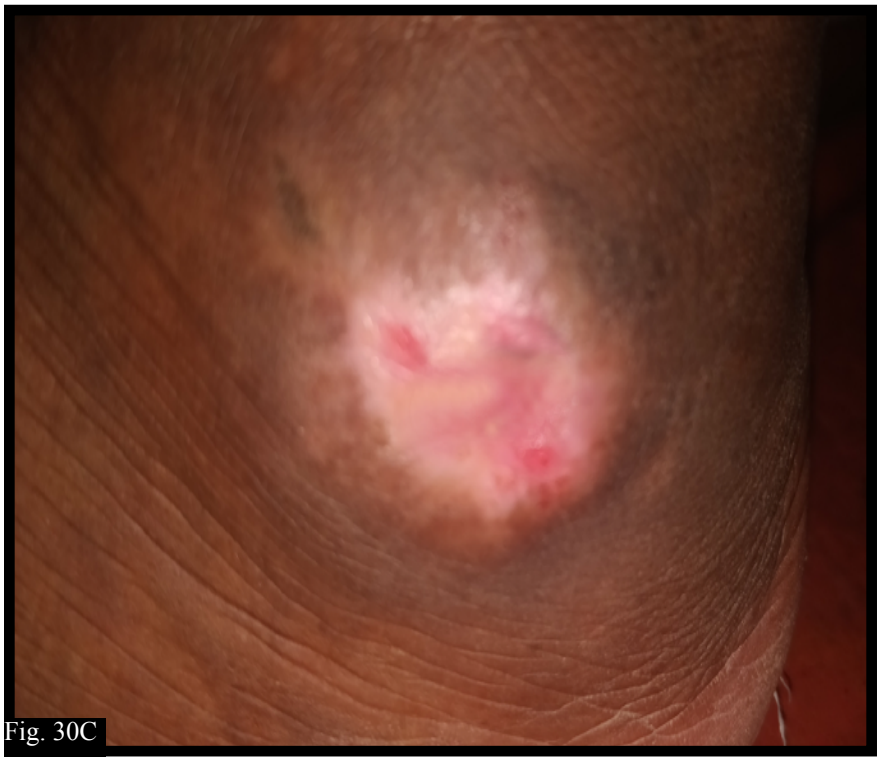


Fig. 30C

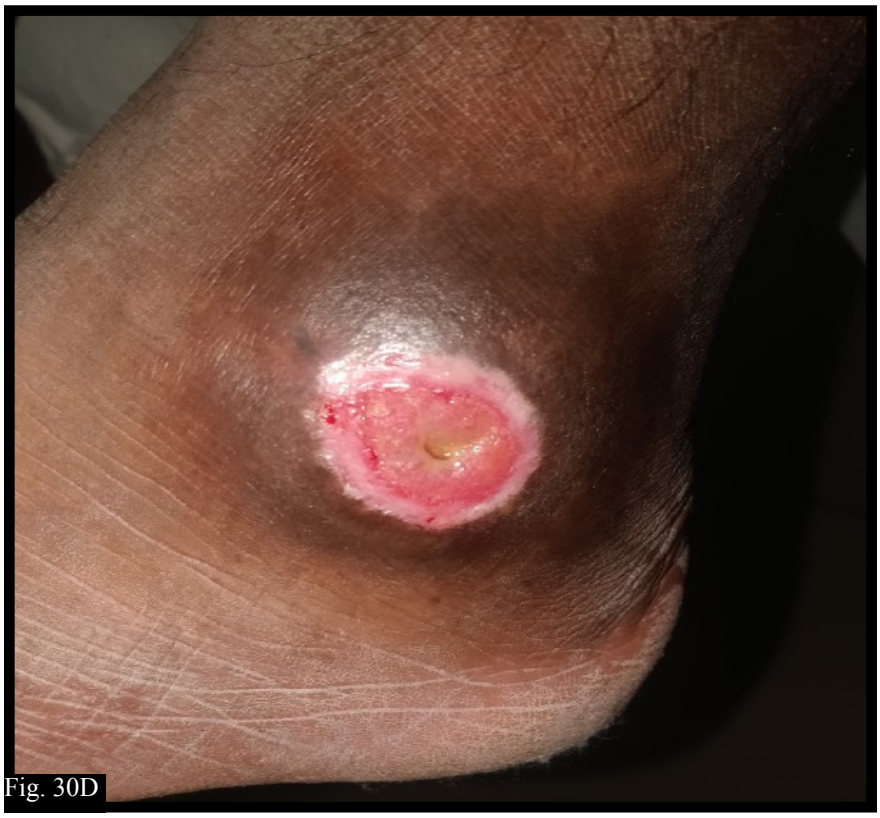


Fig. 30D

Figure 31: Clinical picture of PRP dressing in ulcer due to Hansen's disease: (A) at baseline (B) after 1st PRP dressing (C) after 6th PRP dressing (re-epithelized) (D) recurrence after 3 months

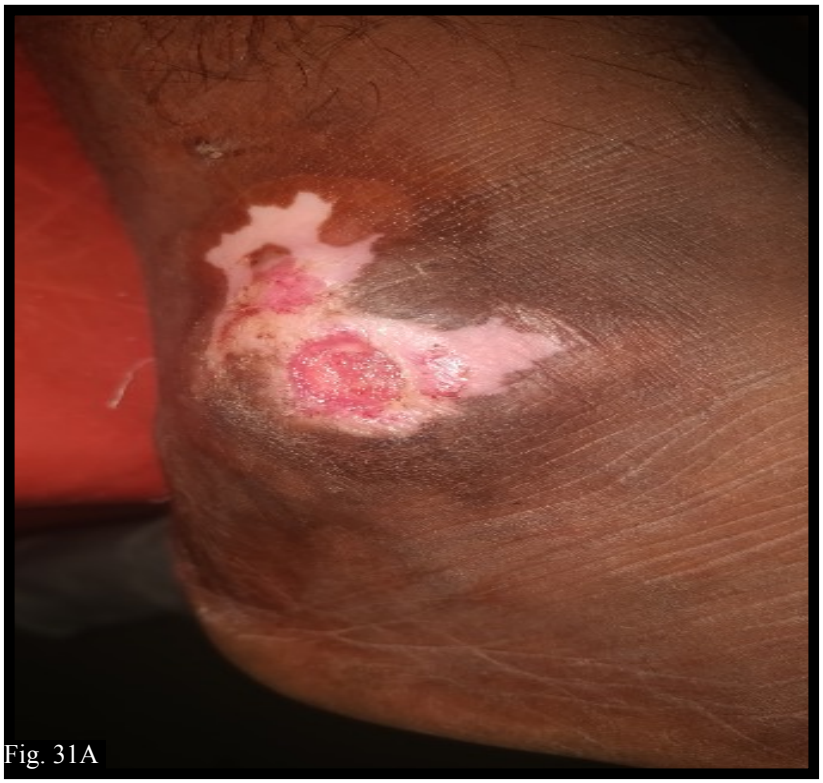


Fig. 31A

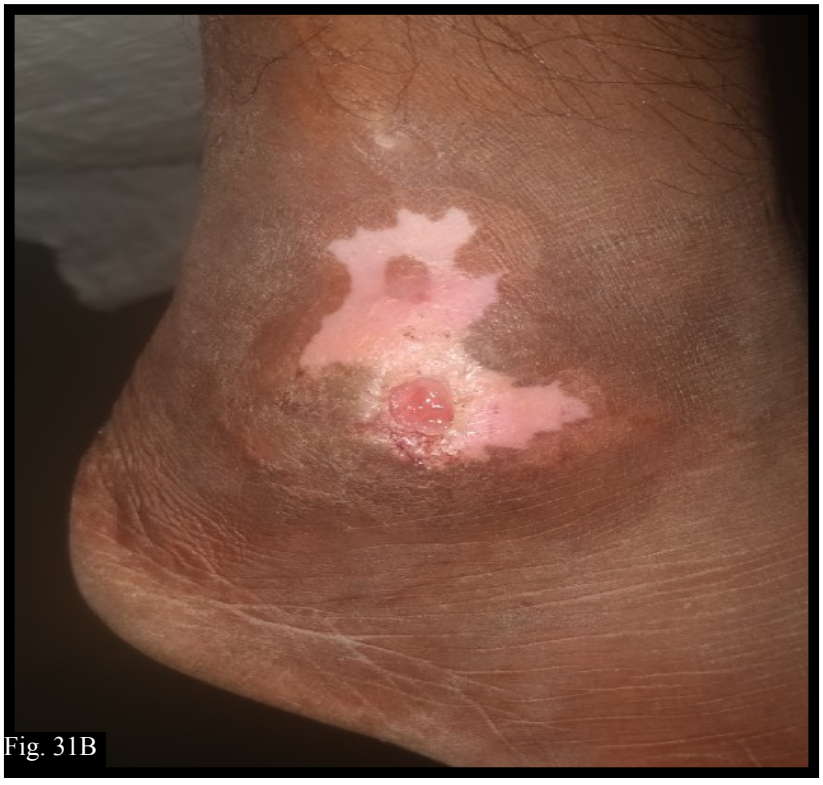


Fig. 31B

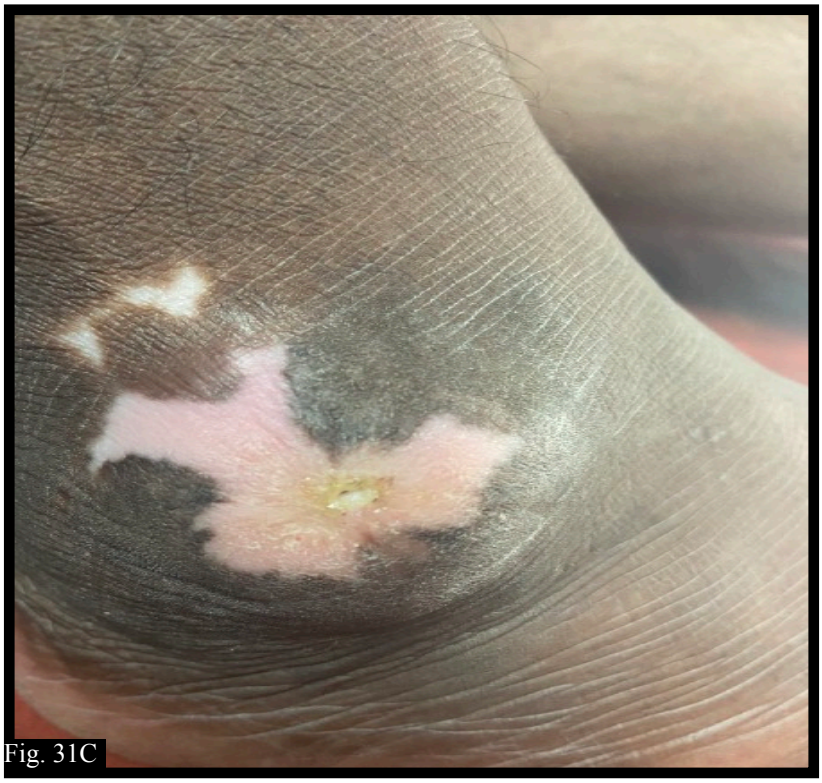


Fig. 31C

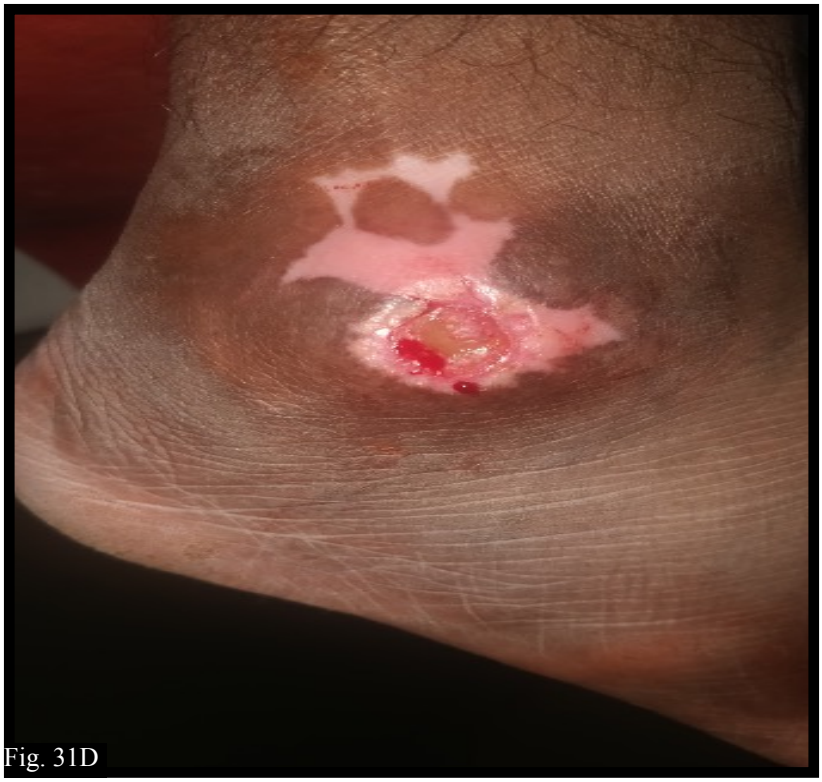


Fig. 31D

DISCUSSION

DISCUSSION

Chronic non healing ulcers are those wounds that show no tendency to heal after 6 weeks of appropriate treatment or those that have not fully healed after 12 months. Symptoms of chronic cutaneous ulcers may include increasing pain (except in trophic ulcers due to Hansen's disease), friable granulation tissue, foul odour, and wound breakdown instead of healing.[14]

Chronic cutaneous ulcers have an impact on almost every aspect of a person's day to day life. Common aspects include persistence of pain, sleep disturbances, restriction of mobility and work capacity, among other complaints. Finances of the patient and their family are also severely affected. Social activities may be restricted due to fear of injury and stigma from society. Thus chronic non healing ulcers are associated with significant morbidity, loss of productivity, and reduced quality of life along with high cost of healthcare. Chronic ulcers of the leg are a common cause of morbidity, and its prevalence in the community ranges from 1.9% to 13.1%.[14]

Our study enrolled 21 patients with a total of 44 chronic cutaneous ulcers. As each ulcer demonstrated individual variation in morphology and response to treatment, hence was treated as an individual case. Out of the 44 ulcer cases 2 cases were lost to follow-up. Thus, 42 ulcer cases were effectively evaluated in the study. The 42 chronic ulcers were alternately allocated into Group A and Group B. The ulcers in both groups were evaluated. Ulcers in Group A were treated with PRF dressings, whereas those in Group B were treated with PRP dressings respectively. In both groups, the dressings were done 1 week apart upto a maximum of 6 dressings or until complete re-epithelization, whichever was earlier. The patients were

followed up at baseline, at every visit and 2 weeks (week 8) after complete re-epithelization or after a maximum of 6 dressings.

Of the 42 ulcer cases evaluated in our study, 15 (35.7%) were seen in male patients and 27 (64.2%) were seen in female patients.

Mean age in the PRF group of our study was 46.2 ± 20.8years, which was comparable to that in the study conducted by Nagaraju U *et al.*[3] and in the PRP group of our study was 48.7 ± 19.1 years, which was similar to that seen in the study conducted by Anandan V et. al.[79]

Of the total number of ulcers in our study, 15 ulcers (71.4%) in Group A (PRF) and 16 ulcer (76.2%) ulcers in Group B (PRP) were seen in patients with Hansen's disease, they were all trophic ulcers, whereas 6 ulcers (28.6%) in the PRF group and 5 ulcers (23.8%) in the PRP group were caused due to other dermatological complaints like infected eczema, systemic sclerosis, keloid, necrolytic acral erythema.

The most common site of ulcers seen in our study in both groups was the right foot, with 12 ulcers (57.14%) seen in Group A and 9 ulcers (42.8%) seen in Group B. Other common sites included left foot (9.5% in Group A & 23.8% in Group B) followed by the right ankle and leg. This was similar to the sites seen in studies conducted by Anandan V et. al.[79] Overall chronic ulcers especially trophic ulcers in Hansen's disease were more common on the lower limbs.

The choice of treatment of the chronic ulcers was independent of both the morphological features, site as well as severity. Each of the ulcers present were alternately allotted into Group A and group B. Mean volume of ulcer at baseline was slightly higher in

Group A (1332.7mm^3) when compared to that in Group B (1090.8mm^3). However, this was not statistically significant.

Mean number of dressings required in each group for re-epithelization was 3.5 in Group A and 3.2 in Group B. This shows that both procedures were similarly efficacious in achieving re-epithelization. This was also true when comparing mean number of dressings required for ulcers due to Hansen's disease (3.3) and those due to non-Hansen's complaints (3.6). However, total number of dressings required for ulcers due to non-Hansen's complaints was lower (upto 5) as compared to the total number of dressings required for ulcers due to Hansen's disease. However, this difference was not statistically significant.

In our study, the improvement in chronic ulcer and response to treatment was assessed by percentage reduction in volume of ulcer per dressing. In Group A, mean percentage reduction in volume of ulcer was between 44.8 - 71.4% in each dressing. In Group B, this range was between 46.1 - 73.8% per dressing. In both groups, there was uniformity in percentage reduction of volume of ulcers per dressing per week, with no significant difference in percentage reduction in any one particular procedure.

The mean percentage reduction of volume of ulcer due to Hansen's disease in group A which underwent PRF dressings was in the range of 10.8% - 71.4% and 55.2% - 78.8% in ulcers due to other dermatological conditions. The least mean percentage reduction ulcer volume was seen in the 4th sitting for ulcers due to Hansen's disease as compared to ulcers due to other dermatological conditions, however this difference was not statistically significant.

In group B, i.e. chronic ulcers undergoing PRP dressings showed mean percentage reduction of ulcer volume in the range of 50.9% - 63% in ulcers due to Hansen's disease and 22% - 87.1% in ulcers due to non Hansen's conditions. Here as well, the difference in percentage reduction in volume of ulcer between the two groups per session was not statistically significant.

Subjective assessment by patients in our study on the basis of the visual analogue scale (VAS) showed high mean VAS at the end of the treatment period with subjects in Group A showing mean VAS of 9.2±1.6 and Group B showing mean VAS of 8.9±0.9. The difference between the two groups was not statistically significant. However, high mean VAS further proves that both forms of procedures were well received by patients for healing of chronic cutaneous ulcers.

Complete re-epithelization was achieved in 20 ulcer cases in group A (95.2%) and 19 ulcer cases in group B (90.4%) out of the total of 21 ulcer cases allotted in both groups. This showed that both procedures were effective in achieving regeneration of tissues in chronic cutaneous ulcers, irrespective of the etiology of ulcers.

Complications were seen in both procedure groups with infection being the most common complication. This was seen in 1 ulcer receiving PRF dressing and 2 ulcers receiving PRP dressing. The procedure had to be abandoned for these ulcers. One ulcer in each group belonged to the same patient with diagnosis of systemic sclerosis, whereas, one ulcer in PRP group belonged to patient with trophic ulcer over left lateral malleolus secondary to Hansen's disease.

Serous and sero-purulent discharge were seen in all ulcers associated with infection, in both groups. Pain was seen in ulcers developing infection in patient with systemic sclerosis. Ulcers were not commonly associated with pain in patients with Hansen's disease. This can be attributed to anaesthesia and hypoaesthesia associated with the disease itself. Chronic ulcers due to other dermatological conditions were commonly associated with mild to moderate degree of pain.

Recurrence was seen in 4 ulcers in group A and 3 ulcers in group B. The ulcers that recurred were at the same site, however varied in size from the previous ulcer. Recurrence was more commonly seen in chronic ulcers due to Hansen's disease.

In the study conducted by Nagaraju U *et al.,* seven treated patients of Hansen's disease with nine non-healing trophic ulcers of more than 6 weeks duration were treated with Platelet Rich Fibrin Matrix (PRF-M) dressing and included patients with a mean age of 38.33 years.[3]

As in our study, the ulcers were treated with PRFM dressings at weekly intervals, repeated once a week for a maximum of five sittings as per requirement and showed a mean percentage improvement in volume of 97.74% at the end of second sitting. All ulcers closed by a maximum of five sittings.[3] In our study the mean percentage reduction in volume of ulcer in each sitting ranged from 10.8 - 71.4% per sitting, in the PRF group. Out of the 21 ulcers in group A, 20 ulcers (95.2%) showed complete re-epithelization.

The study also noted that the ulceration found in Hansen's disease is a result of nerve damage and cutaneous anaesthesia and not as a consequence of the infection itself (2) Similarly in our study, the chronic ulcers noted especially in Hansen's patients were seen in

patients both on active treatment and those who had been released from treatment. Hence, the activity of disease had no bearing on the presence or severity of ulcers.

The study conducted by Shreyas N S *et al.*, where efficacy of PRF therapy was compared with conventional dressing, showed significant differences in both the subjective surface area and the objective surface area improvement with better healing in the PRF group.[2]

The fibrin network in PRF has a homogeneous 3- dimensional organization, even more highly coherent than natural fibrin clots. These preliminary data therefore imply that PRF would not only be a new generation of platelet gel, but a completely usable healing concentrate.[2,52] The leukocytes and platelet rich patch embedded in the fibrin clot can thus provide a way of transferring concentrated cells and signals directly to a surface and would be beneficial for the healing of recalcitrant wounds.

The study further observed that unlike the PRP, PRF does not dissolve quickly after application, hence provides a strong fibrin matrix that is slowly remodelled in a way similar to that of blood clot.[2,52] The study also found that PRF was helpful in volume deficient wounds, where the PRF applied transformed into corresponding adjacent tissue muscle, subcutis and skin. Thus showing that weekly PRF dressing showed better healing as compared to conventional dressing. However, this study had limitations in comparing efficacy of PRP versus PRF.

Our study was able to quantify the percentage reduction in volume of ulcer at each sitting in both groups, hence was able to overcome this limitation of comparing the efficacy of PRP versus PRF. In our study, we found that the percentage reduction in volume of ulcer in

both groups was comparable and both procedures were similar in their efficacy towards healing of the ulcer, irrespective of the etiology of the ulcer.

The study conducted by Anandan V *et al.*, observed the efficacy of PRP dressings on trophic ulcers caused due to leprosy. The methodology and follow-up in the study was comparable to that of our study. Each ulcer was followed up for a maximum of six dressings. This study had a predominance of male patients, which was in contrast with our study which showed predominance of ulcers in female patients.[79] However, mean age of patients (41.9) in this study was similar to our study.

Mean time for ulcer healing with PRP was around 4.38 weeks and 92% patients showed complete re-epithelization within 6 sittings of PRP treatment in the above study (3). Furthermore, studies conducted by Frykberg *et al.,* on 49 patients with 65 non-healing ulcers showed a mean healing time of 2.8 weeks[81] and that by Suryanarayanan S *et al.,* showed a mean duration of healing of 5.6±3.23 weeks among 24 patients.[80] The mentioned studies were done in chronic leg ulcers due to various causes including only a few patients with trophic ulcer secondary to Hansen disease.

Our study had predominance of patients with chronic ulcers due to Hansen's disease along with other dermatological conditions. Our study showed, mean number of weekly PRP dressings done was 3.2 and 95.2% patients in our study undergoing PRP dressings showed complete re-epithelization within 6 sittings.

Although the difference in mean number of dressing between the Hansen's and non Hansen's ulcer was not statistically significant, we observed that a slightly lesser number of dressings (upto 4 weekly dressings) were required in cases of chronic cutaneous ulcers due to

non Hansen's disease. This could be attributed to the decrease in vascularization, growth factors and nutrition in the trophic ulcers seen due to Hansen's disease, which may further delay the process of healing.

Studies conducted by Suthar M *et al.* established safety and efficacy along with improvement in quality of life in patient undergoing weekly PRP dressings.[4]

Studies conducted by Dohan *et al.*, Suchetha *et al.* and Yazawa *et al.* concluded that although PRP had a higher platelet concentration when compared to PRF, superior effects were seen in the use of PRF when compared to PRP as when the growth factors were incorporated into drug delivery systems such as fibrin, the mean concentration of growth factors in the platelet concentrates was three times or more than that observed with conventional platelet- rich plasma. Also in PRF, the growth factors were released in a controlled manner over approximately 1 week. Thus, slower, controlled and consistent release of growth factors from PRFM than PRP, hence showed better healing properties with PRF.[52,82,83]

In contrast, in our study we observed that the mean number of dressings and mean percentage reduction in volume of ulcer in both groups were comparable with no statistically significant difference in outcomes of ulcers in both groups.

Complications and recurrences were also comparable in both groups with no statistically significant difference in number of cases showing complications or recurrences, especially after complete re-epithelization.

CONCLUSION

CONCLUSION

The present study was aimed at assessing the efficacy of Platelet rich fibrin (PRF) versus Platelet rich plasma (PRP) as a regenerative medicine strategy for chronic cutaneous ulcers. A total 42 ulcer cases were evaluated in the study. Among the ulcers, 15 (35.7%) were seen in male patients and 27 (64.2%) were seen in female patients. Mean age of the patients in the PRF group was 46.2 ± 20.8years and in the PRP group was 48.7 ± 19.1 years. In both groups, the predominant cause for chronic cutaneous ulcer was Hansen's disease.

In the present study, patients in group A were treated with weekly PRF dressings and patients in group B were treated with weekly PRP dressings. There was significant improvement in reduction of the volume of ulcer after every follow-up sitting in both groups.

Both groups showed significant reduction in volume of ulcer at each sitting and achieving complete re-epithelization. 20 ulcer cases in group A (95.2%) and 19 ulcer cases in group B (90.4%) out of the total of 21 ulcer cases allotted in both groups.

On comparison of the percentage reduction in volume of ulcer at each visit between the two groups, both groups showed similar efficacy in mean percentage reduction in volume of ulcer.

Subjective improvement in the chronic cutaneous ulcers represented on the visual analogue scale showed high mean VAS at the end of the study in both groups. This showed that both procedures were highly efficacious and well received by patients undergoing the treatment for chronic cutaneous ulcers.

Complications following PRF and PRP dressings included infection associated with mild to moderate pain and sero-purulent discharge, and this was seen in both groups. The procedures had to be abandoned in these cases. The difference in number of cases with

complications was not statistically significant in both groups. No other significant adverse effect or complications were noted in the patients in this study due to the procedures done.

Recurrence of ulcers was noted in patients with Hansen's disease and was seen in similar numbers in both groups. This could be attributed to the nature of the disease and habits of the patients, wherein anaesthesia and hypoaesthesia along with poor nutrition and blood supply to the area may lead to formation and recurrence of ulcers.

Therefore, the study concludes that platelet rich fibrin and platelet rich plasma are similarly efficacious in healing of chronic cutaneous ulcers. They have similar profiles in complications and have similar recurrence rates, which are low. Both the procedure are successful in achieving complete re-epithelization of the ulcer. Thus, PRF and PRP dressings provide a safe, effective and inexpensive modality of therapy and hence can be used as a regenerative medicine strategy in the healing of chronic cutaneous ulcers.

SUMMARY

SUMMARY

A hospital based prospective, double blinded, randomized, comparative study was conducted from October 2018 to July 2020. Out of a total of 21 patients with 44 ulcers, 2 were lost to follow-up at the start of treatment, hence, 42 cases of chronic cutaneous ulcers were evaluated in the study. The enrolled ulcer cases were allotted into 2 groups:

- Group A (PRF dressing)
- Group B (PRP dressings)

A total of 6 PRF/ PRP dressings were performed 1 week apart. The ulcers were evaluated up at baseline, at every follow up and 2 weeks after the sixth session or complete re-epithelization.

- A total of 42 cases of chronic cutaneous ulcers were evaluated in the study.
- Females were predominant in our study.
- Most patients belonged to the middle age group with a mean age of 46.2 ± 20.8 years in group A and 48.7 ± 19.1 years in group B.
- The most common cause of chronic cutaneous ulcers seen in our study was due to Hansen's disease.
- There was no statistically significant difference between the two groups in the mean percentage reduction of volume of ulcer and both groups showed significant reduction in volume of ulcer and re-epithelization at a maximum of 6 sittings.
- Subjective assessment by patients using VAS also showed significant improvement in both groups.
- Most common complications occurring in both groups was infection associated with pain and sero-purulent discharge.

- Recurrence was seen in both groups, predominantly in ulcers due to Hansen's disease. However recurrence rate was low in both groups and the difference in recurrence in both groups was not statistically significant.
- Both procedures i.e. PRF and PRP show similar efficacy as a therapeutic modality in the treatment of chronic cutaneous ulcers and are a safe, effective and inexpensive regenerative medicine strategy for chronic cutaneous ulcers.

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BIBLIOGRAPHY

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ANNEXURE

ANNEXURE I

ETHICAL CLEARANCE CERTIFICATE

B.L.D.E (Deemed to be University)
SHRI.B.M.PATIL MEDICAL COLLEGE HOSPITAL & RESEARCH CENTRE
VIJAYAPUR – 586103

IEC/NO:287/2018
17-11-2018

INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 13-11-2018 at 03-15 PM scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected and revised version synopsis of the Thesis has accorded Ethical Clearance.

Title : Comparison of efficacy of autologous platelet rich fibrin and platelet rich plasma as a regenerative medicine strategy for chronic cutaneous ulcers.

Name of P.G. Student : Dr Vartika Ratan.
Department of Dermatology, Venerology & Leprosy

Name of Guide/Co-investigator: Dr.Arun.C.Inamadar, Professor & HOD
Department of Dermatology, Venerology & Leprosy.

DR RAGHAVENDRA KULKARNI
CHAIRMAN
Institutional Ethical Committee
BLDEU's Shri B.M. Patil
Medical College,BIJAPUR-586103.

Following documents were placed before E.C. for Scrutinization:

1) Copy of Synopsis/Research Project
2) Copy of informed consent form.
3) Any other relevant documents.

ANNEXURE II

PROFORMA

B.L.D.E. (Deemed to be University)

SHRI B. M. PATIL MEDICAL COLLEGE HOSPITAL AND

RESEARCH CENTRE, VIJAYAPUR.

Department of Dermatology, Venereology and Leprosy.

COMPARISON OF EFFICACY OF AUTOLOGOUS PLATELET RICH FIBRIN AND PLATELET RICH PLASMA AS A REGENERATIVE MEDICINE STRATEGY FOR CHRONIC CUTANEOUS ULCERS

SCHEME OF CASE TAKING

1. General information

Name: SL no:

Age:

Sex: Address:

Marital status: married/unmarried Education:

Occupation: Contact no:

Out patient no: Date:

1. Clinical Examination

- General Physical Examination:

Weight : Pallor :

BP : Clubbing :

PR : Icterus :

Cyanosis :

Oedema :

Lymphadenopathy :

Other findings:

- Systemic Examination

  Cardiovascular system :

  Respiratory system :

  Central nervous system :

  Abdominal examination :

- Cutaneous examination:
- Number of ulcers:
- Measurement of ulcer: Length =

  Width =

  Depth =

Volume of ulcer = (Length x Width x 0.7854) x Depth =

- Investigations

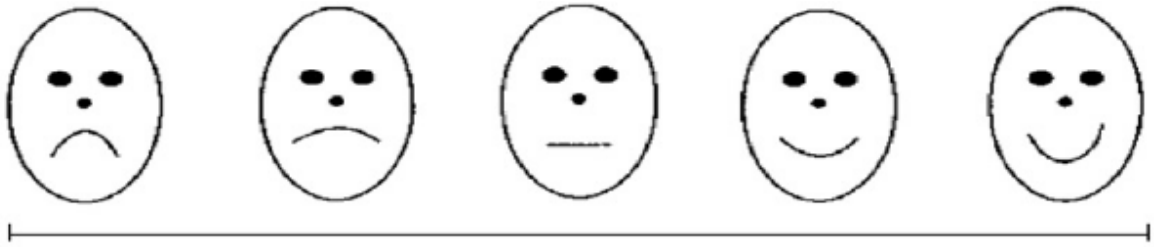
Complete blood count:-	
Total counts	cells/cmm
N/L/E/M/B	%
Hb	gm%
Platelet count	lakhs/cmm
Random Blood Sugar (RBS):-	mg/dl
HIV:-	
HbSAg:-	
S. Protein	
S. Albumin	
RBS	

- Pus Culture & Sensitivity:

	Procedure done	Volume of Ulcer	Percentage Difference in Volume of Ulcer
Baseline			
1st follow up			
2nd follow up			
3rd follow up			
4th follow up			
5th follow up			
6th follow up			
7th follow up			
8th follow up			

Patient self Assessment Scale of Ulcer Reduction.

Visual Analogue Scale



0 cms 2 4 6 8 10 cms

ANNEXURE III

CONSENT FORM

B.L.D.E. (Deemed to be University)

SHRI B. M. PATIL MEDICAL COLLEGE HOSPITAL AND

RESEARCH CENTRE, VIJAYAPUR.

Department of Dermatology, Venereology and Leprosy.

RESEARCH INFORMED CONSENT FOR

TITLE OF THE PROJECT	**:-**	COMPARISON OF EFFICACY OF AUTOLOGOUS PLATELET RICH FIBRIN AND PLATELET RICH PLASMA AS A REGENERATIVE MEDICINE STRATEGY FOR CHRONIC CUTANEOUS ULCERS
PG GUIDE	**:-**	DR. ARUN C.INAMADAR
PG STUDENT	**:-**	DR. VARTIKA RAVI RATAN

PURPOSE OF RESEARCH:-

I have been informed that this project will assess Autologous PRF dressings as a Regenerative Medicine Strategy for chronic cutaneous ulcers.

BENEFITS:-

I understand that my participation in this study will help the investigator to know the effectiveness of autologous PRF versus PRP dressings in chronic cutaneous ulcers.

PROCEDURE:-

I understand that relevant history will be taken and I will undergo detailed clinical examination after which treatment will be given.

RISK AND DISCOMFORTS:-

I understand the possible complications that may occur during and after the procedure, i.e., post procedure pain & swelling.

CONFIDENTIALITY:-

I understand that medical information produced by this study will become a part of my hospital records and will be subjected to the confidentiality and privacy regulation of the said hospital. Information of a sensitive personal nature will not be a part of the medical records, but will be stored in the investigator's research file.

If the data are used for publication in the medical literature or for teaching purposes no names will be used and other identifiers such as photographs and audio or videotapes will be used only with my special written permission. I understand I may see the photographs, videotapes and hear the audiotapes before giving this permission.

REQUEST FOR MORE INFORMATION:-

I understand that I may ask more questions about the study at any time concerned. Dr. Vartika Ratan is available to answer my questions or concerns. I understand that I will be informed of any significant new findings discovered during the course of this study, which may influence my continued participation.

REFUSAL OR WITHDRAWAL OF PARTICIPATION:-

I understand that my participation is voluntary and I may refuse to participate or may withdraw consent and discontinue participation in this study at any time without prejudice. I also understand that Dr. Vartika Ratan may terminate my participation in this study at any time after she has explained the reasons for doing so and has helped arrange for my continued care by my own physician, if this is appropriate.

INJURY STATEMENT:-

I understand that in the unlikely event of injury to me resulting directly from my participation in this study and if such injury were reported promptly, then medical treatment will be available to me, but no further compensation will be provided. I understand that by my agreement for my participation in this study, I am not waiving any of my legal rights.

I have explained to (patient's / relevant guardian's name) the purpose of the research, the procedures required, and the possible risks and benefits to the best of my ability in patient's own language.

_____ _____

Investigator / P. G. Guide Date

I confirm that ……..…(Name of the PG guide / chief researcher) has explained to me the research, the study procedures that I undergo and the possible risks and discomforts as well as benefits that I may experience. I have read and I understand this consent form. Therefore, I agree to give my consent for my participation as a subject in this research project.

_____ _____

Participant / guardian Date

_____ _____

Witness to signature Date

ANNEXURE IV

KEY TO MASTERCHART

PRF	PLATELET RICH FIBRIN
PRP	PLATELET RICH PLASMA
RE	RE-EPITHELIZED
ST	STABLE
IF	INFECTION
ABD	ABANDONED
P	PAIN
D	DISCHARGE
LF/U	LOSS TO FOLLOW-UP
VAS	VISUAL ANALOGUE SCALE

ANNEXURE V

Sr.no	Initials	Age	Sex	Diagnosis	Site	Procedure done	Initial vol. of ulcer in mm3	No. of PRF/PRP dressings done	Percentage reduction after each dressing							Status at 2 week follow-up	Other complaints	Recurrence	VAS
									1st	2nd	3rd	4th	5th	6th	7th				
1a	MK1018_01	45y	M	Infected eczema	Rt great toe	PRF	1407.43	3	81.2	97.32	RE					ST	N	N	9
1b	MK101801	45y	M	Infected eczema	Lt sole	PRP	7238.24	5	48.95	66.4	93.16	59.23	RE			ST	N	N	9
2a	MK1018_02	45y	M	Infected eczema	Rt sole	PRF	2695.49	5	55.24	44.14	90.21	75	RE			ST	N	N	9
2b	SB101802	50y	F	Systemic sclerosis	Lt great toe	PRP	3110.18	3	14.15	19.52	ABD					IF	PD	-	-
3a	SB1018_03	50y	F	Systemic sclerosis	Lt heel	PRF	4043.23	2	37.06	ABD						IF	PD	-	-
3b	MB111803	55y	F	Hansen's disease	Rt foot	PRP	534.07	5	75.8	39.1	57.3	81.8	RE			ST	N	N	10
4a	GC1118_04	80y	F	Hansen's disease	Rt great toe	PRF	47.124	1	RE							ST	N	N	10
4b	MB111804	55y	F	Hansen's disease	Rt 5 toe	PRP	42.41	2	88.89	RE						ST	N	N	10
5a	GC1118_05	80y	F	Hansen's disease	Rt 3 toe	PRF	173.57	2	78.61	RE						ST	N	N	10

5b	GC111805	80y	F	Hansen's disease	Rt 2 toe	PRP	109.95	1	RE							ST	N	N	10
6a	GC1118_06	80y	F	Hansen's disease	Lt 5 toe	PRF	176.71	3	81.66	59.66	RE					ST	N	N	8
6b	GC111806	80y	F	Hansen's disease	Lt 4 toe	PRP	204.2	2	86.7	RE						ST	N	N	10
7a	MS1118_07	16y	M	Hansen's disease	Lt hand	PRF	863.49	4	45.4	50.1	77	RE				ST	N	N	8
7b	GC111807	80y	F	Hansen's disease	Lt 3 toe	PRP	39.27	1	RE							ST	N	N	10
8a	PT1119_08	27y	F	Hansen's disease	Rt foot	PRF	3298.68	1	RE							ST	N	N	10
8b	PT111808	27y	F	Hansen's disease	Rt great toe	PRP	439.82	4	-87.6	77.1	75	RE				ST	N	Y	7
9a	SG1218_09	30y	F	Hansen's disease	Rt sole	PRF	1121.55	4	71.98	77.5	82.22	RE				ST	N	N	8
9b	SG121809	30y	F	Hansen's disease	Lt hand	PRP	816.81	2	94.2	RE						ST	N	N	8
10a	SG1218_10	30y	F	Hansen's disease	Rt 3 finger	PRF	150.79	3	73.95	76.01	RE					ST	N	Y	7
10b	SG121810	30y	F	Hansen's disease	Rt foot	PRP	235.62	3	76.5	91.07	RE					ST	N	N	7
11a	TG0119_11	65y	F	Hansen's disease	Rt foot	PRF	816.81	2	95.19	RE						ST	N	N	8
11b	MB011911	55y	F	Hansen's disease	Lt 4 finger	PRP	153.93	2	87.76	RE						ST	N	N	10
12a	TG0119_12	65y	F	Hansen's disease	Rt heel	PRF	659.73	2	93.33	RE						ST	N	N	8
12b	MD031912	65y	M	Hansen's disease	Lt lat. malleolus	PRP	2827.44	3	4.66	34.9	ABD					IF	PD	-	4

13a	MD0319_13	65y	M	Hansen's disease	Rt lat. malleolus	PRF	282.74	3	10.55	15.52	RE					ST	N	Y	8
13b	CC061913	55y	M	Hansen's disease	Rt upper sole	PRP	1696.46	4	93.51	44.28	38.47	RE				ST	N	N	10
14a	CC0619_14	50y	M	Hansen's disease	Rt heel	PRF	3820.18	6	56.82	11.61	66.32	10.39	69.64	RE		ST	N	N	10
14b	GG071914	80y	F	Hansen's disease	Rt heel	PRP	1372.87	3	69.1	87.03	RE					ST	N	N	10
15a	SG0819_15	30y	M	Hansen's disease	Lt lat. malleolus	PRF	904.78	7	84.2	20.88	12.49	11.11	73.21	60.01	RE	ST	N	Y	9
15b	RL071915	64y	M	Hansen's disease	Lt foot	PRP	904.78	3	91.49	51.01	RE					ST	N	N	10
16a	IH0819_16	19y	F	Keloid	Rt foot	PRF	2827.44	5	56	26.13	64.44	82.69	RE			ST	N	N	10
16b	SG081917	30y	M	Hansen's disease	Rt lat. malleolus	PRP	306.3	6	67.69	23.81	79.17	20	62.5	RE		ST	N	Y	8
17a	MD1019_18	58y	M	Hansen's disease	Rt foot	PRF	923.63	4	54.08	76.66	68.25	RE				ST	N	N	9
17b	TG091918	58y	F	Hansen's disease	Rt heel	PRP	336.93	4	90.21	28.56	73.34	RE				ST	N	N	10
18a	JS1119_19	25y	F	Nec. acral erythema	Rt lower leg	PRF	339.29	4	55.55	43.74	33.34	RE				ST	N	N	10
18b	MD101919	58y	M	Hansen's disease	Rt great toe	PRP	381.7	3	83.95	65.39	RE					ST	N	N	10
19a	JS1119_20	25y	F	Nec. acral erythema	Rt upper leg	PRF	351.85	4	46.42	70	79.16	RE				ST	N	N	10

19b	JS111920	25y	F	Nec. acral erythema	Rt foot	PRP	395.84	3	86.89	33.33	RE					ST	N	N	10
20a	MB1219_21	55y	F	Hansen's disease	Rt foot	PRF	1102.7	4	34.18	87.87	60.7	RE				ST	N	N	10
20b	JS111921	25y	F	Nec. acral erythema	Rt calf	PRP	197.92	2	90.48	RE						ST	N	N	10
21a	SG0220_22	31y	M	Hansen's disease	Rt lat. malleolus	PRF	1979.2	4	94.28	61.11	71.44	RE				ST	N	Y	9
21b	JS111922	25y	F	Nec. acral erythema	Lt lat. malleolus	PRP	150.79	4	96.5	-31.21	80.96	RE				ST	N	N	8