"AUTOLOGOUS PLATELET RICH PLASMA IN TREATMENT OF CHRONIC CUTANEOUS ULCERS"

By

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MASTER OF SURGERY

IN

GENERAL SURGERY

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Dr.Manisha.Victor.

LIST OF ABREVATIONS

PRP	: Platelet Rich Plasma
PPP	: Platelet poor plasma
DFU	: Diabetic foot ulcers
WBC	: White blood cell
RBC	: Red blood cell
ECM	: Extracellular matrix
TcO2	: Transcutaneous oxygen tension
TNF-α	: Tumor necrosis factor alpha
IL-1	: Interleukin 1
HBOT	: Hyperbaric oxygen therapy
TPN	: Topical negative pressure
PDGF	: Platelet Derived Growth Factor
EGF	: Epidermal growth factor
IGF	: Insulin growth factor
KGF	: Keratinocyte growth factor
TGF	: Transforming growth factor
VEGF	: Vascular endothelial growth factor
PDAF	: Platelet derived angiogenesis factor
EDTA	: Ethylenediaminetetraacetate
GM-CSF	: Granulocyte macrophage colony stimulating factor
FDA	: Food and drug administration
EMEA	: Europe Middle East and Africa
SD	: Standard deviation
HbA1c	: Glycated hemoglobin

ABSTRACT

BACKGROUND: Chronic ulcers are most common problem affecting the population worldwide. Chronic wounds fail to process through the expected healing process in timely manner. Plasma rich plasma is considered to be advanced therapy for treatment of chronic wounds.[1]

OBJECTIVES OF THE STUDY: To compare the clinical efficacy and local injection of platelet rich plasma with conventional dressing in treatment of chronic cutaneous ulcers.

MATERIALS AND METHOD: All patients presenting to B.L.D.E (Deemed to be) University's Shri B.M.Patil Medical College Hospital and Research Centre Vijayapur and admitted patients in whom the diagnosis of CHRONIC CUTANEOUS ULCER from OCTOBER 2016 to JUNE 2018 were included in the study. A prospective interventional study was conducted with 90 patients alternatively assigned to each group i.e., 45 patients to local injections of autologous platelet rich plasma and 45 patients to conventional dressing with 10% povidone iodine solution. All patients were examined, necessary investigations were done and appropriate treatment was given. All cases were followed up till discharge of the patient from the hospital or till closure of wound. 'Primary efficacy end point' was complete ulcer closure. 'Secondary efficacy end points' include reduction in ulcer surface area over time, time to achieve ulcer closure by either skin grafting or secondary suturing. All the data was analyzed using the Z-test, student's T-test and the results were tabulated. A "p value" of <0.05 was considered statistically significant.

RESULTS: Most of the patients included in the study were males and majority of them presented with ulcer. The efficacy of the dressing was compared as the

percentage of reduction in the surface area of the ulcer, percent of ulcer surface area covered by granulation tissue and mean duration to outcome in the form of skin grafting, secondary suturing or healing by secondary intention. Granulation tissue fill up of the ulcers and wound contraction was better in local injection of autologous platelet rich plasma group as compared to conventional dressing group.

CONCLUSION: Local injection of autologous platelet rich plasma can be considered as a superior option in the management of chronic ulcers. But we advocate further studies with larger sample size to substantiate the findings we made

KEY WORDS: Chronic ulcers, PRP.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF CHARTS

LIST OF FIGURES

LIST OF PICTURES

INTRODUCTION

Chronic wounds are catastrophic health problem worldwide. These stubborn wounds fail to heal in an expected manner of healing process.

The overall increase in the incidence of chronic wounds makes them a tremendous socioeconomic burden globally.

Wound healing is a complex and dynamic process.[1]

Once a wound begins healing, normally the process resolves with complete wound closure. However, healing of acute or chronic wounds can become impaired by patient factors (i.e., co-morbidities) and/or wound factors (i.e., infection)[1]. Wound with impaired healing is difficult to treat because good standard wound care does not always provide an improved healing outcome and often more advanced therapies are employed. [1]

The standard management includes advance therapeutics with drugs (antibiotics), intense local dressings (such as negative pressure/antimicrobial) and multiples surgical interventions/reconstructions. Such intervention and modalities requires experts and large resources. Still outcomes are unpredictable and associated with morbidities. [2]

Platelet rich plasma is considered to be advanced therapy for chronic and acute wounds.[1]

The curative properties of platelet rich plasma rely on the fact that platelets are a physiological reservoir of growth factors, which have an active role in tissue regeneration.[3]

Platelets are anucleated cell fragments that originate from megakaryocytes in the bone marrow. [6]

It is well known that platelets contain a great variety of growth factors, with healing functions. [3]

PRP is a volume of autologous plasma that has a platelet concentration above baseline i.e., five times more than the normal platelet counts. [4]

When concentrating platelets, 7 fundamental protein growth factors are concentrated.[5]

Continuous release of these growth factors has been proposed to promote angiogenesis both in vitro and in vivo. [6]

Release of these angiogenic factors in platelet derived fraction preparations could be useful in tissue regeneration and wound healing. [6]

Platelets also regulates hemostasis through vascular obliteration and fibrin clot formation [6]

Among the three organelles described in platelets, namely lysosomes, alpha granules and dense granules. The biggest compartments for protein storage are alpha granules.[6]

High leukocyte concentration of PRP has an added antimicrobial effect. [7]

Since PRP is an autologous blood product, it carries no risk of transmitting infection disease.[7]

Hence, such a physiological mixture of growth factors may be advantageous clinically to achieve wound healing. [10]

This study is undertaken to evaluate the safety and clinical efficacy of local injection of autologous platelet rich plasma with the conventional type of dressing in management of chronic cutaneous ulcers.

AIM & OBJECTIVES OF THE STUDY:

To evaluate the safety and efficacy of local injections of Autologous Platelet Rich Plasma in treatment of chronic cutaneous ulcers.

REVIEW OF LITERATRE

Chronic wounds are significant burden on healthcare facilities globally. The management and treatment of chronic wounds are demanding to health care providers. The subset of chronic wounds and the complications associated with them continue to progress rapidly despite enormous progress in science of wound healing.

Clinically the process of wound healing is important to understand from several prospective. These include:

Development of precise, least traumatic surgical technique; the clear understanding of newer developments and anti-infective therapies affect wound healing.[11]

Optimal outcome of wounds healing depends on complete evaluation of the patient and of the wound and application of best practices and techniques. [12]

As noted by John Hunter (1728-1793), a keen observer of biological phenomena, "… the injury alone has in all cases a tendency to produce the disposition and means of a cure."[12]

PROCESS OF NORMAL WOUND HEALING:[11,12,13,14]

Wound healing is a mechanism wherein the body attempts to restore the integrity of injured part.

"It is achieved through four highly integrated and overlapping

Bio-physiological phases namely:

1. Hemostasis
2. Inflammation proliferation
3. Proliferation
4. Tissue remodeling/ resolution".[11]

The series of events associated with wound healing begins at the moment of injury. For a wound to heal successfully, all four phases must occur in proper sequence and time frame and continue for a specific duration at an optimal intensity (that takes almost a year to complete).

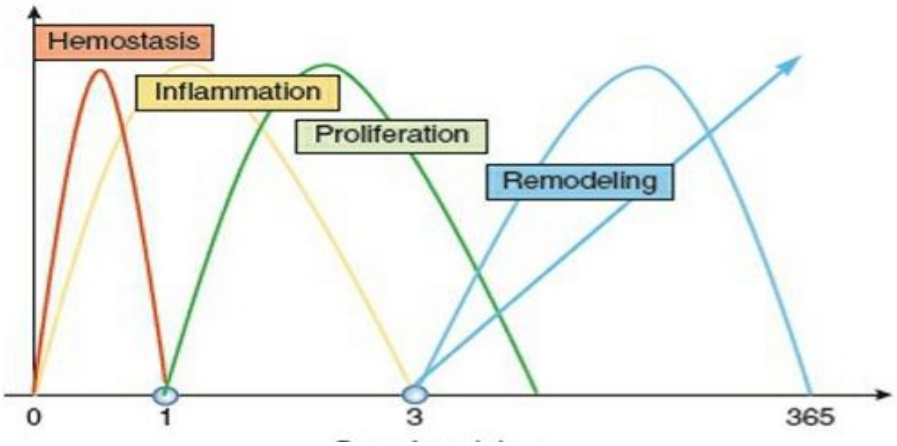

Figure 1: Four phases of wound healing plotted against "time" on X-axis. [11]

Table 1	Cellular and Biological Events that Frame the Normal Wound Healing Process
PHASE	**Cellular and Biophysiologic Events**
Hemostasis	Vascular constriction Platelet aggregation, degranulation, and fibrin formation (thrombus)
Inflammation	Neutrophil infiltration Monocyte infiltration and differentiation to macrophage Lymphocyte infiltration
Proliferation	1. Re-epithelialization 2. Angiogenesis 3. Collagen synthesis 4. ECM formation
Remodeling	1. Collagen remodeling 2. Vascular maturation and regression

Components of normal wound healing [11]

- **HEMOSTATIC PHASE:**

This phase immediately begins following wounding of the tissue.

Circulating factors that initiate the inflammatory phase of healing are platelets, plasma proteins and leukocytes.

Platelets are anucleated cells produced in bone marrow by megakaryocytes. The plasma membrane of each platelet contains specific receptors known as glycoprotein Ia/Iia.[16]

The platelets contain granules with important factors for hemostasis and inflammation. Platelets bind and anchor to vascular endothelium (Type IV). Together platelet activation occurs and platelets change from round shape to flattened configuration and discharge contents of cytoplasm which is known as "platelet release reaction"[11].

These bioactive factors serve a dual purpose in hemostasis and wound healing.

This phase is characterized by increased vascular permeability, chemotaxis, secretion of cytokines and growth factors into the wound.

It represents an attempt to arrest bleeding and maintaining milieu interior.

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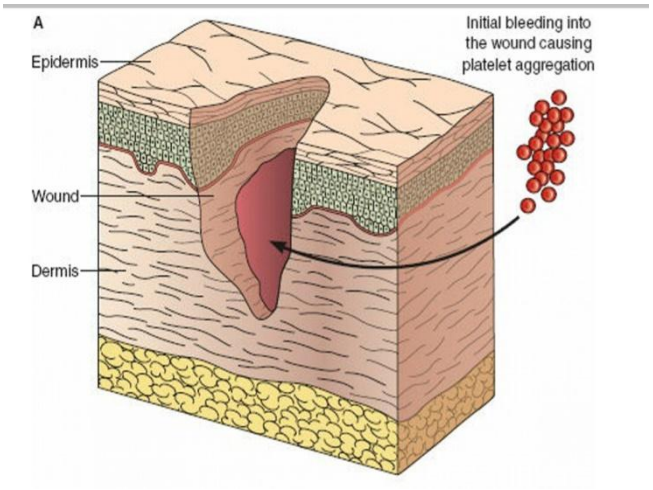

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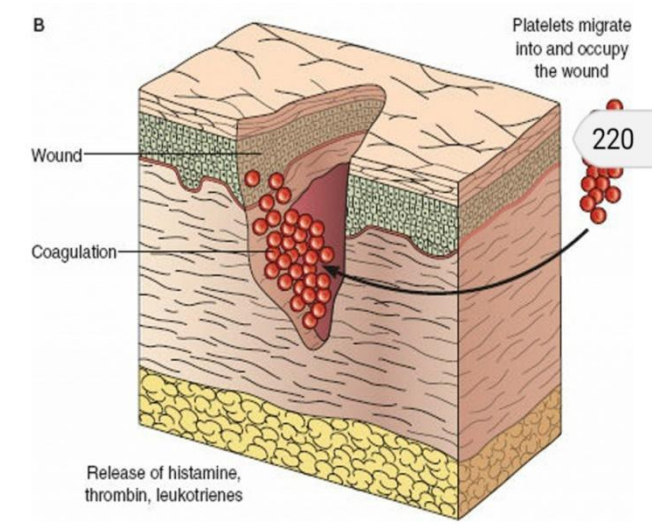


Figure 2 and 3: Diagrammatic wound healing model. Platelets are the first cells to arrive at the wound and are critical to create a clot and hemostasis.[(11)]

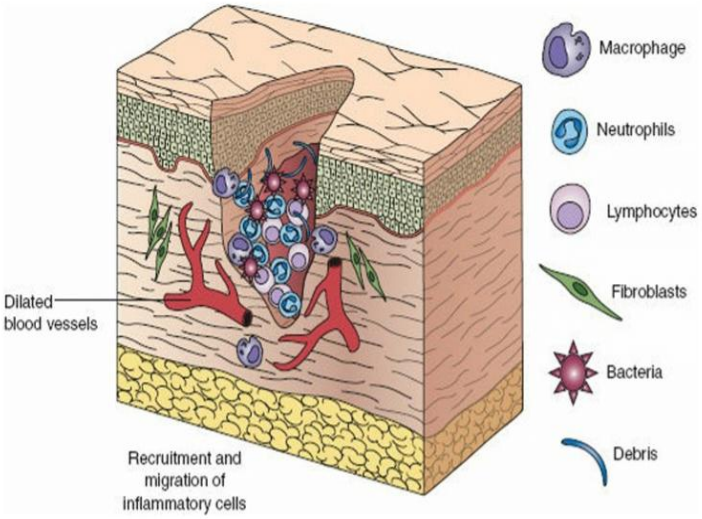
- **INFLAMMATION:**

Activated WBC migrate to wound site to engulf debris and injured tissue. Chemical mediator Leukotaxine attract WBCs.

First polymorpho-nuclear neutrophil dominate. By 5th day, granulocytes die and monocytes predominate to continue scavenging activity.(14)

Monocytes must be present at the wound site to create a normal fibroblast production. Depression of monocytes will delay wound healing.(14)

These cells secrete cytokines and growth factors which function in rapidly amplifying process that affect all aspects of healing.



6 Phase 2 – Inflammation

Figure 4: Phase of inflammation(11)

- **PROLIFERATION:**

Primarily consists of epithelization mainly occur by proliferation and migration of marginal basal cells.(14)

Marginal basal cell loose there attachment to underlying dermis, Enlarge and migrate. Basal cells in wound zone undergo rapid mitotic division.(14)

Epidermis adjacent to wound begins to proliferate on day 1.

No regeneration of hair follicles, sweat and sebaceous glands in new epidermis.(14)

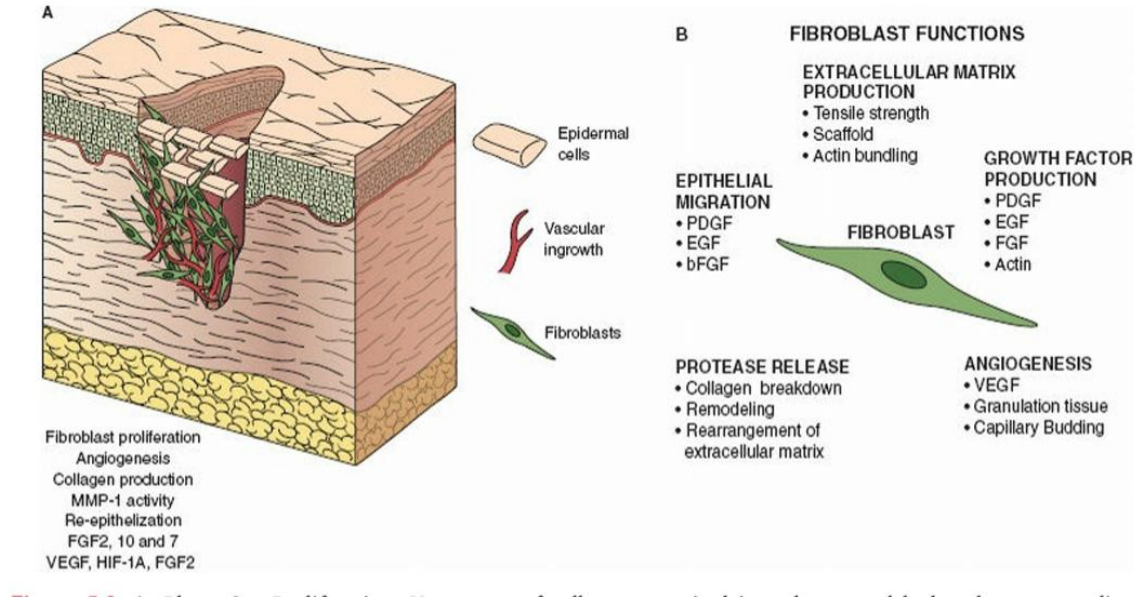


Figure 5

A. Phase of proliferation. Vast array of cells are recruited into the wound bed and carry out diverse functions including proliferation and deposition of ECM.

B. Fibroblast function.

- **GRANNULATION TISSUE FORMATION:**

  Proliferation and migration of surrounding connective tissue.

  Fibroblast start synthesizing extra-cellular matrix.

- **STAGE OF VASCULARIZATION:**

  Formation of living granulation tissue is called “organization”. Wound clot is invaded by macrophages which lead to capillary loop formation.

  Capillary loops undergo canalization to form vascular arcade.

  Which with time mature and acquire muscle coat to form arterioles.

- **COLLAGEN SYNTHESIS:**

  It is brought about by fibroblasts. Collagen is nothing but extracellular secretion of fibroblasts.

  Collagen is made up of glycine, lysine, hydroxyprolene and proline.

  Initially tropocollagen is formed which later condenses to form fibril. Fibril condenses to from collagen.

  Collagen is not inert and undergoes constant turnover under the influence of enzyme collagenase.

  TYPES OF COLLAGEN: [14]

  Collagen I: Tendon, bone, skin

  Collagen II: Cartilage

  Collagen III: Fetal dermis, aorta, esophagus, uterus

  Collagen IV: Immature scar

- **REMODELING[14]**

  Also called as phase of maturation.

  It begins by six weeks and lasts for two years.

  Maturation of collagen is brought about by cross linking which is responsible for tensile strength of scar.

  Normal dermis contain 80% of collagen type I and 20% of type III, while scar contains 50% of collagen type I and 50% of collagen type III.

  Hydroxylation of lysine and proline will produce collagen type I which requires Vitamin C Iron and α-ketogluteric acid.

  STRENGTH OF SCAR: 3% by 1 week

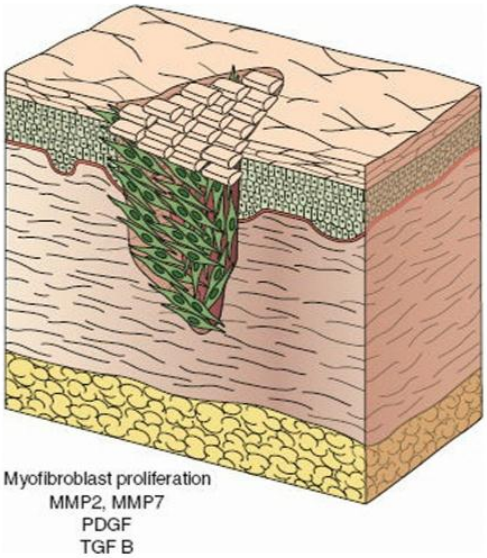
  20% by 3 weeks

  80% by 12 weeks.[14]

- **VASCULAR MATURATION AND REGRESSION:**

  Arterioles and venules are formed.

  Some undergo dysplasia which leads to end artery obliterians and devascularization 6.



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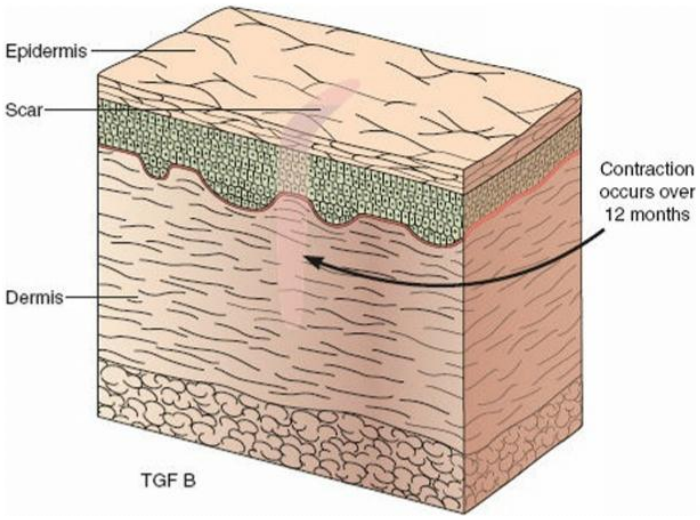


Figure 6 and 7: phase of remodeling. [11]

- **FACTORS AFFECTING NORMAL WOUND HEALING:[14]**
- **GENERAL FACTORS:**

1. Age : young age – better wound healing
2. Nutrition : a. Protein deficiency cause impairment of granulocyte action and collagen formation.

b. Sulfur containing amino-acid determine tensile strength of the scar

c. Vitamin C important for hydroxyltion of amino acids for synthesis of collagen

d. Vitamin A is essential for re-epithelization.

c. Zinc is an important co-emzyme for DNA, RNA synthesis.

d. Metallo-enzymes.

3 Hematological: Anemia, granulocytopenia, immunodeficiency.

4. Diabetes : Microangiopathy

Hyperglycemia

Atherosclerosis

Decreased chemotaxis

Decreased phagocytosis

5. Corticosteroids: Have anti-inflammatory effect

Decrease protein synthesis

Inhibit fibroblast activity

Decrease capillary budding,

Decrease epithelization.

6. Cytotoxic drugs: inhibit cell division
7. Radiation

8. Uremia
9. Malignancy

- **LOCAL FACTORS:**

1. Position of wound: parallel to Langer lines heal faster
2. Blood supply: directly proportional to wound healing
3. Tension: decreases wound healing
4. Infection: delay fibroblast action(fibroblast require alkaline medium for collagen synthesis)
5. Wound over joints have poor wound healing
6. Foreign body at wound site cause inflammation
7. Necrosis
8. Local radiation: decreases vascularization and fibroblast activity
9. UV light: Promote wound healing

- **CHRONIC WOUND:**[11,12,13,14,15]

"Chronic wounds are defined as wounds that have failed to proceed through orderly process of healing that produce satisfactory anatomic and functional integrity".[12]

Wounds heal within the time of 4-6 weeks, those that do not are termed at chronic wounds.[11]

Non healing ulcers and wounds represent failure to achieve complete epithilization in appropriate temporal sequence and tissue repair.[15]

Unresponsiveness to normal regulatory signals have implicated in predictive factor for chronic wounds.

It may be due to-

- Failure of synthesis of normal growth factors
- Excessive breakdown of growth factors within the wound due to increased proteolytic enzymes.
- Failure of function of normal anti-protease inhibitor enzymes.[12]

Often chronic wounds stall in inflammatory phase of healing.[15]

It is due to persistent proinflammatory state and paradoxically leads to increased degradation of matrix.[11]

They have high pro-oxidant environment along with bacterial colonization, necrotic tissue, foreign body, localized tissue hypoxia.[13]

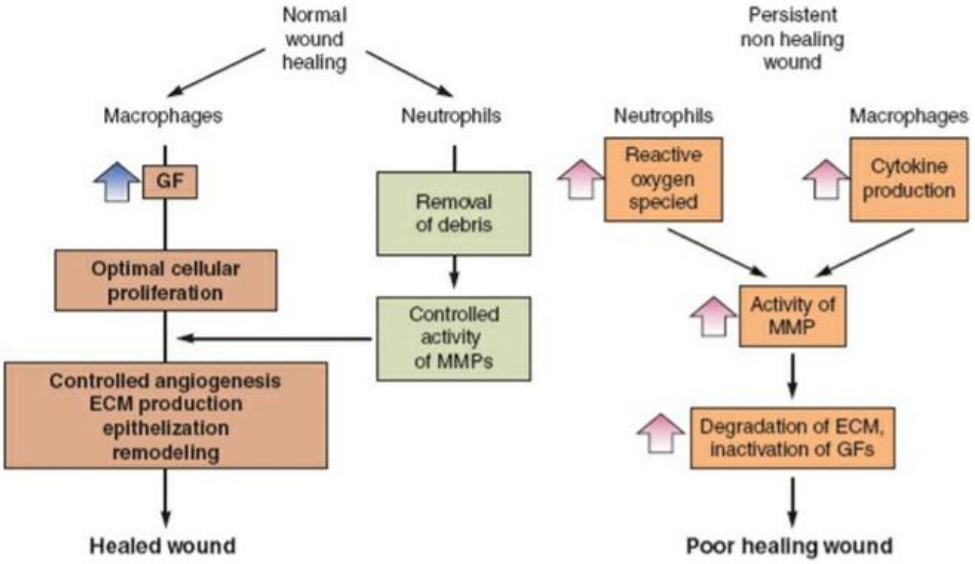


Figure 7: Flowchart representing normal and delayed wound healing.[11]

Chronic wounds have been classified as:

- Vascular ulcers (venous and arterial)
- Diabetic ulcers
- Pressure ulcers[15]

MOST COMMON CAUSE OF CHRONIC WOUNDS:

DIABETES:

Diabetic foot ulcer (DFU) is a major complication of diabetes mellitus.

This medical condition affects more than 15% of population worldwide.

Recent study showed that up to 88% of all lower limb amputation are related to diabetic foot ulcer (Alvarsson et al., 2012).[17]

Diabetic associated large vessel occlusion and end-organ microangiopathy each lead to tissue ischemia and infection.[13]

Diabetic sensory neuropathy lead to repeated unnoticed trauma and constant pressure on the wound.[13]

Tissue hypoxia is well demonstrated by reduced dorsal foot transcutaneous oxygen tension (TcO2)[13]

Also the thickened basement membrane decrease perfusion of tissues.

VEGF up regulation in patients with diabetes impaired.[13]

Hyperglycemia further increases pro-inflammatory mediators i.e.,

TNF-α, IL-1.[13]

There is also loss of balance between metalloprotinases and MMP inhibitors (muller et al., 2008) accelerating ischemia.[17]

These changes in structure and functions of cells at wound site lead to delayed healing in DFUs.

Hence treatment of DFUs remain a challenge.[17]

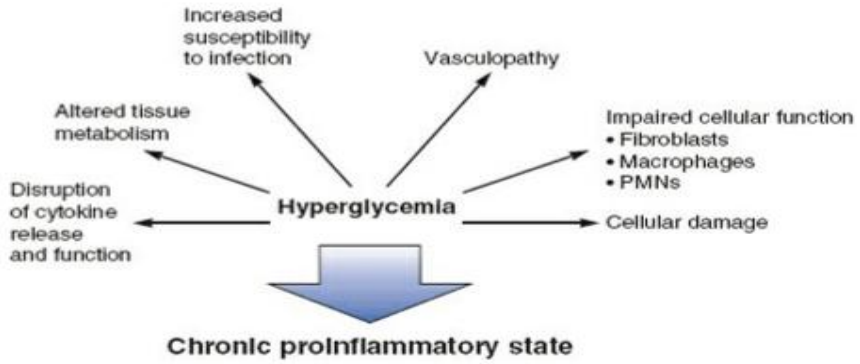


Figure 8: Cellular mechanism that impair healing of diabetic wound.[11]

- **VASCULAR ULCERS:**

Ischemic arterial ulcers: Occur due to loss of blood supply to the extremities. The ulcers are associated with other features of chronic limb insufficiency such as claudication, rest pain, pulselessness.

On examination pulses are feeble or absent with decreased ABPI and poor unhealthy granulation tissue over the ulcer bed.[12]

Management of arterial ulcers require revascularization as well as wound care.[12]

On establishing adequate blood supply, these wound heal progressively.[12]

Venous stasis ulcers: at the microvasculature level, there is alteration in capillary permeability due to sustained venous hypertension which facilitates leak of fibrinogen into the subcutaneous tissues. Further fibrinogen is polymerized to fibrin cuffs which lead to peri-vascular cuffing that reduce oxygen delivery causing ulcerations.

Also there is high oxidative stress due to neutrophil entrapment and release in proinflammatory cytokines.

Together they lead to ulcers that fail to re-epithelize despite the presence of adequate granulation tissue in the ulcer bed.

The mainstay of treatment for venous ulcers in compression therapy.[12]

PRESSURE ULCERS:

Also called decubitus ulcers. "It is defined as a localized area of tissue necrosis that develops when soft tissue is compressed between a bony prominence and an external surface"[12]

Excessive pressure causes collapse of capillaries and impairs delivery of nutrients and oxygen to the tissue.

Ulcer formation is further favored by moisture, friction and shear forces and co-morbidities associated such as immobility, obesity.

There are four stages of pressure ulcer formation:

Stage I – non-blanching erythema of intact skin

Stage II – partial thickness skin loss involving epidermis or dermis

Stage III – Full thickness skin loss but not through the fascia.

Stage IV – Full thickness skin loss with extensive involvement of muscle and bone.

Treatment of pressure ulcers is multidisciplinary involving wound care teams with therapist, nutritionists.

Recurrence rates are very high.[12]

INFECTION:

Infection is the common cause of delayed wound healing. Particularly beta-hemolytic streptococci prevents wound from healing by any means.

Bacterial infection prolongs the inflammatory phase and interferes with epithilization, wound contraction and collagen deposition.

Bacterial endotoxins continue the process of phagocytosis and collagenase release, which degrade the collagen in the wound and surrounding structure.[13]

WAGNER CLASSIFICATION SYSTEM FOR ULCERS

Grade 0	Foot symptoms like pain only
Grade 1	Superficial ulcers
Grade 2	Deep ulcers
Grade 3	Ulcer with bone involvement
Grade 4	Forefoot gangrene
Grade 5	Full foot gangrene

DRESSING IN CHRONIC ULCERS:

Wound dressings represent an integral part of the management of chronic ulcers.

Dressings should alleviate symptoms, provide wound protection and encourage healing.

In choosing a dressing for a chronic ulcer, several factors have to be taken into account. Infection need to be controlled.

A dressing must be comfortable and acceptable for the patient. Ideally, the dressing should also aid in the management of the infection itself and promote healing of the ulcer.

Desirable characteristics for wound dressings must incorporate the principles of wound healing.

These dressings must also accommodate practical issues such as allowing providing mechanical protection at the same time they must also be cost effective , should be hypoallergenic.

Various types of non-adherent or saline-soaked gauze dressings are often regarded as standard treatment for chronic ulcers.

These dressings are designed to be atraumatic and to provide a moist wound environment. These simple, relatively inexpensive dressings are not designed specifically for managing infection but can be safely used in conjunction with antibiotic treatments.

- **HYDROCOLLOIDS**

Hydrocolloid dressings are semipermeable to vapour, occlusive to wound exudate and absorbent. They are usually presented as an absorbent layer on a film or foam. Examples of commercially available products include Duoderm (Convatec), Granuflex (Convatec), and Comfeel (Coloplast). They are found to be the second most popular choice of dressing (behind non-adherent) for chronic ulcers, espicially diabetic ulcer. Despite their popularity, their use on infected wounds is controversial. Hydrocolloid dressing creates a hypoxic and moist environment that may also facilitate autolysis of necrotic material. Their use for highly exudative wounds can lead to maceration of the surrounding skin.

Most authorities, however, have expressed concern that hydrocolloids may increase the risk of infection developing within a wound .

Hydrocolloid dressings are designed to be left on the wound for prolonged periods (1week), hence they are useful in clean ulcers but their role in infected ulcers is controversial as infected wounds require repeated inspection of wound.

- **HYDROGELS:**

Hydrogels are designed to facilitate autolysis of necrotic tissue and they donate moisture to extensively dry wounds. They can lead to maceration when applied to wounds that show moderate to severe exudate. Examples include Aquaform (Maersk Medical)and Intrasite.

- **IODINE PREPRATION:**

Antiseptics such as iodine-based preparations are commonly used on wounds, although there is no evidence to support a beneficial effect. Typically they are applied to locally infected wounds, usually in combination with systemic antibiotics.

Iodine comes in 2 main preparations: cadexomer-iodine and povidone iodine. Iodine is bactericidal in vitro, with maximal activity at 0.1%–1% povidone iodine has long been used as a skin antiseptic, but its antimicrobial effect on wounds is debatable. Furthermore, some data have shown iodine solutions to be toxic to fibroblasts and keratinocytes.

A randomized controlled trial of cadexomer-iodine versus saline-soaked gauze on clean foot ulcers showed no significant difference in healing between the groups. Certain iodine dressings are highly absorbent and therefore useful in preventing skin excoriation in moderately exudating ulcers. In our own clinical practice, Cadexomer-iodine pastes are used for wounds and povidone-iodine gauze for superficial ulcers. Despite the lack of evidence, many consider iodine preparations to be appropriate dressings for infected diabetic foot ulcers.

Povidone iodine preparation was used in control group in this study.

- **SILVER-IMPREGNATED DRESSING**

The use of silver as a topical antimicrobial for acute and chronic wounds is well established. It has been traditionally delivered as silver nitrate or as silver sulfadiazine. Silver nitrate has cytotoxic effects on host cells, a property often exploited in the treatment of hypergranulating tissue, but its application can be uncomfortable. Silver sulfadiazine, which has the antimicrobial actions of both silver and sulfadiazine, is used on burns and chronic wounds and is generally well tolerated.

The antimicrobial effects of silver are complex, including direct inhibition of bacterial cell respiration, inactivation of intracellular enzymes and alterations to the cell membrane. Silver-coated dressings that use elemental silver may be more efficacious at killing bacteria than is silver sulfadiazine or silver nitrate. New silver-impregnated dressings may be suitable for use for infected diabetic foot ulcers. Examples include Megaheal and Hydroheal.

However, reports suggest of accelerated wound reepithelialization.

- **BIOLOGICAL DRESSING:**

Biological Therapy (e.g., bilayered keratinocytes and fibroblasts and platelet derived growth factor) are used when patients fail to improve after the approaches described above have been applied for 3 weeks. Biological therapy should be implemented only if wound size cannot be decreased by more than 10 percent within a 3-week time period.

Chronic ulcers exhibit a decreased production of growth factors within the wound. Cell therapy, also known as biological therapy, presents an appropriate treatment option in some cases. Biological therapy is an ideal treatment for chronic ulcers because it adds cells that release growth factors to a growth factor dependent environment, increases cytokines and matrix proteins and promotes angiogenesis. Thus accelerating healing time decreases the risk of wound infection.

The biological therapy consists of “The bilayer biologically active skin construct”, composed of a surface layer of allergenic human keratinocytes over a layer of allogeneic human fibroblasts suspended within a collagen matrix. The “Bilayer cell therapy” has been shown to increase the healing rate of diabetic foot ulcers not complicated by osteomyelitis or ischemia. Fibroblasts synthesize collagen and secrete

a matrix of growth factors and matrix proteins in physiological concentrations essential for wound healing and epithilization.

HYPERBARIC OXYGEN THERAPY:

Hyperbaric oxygen therapy is based on the premise that the delivery of supraphysiological concentrations of oxygen to diseased tissues will result in beneficial physiological changes.

The therapy is based on achieving an atmospheric pressure of 2–3 atmospheres pressure which is administered using a sealed polyethylene bag over the affected area and administering 100 percent oxygen to a pressure between 20 and 30mmHg. Treatment lasts for 2 to 2 ½ hours.

HBOT can be offered to patients who have diabetic foot ulcers for whom atleast 30 days of standard wound care has failed and who have a Wagner grade III lesion or higher. (meaning the ulcer must penetrate to tendon, bone or joint and may be associated with deep abscess, osteomyelitis, gangrene, or septic arthritis) In treatment ulcer, it is believed both that the function of phagocytic cells is improved, assisting in the fight against any infection and that wound healing is independently aided through effects on cellular processes. Thus, it has been suggested that HBOT is useful for the treatment of infection and for the healing of chronic wounds.

- **TOPICAL NEGATIVE PRESSURE:**

The practice of exposing a wound to sub-atmospheric pressure for an extended period to promote debridement and healing was first described by Fleischmann et al in 1993 following the successful use of this technique in 15 patients with open fractures. The science behind topical negative pressure dressings is to apply a sub-atmospheric pressure over the wound bed and maintain the negative pressure environment by means of a semi permeable occlusive coverage. Since the wound is

occluded from the surrounding environment it is also called " Limited access dressing".

Usage of a subatmospheric pressure causes,

- Fourfold increase in blood flow in the local wound environment. (As measured by a laser Doppler technique.)
- Induces mechanical stress which causes an increase in cellular activity.
- Increase in the rate of granulation tissue formation and reduction in the bacterial load in the wound.
- Clinically TNP removes large amounts of fluid from wounds especially acute wounds. The resulting reduction in oedema is thought to aid in the enhancement of blood and nutrient flow into the wound.
- The mechanism behind the ability of the TNP to decrease bacterial count may be attributable to three properties increased blood flow, decreased interstitial edema and removal of harmful enzymes in wound.

AUTOLOGOUS PLATELET RICH PLASMA

Also called as PRP, maybe defined as "A component of plasma fraction of autologous venous blood with platelet counts in the range between 4 to 6 above the baselines considered to be therapeutic benefit (I million platelets/L)".[6]

A sample of normal blood will contain 93% of red blood cells, 6% of platelets and 1% of white blood cells. In PRP the ratio of RBC to platelets is reversed, thereby increasing the factors that would be more useful in healing.[22]

"It is an endogenous therapeutic technology that has been gaining popularity in regenerative medicine due to its potential to stimulate and accelerate tissue healing".[19]

Platelets were discovered in 1882 by Giulio Bizzozero. Though for many years the dynamic and multifunctional nature of platelets remained unknown.[16]

Platelets are anucleated, discoid shaped cells that are derived from megakaryocytes in the bone marrow through controlled fragmentation.[18]

Diameter of mature platelet is 2-5μm and half-life of platelets is 5-9 days. The normal platelet count in blood is $150\text{-}400x10^3$ per cubic mm of blood. An average healthy adult can produce10^{11} platelets per day.[16]

Platelets are peculiar in their structure. Although they are anucleated yet have well defined mitochondria. The plasma membrane of platelet is bilayered and contain phospholipid which is a site for various surface receptor expression which help in signaling and intracellular trafficing.[16]

They are specialized blood cells that release contents of their intracellular granules in response to activation.[18]

The named biological markers on platelet plasma membrane are CD36, CD41, CD42a, CD42b, CD61, CD63 IIbIIIa and GLUT-3.

Majorly platelet activity is associated with initiation of coagulation cascade to achieve hemostasis. But they also play many important roles in pathophysiological states.[16]

The surface receptors of platelets also contain α granules which participate in extensive functions such as coagulation, inflammation, atherosclerosis, angiogenesis, wound repair and tumorogenesis.[16]

Platelets have two major storage granules, which store biologically active molecules that involve in initiation of coagulation and recruiting other cells during inflammation.[16]

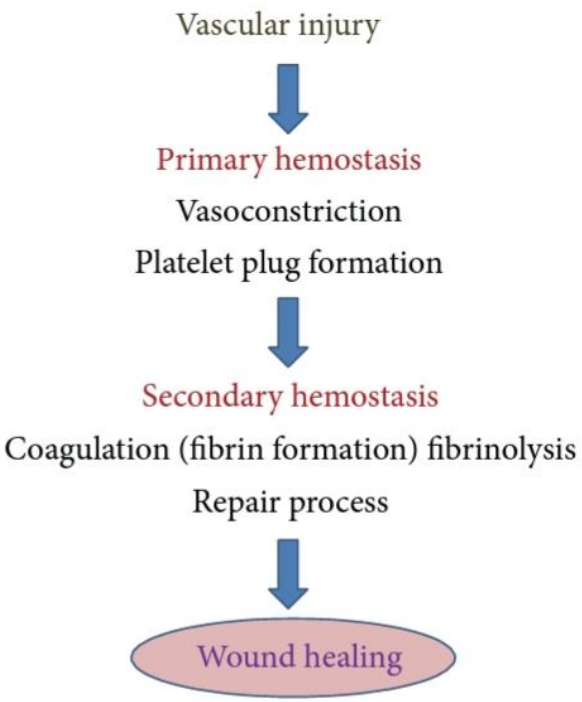


Figure 9: Pathway illustrating hemostasis.[16]

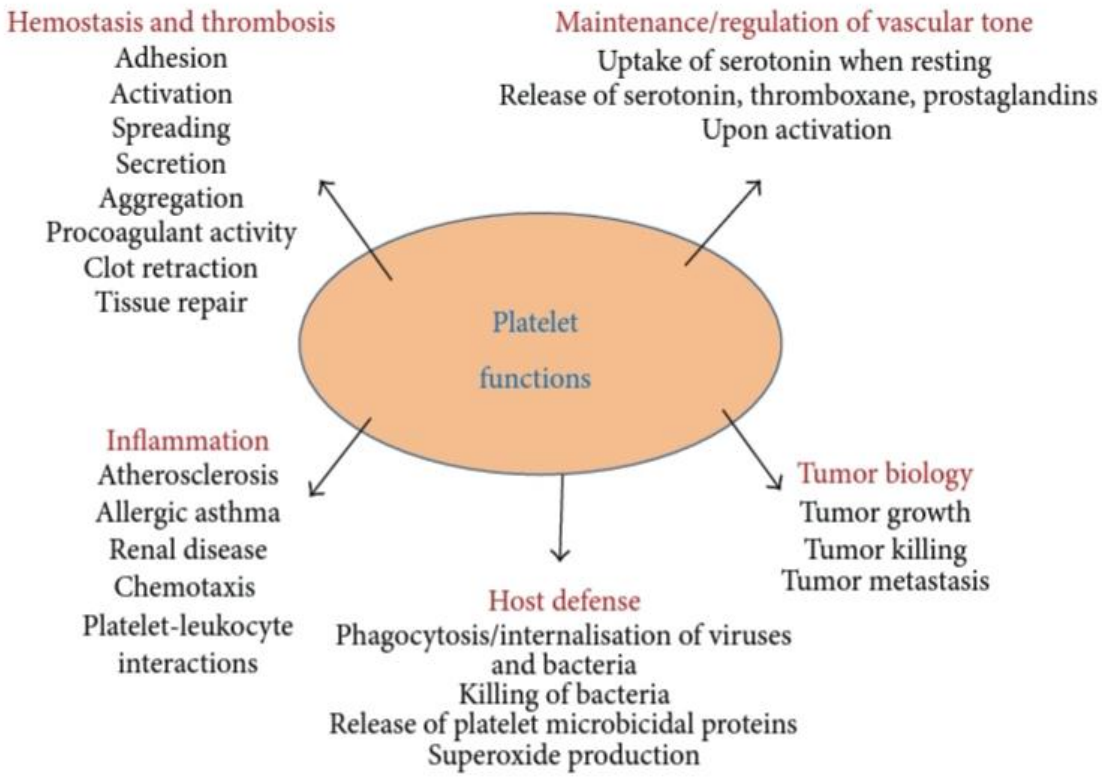


Figure 10: The multifunctional platelet (Harrison, 2005 [44][(16)]

- **PLATELET GROWTH FACTORS:**

"Growth factors are solube and diffusible polypeptide substances that regulate growth, defferentiation, proliferation, and cellular metabolism of numerous cell types."[(19)]

There are not less than sixty different biologically active substances in platelets that alleviate in tissue repair and healing like chemotaxis, cell proliferation, angiogenesis, immune modulation, cell differentiation, anti-bacterial activity, intracellular matrix deposition and remodeling.[(18)]

Few of the named important growth factors that are released by activated platelets are-

PDGF (platelet-derived growth factor)

PDEGF (platelet derived epidermal growth factor)

TGF α and β (transforming growth factor)

EGF (epidermal growth factor)

IGF (insulin growth factor)

KGF (keratinocyte growth factor)

IL-8 (interleukin)

TNF-α (tumor necrosis factor)

GM-CSF (granulocyte macrophage colony stimulating factor).

"So far only PDGF has been approved by the United States Food and Drug Administration (FDA) and by European Authorities (EMEA) for clinical application in patients."[19]

List of growth factors in Platelet Rich Plasma	
Growth Factor	*Effect*
PDGF	Macrophage activation and angiogenesis Fibroblast chemotaxis and proliferative activity Enhances collagen synthesis Enhances the proliferation of bone cells
TGF-Beta	Enhances the proliferative activity of fibroblasts Stimulates biosynthesis of type I collagen and fibronectin Induces osteoclast formation and bone resorption
IGF-I	Chemotactic for fibroblasts and stimulates protein synthesis Enhances bone formation by proliferation and differentiation of osteoblasts
PDEGF	Promotes wound healing by stimulating the proliferation of keratinocytes and dermal fibroblasts
PDAF	Induces vascularization by stimulating vascular endothelial cells
PF-4	Stimulates the initial reflux of neutrophils into wounds A chemoattractant for fibroblasts A potent antiheparin agent
EGF	Cellular proliferation Differentiation of epithelial cells
VEGF	Angiogenesis Migration and mitosis of endothelial cells Creation of blood vessel lumen Creates fenestrations Chemotactic for macrophages and granulocytes Vasodilation (indirectly by release of nitrous oxide)
PDGF = Platelet-Derived Growth Factor; TGF = Transforming Growth Factor; IGF = Insulin Growth Factor; PDEGF = Platelet-derived endothelial growth factor; PDAF = Platelet-derived angiogenesis factor; PF-4 = Platelet Factor 4; EGF = Endothelial Growth Factor; VEGF = Vascular Endothelial Growth Factor;	

During the process of wound healing, the growth factors in platelet granules act as messenger to regulate well-organised and complex series of events which involve cell-cell, cell-matrix interactions which play an important role during various phases of wound healing.[18]

"Autologous platelet derived wound healing factors were proposed to regulate wound healing of chronic cutaneous ulcers by promoting the formation of granulation tissue in the early healing phase. This conclusion was based on randomized, prospective, double-blind, placebo-controlled studies, who showed improved healing compared to usual treatments."[20]

Platelet rich plasma functions as growth factor delivery system in high concentrates and also function as tissue sealant system.

The wound repair is brought about by degranulation of α-granules which releases locally active growth factors.

These active growth factors accelerate wound healing by attracting undifferentiated immune cells into newly formed collagen matrix which further undergo cell division and de-differntiation.

Platelets also suppress cytokine release and halt inflammation by interacting with macrophages and promote healing and regeneration.[19]

PRP further promote neoangiogenesis and re-epithilization.

"Platelets in PRP also play a role in host defense mechanism at the wound site by producing signaling proteins that attract macrophages.

PRP contain small number of leukocytes that synthesize interleukins as a part of a non-specific immune response. Previous studies of PRP have demonstrated antimicrobial activity against *Escherichia coli, Staphylococcus aureus,* including methicillin-resistant *Staphylococcus aureus, Candidia albicans, Cryptococcus neoformans".*[20]

Pain reduction following platelet growth factor application was mentioned in study by Crovetti et al.[18]

“The curative properties of PRP explained by the fact that platelets are physiological reservoir of growth factors, that have an active role in tissue regeneration”[21]

MATERIALS AND METHODS

- **SOURCE OF DATA:** This study was carried out in the Department of General Surgery, B.L.D.E (Deemed to be) University Shri B.M Patil Medical College, Hospital and Research centre, Vijayapur.
- **STUDY PERIOD**: One and half years, from October 2016 to june2018.
- **STUDY DESIGN**: Prospective, comparative study of efficacy of local injection of platelet rich plasma in wound healing versus conventional dressing with 10% povidone iodine solution in wound healing.
- **STUDY SAMPLE**: Total of 90 with 45 in each group i.e., 45 patients in study group and 45 patients in control group.
- **APPROVAL**: Study was approved by the institutional medical ethics committee and written informed consent was obtained from all patients participating in the study.
- **STUDY POPULATION:** Patients came with chronic ulcers Wagener's grade I-III ulcers during the study period were initially subjected for detection of general health of the patient and culture of wounds and other routine investigations.
    - And while allocating cases, age of patient and size of the ulcer was matched.
    - A proforma was used to collect all the relevant data from the patients.
    - Detailed history was taken; thorough clinical examination and investigations were performed on all the patients included in the study.
    - All the cases were followed up to discharge and subsequently for a follow up till wound healing.

- "Primary efficacy end point" was complete ulcer closure and "Secondary efficacy end point" was time taken to achieve ulcer closure by either secondary suturing or skin grafting.

- **INCLUSION CRITERIA:**
  - Highest dimensions within 5cm breadth, 5cm Width, 0.5cm Depth
  - **Wagner's Classification Of Diabetic Ulcer Upto Grade-III**
  - Traumatic Non-Healing Ulcers
  - Bedsores
  - Venous Ulcers
  - Other non-specific ulcers.

- **EXCLUSION CRITERIA:**
  - Anemia – Hb <10gm% in adults, <12gm% in children <14 years
  - Uncontrolled Diabetes
  - Gross Nutritional Deficiency BMI <18 , Albumin <2gm/Dl of blood
  - Dyslipidemia
  - **Wagner's Classification Of Ulcer Grade-IV or More**
  - Patient on immunosuppressive drugs
  - Known malignancies
  - Patient with bleeding disorders.
  - Radiotherapy to local area of ulcer.

METHOD OF COLLECTION OF DATA

- All eligible patients admitted in the Department of General Surgery in Shri B. M Patil medical college with ulcer during the study period from October 2016 to June 2018 were initially evaluated for the presence of chronic ulcers and

co-morbidities associated with the patient. Routine investigations were done and swab culture were sent from the ulcer for organisms. Ulcer dimensions were measured.

- The study subjects will be randomly divided into two groups, Local injection of platelet rich plasma (A group) and Povidone iodine dressing group (B group).
- Study group were treated with local injection of PRP
- Control group was treated with mechanical debridement and dressed with 10% Povidone Iodine.
- PRP was injected every 4^{th} day for 4 times and every alternate day normal saline dressing was done while Povidone iodine dressing was done in control group on daily basis.
- Measurements from the ulcer were taken on every 4^{th} day till 16 days.
- Statistical analysis was done by using Fisher exact test, Chi square test and p value <0.05 was considered significant.

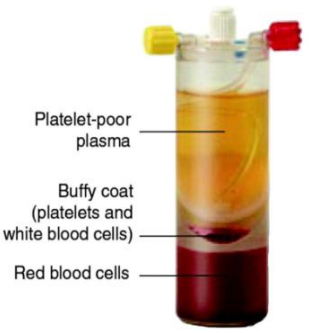
METHOD OF AUTOLOGOUS PRP PREPRATION

This therapy uses autologous PRP as a definitive therapy for chronic wound management.

Definition- PRP defined as a component of plasma fraction of autologous venous blood with platelet counts in the range between 4-6 times above baselines.

1. Preparation of PRP –

- Under all aseptic precautions, 8-10 ml of venous blood was freshly drawn from the patient.
- Then 3 ml blood is then transferred to 3 EDTA test tubes each and the tubes were shaken well to prevent the blood from clotting and the tubes were transferred to ward laboratory.
- A double spin technique was used, initially the test tubes were centrifuged at **2000 rpm** for 15 minutes. By this RBCs will settle in the lower portion of the test tube and plasma in the upper part.
- Plasma is extracted and collected in separate EDTA test tube and further re-centrifuged at **1200 bpm** for 10 mins
- The plasma then separates into upper buffy coat which is platelet poor plasma (PPP) and the lower 2-4 ml layer containing platelet rich plasma (PRP).

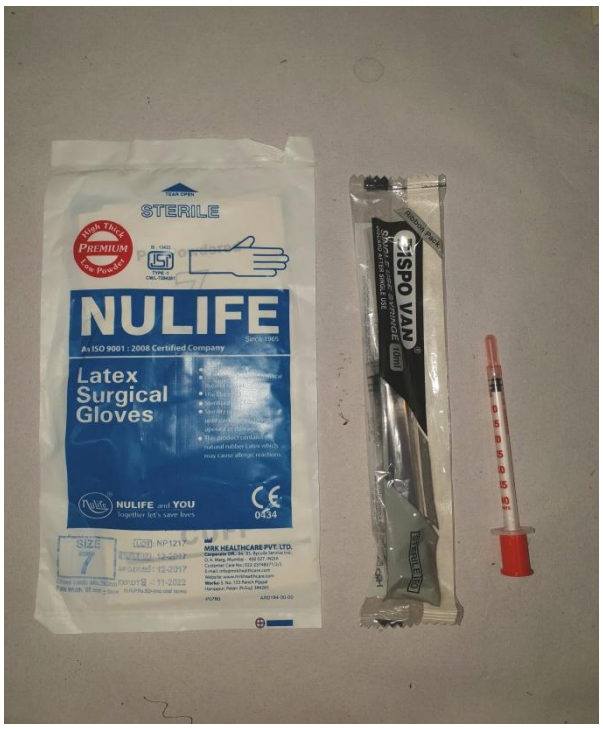


Picture 1: Layers of blood components after centifugation

- The centrifuge machine used is simple non-cooled one.
- The process is performed at a room temp of 22-24°C
- This autologous PRP is then transferred to a insulin syringe and locally infiltrated in the wound margins, just like a local infiltration of local anesthesia. At a distance of approximately 0.2 ml/cm with equal quantity.
- The process was repeated every 4th day for minimum of 4 cycles.
- Local dressing was performed on alternate days, with moist saline only.
- From infected wounds swabs were taken for culture and sensitivity every week, to monitor infection status of the wound.
- Patient was thoroughly examined for general condition and local wound condition and ulcer size (length, breath, depth) was measured.
- Every 4th day, the ulcer area and volume was calculated and photographed.
- Wound area was calculated using the formula length in greatest dimensions multiplied with breadth in greatest dimension.
- For an ellipse wound the formula used was Length x breadth x 0.7854
- (An ellipse is closure to a wound shape then square or rectangle)
- The use of an ellipse for calculating wound measurement has been used in RCTs in wound healing literature. [8, 9]
- Treatment outcome will be defined as a percentage of change in the area, which will be calculated as initial measurement minus assessment day measurement divided by initial measurement.

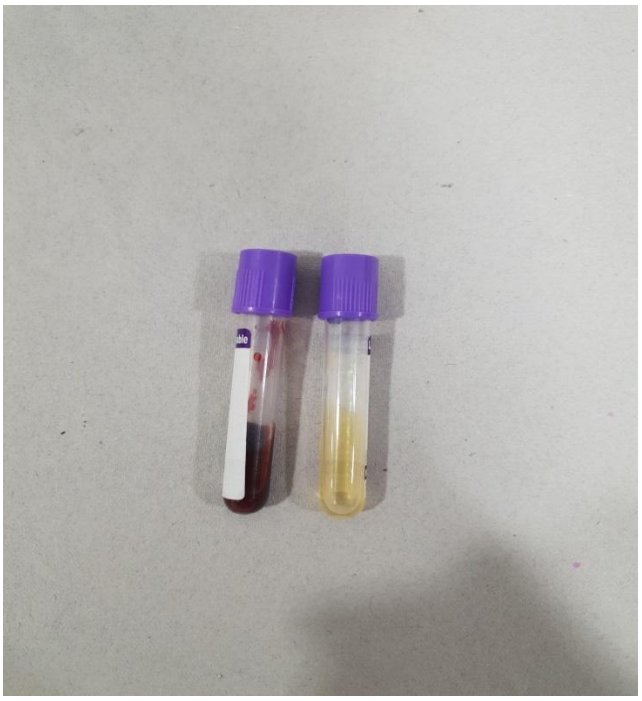
$$\textbf{HEALING IN \%} = \frac{\textbf{Initial measurement} - \textbf{Final measurement}}{\textbf{Initial measurement}} \times 100$$

Picture 2: Centrifuge machine



Picture 3: Sterile gloves, 10cc syringe, insulin syring

Picture 4 : Whole blood collected in EDTA Tube



Picture 4: Separated RBC concentrate and PRP

RESEARCH HYPOTHESIS

Autologous Platelet rich plasma is a safe, simple, biocompatible technique as a definitive management of chronic cutaneous ulcers without any adverse events.

SAMPLING:

Study period from OCTOBER 2016 to JUNE 2018. All the patients admitted during this period, who fulfill the inclusion criteria, will be included in this study.

ESTIMATION OF SAMPLE SIZE:

It is a interventional comparative study.

With anticipated mean difference of average duration of wound healing between the two study groups as 5.1 weeks and Anticipated SD as 5.3 weeks.

With this the minimum sample size for both the groups is 90 and per each group is 45.

With 95% Power and 99% Confidence level obtained from reference study. [4]

Formula Used

$$n = \frac{(Z_\alpha + Z_\beta)^2 2SD^2}{MD^2} \qquad n = 90$$

WHERE

- Z = Z statistic at a level of significance
- MD = Anticipated Mean difference = 5.1
- SD = Anticipated Standard deviation = 5.3
- Student's t – test used to compare the subcutaneous injection of PRP and standard wound management techniques based on the healing results under study.

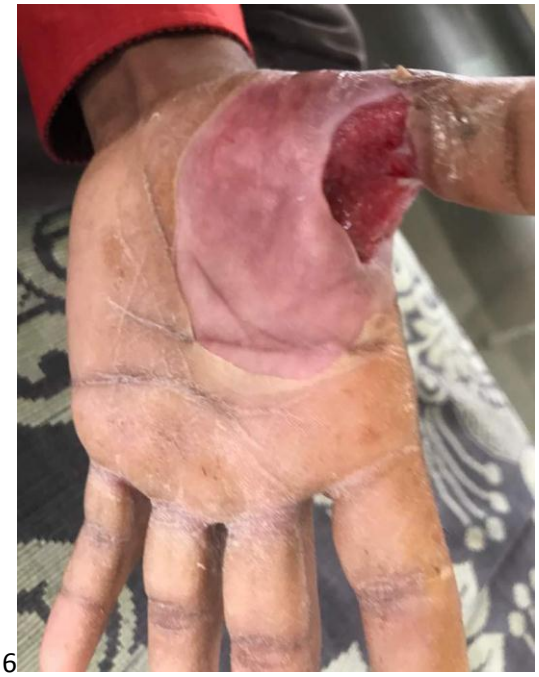
Hence the samples size of two groups is 90.

Statistical Analysis:

Data will be analyzed using-

- Mean ± SD
- Percentages
- Z proportion test
- T-test for comparison of mean
- Graphical presentation

PHOTOGRAPHS OF RESULTS WITH PRP INJECTIONS

6 

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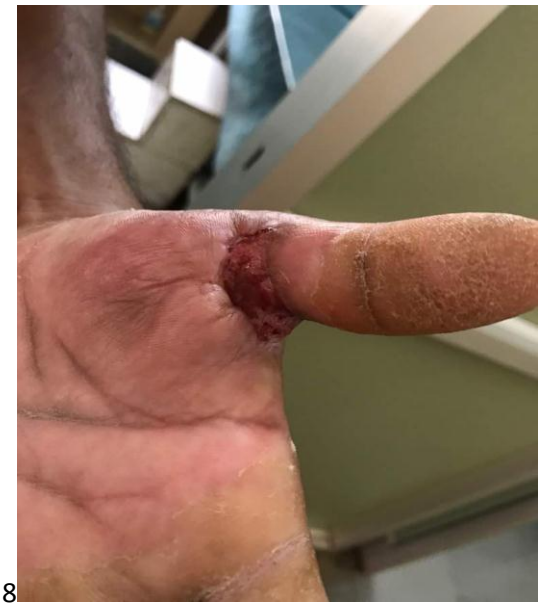
8 

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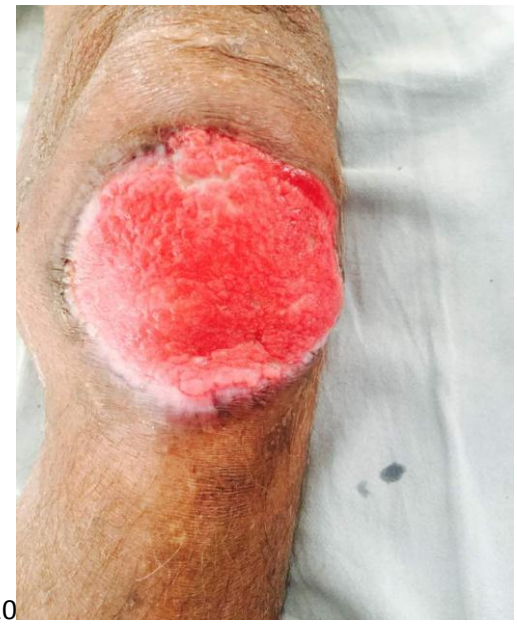
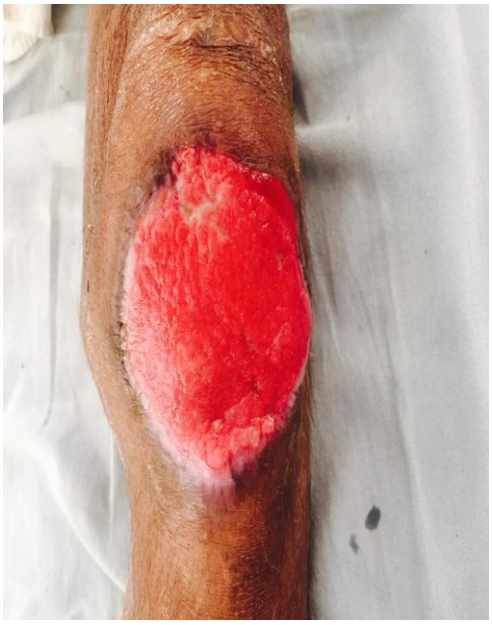
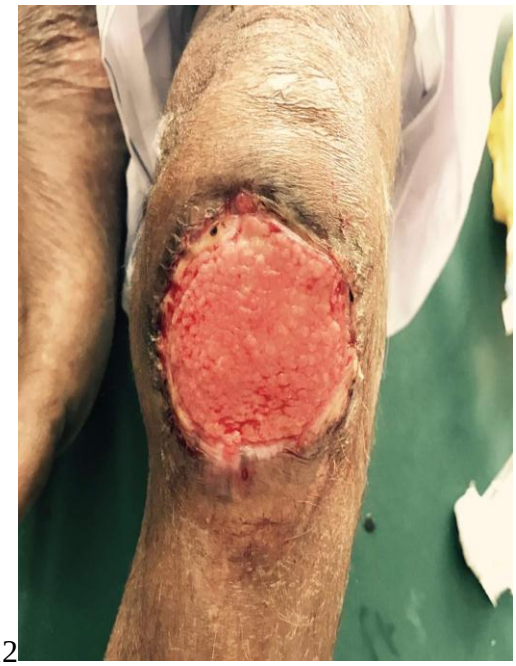
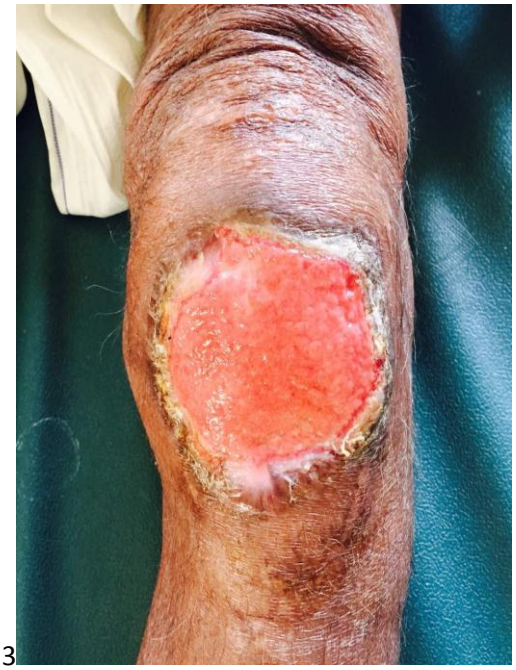
Picture 6, 7, 8, 9, showing progressing healing of chronic ulcer over left hand following 4 cycles of PRP. End result: complete wound closure in 20 days.

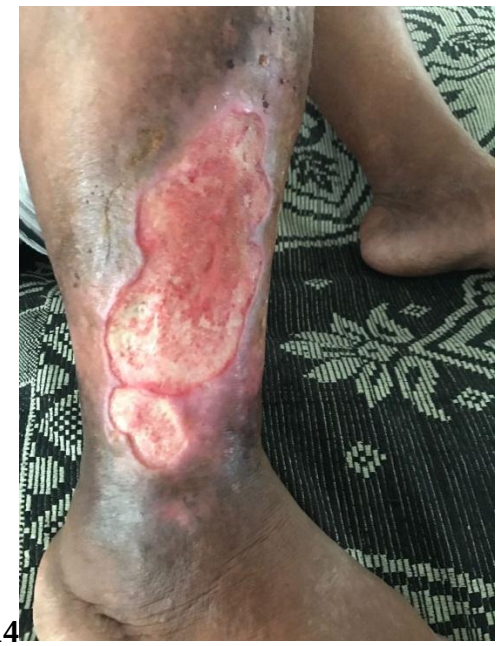
10 

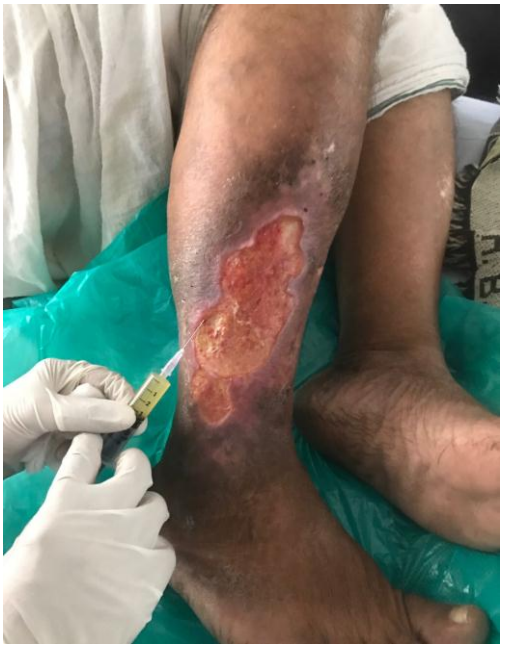
11 

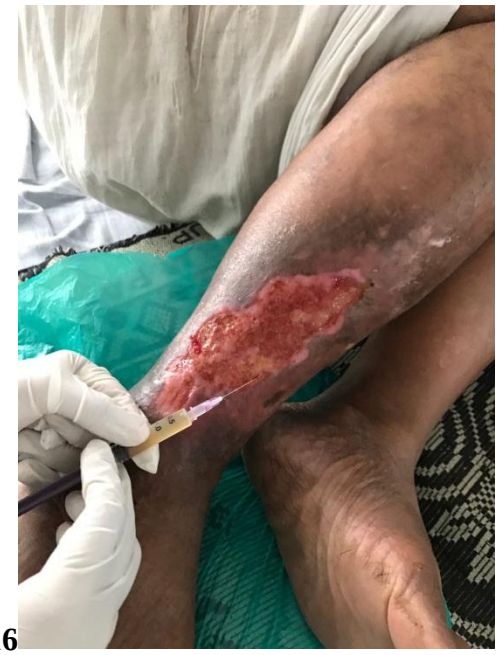
12 

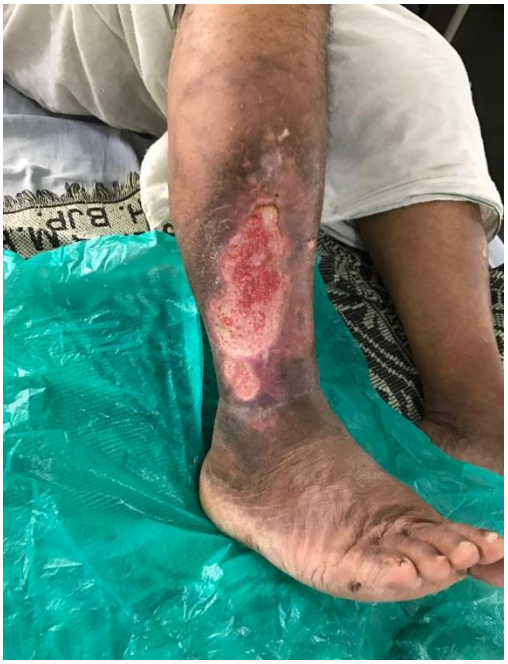
13 

Picture 10, 11, 12, 13 showing wound contraction of 45% in a chronic ulcer over left knee following 4 cycles of PRP. Duration of ulcer-3 mont

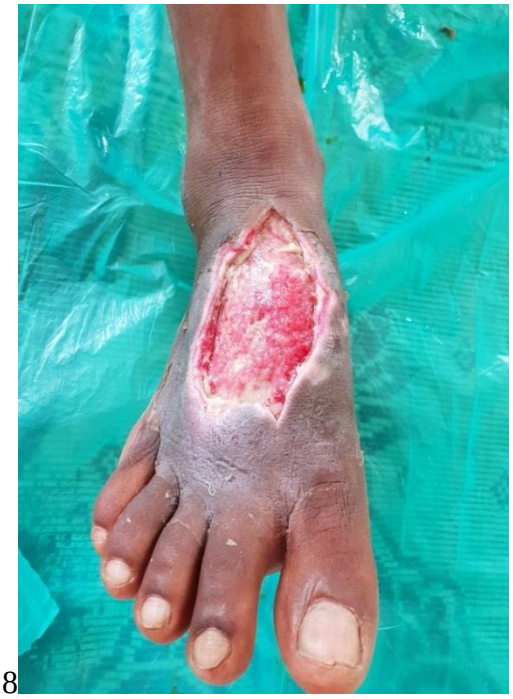
14 

15 

16 

17 

Picture 14, 15,16,17, show serial progression of chroinc ulcer of 6 months duration with PRP injection brought healthy granulation tissue.

18 

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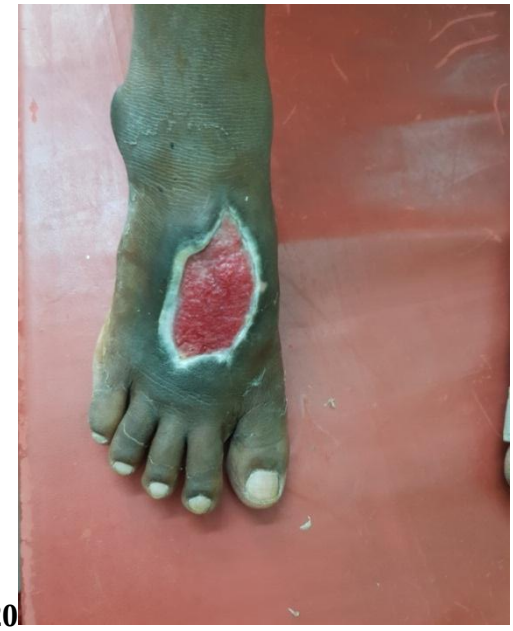

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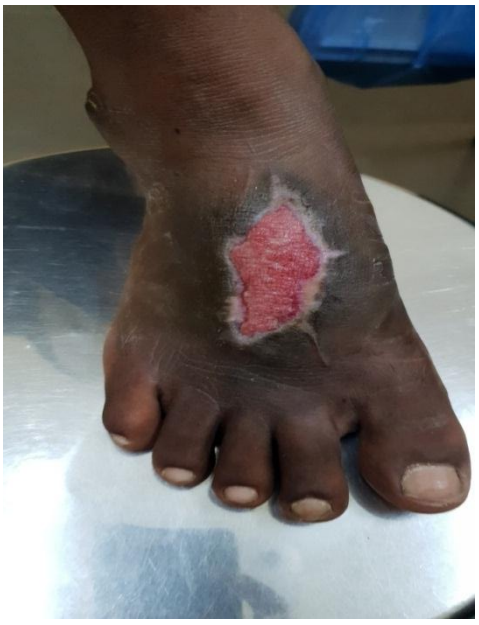

21

Picture 18, 19, 20, 21 : Show contraction of wound by >50% with 4 cycles of PRP in a chronic ulcer over dorsum of right foot.

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Picture 22, 23, 24: Show contraction of wound by >40% with early granulation tissue following 4 cycles of PRP injections in chronic ulcer over right arm in diabetic patient.

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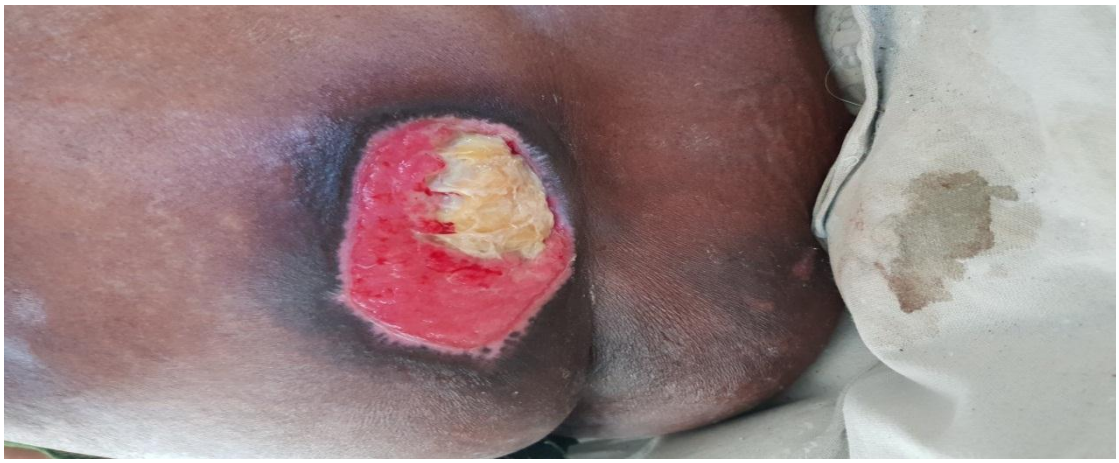

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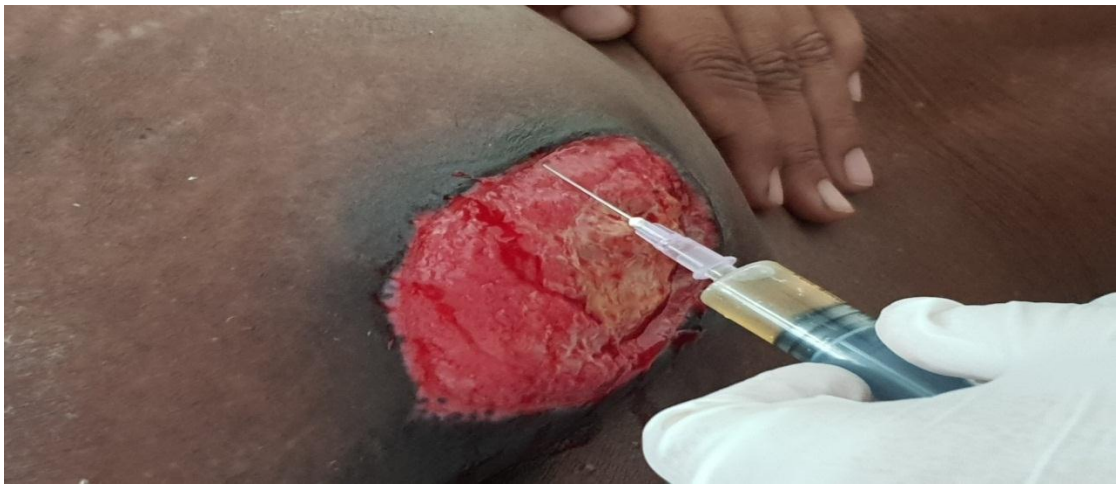

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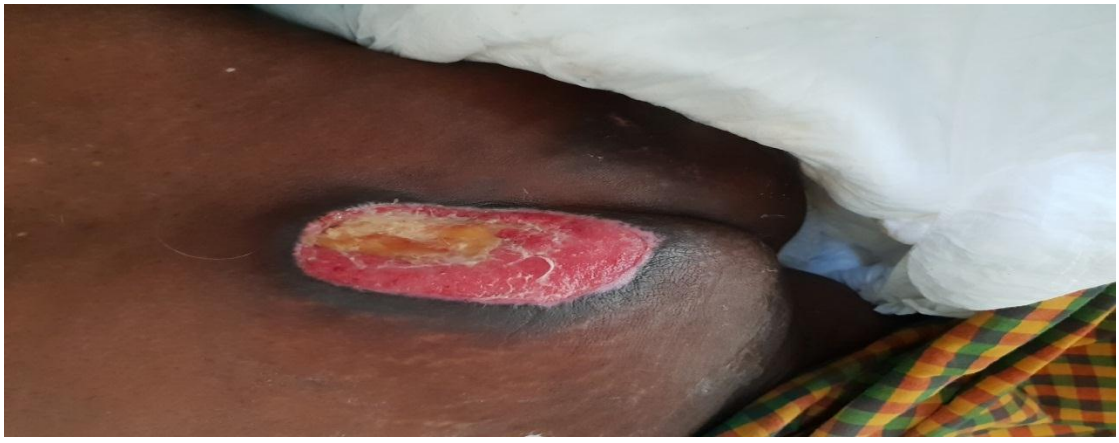

Picture 25, 26, 27: show appearance of healthy grannulation tissue following local injections of PRP in a bedsore over gluteal region of 6 months of duration.

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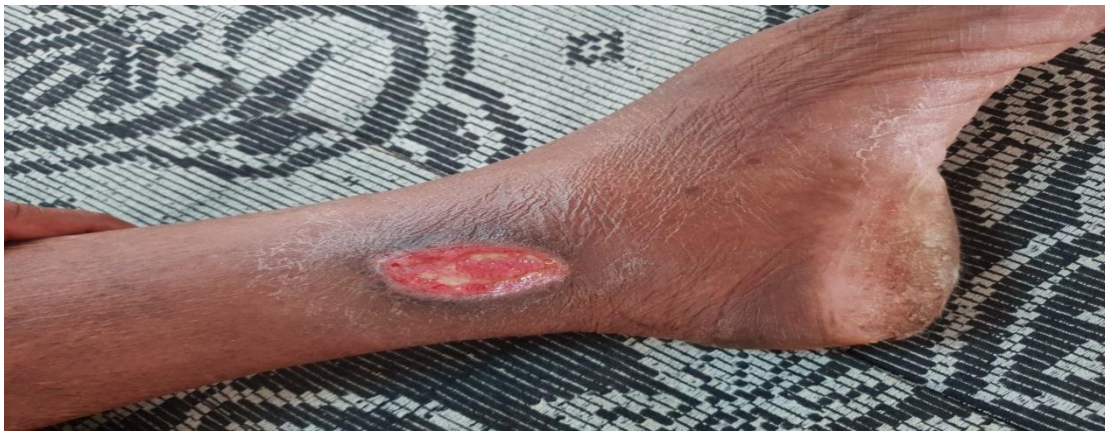

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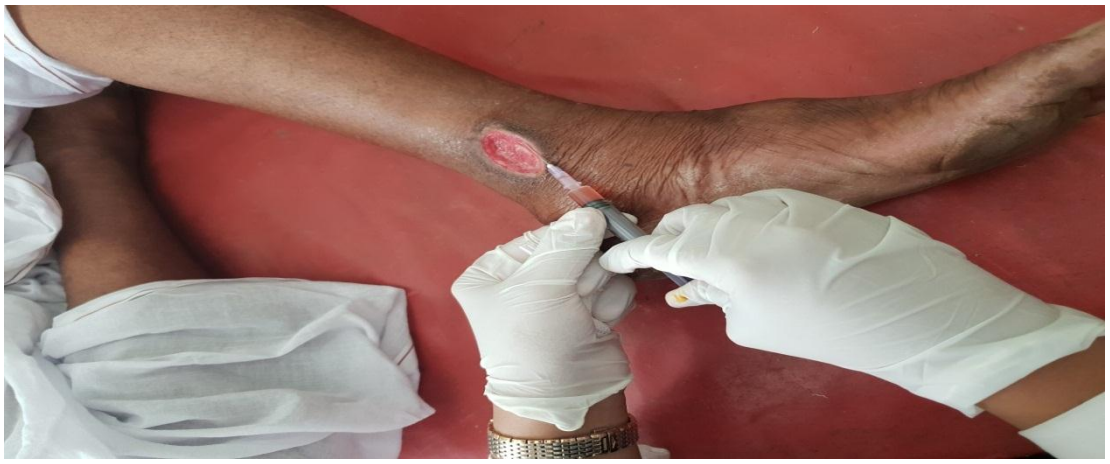

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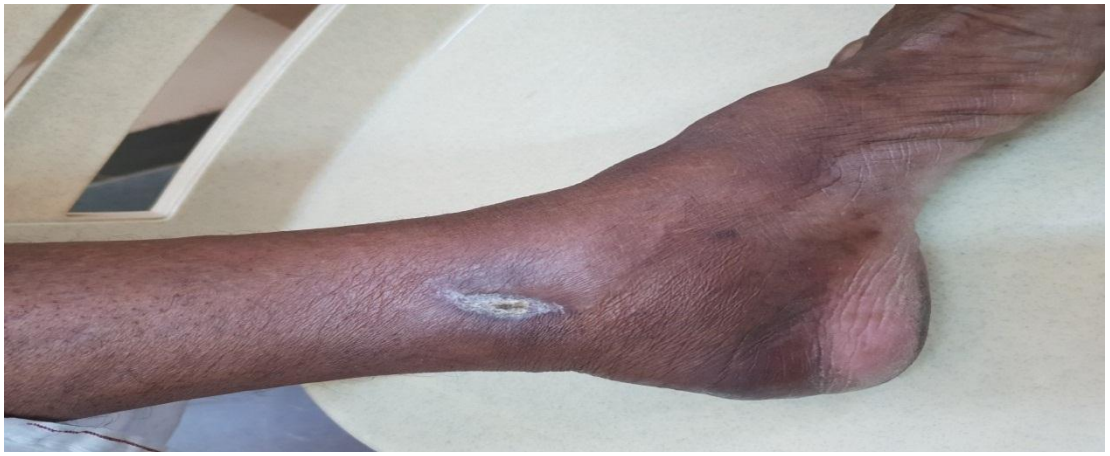


Picture 28, 29, 30: show complete closure of wound in 16 days following PRP injection in an ulcer over left ankle region.

RESULTS

TABLE 1 : DISTRIBUTION OF AGE BETWEEN PRP GROUP (A) AND CONTROL GROUP (B)

Age (Years)	PRP GROUP	%	CONTROL GROUP	%	Total	%	Chi square test
< 40	11	24.4	7	15.6	18	20	
40 – 49	8	17.8	5	11.1	13	14.44	
50 – 59	10	22.2	9	20.0	19	21.11	
60 – 69	11	24.4	14	31.1	25	27.78	
70 – 79	5	11.1	6	13.3	11	12.22	P=0.2981
80+	0	0	4	8.9	4	4.44	NS
Total	45	100.0	45	100.0	90	100	

CHART 1: DISTRIBUTION OF AGE BETWEEN PRP GROUP AND CONTROL GROUP

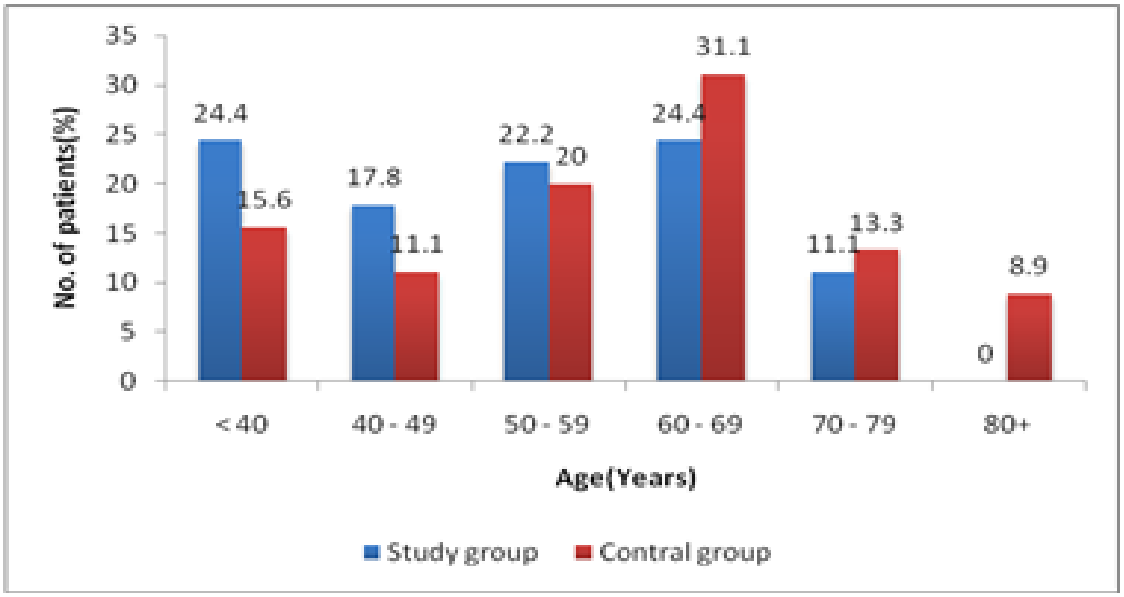


Table 1 and chart 1 show age distributiom between PRP group and control group with MD with percentage distribution of age is maximum in between 60-69years (24.4%) in PRP group and (31.1%) in control group.

TABLE 2: DISTRIBUTION OF SEX BETWEEN PRP AND CONTROL GROUPS.

Gender	Study group	%	Control group	%	Total	%	Chi square test
Male	37	82.2	31	68.9	68	76	P=0.1411 NS
Female	8	17.8	14	31.1	22	24	
Total	45	100	45	100	90	100	

CHART 2: DISTRIBUTION OF SEX BETWEEN PRP AND CONTROL GROUPS.

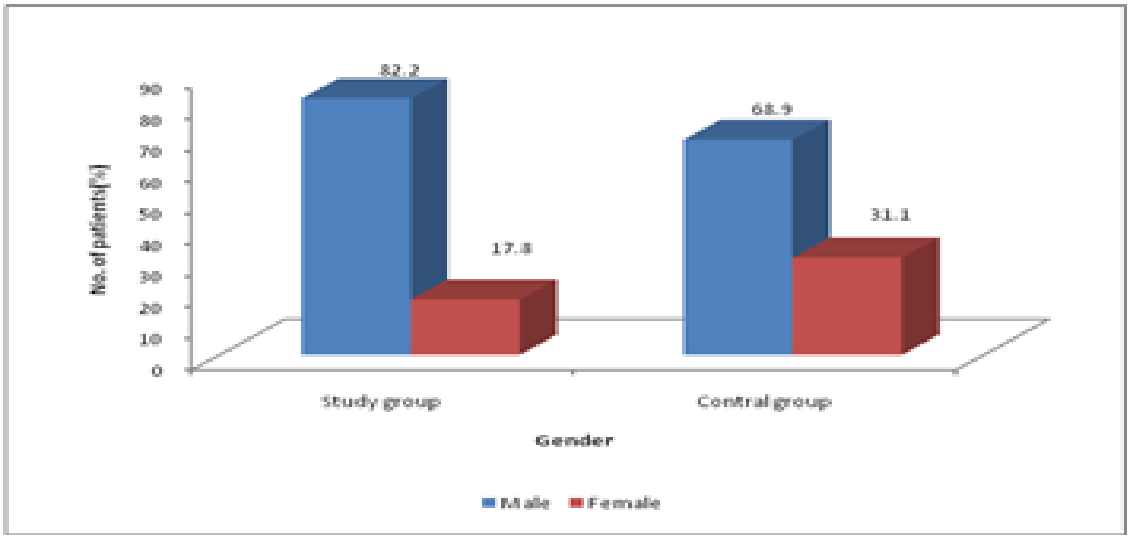


Table 2 and chart 2 show sex distribution among two groups. In this study both the groups were male predominant i.e., 76%

TABLE 3: DISTRIBUTION OF SITE BETWEEN PRP AND CONTROL GROUPS.

Site of Ulcer	Study group	%	Control group	%	Total	%	Chi square test
Lower Extremity	38	84.4	35	78	73	81	P=0.4191 NS
Upper Extremity	7	15.6	10	22	17	19	
Total	45	100	45	100	90	100	

CHART 3: DISTRIBUTION OF SITE OF ULCER BETWEEN PRP AND CONTROL GROUPS.

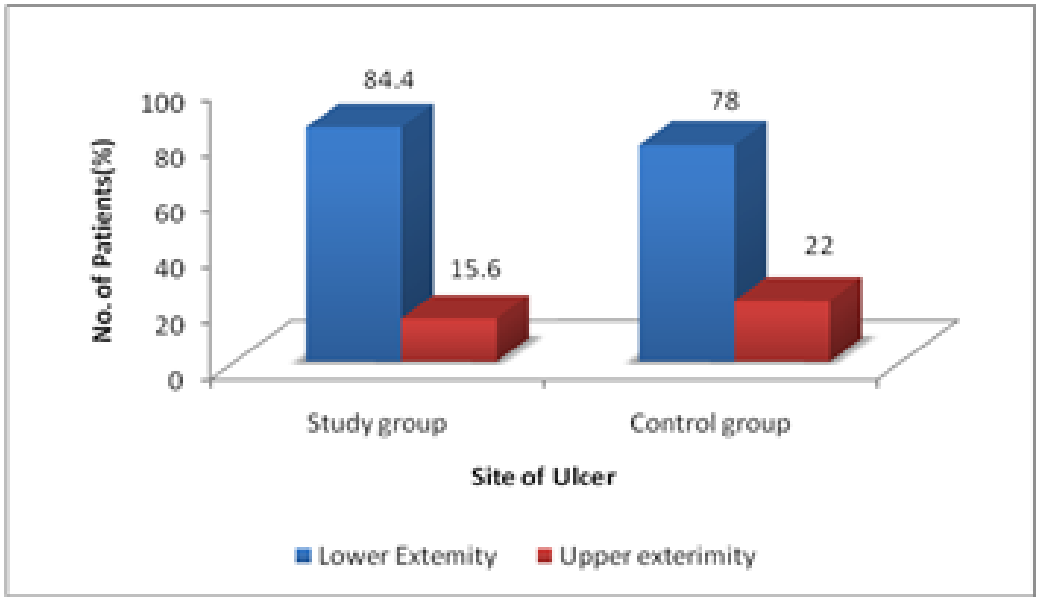


Table 3 and chart 3 shows site distribution of ulcers in both groups. In this study lower extrimity ulcers were predominant in both groups i.e., 81%.

TABLE 4: DISTRIBUTION BETWEEN DURATION OF ULCER BETWEEN PRP AND CONTROL GROUP:

Duration	Study group	%	Control group	%	Total	%	Pooled chi square test
<1 MONTH	0	0	3	6.7	3	3.33	
1-2 MONTHS	37	82.2	37	82.2	74	82.22	
>2 MONTHS	8	17.8	5	11.1	13	14.45	P=0.1508 NS
Total	45	100.0	45	100.0	90	100	

CHART 4: DISTRIBUTION BETWEEN DURATION OF ULCER BETWEEN PRP AND CONTROL GROUP:

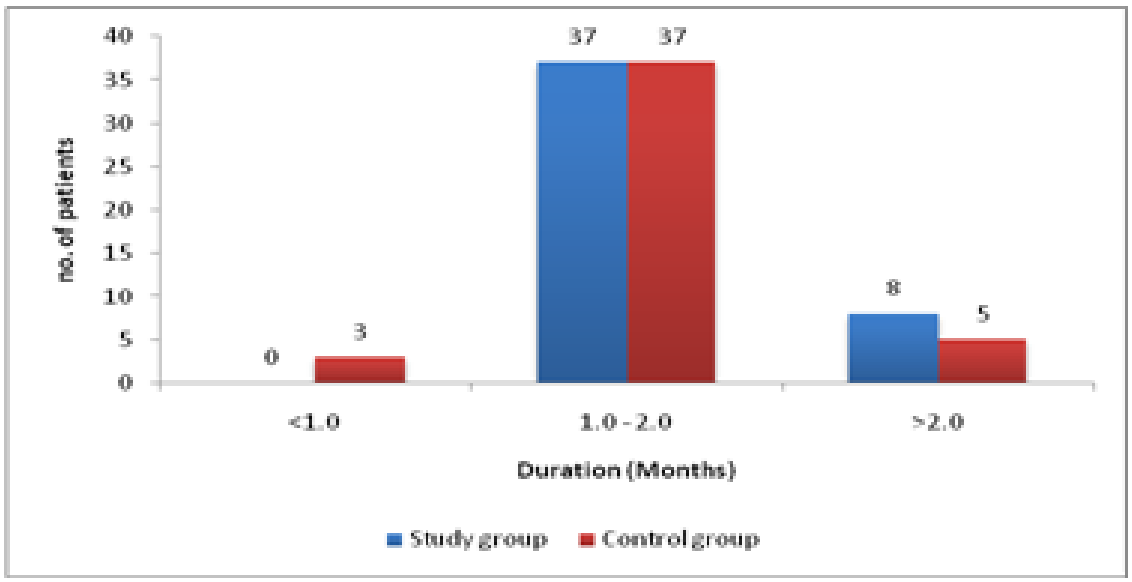
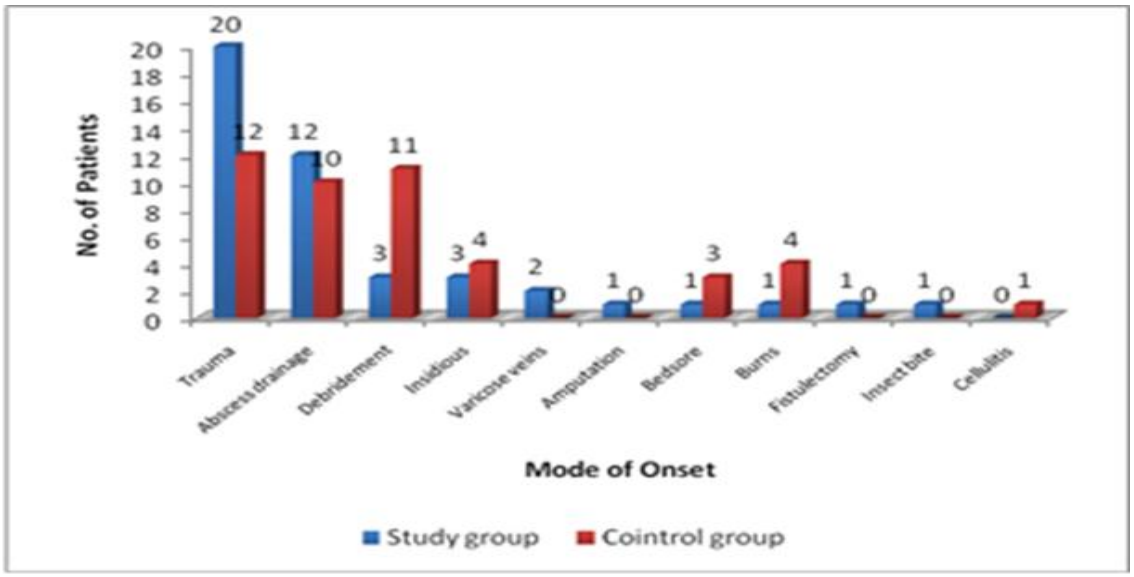


Table 4 and chart 4 show distribution of duration of ulcer between both groups, maximum patients had ulcers of duration between 1-2months i.e., 82.2%

TABLE 5: DISTRIBUTION OF MODE OF ONSET BETWEEN PRP GROUP AND CONTROL GROUP.

Mode of Onset	Study group	%	Control group	%	Total	%	Chi square test
Trauma	20	44.4	12	26.7	32	35.55	
Abscess drainage	12	26.7	10	22.2	22	24.44	
Debridement	3	6.7	11	24.4	14	15.5	
Insidious	3	6.7	4	8.9	7	7.77	
Varicose veins	2	4.4	0	0	2	2.22	
Amputation	1	2.2	0	0	1	1.11	P=0.1081 NS
Bedsore	1	2.2	3	6.7	4	4.44	
Burns	1	2.2	4	8.9	5	5.55	
Fistulectomy	1	2.2	0	0	1	1.11	
Insect bite	1	2.2	0	0	1	1.11	
Cellulitis	0	0	1	2.2	1	1.111	
Total	45	100.0	45	100	90		

CHART 5: DISTRIBUTION OF MODE OF ONSET BETWEEN PRP GROUP AND CONTROL GROUP.



Trauma was most common mode of onset in both the groups i.e., 35% average.

TABLE 6: DISTRIBUTION OF CO-MORBIDITIES BETWEEN PRP AND CONTROL GROUP.

Co-Morbidities	Study group	%	Control group	%	Total	%	Pooled chi square test
Diabetic	17	37.8	20	44.4	37	41.11	
Hypertension	2	4.4	5	11.1	7	7.78	
Varicose veins	6	13.3	2	4.4	8	8.89	
Diabetic & HTn	2	4.4	1	2.2	3	3.33	
Diabetic & Hypertension	0	0	1	2.2	1	1.11	
Foot Drop	0	0	1	2.2	1	1.11	
Nil	18	40	15	33.3	33	36.66	P=0.4082 NS
Total	45	100.0	45	100	90	100	

CHART 6: DISTRIBUTION OF CO-MORBIDITIES BETWEEN PRP AND CONTROL GROUP:

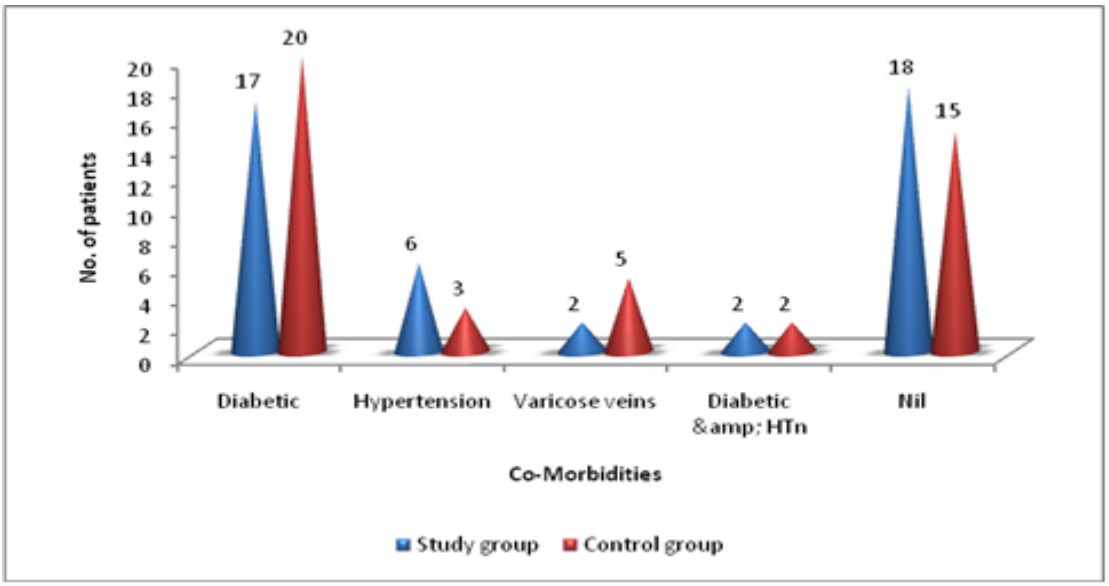


Table 6 and chart 6 showing distribution of co-morbidities between both the groups. In this study 37.8% and 44.1% were diabetics were diabetics with mean of 41.4%.

TABLE 7: DISTRIBUTION OF CULTURE GROWTH BETWEEN PRP AND CONTROL GROUP.

Culture from wound	Study group	%	Control group	%	Total	%	Chi square test
Sterile	28	62.2	25	55.6	53	58.89	P=0.5737 NS
S. aureus	10	22.2	9	20	19	21.11	
Acinobacter	3	6.7	3	6.7	6	6.67	
Citrobacter	3	6.7	4	8.9	7	7.78	
Klebsiella	1	2.2	4	8.9	5	5.56	
Total	45	100.0	45	100	90	100	

CHART 7: DISTRIBUTION OF CULTURE GROWTH BETWEEN PRP AND CONTROL GROUP.

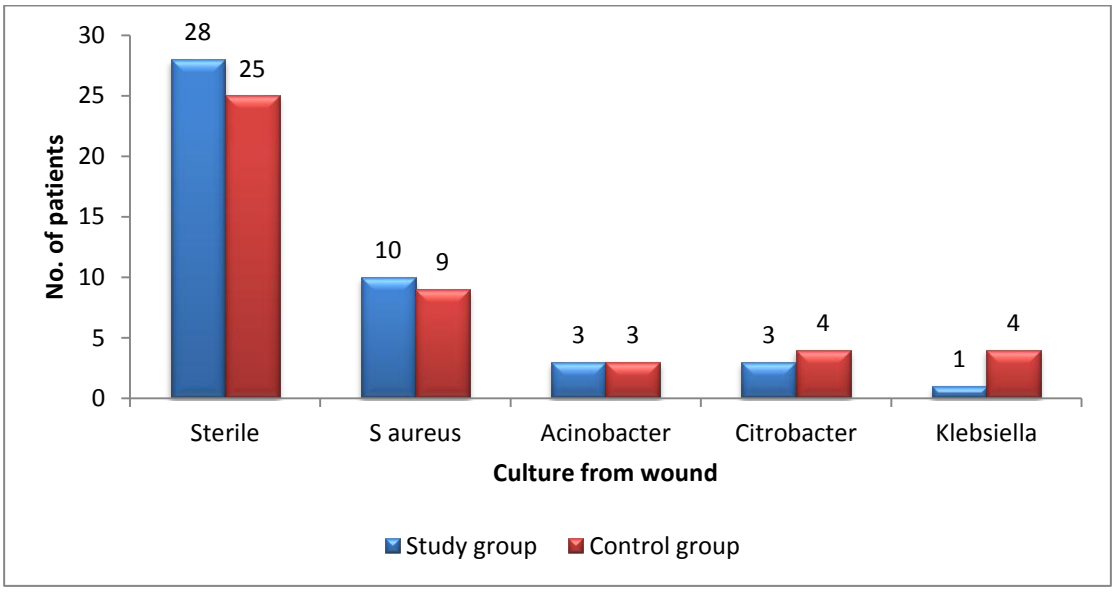


Table 7 and chart 7 show the organisms isolated between both the groups. In this study most of the ulcers were consided once the culture was sterile with mean of 58.89%.

TABLE 8: DITRIBUTION OF WAGNER GRADE OF ULCER BETWEEN PRP AND CONTROL GROUP:

WAGNER'S ULCER GRADING	Study group	%	Control group	%	Total	%
I	15	33.3	3	6.7	18	20
II	17	37.8	28	62.2	45	50
III	13	28.9	14	31.1	27	30
Total	45	100.0	45	100.0	90	100

CHART 8: DITRIBUTION OF WAGNER GRADE OF ULCER BETWEEN PRP AND CONTROL GROUP:

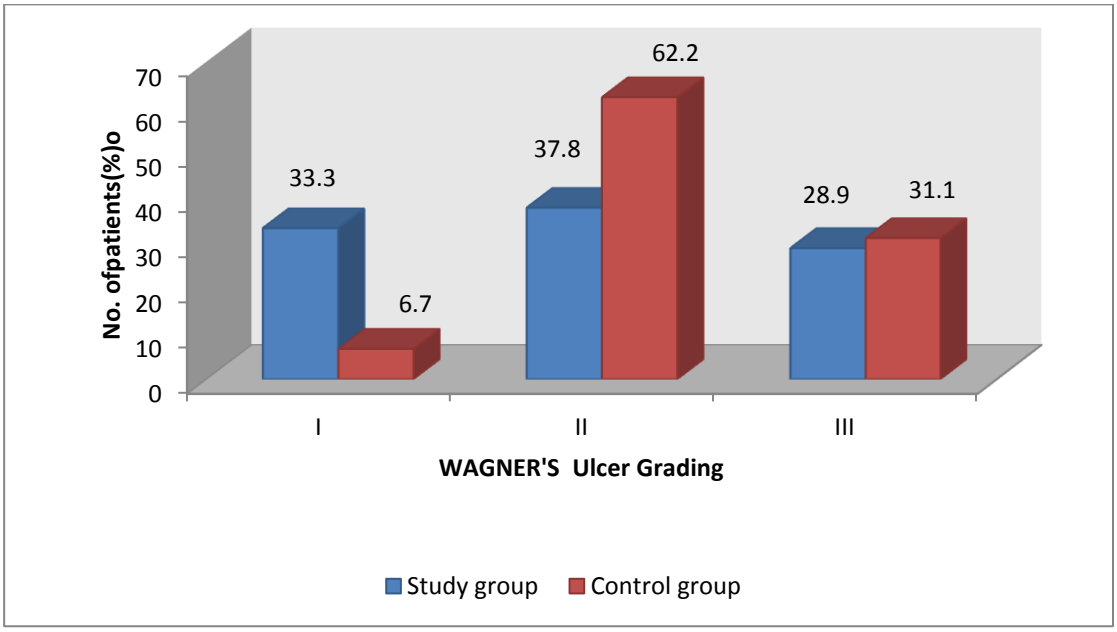


TABLE 9: DISTRIBUTION OF GRANULATION TISSUE BETWEEN PRP AND CONTROL GROUP.

GRANNULATION TISSUE	Study group	%	Control group	%	Total	%	Chi square test
Satisfactory	31	68.9	17	37.8	48	53.33	P=0.0031*
Unsatisfactory	14	31.1	28	62.2	42	46.67	
Total	45	100.0	45	100.0	90	100	

CHART 9: DISTRIBUTION OF GRANULATION TISSUE BETWEEN PRP AND CONTROL GROUP.

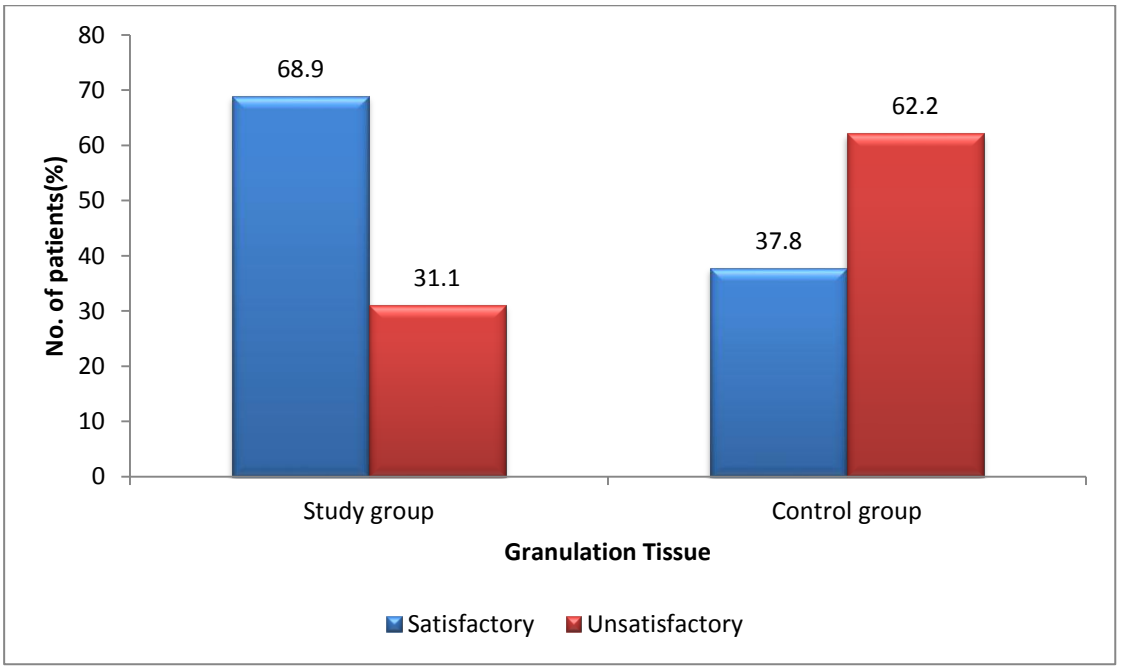


Table 8 and chart 8 show distribution of granulation tissue among two groups. A significant satisfactory granulation tissue was observed in PRP group with P value of 0.0031 when compared to conventional dressing.

TABLE 10: COMPARISION OF HEALING PROGRESS IN % BETWEEN PRP AND CONTROL GROUP.

Healing Progress in %	Study group	%	Control group	%	Total	Chi square test
20-30	5	11.1	10	22.2	15	
30-40	18	40	27	60	45	
40-50	14	31.1	7	15.6	21	P=0.00105*
50+	8	17.8	1	2.2	9	
Total	45	100.0	45	100	90	

CHART 10: COMPARISION OF HEALING PROGRESS IN % BETWEEN PRP AND CONTROL GROUP.

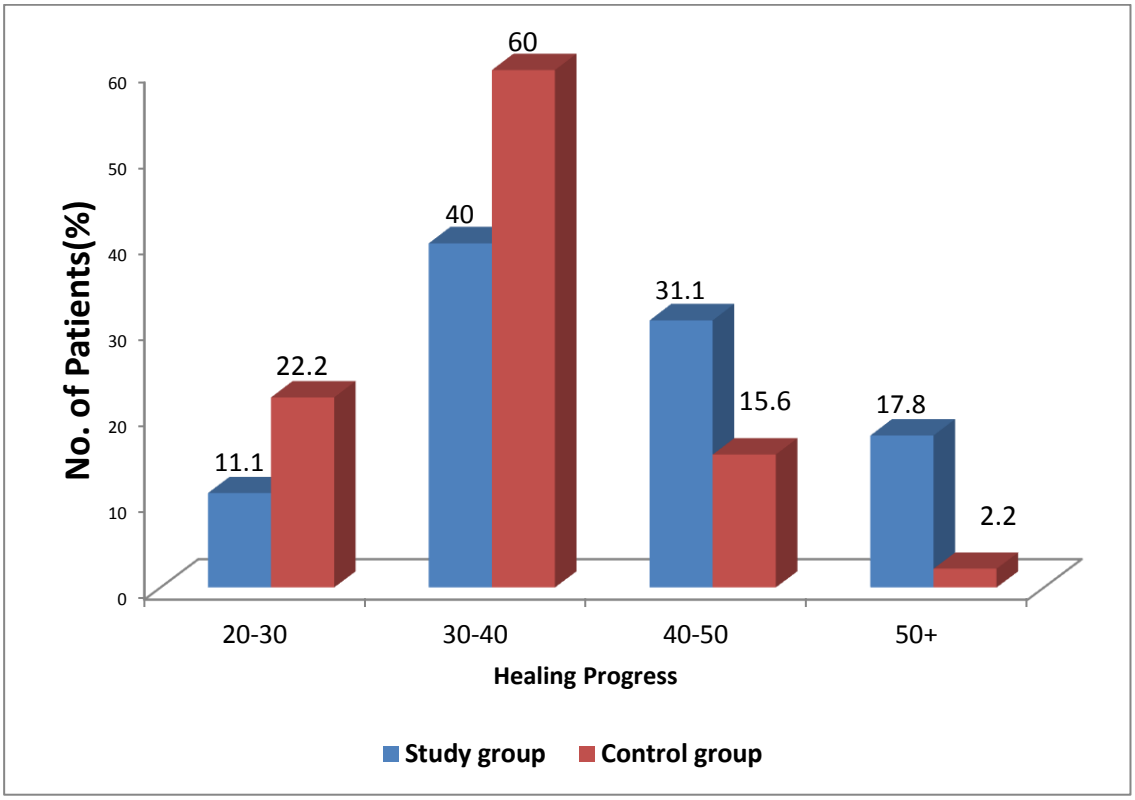


Table 10 and chart 10 showing comparison between healing progress in % among both the groups after 20 days of observation. In this study healing of the ulcers were maximum in PRP group with 31.1% patients having >50% healing in PRP group with significant P value of 0.0015%

TABLE 11: DISTRIBUTION OF 3 MONTHS FOLLOW-UP BETWEEN PRP AND CONTROL GROUP:

3 MONTH FOLLOW-UP	Study group	%	Control group	%	Total	%
Contraction Of Wound	19	42.2	8	17.8	27	30
Skin Grafting	8	17.8	14	31.1	22	24.44
Suturing	12	26.7	17	37.8	29	32.22
Did not follow up	6	13.3	6	13.3	12	13.34
Total	45	100.0	45	100	90	100

CHART 11: DISTRIBUTION OF 3 MONTH FOLLOW-UP BETWEEN PRP AND CONTROL GROUP:

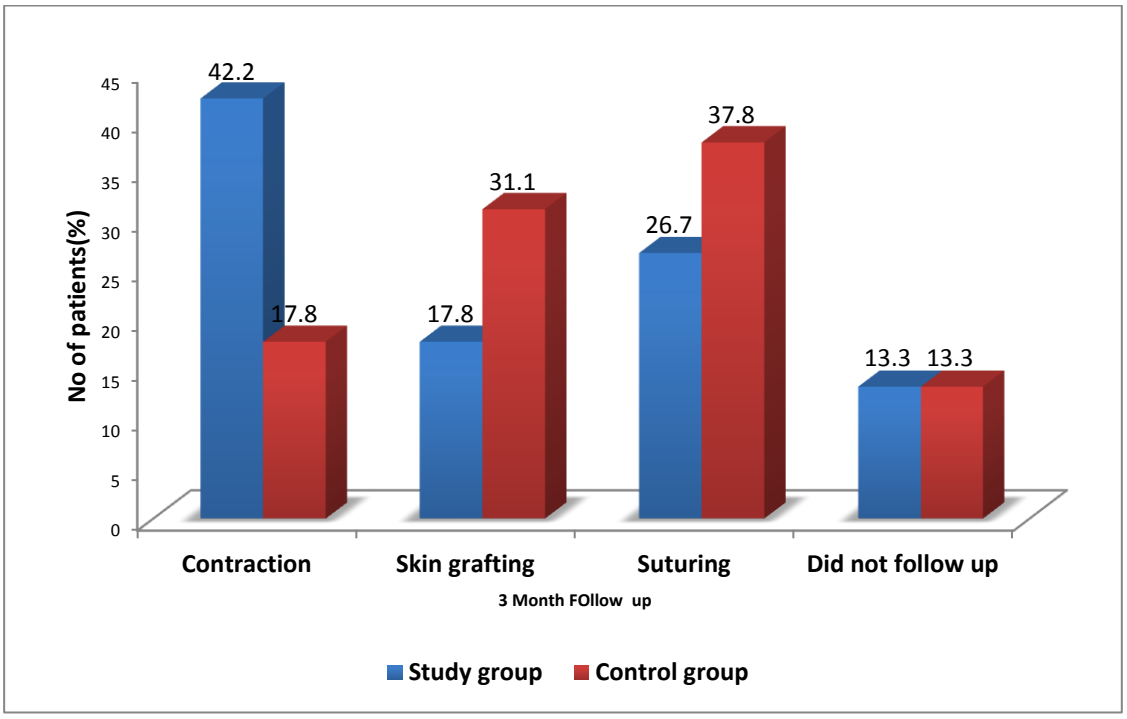


Table 11 and chart 11 showing 3 months follow up in both the groups with 42.2% ulcers healing completely by secondary intension in PRP group when compared to control group i.e., 17.8%.

TABLE 12: ASSOCIATION BETWEEN WAGNER'S ULCER GRADING AND HEALING PROGRESS IN % IN PRP GROUP:

WAGNER'S Ulcer Grading	Healing Progress					Chi square test
	20.00 – 30 N(%)	30.00 – 40 N(%)	40.00 – 50 N(%)	50.00+ N(%)	Total N(%)	
I	0	0	7(50)	8(100)	15(33.3)	P=0.001*
II	0	10(55.6)	7(50)	0	17(37.8)	
III	5(100)	8(44.4)	0	0	13(28.9)	
Total	5(100)	18(100)	14(100)	8(100)	45(100)	

CHART 12: ASSOCIATION BETWEEN WAGNER'S ULCER GRADING AND HEALING PROGRESS IN % IN PRP GROUP:

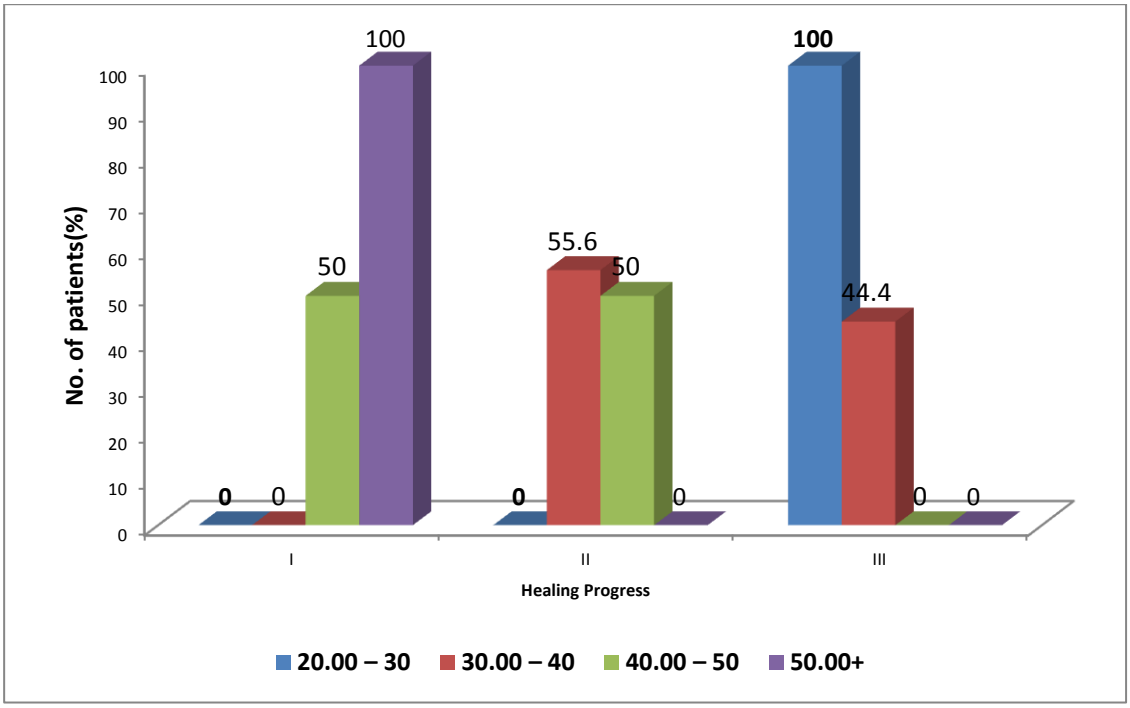


TABLE 13: ASSOCIATION BETWEEN WAGNER'S ULCER GRADING AND HEALING PROGRESS IN % IN CONTROL GROUP:

WAGNER'S Ulcer Grading	Healing Progress					Chi square test
	10-20 N (%)	20.00 – 30 N (%)	30.00 – 40 N (%)	40.0+ N (%)	Total N (%)	
I	0	2(7.4)	1(14.3)	0	3(6.7)	P=0.218 NS
II	9(90)	13(48.1)	5(71.4)	1(100)	28(62.2)	
III	1(10)	12(44.4)	1(14.3)	0	14(31.19)	
Total	10(100)	27(100)	7(100)	1(100)	45(100)	

CHART 13: ASSOCIATION BETWEEN WAGNER'S ULCER GRADING AND HEALING PROGRESS IN % IN CONTROL GROUP:

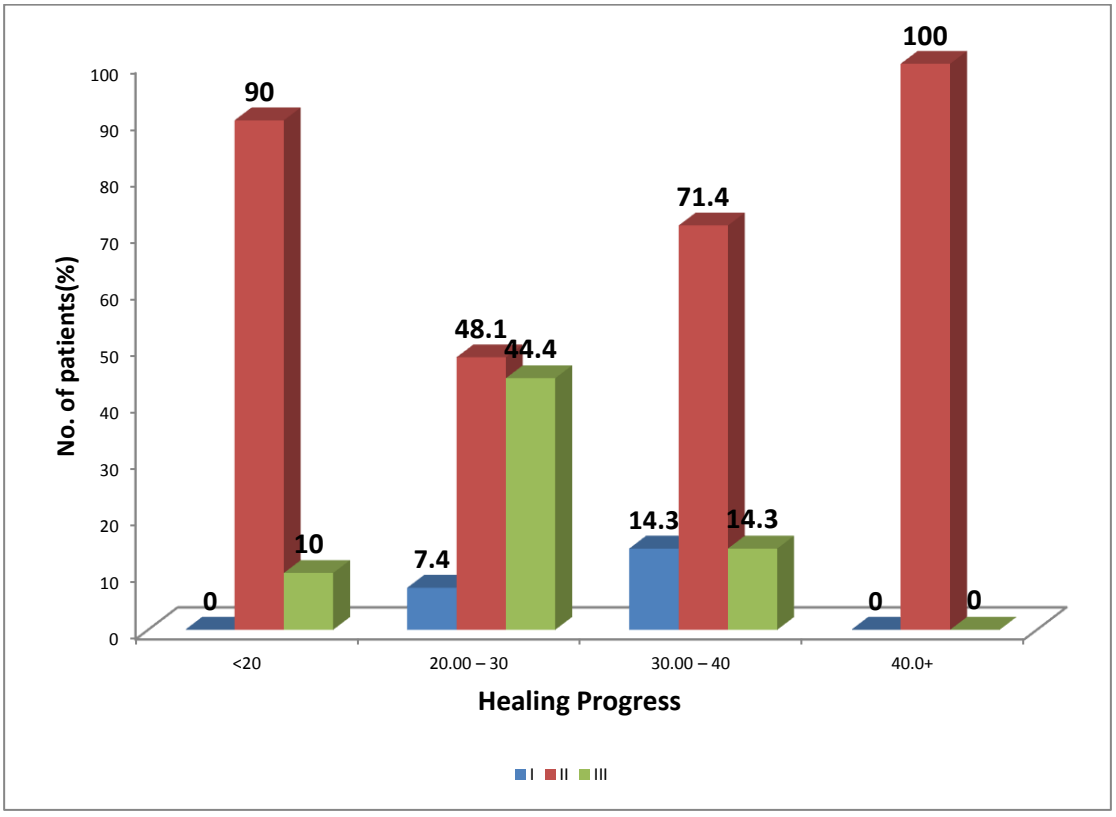


TABLE 14: ASSOCIATION BETWEEN CO-MORBIDITIES AND HEALING PROGRESS IN PRP GROUP

Co-Morbidities	Healing Progress					Chi square test
	20.00– 30 N(5)	30.00 – 40 N(%)	40.00 – 50 N(%)	50.00+ N(%)	Total N(%)	
Diabetic	4(80)	6(33.4)	4(28.6)	3(37.5)	17(37.8)	P=0.662 NS
Hypertension	0	0	1(7.1)	1(12.5)	2(4.4)	
Diabetic and Hypertension	0	2(11.1)	0	0	2(4.4)	
Nil	1(20)	7(38.9)	8(57.1)	2(25)	18(40)	
Varican Veins	0	3(16.7)	1(7.1)	2(25)	6(13.3)	
Total	5(100)	18(100)	14(100)	8(100)	45(100)	

CHART 14: ASSOCIATION BETWEEN CO-MORBIDITIES AND HEALING PROGRESS IN PRP GROUP

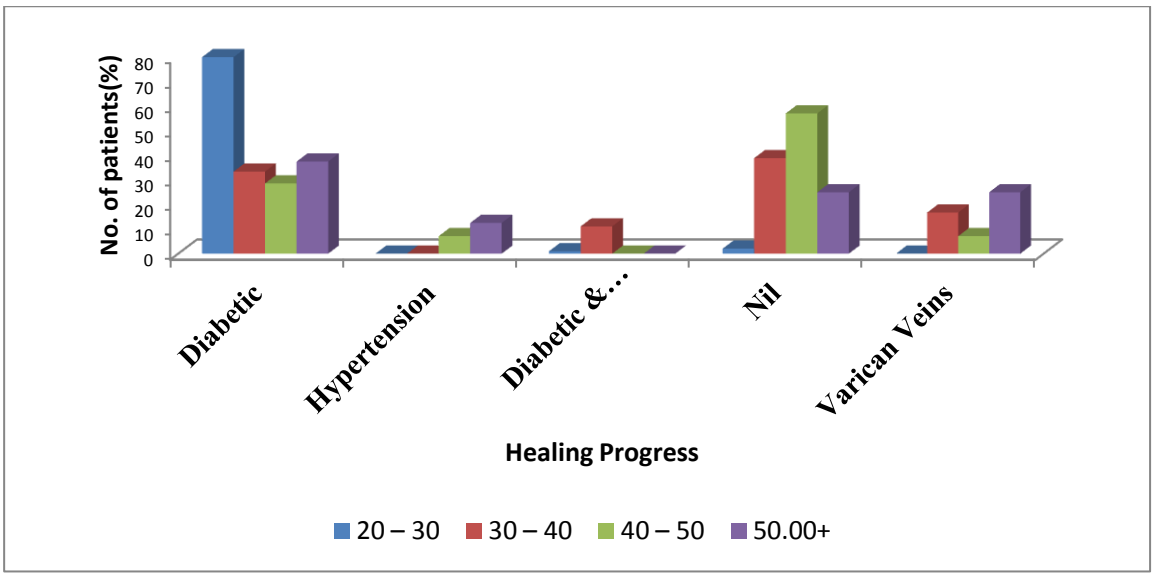


TABLE 15: ASSOCIATION BETWEEN CO-MORBIDITIES AND HEALING PROGRESS IN CONTROL GROUP:

Comorbidities	Healing Progress					Chi square test
	10-20 N(%)	20.00 – 30 N(5)	30.00 – 40 N (%)	40+ N(%)	Total N(%)	
Diabetic	5(50)	14(51.8)	2(28.6)	0	21(46.6)	P=0.120 NS
Hypertension	2(20)	2(7.4)	1(14.3)	0	5(11.1)	
Diabetic and Hypertension	0	2(7.4)	0	0	2(4.4)	
Nil	3(30)	9(33.3)	3(42.9)	0	15(33.3)	
Varicose Veins	0	0	1(14.3)	1(100)	2(4.4)	
Total	10(100)	27(100)	7(100)	1(100)	45(100)	

CHART 1: ASSOCIATION BETWEEN CO-MORBIDITIES AND HEALING PROGRESS IN CONTROL GROUP:

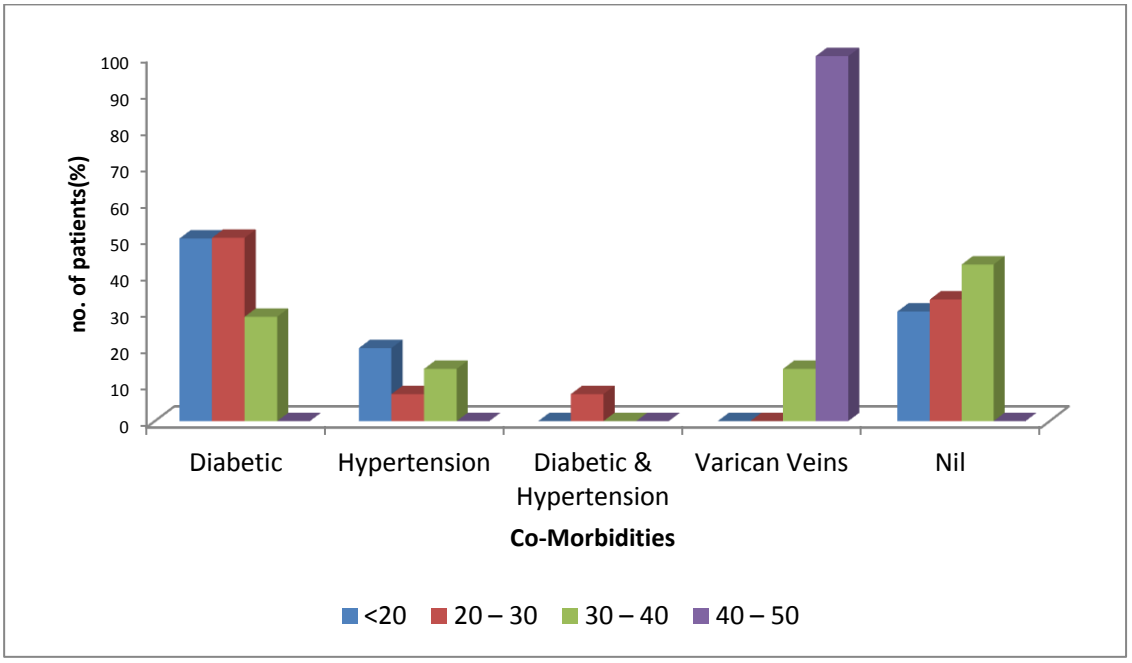


Table 14,15 and chart 14,15 are showing the association between co-morbidities associated with the patient and its association to healing progress of the ulcer. With P value of 0.0662 in PRP group and 0.120 in control group respectively. In this study there was negligible association seen between Diabetes mellitus and healing progress in both the groups i.e., ulcers in patients with type 2 DM had poor healing outcome when compared to non-diabetics.

TABLE 16: ASSOCIATION BETWEEN WAGNER'S ULCER GRADE WITH HEALING PROGRESS IN % IN PRP GROUP.

WAGNER'S Ulcer Grade	Healing Progress					Chi square test
	20.00 – 30 N(%)	**30.00 – 40 N(%)**	**40.00 – 50 N(%)**	**50.00+ N(%)**	**Total N(%)**	
I	0	0	7(50)	8(100)	15(33.3)	P=0.001*
II	0	10(55.6)	7(50)	0	17(37.8)	
III	5(100)	8(44.4)	0	0	13(28.9)	
Total	5(100)	18(100)	14(100)	8(100)	45(100)	

CHART 16:ASSOCIATION BETWEEN WAGNER'S ULCER GRADE WITH HEALING PROGRESS IN % IN PRP GROUP.

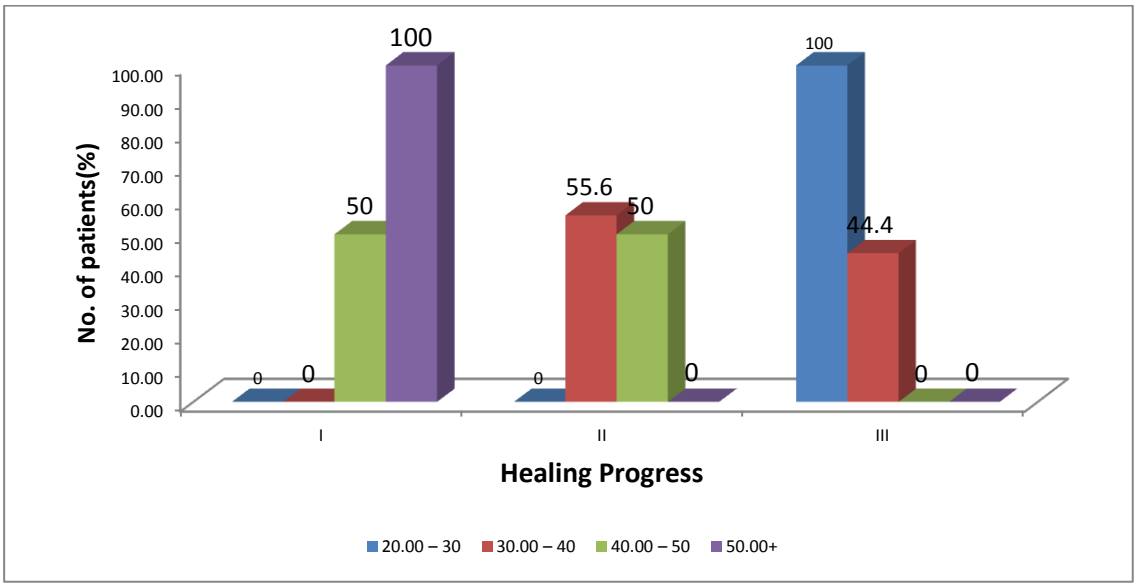


TABLE 17: ASSOCIATION BETWEEN WAGNER'S ULCER GRADE WITH HEALING PROGRESS IN % IN CONTROL GROUP.

WAGNER'S Ulcer Grade	Healing Progress					Chi square test
	10-20 N (%)	20.00 – 30 N (%)	30.00 – 40 N (%)	40.0+ N (%)	Total N (%)	
I	0	2(7.4)	1(14.3)	0	3(6.7)	P=0.218 NS
II	9(90)	13(48.1)	5(71.4)	1(100)	28(62.2)	
III	1(10)	12(44.4)	1(14.3)	0	14(31.19)	
Total	10(100)	27(100)	7(100)	1(100)	45(100)	

CHART 17:ASSOCIATION BETWEEN WAGNER'S ULCER GRADE WITH HEALING PROGRESS IN % IN CONTROL GROUP.

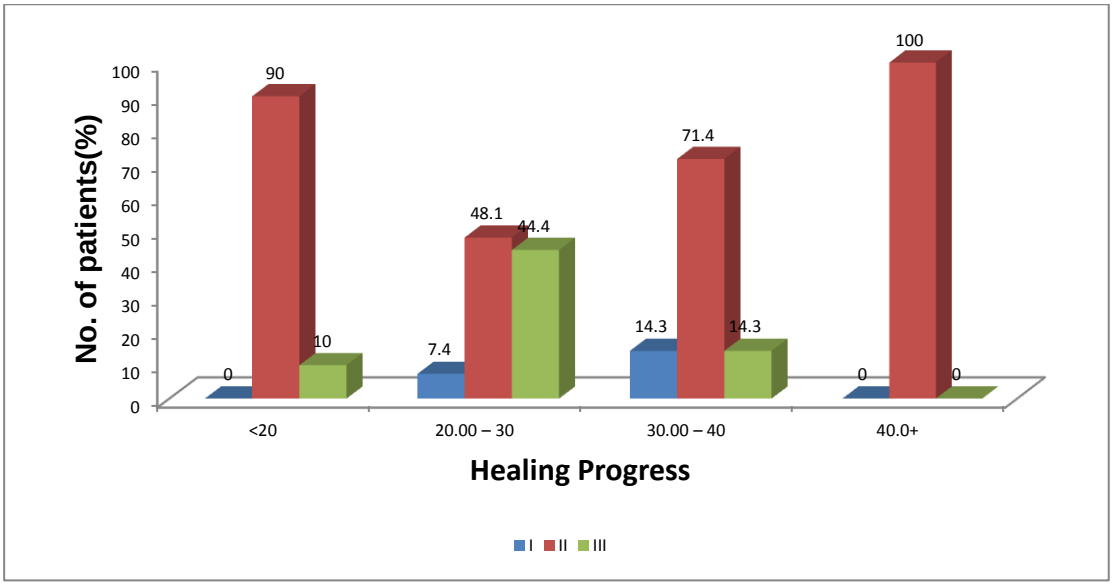


Table 16,17 and chart 16,17 are showing association between Wagner ulcer grade with healing progress in % in both PRP and control groups. There is sigificant association between ulcer grade and healing % in PRP group with P valuve of 0.001 and 0.218 in control group. >50% healing was seen in 8 cases of grade I ulcer, 40-50% healing results were seen in Grade II ulcers.

DISCUSSION

Worldwide accepted standard therapy for chronic ulcers are debridement of wound surgically/chemically, local wound dressings with topical bacteriostatic and bactericidal agents such as povidone iodine, silver preparations etc., which are thought to accelerate granulation tissue and promote wound healing. The prevalence and incidence of chronic ulcers and the complications associated with them continue to escalate even with proper and timely interventions. Platelet-rich plasma (PRP) is an autologous product, extracted from the patient's plasma, which includes a high platelet concentration in a fibrin clot that can be easily applied to the ulcer area. The fibrin clot is absorbed during wound healing within days to weeks following its application. Most of the studies on PRP reporting studies closure time and higher healing percentage in patients using PRP and platelet derived products. This study compares the efficacy of local injections of autologous platelet rich plasma with conventional dressing in treatment of chronic ulcers.

A total of 90 patients with chronic cutaneous ulcers of Wagner's grade between I-III were randomly divided into PRP group and conventional dressing group with 45 patients each.

In this study most of the patients were in the age group of 60-69 years i.e., 11 out of 45 (24.4%) in PRP group and 14 out of 45 (31.1%) in control group (table 1 and chart 1).

In this study both the groups were male predominant i.e., 37 (82.2%) in PRP group and 31 (68.9%) in control group accounting to 76%. (Table 2)

Site of ulcer (Table 3) was more on lower extremity in both the groups. i.e., 38 patients (84.4%) in PRP group and 35 patients (78%) in control group. The ulcers

were invariably seen oven the dorsal and plantar aspects of foot and at the ankle region.

Most common mode of onset in the study was trauma in both the groups. 20 patients (44.4%) in PRP group and 12 patients (26.7%) in control group with average of 35.55%. ulcers following abscess drainage was seen in 12 (26.7%) in PRP group and 10 (22.2%) in control group. Other mode of onset were insidious more commonly due to scratching, long standing varicose veins. Immobilization leading to bedsores were seen in 1 patient in PRP group and 3 patients in control group.(Table 4)

Driver et al. (2006) carried out the first reported prospective, randomized, controlled multicenter trial in the United States regarding the use of PRP for treatment of diabetic ulcers. It included 32 patients. The authors found that 68.4% in PRP group and 42.9% in control groups had complete closure of wounds with P value of 0.036 which was significant.

Sarvajnamurthy S et al. In 2013 studied on 12 patients with 17 chronic venous ulcers were treated with PRP application and treatment outcome was measured by percentage of improvement in area and volume of the ulcer. The mean duration of healing of the ulcers was 5.1 weeks and 100% improvement in the area of the ulcers was seen in 13 (76%).

In this study Wager's grade II ulcers were most commonly considered in both PRP group and control group i.e., 17 (37.8%) in PRP group and 28 (62.2%) in control group with mean average of 50%.

Most of the ulcers in the study were included once the culture from the wound swab was sterile i.e., 28 (62.2%) in PRP group and 25 (55.6%) in control group and the others comprised of S.aureus, Acinobacter and Citrobacter.

Coming to the healing progression in percentage, clearly healing was better

and faster in PRP group when compared to conventional dressing with 14 patients 40-50% healing in PRP group when compared to conventional group i.e., 7 patients (15.6%) and >50% healing seen in 8 (17.8%) of PRP group with high significant P value of 0.00105.

In a study by Li L et al. (wound repair regen 2015) which was a prospective, randomized controlled trial showed that standard treatment plus autologous platelet rich plasma was statistically more effective than standard treatment. The subjects defined as healing grade 1 were 85.4% while 67.3% in control group with significance p value.

In this study, appearance of healthy granulation tissue was distinctively high and early in PRP group i.e., 31 (68.9%) patients had satisfactory granulation tissue when compared to the study group which had healthy granulation tissue in 17 patients (37.8%) after treatment with povidone iodine dressing.

In a meta-analysis performed by CARTER et al. 2011 evaluated time to heal in PRP subjects versus saline dressings. Two RCTs showed statistically significant difference in wound area reduction compared to saline gauze dressings.

Similarly, a non RCT, comparative study showed significant area and depth reductions with 2.5-3.5 fold decrease in time to reach 50% compared to pre-treatment moist wound care controls.

Table16,17 and chart 16,17 are showing association between Wagner ulcer grade with healing progress in % in both PRP and control groups. There is significant association between ulcer grade and healing % in PRP group with P value of 0.001 and 0.218 in control group. >50% healing was seen in 8 cases of grade I ulcer, 40-50% healing results were seen in Grade II ulcers.

In a study by JM de Leon et al.(2011) near physiological concentration of

PRP-gel in treatment of chronic ulcers which showed positive response occurred in 96.5% of wounds within 2.2weeks. in 47.5% area reduction occurred and 90.5% of wounds had 63.6% volume reduction. Rapid treatment response was observed in 275 of 285 patients. The magnitude of response was consistently high, with statistically significant outcomes reported for various groups.

In relation to associations between the study groups, there was significant association with Wagner's ulcer grade and healing progress in % with high P value of 0.001 in PRP group and significance of P being 0.218 in control group.

There was no significant association seen between the mode of onset with and healing progress between both the groups.

There was no significant association seen between the culture from the wound and healing progress in both the groups.

There was no association seen between co-morbidities of the patient with the healing progress in both the groups.

CONCLUSION

- All the patients with chronic cutaneous ulcers of size less than 5cmx5cm in greatest dimensions and ulcer grade between I-III of Wagener's grading were managed with local injections of autologous PRP and were compared to the ulcer management with conventional dressing i.e 10% povidone iodine solution. Serial examination of ulcers have shown significant reduction in the ulcer size with appearance of healthy granulation tissue, improvement in mean duration of wound healing and contraction of wound in patients treated with local injections of autologous PRP.

- Local injections of autologous PRP in chronic cutaneous ulcers is more effective when compared to conventional dressing with povidone iodine dressing in achieving faster and complete wound healing and promoting growth of healthy granulation tissue.

- Since the PRP is derived from patients own blood, the risk of complications were nil.

- However, additional successful clinical evidence is required with validated laboratory findings to establish local injection of autologous PRP as one of the most effective alternative topical agents in treatment of chronic ulcers.

SUMMARY

❖ This study was performed in Shri B.M Patil Medical College in the department of general surgery during the study period of October 2016 to June 2018, to study the efficacy of local injection of PRP compared to conventional dressing with 10% povidone iodine solution in treatment of chronic cutaneous ulcers. Total of 90 patients with chronic ulcers were enrolled into the study and randomly divided into PRP group and control group. With 45 patients in each group. PRP was injected on every 4^{th} day for 4 cycles and regular dressing was done with 10% povidone iodine in control group.

❖ In our study we found that-

- 11 patients (24.4%) and 14 patients(31.1%) were in the age group of 60-69years.
- Both the groups were male predominant i.e., 37 patients (82.2%) in PRP group and 31 patients (68.9%) in control group which accounted to 76%.
- Most common mode of onset in the study was trauma in both the groups. 20 patients (44.4%) in PRP group and 12 patients (26.7%) in control group with average of 35.55%. ulcers following abscess drainage was seen in 12 (26.7%) in PRP group and 10 (22.2%) in control group.
- Wager's grade II ulcers were most commonly considered in both PRP group and control group i.e., 17 (37.8%) in PRP group and 28 (62.2%) in control group with mean average of 50%.
- Culture from the wound swab was sterile i.e., 28 (62.2%) in PRP group and 25 (55.6%) in control group and the others comprised of S. aureus, Acinobacter and Citrobacter.

- Healing was better and faster in PRP group when compared to conventional dressing with 14 patients 40-50% healing in PRP group when compared to conventional group i.e., 7 patients (15.6%) and >50% healing seen in 8 (17.8%) of PRP group with high significant P value of 0.00105.
- Appearance of healthy granulation tissue was distinctively high and early in PRP group i.e., 31 (68.9%) patients had satisfactory granulation tissue when compared to the study group which had healthy granulation tissue in 17 patients (37.8%) after treatment with povidone iodine dressing.
- There is significant association between ulcer grade and healing % in PRP group with P value of 0.001 and 0.218 in control group. >50% healing was seen in 8 cases of grade I ulcer, 40-50% healing results were seen in Grade II ulcers.

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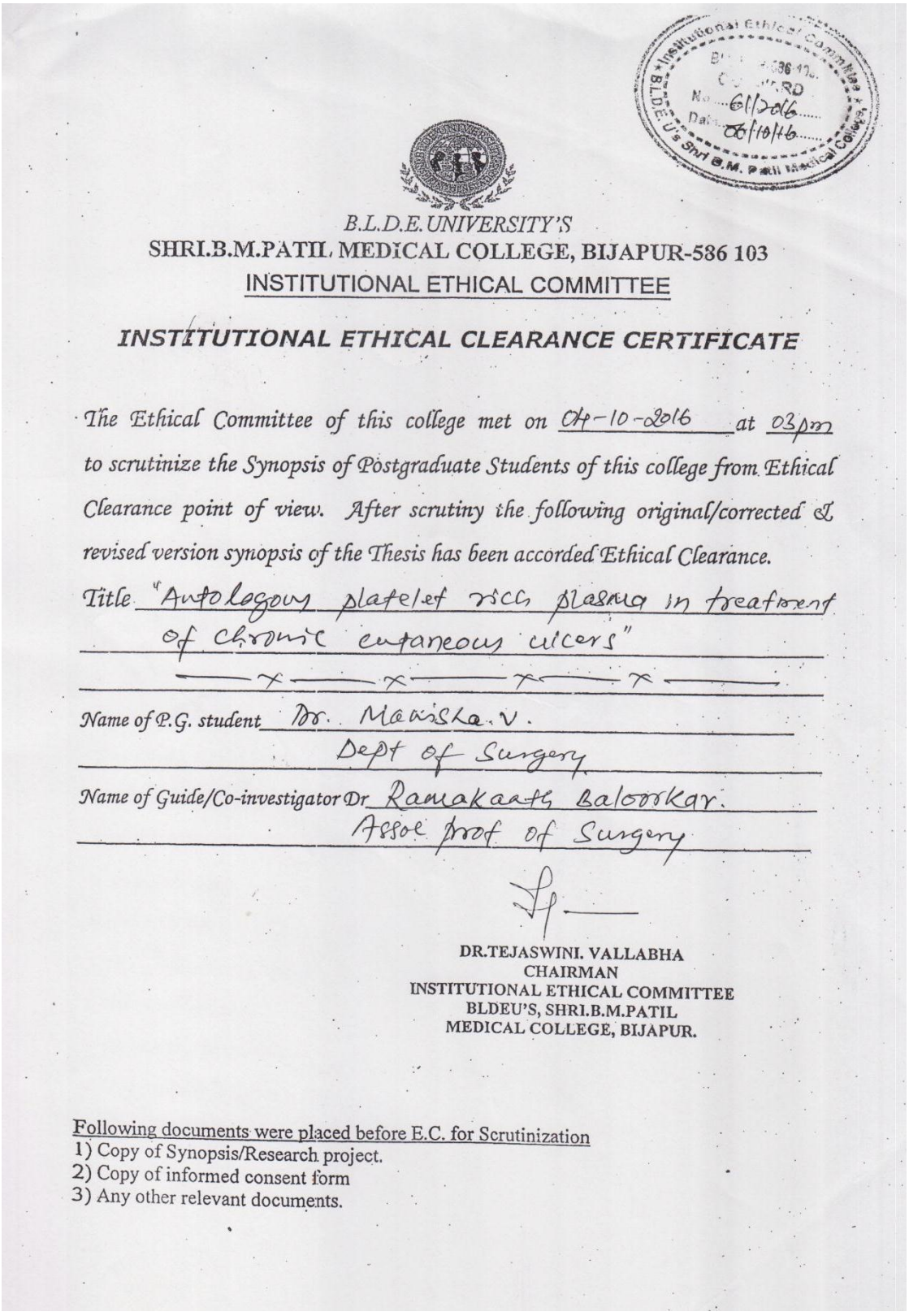
ANNEXURES

ETHICAL CLEARANCE CERTIFICATE



B.L.D.E. UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103
INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 04-10-2016 at 03pm to scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis has been accorded Ethical Clearance.

Title "Autologous platelet rich plasma in treatment of chronic cutaneous ulcers"

Name of P.G. student Dr. Manisha. V.
Dept of Surgery

Name of Guide/Co-investigator Dr Ramakanth Balooskar.
Asso prof of Surgery

DR.TEJASWINI. VALLABHA
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.

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.

INFORMED CONSENT FORM

B.L.D.E (DEEMED TO BE) UNIVERSITY's

SHRI B.M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE, VIJAYAPUR – 586103, KARNATAKA.

TITLE OF THE PROJECT: AUTOLOGOUS PLATELET RICH PLASMA IN TREATEMENT OF CHRONIC CUTANEOUS ULCERS.

PRINCIPAL INVESTEGATOR: **DR MANISHA.V**
Department of General Surgery

PG GUIDE: **DR. RAMAKANTH BALOORKAR** **MS**
Associate Professor of Surgery
Shri. B. M. Patil Medical College & Hospital Vijayapur

PURPOSE OF RESEARCH:

I have been informed that this study will analyze the usefulness of local injection of Platelet rich plasma in healing of chronic cutaneous ulcers.

I have been explained about the reason for doing this study and selecting me/my ward as a subject for this study. I have also been given free choice for either being included or not in the study

PROCEDURE:

I understand that relevant history will be taken. I will undergo detailed clinical examination after which necessary investigations will be done whenever required, which would help the investigator for appropriate management.

RISKS AND DISCOMFORTS:

I understand that I/my ward may experience some pain and discomfort during the examination or during my treatment. This is mainly the result of my condition and the procedure of this study is not expected to exaggerate these feelings which are associated with the usual course of treatment.

BENEFITS:

I understand that I/my wards participation in this study will help to analyse the effectiveness of PRP in healing of chronic cutaneous ulcers.

CONFIDENTIALITY:

I understand that medical information produced by this study will become a part of this Hospital records and will be subjected to the confidentiality and privacy regulation of this 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 and identified only by a code number. The code key connecting name to numbers will be kept in a separate secure location.

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

REQUEST FOR MORE INFORMATION:

I understand that I may ask more questions about the study at any time. DR.MANISHA V 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 might influence my continued participation.

If during this study, or later, I wish to discuss my participation in or concerns regarding this study with a person not directly involved, I am aware that the social worker of the hospital is available to talk with me and that a copy of this consent form will be given to me for careful reading.

REFUSAL OR WITHDRAWL OF PARTICIPATION:

I understand that my participation is voluntary and I may refuse to participate or may withdraw consent and discontinue participation in the study at any time without prejudice to my present or future care at this hospital.

I also understand that DR MANISHA V. will terminate my participation in this study at any time after he has explained the reasons for doing so and has helped arrange for my continued care by my own physician or therapist, if this is appropriate

.**INJURY STATEMENT:**

I understand that in the unlikely event of injury to me/my ward, resulting directly to my participation in this study, if such injury were reported promptly, then medical treatment would be available to me, but no further compensation will be provided.

I understand that by my agreement to participate in this study, I am not waiving any of my legal rights.

I have explained to _____ the purpose of this research, the procedures required and the possible risks and benefits, to the best of my ability in patient's own language.

Dr. RAMAKANTH BALOORKAR	Dr .MANISHA
(Guide)	(Investigator)

PROFORMA

CASE NO:

- Name : IP No :
- Age/sex: DOA:
- Occupation:
- Address:
- CHIEF COMPLAINTS:

WOUND:

- Mode Of Onset
- Duration
- Number
- Site
- Size and Extent
- Associated Pain
- Discharge
- Others
- Any Associated Disease
- Past History Of Similar Wound
- Personal History

PAST HISTORY:

- Diabetes Mellitus
- Hypertension
- Peripheral vascular disease

PERSONAL HISTORY:

- Diet
- Sleep
- Appetite
- Bowel & bladder

7. GENERAL PHYSICAL EXAMINATION:

- Mental Status Weight:
- Built Height:
- Nourishment BMI:
- Hydration status
- Pallor
- Pulse
- Blood Pressure
- Any Obvious Deformity

A. <u>LOCAL EXAMINATION :</u>

INSPECTION-

- NUMBER
- SITE
- SIZE
- SHAPE
- EXTENT
- MARGIN
- EDGE OF THE ULCER

- FLOOR OF THE ULCER
- DISCHARGE:
    - Amount
    - Character
    - Odour
- ADJACENT AREA:
    - Any Swelling
    - Any Skin Change
    - Any Secondary Changes

PALPATION:

- TEMPERATURE
- TENDERNESS
- SIZE
- MARGIN
- EDGE
- MOBILITY
- DEPTH
- BLEEDING

B. <u>EXAMINATION OF LYMPH NODES</u>

C. <u>EXAMINATION OF VASCULAR SYSTEM</u>

D. <u>EXAMINATION OF NERVE SUPPLY OF THE LIMB</u>

SYSTEMIC EXAMINATION

PER ABDOMEN:

Inspection:

Palpation:

Percussion:

Auscultation:

R*ERSPRATORY SYSTEM*:

Inspection

Palpation

Percussion

Auscultation

CARDIO-VASCULAR SYSTEM:

Inspection

Palpation

Percussion

Auscultation

C*ENTRAL NERVOUS SYSTEM:*:

Higher Mental functions

Diagnosis

INVESTIGATIONS:

- Hemoglobin
- Total Count
- Differential Count
- Blood Urea

- Serum Creatinine
- BLOOD SUGAR RBS, FBS, PPBS (if diabetic)
- Urine For Ketone Bodies (If Diabetic)
- HbA_1C (If Diabetic)
- Urine Routine
- Culture Sensitivity Of Discharge
- X-Ray of bone Or Joint Involved

COMMENTS:

PRE-TREATEMENT MEASUREMENTS:

SERIAL MEASUREMENTS DURING THE TREATEMENT:

FINAL MEASUREMENTS:

PROGRESSION OF HEALING: (In terms of %)

INFERENCE: