EFFICACY OF AUTOLOGOUS PLATELET GEL VERSUS THE REGULAR DRESSING IN EPITHELIALIZATION AND WOUND REDUCTION IN CHRONIC ULCERS OF THE LOWER LIMB"- A RANDOMIZED CONTROL TRIAL AT KLES' DR. PRABHAKAR KORE HOSPITAL AND MRC, BELGAUM.

Submitted by:

DR. TEJAS. CHIRANJEEVI

DISSERTATION

Submitted to the

KLE UNIVERSITY, BELGAUM

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In partial fulfillment of the requirements for the degree of

M.S. IN GENERAL SURGERY

Under the Guidance of:

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KLE UNIVERSITY, BELGAUM, KARNATAKA

DECLARATION

I hereby declare that this dissertation entitled **"EFFICACY OF AUTOLOGOUS PLATELET GEL VERSUS THE REGULAR DRESSING IN EPITHELIALIZATION AND WOUND REDUCTION IN CHRONIC ULCERS OF THE LOWER LIMB"- A RANDOMIZED CONTROL TRIAL,** is a bonafide and genuine research work carried out by me under the guidance of **Dr. V. M. UPPIN$_{MS}$**, Professor , Department of Surgery, Jawaharlal Nehru Medical College, Belgaum-590010.

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CERTIFICATE

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LIST OF ABBREVIATIONS USED

1.	BP	: Blood Pressure
2.	cPRP	: Concentrated Platelet rich plasma.
3.	DM	: Diabetes Mellitus.
4.	ECM	: Extra Cellular Matrix
5.	FDA	: Food and Drug Administration.
6.	grp	: Group.
7.	Hb	: Hemoglobin.
8.	IGF-I	: Insulin like Growth Factor-I.
9.	mm	: Millimeter
10.	NS	: Normal Saline
11.	PDAF	: Platelet Derived Angiogenesis Factor.
12.	PDGF	: Platelet derived Growth Factor.
13.	PF-4	: Platelet Factor-4.
14.	PR	: Pulse Rate
15.	PRP	: Platelet Rich Plasma.
16.	PS	: Pressure Sore.
17.	PVD	: Peripheral Vascular Disease.
18.	SD	: Standard Deviation
19.	TGF-β	: Transforming Growth Factor-β.
20.	Vv	: Varicose vein.

ABSTRACT

Background and objectives:

There are various modalities of treatment in treating chronic ulcers. One of them being the use of platelet derived growth factors. It is shown that chronic ulcers are deficient in growth factors. On application of these factors over the ulcer, the wounds healed faster. This study was conducted to test the efficacy of autologous platelet gel prepared from patients own blood in epithelialization and wound size reduction of chronic ulcers of lower limb.

Methods:

This randomized control study was done for a period of one year. 40 cases were randomized into two groups. Study group (PDGF grp) received autologous platelet gel as ulcer dressing and control group (NS grp) received normal saline dressing. Dressings were done on day 1, 4, 7, 10, 14. The wounds edges were traced over a graph sheet to estimate the size reduction.

Results:

At the end of the study the mean reduction in ulcer size was 463.50 $\pm$ 249.40 mm^2 in the group treated with PDGF and 609.10 $\pm$ 334.8 mm^2 in the group treated with NS, which was statically significant. The study shows that the reduction achieved in ulcer size was (206.80 $\pm$ 119.09 mm^2) in PDGF grp and (120.15 $\pm$ 71.00 mm^2) NS grp which is statistically significant (P= 0.0118). The percentage of area reduction was (30.56 $\pm$ 6.67) in PDGF and (16.76 $\pm$ 3.49) in NS with P value being 0.0001.

Conclusions and interpretation:

PDGF showed faster and better healing rates. In individual subgroups like ulcers due to diabetes mellitus, venous ulcers, and pressure sores there was more than 10% better healing rate compared to the conventional dressing except for ulcers secondary to peripheral vascular disease. Ulcers of peripheral vascular disease etiology did not show significant decrease in either group. There were no adverse effects of PDGF application.

Key words:

Chronic ulcers; Autologous platelet gel; growth factors; wound reduction.

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INTRODUCTION

Wounds which do not heal and become chronic ulcers represent a heavy burden to both the patient and the service provider. There has been an extensive investigation over the distribution of growth factors in chronic wounds such as diabetic ulcers, venous ulcers, and pressure sores[1].

Historically, wounds were treated with homespun remedies derived from ritualistic teachings and in part from careful observations .The "three healing gestures" were described (circa) 2200 BC on an ancient clay tablet[2] : 1) washing the wound, 2) making plasters (mixtures of herbs, ointments, and oils that were applied to wounds to aid in the healing process), 3)bandaging the wound. References to early wound care are seen in the Bible, "…went to him, and bound his wounds, pouring oil and wine" (Luke, 10:34); ancient Assyrian writings, "…the surface of the sick part with butter you shall anoint…"; and ancient Greek texts, "with bandage firm, Ulysses' knee they bound" (Homer, *The Odyssey*).

Studies on the wound fluids suggested that growth factor levels were reduced in chronic wounds and perhaps the level of protease activity was high[3,4]. There may thus be an intrinsic molecular defect in chronic wounds which prevents their healing. Platelet extract from the patients own blood has been used in trials on chronic wounds. It has shown impressive results in both healing percentage and time to full epithelialization[5]. Platelets contain number of cytokines which may be implicated in these effects.

A larger and better controlled trial of PDGF-BB in pressure sores also produced impressive results and gave an indication of effective dose levels [6]. The

usual wound care involving the use of non-biological occlusive dressings in the form of povidone iodine when tested in various dilutions was toxic on fibroblasts and hence delayed wound healing and reduced wound strength[7]. The wet to dry debridement technique which was standard in the treatment of ulcers, showed that the newly formed granulation tissue and epithelium formed are removed as it gets adhered to the gauze[8].

Chronic wounds often exhibit chronic inflammatory phenotype. Distribution and composition of growth factors and extracellular matrix molecules are different in chronic wounds than in the acute ones[1]. It is likely that effective therapies for stimulating healing of chronic wounds will involve therapeutic manipulation on the profile of growth factors needed. Platelets are known to release certain factors from the α granules, which include platelet derived growth factor(PDGF), platelet derived angiogenesis factor, epidermal growth factor(EGF), platelet factor-4, transforming growth factor-β (TGF- β), acidic fibroblastic and basic fibroblastic growth factor(bFGF). All these factors act locally on the wound thereby hastening the healing process.

As the therapeutic role of PDGF in wound healing has not been clearly addressed previously and as there are no randomized clinical trials addressing this issue, an attempt has been made in this study to know the therapeutic efficacy of PDGF in wound healing while comparing it with the conventional dressing.

AIMS AND OBJECTIVES

Objective of study is to test the efficacy of autologous platelet gel in epithelialization and wound reduction in chronic wounds of the lower limb in comparison with conventional method of treatment**.**

REVIEW OF LITERATURE

"Nowhere is the gap between basic research and clinical application more glaring than in the biology of wound healing". Earl A. Peacock Jr (1938)[1].

"A wise physician skilled our wounds to heal is more than armies for the common weal".-Homer.

The consensus definition of a wound, agreed by specialists from different disciplines involved with wound healing, is that it is disruption of normal tissue structure and function[1]. Wounds can result from injurious processes beginning either internally or externally to the involved organs. The difference between acute and chronic wounds is that in the former healing occurs through an orderly and timely process leading to restoration and functional integrity, while on other hand chronic wounds fail to follow this course of healing[1].

The process of wound healing has been defined by Davidson as a succession of cellular events which are co-ordinated by the release and recognition of soluble mediators[1]. The gap between the basic wound healing research and its clinical application is at last narrowing and the challenge of Peacock is being answered.

Patho-physiology of wound healing:

Wound healing is a complex biological process well characterized at structural level but poorly understood at the molecular level. The process can be organized into three phases: inflammation, fibroplasia, and remodeling (fig 1)[9] Failure in the orderly process of wound healing leads to chronic ulcer formation. The phases of wound healing are as follows:

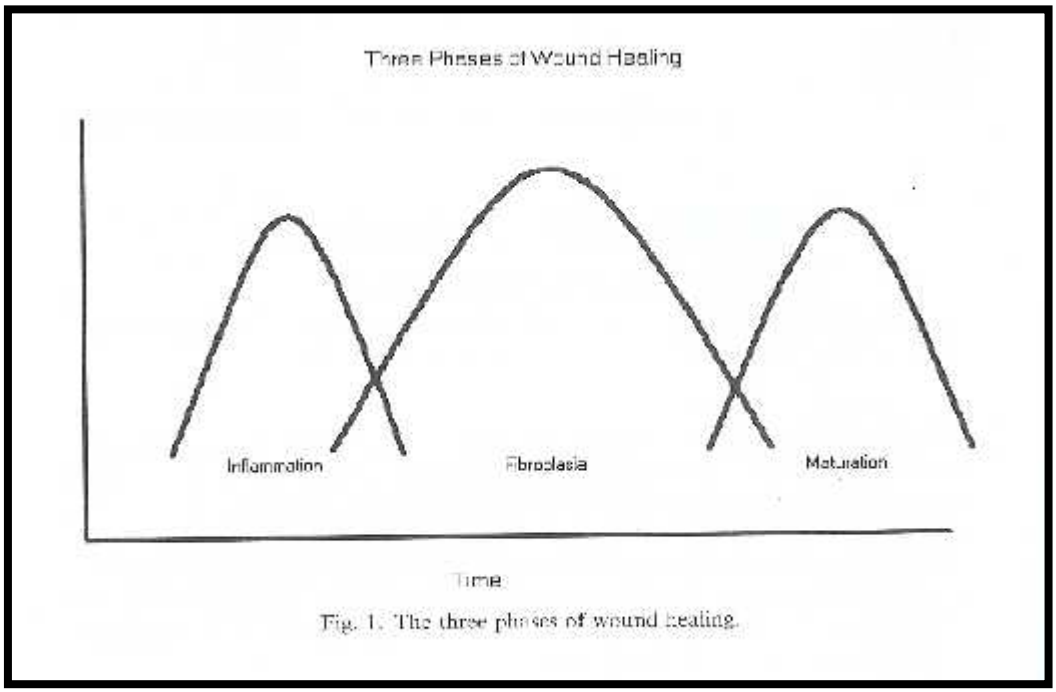


Fig. 1. The three phases of wound healing.

Phases of wound healing

1. Lag phase / Inflammatory or Exudative phase.

It is characterized by inflammation of wound and mobilization of the cells which will synthesize granulation tissue. Lag phase was so entitled not because it is a phase of inactivity in wound repair, but simply because there is no significant increase in the mechanical strength of the wound. It serves as a foundation on which wound healing takes place.

2. Proliferative or Granulation phase

Granulation tissue is formed in the wound; collagen and mucopolysaccharides are synthesized by the granulation tissue, and there is an increase in the mechanical strength of the wound.

3. Wound contraction (Matrix formation) or Remodelling phase

Cells in the wound diminish in number but there is extensive remodelling of wound collagen and a further increase in the mechanical strength of the wound [10, 11]

Inflammation / Exudative Phase (2-5 days):

At Zero to 48 hours post injury, bleeding is the first response. Platelets attracted in this process promote haemostasis by accelerating fibrin deposition and formation of a platelet plug. Platelets also release cytokines, which recruit neutrophils, and include the chemokines interleukin-8 (IL-8), platelet factor-4 (PF-4) and β-thromboglobulin (β-TGH), as well as transforming growth factor-β(TGF-β) and platelet-derived growth factor (PDGF)[12]. Neutrophils are not required for normal repair in the absence of infection but aid in the initial removal of the fibrin clot. After 24 hours, monocytes start to infiltrate the wound, largely under the influence of the cytokines TGF- β and PDGF and the chemokine, monocyte chemotactic protein-1 (MCP-1)[10, 13]. Monocytes have two primary roles within the wound; first to continue cleaning debris from the site of injury, and second, to produce further cytokines that attract those cell types capable of laying down granulation tissue, that is, fibroblasts and endothelial cells.

During this period there is also proliferation of epithelialcells at the epidermal-dermal junction, which migrate toward the midline re-forming a thin epidermal layer under the surface clot [14].

Proliferative or Granulation Phase:

This phase of healing is also typified by the gradual appearance of granulation tissue, which consists of newly developed blood vessels with surrounding fibroblasts and additional elements of extra cellular matrix, creating a pink, velvety appearance. Collagen deposition from fibroblasts is largely under the control of TGF-β, whereas neovascular development is stimulated by vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) - all factors secreted by monocytes. Towards the end of the fifth day, neovascularization is maximal with a gradual increase in the amount of granulation tissue, which begins to fill the defect [11, 12, 14].

Formation of granulation tissue

New stroma, often called granulation tissue, begins to invade the wound space approximately four days after injury. Numerous new capillaries endow the new stroma with its granular appearance. Macrophages, fibroblasts and blood vessels move into the wound space at the same time. The macrophages provide a continuing source of growth factors necessary to stimulate fibroplasia and angiogenesis; the fibroblasts produce the new extracellular matrix necessary to support cell ingrowth and blood vessels carry oxygen and nutrients necessary to sustain cell metabolism.[11,13]

Growth factors especially PDGF and TGF β stimulate fibroblasts to proliferate and migrate into the wound space. The structural molecules of the newly formed extracellular matrix, termed the 'Provisional Matrix', contribute to the formation of granulation tissue by providing a conduit for cell migration. These molecules include:

- Fibrin
- Fibronectin
- Hyaluronic acid

The appearance of fibronectin and the appropriate receptors that bind fibronectin, fibrin or both on fibroblasts appear to be the rate limiting step in the formation of granulation tissue[13]. The fibroblasts are responsible for the synthesis, deposition and remodelling of the extracellular matrix. Conversely the extracellular matrix can have a feed back effect on the ability of fibroblasts to remodel [10].

Cell movement into a blood clot of cross-linked fibrin or into tightly woven extracellular matrix requires an active proteolytic system that can cleave a path for cell migration. A variety of fibroblast derived enzymes including plasminogen activator and collagenases are potential candidates for this task.

After migrating into wounds, fibroblasts commence the synthesis of extracellular matrix. The provisional matrix is gradually replaced with a collagenous matrix. Once an abundant collagen matrix has been deposited, the fibroblasts stop producing collagen and the fibroblast rich granulation tissue starts getting replaced by a relatively acellular scar.

Neovascularization

The formation of new blood vessels is necessary to sustain the newly formed granulation tissue. Angiogenesis is a complex process that relies on extracellular matrix in the wound bed as well as migration and mitogenic stimulation of endothelial cells.

Induction of angiogenesis has been attributed to molecules like TGF-β, angiogensin, angiotropin, and vascular endothelial growth factor. Low oxygen tension and elevated lactic acid may also stimulate angiogenesis. Many of these molecules mentioned above appear to induce angiogenesis by stimulating the production of

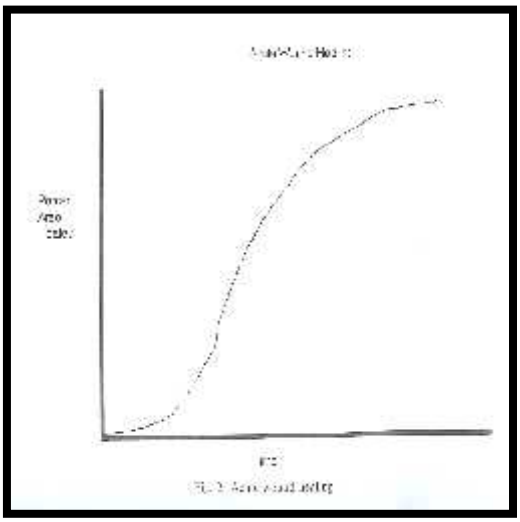
a. Basic fibroblast growth factor ➔ active during first three days of repair.
b. Vascular-Endothelial cell growth factor ➔ critical during formation of granulation tissue on days 4 through to 7.

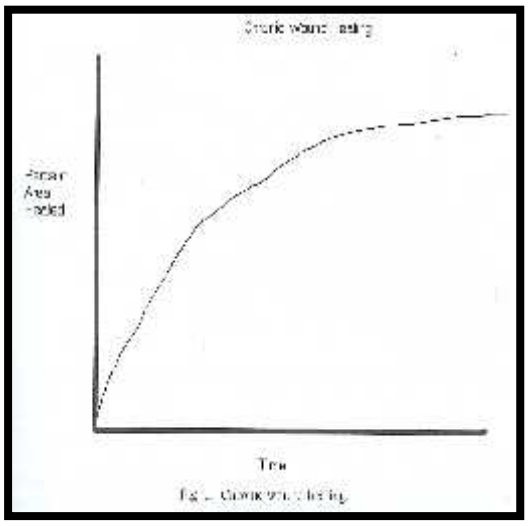
Remodelling (3 weeks – 2years)

Two weeks post injury → Granulation tissue begins to be remodelled and its vascularity decreases as the amount of collagen increases. Reduced concentrations of growth factors involved in earlier phases of wound healing and the increased expression of others, including high level of TGF-β_1, initiate the differentiation of fibroblasts into myofibroblasts which contain increased numbers of actin filaments. Collagen produced from fibroblasts is initially laid down in a vertical manner, but gradually changes its orientation to align across a defect, leading to increased wound strength.

For the first 6 weeks, new collagen production dominates the wound healing process, deposited randomly in acute wound granulation tissue. As the wound matures, collagen is remodeled into a more organized structure with increased tensile strength. Gradually, type I collagen replaces type III until the normal skin ratio of 4:1 is achieved. As remodeling continues, matrix metalloproteinase collagenolysis achieves a steady state with collagen synthesis. Tensile strength plateaus at 80% of the original strength approximately 1 year post injury [15, 16, 17.] Healing curves can be constructed for both acute and chronic wounds (fig 2 & 3)[18]. A healing curve provides an overview of the healing process, expressed as continuum. Many local and systemic factors can disrupt the process of wound healing and alter the healing trajectory. When healing occurs, the effect of the interaction can be measured as a curve of healing versus time. This curve or trajectory provides an overview of the

healing process for any wound and can be used to provide information of the healing process[15].





Measuring time to complete healing does not take into account differences in wound size. Differences in wound size and the impact on healing can be lessened, perhaps by using wound perimeter. The healing of chronic wounds using trajectories have been studied for diabetic, pressure, and venous stasis ulcer. It is interesting to note that when the "normal" wound healing trajectory is constructed for each of these disease processes, the curves are quite similar and can be superimposed[15]. Healing trajectories mimic one another. These findings suggest that there is such a thing as normal healing and that occurs in an orderly fashion at a relatively fixed rate, despite the etiology of the wound. Any impairment will shift the trajectory to right, which form the basis of chronic wounds[15] and intervention will shift the trajectory to left.

Role of growth factors:

Growth factors are peptide signaling molecules[1]. They play a critical role in the orchestration and integration of all cellular and molecular events during wound

healing. They affect all cells involved in the wound healing process: inflammatory cells, keratinocytes, endothelial cells, and fibroblasts, and both individually or collectively exert selective effects on such cells, including stimulation or inhibition of cell division, migration, differentiation, and extra cellular matrix synthesis.

There are five known super families of growth factors. The growth factors, along with their receptors, vary in structure and cell pathway activation between families and within each family. Most growth factors undergo post translational modification before they are released in active state[19]. Growth factor receptors are transmembrane glycoprotein whose effects are seen largely through kinase domains and phosphorylation reactions.

Platelets release large amount of platelet derived growth factors (PDGF) and transforming growth factor (TGF)-β, VEGF vascular endothelial growth factor and smaller amounts of epidermal growth factor (EGF). The PDGF super family of growth factors comprises PDGF and VEGF. These two proteins are similar in structure but bind to different receptors and have different cellular effects. The effect of PDGF stimulation is seen in cells of mesoderm in origin, whereas VEGF has primary effect on endothelial cells[19].

Originally shown to be mitogen for fibroblasts, smooth muscle cells, and glial cells[20], PDGF is the first and, to date, only growth factor to be given US Food and Drug administration approval for clinical use[20]. It first was isolated from human platelets but since has been shown to be secreted from the many cell types, including monocytes, macrophages, fibroblast, smooth muscle cells and endothelial cells. In vitro studies have shown that PDGF stimulates chemotaxis, proliferation, and new gene expression in these cells[21]. It is known to exist in five isoforms: PDGF-AA,

PDGF-AB, PDGF-BB, PDGF-CC and PDGF-DD. PDGF plays a key role throughout the wound healing process. Over the first several days after an injury, its release from platelets and endothelial cells allows for directed and sequential migration of neutrophils, macrophages, and fibroblasts into and around damaged tissues.

When at the wound site, continued stimulation of the newly arrived cells by PDGF results in endogenous production of the growth factor and provisional ECM synthesis, fibroblast proliferation, and eventually collagen production. This stimulation continues for 2-3 weeks, after which PDGF contributes to the remodeling of the wound by helping to orchestrate active collagen turnover and cross-linking[21].

Because of its ability to enhance the cellular response to a wound and to contribute to collagen production, it was speculated that exogenous application of PDGF could amplify this effect through the autocrine feedback loop[22]. Early animal studies supported this claim; treatment of incisional wounds in rats with a single dose of recombinant human PDGF produced an increase in the inflammatory response, wound cellularity, granulation tissue formation, neovascularization, and rate of epithelialization over normal control wounds[23]. Nonetheless, the remarkable impact of a single topical application at the time of wounding and the absence of a deleterious effect on scarring suggested that, PDGF was a highly promising candidate for therapeutic testing[23]. PDGF's success may be due in part to its resistance to proteases and the use of agent in an appropriate patient population. It is known to be a stable protein. Eight cysteine residues per polypeptide chain, each linked by a disulfide bond, confer resistance to changes in heat and pH and to the effects of proteases[21], making it a good substance for introduction into a hostile, and matrix metalloproteinase-rich environment.

With new and improving methods of delivering growth factors to a wound, focus now will turn to which combinations of factors are needed for the various forms of pathologic healing and at what levels, their expression most would improve patient's ability to heal.

Definition of an ulcer: An ulcer is defined as break in the continuity of an epithelial surface, characterized by progressive destruction of the surface epithelium.

Acute wounds: is defined as the traumatic loss of normal structure and function to recently uninjured tissue after a noxious insult[24].

Chronic wounds: wounds ;≥ 4 weeks duration, is known as chronic wounds. Disruption in the event of healing regulated by process of cellular, humoral, and molecular events and resulting in a time dependent but predictable and orderly pattern of tissue repair[18].

Characteristics of chronic wounds: - floor is covered with pale granulation tissue, scanty discharge, indurated base, edge and the surrounding skin.

Types of chronic leg ulcers:

1) Diabetic ulcer
2) Venous ulcer
3) Pressure sore
4) Arterial ulcer
5) Traumatic ulcer

1) Diabetic ulcer: The incidence of diabetes and complications are on rise. In the study of Framingham, the prevalence of diabetes has risen from 0.9% in 1958 to 3%

in 1993. India has dubious distinction of highest number of diabetics in the world. In the year 1995 there were 19.4 million diabetics which is expected to rise to 57.2 million by 2025[25].

Diabetic foot being one of the most common complications, where 15% of all diabetics develop diabetic ulcers, the most common site being the foot. Every 2% raise in glycosylated hemoglobin increases the risk of lower extremity ulcers by 1.6 times and lower extremity amputation by 1.5 times[26.]

In addition, the microcirculation is altered in such a manner that, following injury, patients with type1 insulin dependent diabetes fail to elicit a hypersensitivity response resulting in impaired neo-vascularization, which is essential in healing process. In implanted Hunt-Schilling wound chambers in diabetic rats, PDGF restores granulation tissue formation and angiogenesis to normal[27].

2) <u>Venous ulcer</u>: In the venous stasis ulcer, chronic passive venous congestion of the lower extremities results in local hypoxia. One current hypothesis of the pathogenesis of these wounds includes the impediment of oxygen diffusion into the tissue across thick perivascular fibrin cuffs. Another belief is that macromolecules leaking into the perivascular tissue trap growth factors needed for the maintenance of skin integrity. Additionally, the flow of large white blood cells slows due to venous congestion, occluding capillaries, becoming activated, and damaging the vascular endothelium to predispose to ulcer formation[1]. The activity of growth factors in such chronic wounds may be different, being rapidly activated by altered wound pH and or hypoxia. Degraded by wound proteases, activated or inactivated by oxygen free radicals[28.]

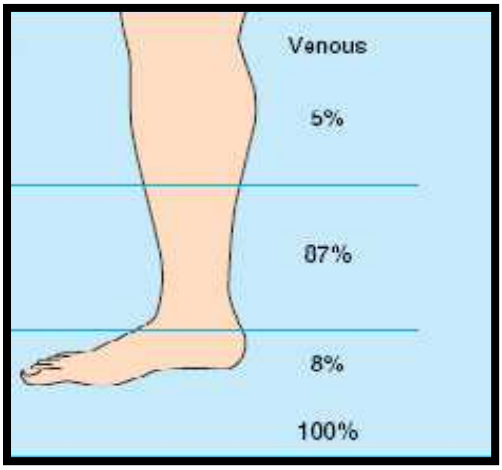


Fig 4: Sites of venous ulcers

3) <u>Pressure sore</u>: these can be defined as tissue necrosis with ulceration due to prolonged pressure. Less preferable terms are bed sores, pressure ulcers and decubitus ulcers. They are regarded as preventable but occur in approximately 5% of all hospitalized patients (range 3% to 12%). There is higher incidence in paraplegic patients, in the elderly and severely ill patients[29].

3) <u>Arterial ulcer</u>: Ulcers are due to peripheral arterial disease and poor peripheral circulation. These are often seen in older people and are episodes of trauma and infection of the destroyed skin over a limited area of the leg and foot. The ulcers tend to be punched out and destroy deep fascia with history of intermittent claudication with discoloration of one or more toes. Fig 5 shows the common sites of arterial ulcers.

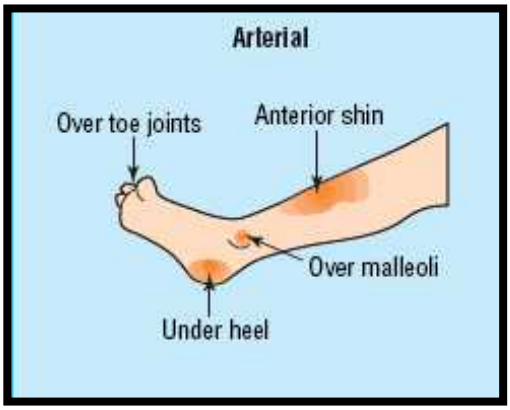


Fig 5: Common sites of arterial ulcers.

Management of chronic wounds: Wound dressings have been used since antiquity to facilitate the healing process. A material which when applied to the surface of a wound, provides and maintains an environment in which healing can take place at the maximum rate; Thomas (1986)[1]. The first antiseptic dressing was introduced by Lister in 1867 who soaked the lint and gauze in carbolic acid[8].

Features desired in a dressing regardless of its structure and the type of wound is as follows,

i) protect wound from bacteria
ii) absorb exudates from the wound
iii) prevent heat and fluid loss from the wound
iv) provide compression to minimize edema and obliterate the dead space
v) be non-adherent to limit wound disruption
vi) create a warm, moist occluded environment to minimize pain and maximize the epithelialization

Types of dressing:

1] Non-adherent fabrics.

2] Absorptive: gauze, foams.

3] Occlusive:

Non-biological: films, hydrocollids, alginates, hydrogels.

Biologic: homograft, xenograft, amnion, skin substitutes

4] Creams, ointments and solutions: antibacterial, enzymatic, others.

5] Mechanical devices.

6] Gene therapy: growth factors, tissue cultures.

Action of saline dressing: normal saline dressing keeps the environment moist for proper healing. Normal saline dressing acts as an osmotic dressing, with time the concentration of the saline increases due to evaporation altering it from an isotonic to hypertonic dressing which in turn decreases the fluid from the wound keeping it moist[30]. Wound heals quicker in moist environment and dressing are used to absorb excess fluid or retain fluid in an otherwise, dry wound in order to achieve a moist wound environment[31].

METHODOLOGY

This is a prospective randomized controlled study, to test the efficacy of autologous platelet gel versus the conventional method of dressing in epithelialization and wound reduction in chronic ulcers of the lower limb. This study was conducted at KLES' Dr Prabhakar Kore Hospital and Medical Research Centre Belgaum for a period of one year from January 2007 to December 2007 in the department of surgery.

20 cases were studied for chronic ulcers of the lower limb with autologous platelet gel and 20 for conventional method who received normal saline as dressing for chronic ulcers of the lower limb.

Patients were randomized according to random number table

1. Sample Size: 40 cases

20 patients received PDGF and 20 patients received conventional method (Wet saline dressing)

2. Inclusion criteria

a) Age group 35-70 years

b) Ulcer of the lower limb $\geq$ 4 weeks duration

c) Hb%- >10 gm%

d) Platelet count >1, 50,000

e) HbA1c- 7-9 if diabetic

3. Exclusion criteria: Ulcers with evidence of malignancy, active pus discharge, slough.

Study type: Prospective observational randomized study

Method of collection of data:

Detailed history was taken in all cases regarding the duration, mode of onset, progression, and associated symptoms. Also the etiological factor responsible was elicited in the history.

Ulcer examination was done in all this patients and wound was assessed of its characteristics and photographed. Ulcer was assessed by the investigator at the beginning of the study and at the end of the study; investigator being the staff and residents in the unit excluding the guide. Size of the wound was charted by tracing the edge of wound over the graph on day 1, 4, 7, 10, and 14.

Wound characteristics (Table 1)

Characteristics	**YES**	**NO**
Periwound edema		
Periwound erythema		
Wound purulence		
Limb pitting edema		
Limb brawny edema		
Wound granulation		
Wound Size		
Peripheral temp		

All the patients underwent following investigations

1] Hb%

2] Platelet count

3] Total count

4] HbA1c

5] Serum creatinine

6] Blood urea

Dressing technique:

After allotting the dressing with the help of random number table, the following procedure was performed.

For conventional dressing:

A chronic ulcer with no active pus discharge and slough was cleaned with the 0.9% saline solution and was covered with pad and roller bandage

For Autologous Platelet gel:

Autologous platelet gel was prepared from patients own blood and placed over the ulcer bed after cleaning with 0.9% saline and dressing done using pad and roller bandage.

The dressing was changed every 3rd day; similar 4 dressings done to all the patients to both the groups and the outcome is measured on 14th day.

Outcome is measured in terms of wound reduction between the two groups. Data were tabulated and the 2 groups were compared with reference to area and percentage of reduction.

Statistical analysis: Unpaired students "t" test and chi square tests were used to find out the statistical significance. A 'P'<0.05 was taken as significant

PREPARATION OF PRP GEL

Armentarium:

1. Centrifugation Machine
2. Water Bath with Temperature Control
3. Vacutainers.
4. Vacutainer Needle & Holder
5. Glass Petri Dish
6. Glass Test Tubes
7. Syringes (2 ml & 5 ml)
8. C.P.D.A. Anti-coagulant
9. 10 % $Cacl_2$ / Ca gluconate
10. Tournique

1st STEP: Collection of blood.

Under all aseptic techniques, 12-16 ml of blood was drawn intravenously from the anticubital region of patients forearm using vacutainer needle and vacutainers containing CPDA (4ml each). The vacutainers were thoroughly shaken to ensure mixture of anti coagulant with the drawn blood.

2nd STEP: Preparation of Platelet Rich Plasma (Fig 6)

1) 15-16 ml blood collected in vacutainer containing C.P.D.A. anti-coagulant. The whole blood is then centrifuged at 1000 r.p.m. for 10 mins. The supernatant formed is PRP.

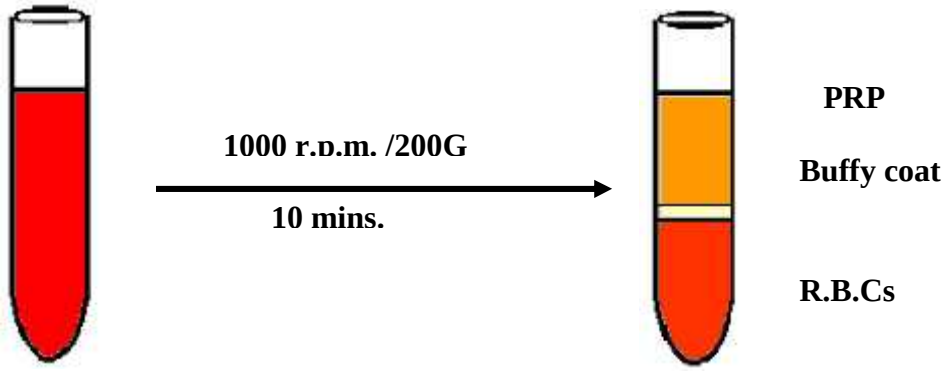


Fig. 6 : Preparation of platelet rich plasma (PRP)

2) **Preparation of concentrated PRP cPRP**

PRP, Buffy coat and upper1-2 mm of R.B.C. layer is collected in a fresh vacutainer and again centrifuged at 1000 r.p.m. for 10 mins. The upper half of the supernatant is discarded and the lower half is mixed thoroughly to yield cPRP (Fig. 7)

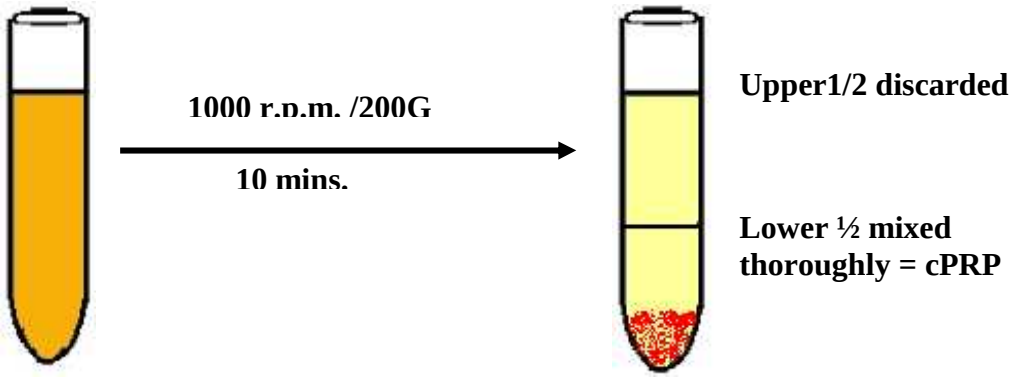


Fig. 7 : Preparation of concentrated PRP cPRP

3rd STEP: Preparation of autologous thrombin.

2.5 ml of PRP thoroughly mixed with 0.08 ml of 10% $Cacl_2$ or Calcium gluconate. This resulted in a clot formation and thrombin formation.

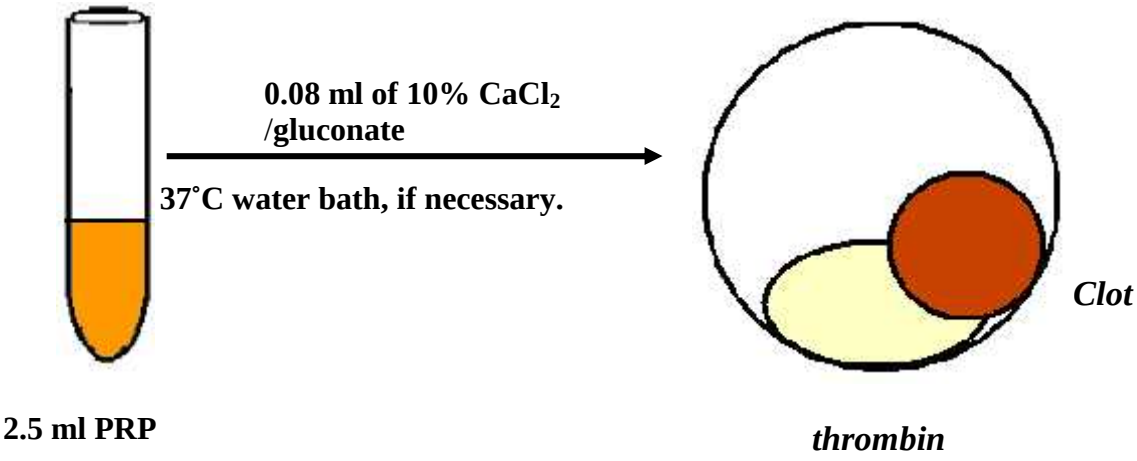


Fig. 8 : Preparation of autologous thrombin

The clot is discarded and the thrombin is used for the preparation of concentrated Platelet rich Plasma (cPRP).

4th STEP: PRP Gel Preparation

0.5 ml of autologous thrombin is added to cPRP and placed in water bath or thawing bath at 37˚C for 2-3 min to yield a gel, which is transparent in color.

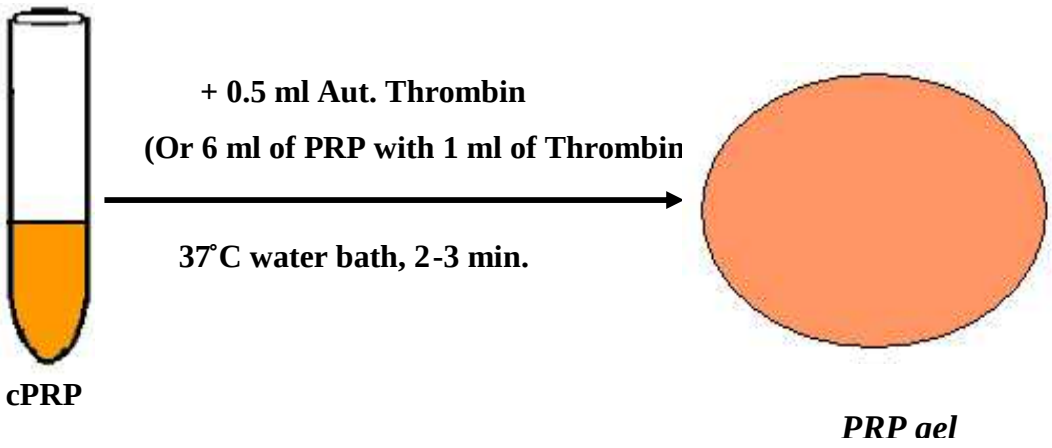


Fig. 9: PRP Gel Preparation

RESULTS

The present study was conducted in KLES Dr Prabhakar Kore Hospital and Medical research centre, Belgaum and the findings are tabulated as below. During the study year from January 2007 to December 2007, 40 patients with chronic ulcers of the lower limb were randomized into study (PDGF) and control (normal saline) group. These groups were studied for the effect of conventional dressing versus PDGF on epithelialization and wound reduction.

A total of 40 patients satisfied the selection criteria, analysis was done by using students paired‘t’ test for continuous variables within the groups and unpaired ‘t’ test for continuous variables between cases and controls.

Table no. 2 Social Demography

Variables		**PDGF**		**NS**		**P**
		Mean	**SD**	**Mean**	**SD**	
Age		53.30	7.34	56.85	7.61	0.0562
Sex	M (N)	17	85%	16	80%	0.677
	F (N)	3	15%	4	20%	

The baseline characteristics like age and sex were similar between the two arms.

Table no. 3 Platelet Count

	PDGF		NS		P
	Mean	**SD**	**Mean**	**SD**	
Platelet Count	193900.00	29673.49	194750.00	32463.54	0.8725

The platelet counts were also similar between the two groups.

Table no. 4 Wound Area In mm^2

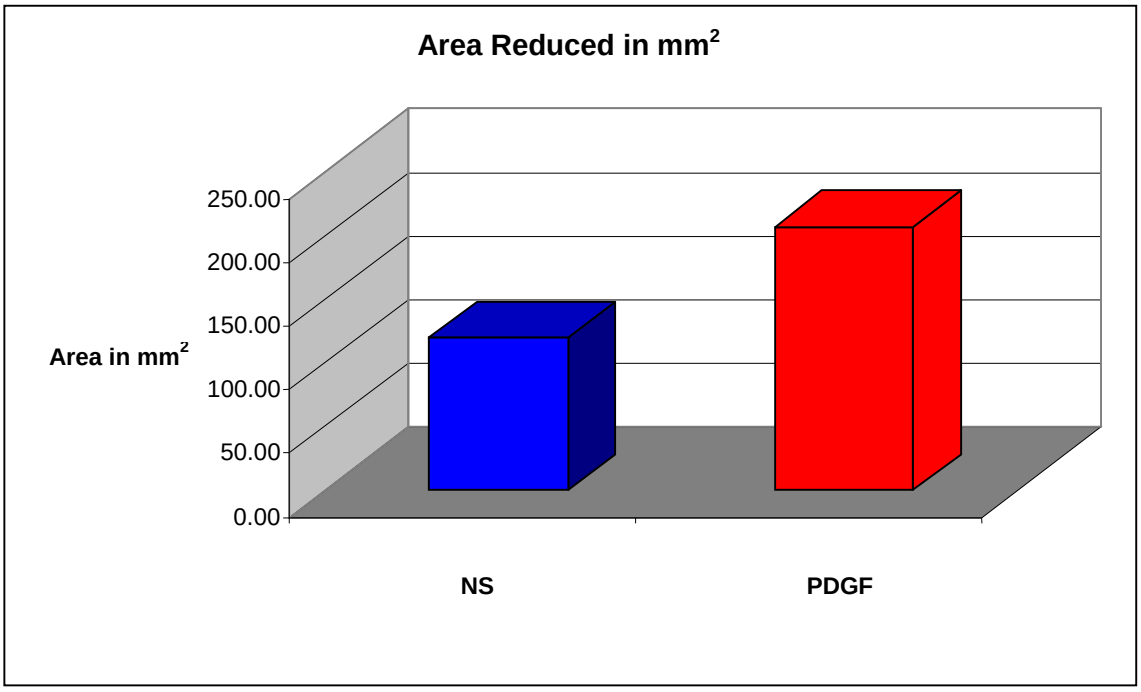
	Before		After		P
	Mean	**SD**	**Mean**	**SD**	
PDGF	670.30	361.46	463.50	249.40	0.001
NS	729.25	397.48	609.10	334.80	

The mean area at the beginning of the study was 670.30 $\pm$ 361.46 mm^2 in the PDGF and 729.25 $\pm$ 397.48 mm^2 in the NS group. There was no significant difference in the mean area between the two groups (P= 0.5389) at the beginning of the study. At the end of the study the mean area was 463.50 $\pm$ 249.40 mm^2 in the group treated with PDGF and 609.10 $\pm$ 334.8 mm^2 in the group treated with NS. There was significant difference in the mean area between the two groups (P= 0.001) at the end of the study.

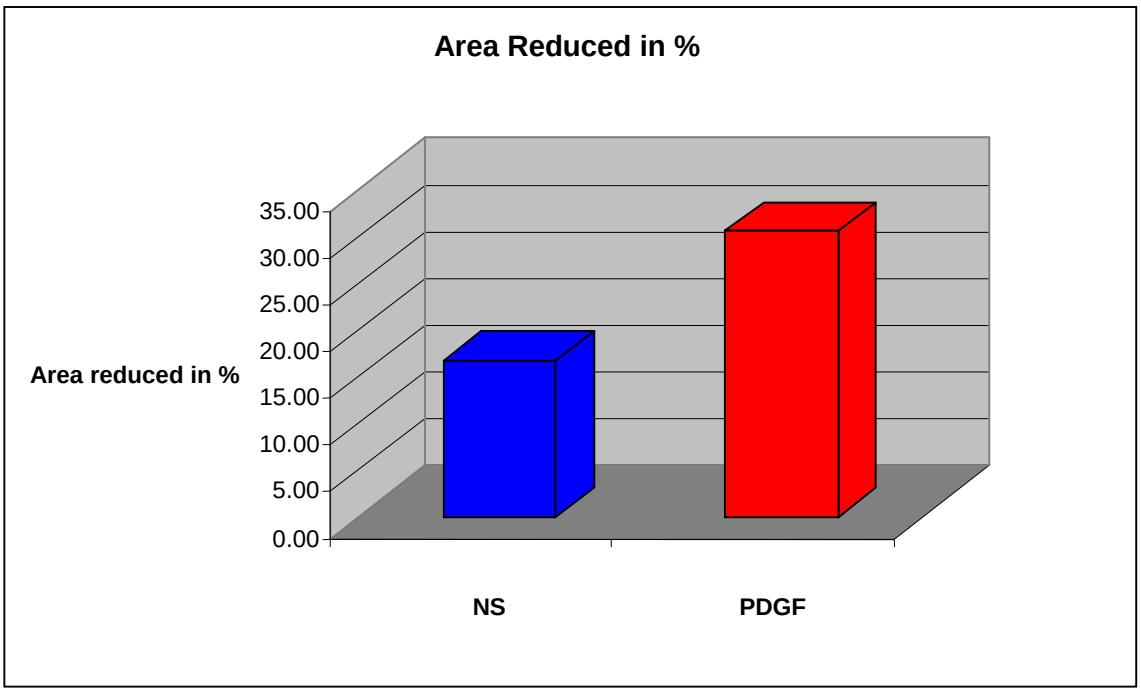
Table no. 5 Final area

	PDGF		NS		P
	Mean	**SD**	**Mean**	**SD**	
Final Area in mm^2	206.80	119.09	120.15	71.00	0.0118
% Area Reduction	30.56	6.67	16.76	3.49	0.0001

Graph no. 1. Area reduced



Graph .2 Area reduced in percentage

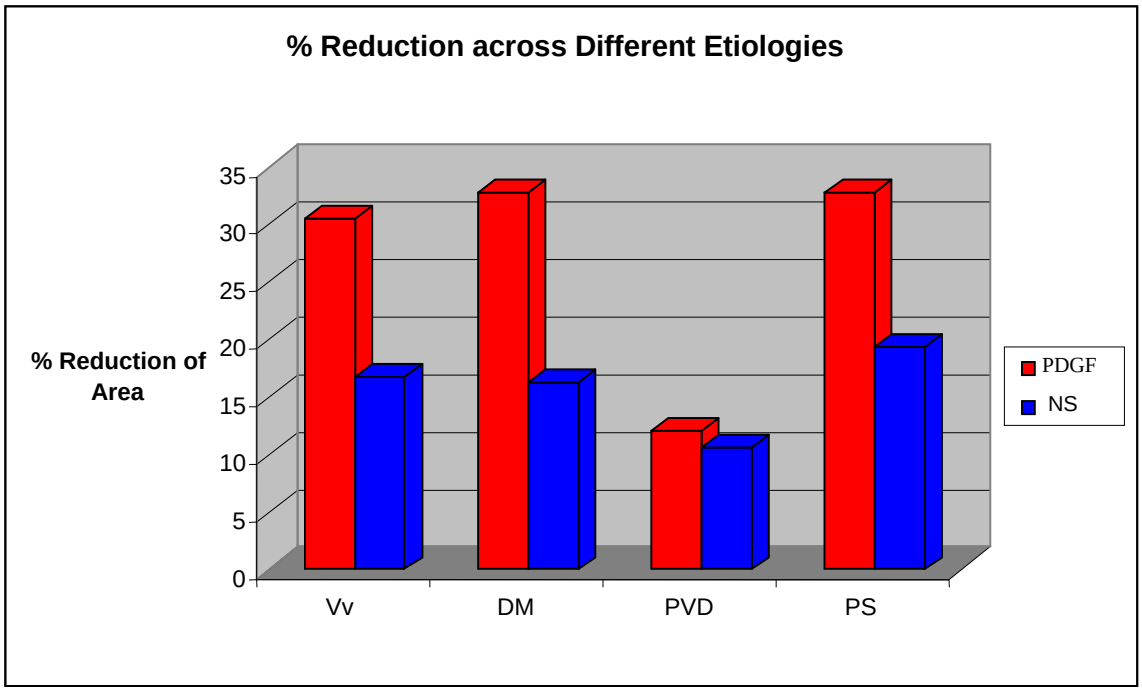


The study shows that the final area achieved between the two groups were (206.80 ± 119.09 mm^2) in patients treated with PDGF and (120.15 ± 71.00 mm^2) in patients treated with NS which is significant (P= 0.0118). The percentage of area reduction was (0.56 ± 6.67) in PDGF and (16.76 ± 3.49) in NS group, which is statistically significant (P=0.0001).

Table 6. % Reduction across Different Etiologies.

Variables	% reduction in PDGF	% reduction in NS	P Value
Vv	30.56 ± 6.67	16.76 ± 3.49	0.001
DM	32.89 ± 2.47	16.29 ± 3.87	0.001
PVD	12.12 ± 0.70	10.56 ± 12.12	0.873
PS	32.86 ± 1.52	19.30 ± 0.98	0.002

Graph 3: % Reduction across Different Etiologies



When the results were analyzed according to the etiology of the ulcer, it was found that in ulcers due to varicose veins the area reduced was 30.56 $\pm$ 6.67 mm^2 in patients treated with PDGF and 16.76 $\pm$ 3.49 mm^2 in patients treated with NS. Similarly in diabetic ulcers the area reduced was 32.89 $\pm$ 2.47 mm^2 in PDGF and 16.29 $\pm$ 3.87 mm^2 in NS group; in ulcers due to PVD, the area reduced was 12.12 $\pm$ 0.70 mm^2 in PDGF group and 10.56 $\pm$ 12.12 mm^2 in NS group; in pressure sore the area reduced was 32.86 $\pm$ 1.52 mm^2 in PDGF treated patients and 19.30 $\pm$ 0.98 mm^2 in pts treated with NS. The P value in varicose ulcer and diabetic ulcer patients were significant (P=0.001) and (P=0.002) in patients with pressure sore. One significant finding noted was, patients with ulcers due to PVD who were treated with PDGF in study group and NS in control group did not show any statistically significant reduction in the wound area (P=0.873).

There were no adverse events or reactions noticed in either of the groups during the study period.

DISCUSSION

The first indication that platelet contained mitogen, for vascular smooth muscle and fibroblasts in the process of wound healing came from comparisons of serum and plasma-induced stimulation of fibroblast growth in culture. Rutherford and Ross showed that the growth stimulating activity for proliferation of smooth muscle cells and fibroblasts is necessary after carefully removing the platelet-poor plasma[32]. These in vivo properties suggest that PDGF, derived from platelets at the site of injury, may play an important role in the initiation of repair process of wounds. Autologous platelet gel has been used with apparent clinical success in otolaryngology, neurosurgery, oral and maxillofacial surgery[33].

In the present study, an attempt has been made to establish better healing rates with use of PDGF in chronic ulcers of lower limb. In this study the baseline characteristics such as age, sex and location of the ulcer were similar in the patients who received PDGF dressing in study group and in patients who received NS dressing in control group.

Previous studies have shown that, with a mean platelet count value of 2, 30,000 the PRP yield was good and it showed better healing capabilities among the soft tissues[34]. In the present study the mean platelet count was 2, 00,000 in both PDGF group and NS group but the PRP yield was not measured.

In this study, initial area of the ulcer (in mm^2) was similar between the two groups. However, the final area of the ulcer (in mm^2) was significantly reduced in patients with PDGF group as compared to the patients in NS group at the end of the

study. The percentage reduction in the area of the ulcer was more in the PDGF group as compared to the NS group and this difference was statistically significant.

This study was based on the study conducted by Steed et al. An initial multi centre phase II study led by Steed[35] indicated that the number of patients who went on to heal completely over the 20 week duration of the trial doubled in the treatment group compared with controls. Three pivotal phase III trials were conducted which led to the approval by the FDA 1997 for use of growth factors[35]. When the results from all phase III trials were combined, with no exclusions, PDGF gave a persistent 10% overall increase in the rate of complete healing.

However when the subgroups were analyzed for reduction in size depending on etiology, we found that there was significant reduction in size of ulcers of all etiologies except those due to PVD. In patients with PVD, there was no significant reduction in size between the two groups. Since the number of patients presenting with ulcer secondary to PVD were less, more studies are needed to test and validate the significance using PDGF in chronic ulcers secondary to PVD.

More recently, Stadelman has stated that hypoxia is a significant contributing factor in the formation and failure to heal vascular ulcers. Experimentally, it has been demonstrated that growth factors can be ineffective in augmenting wound repair in ischemic, hypoxic wounds. These observations led Zhao, et al.[36], to suggest that growth factors may need to be used with supplemental hyperbaric oxygen to achieve optimal benefit in ischemic wounds.

Overall this study shows that PDGF is a safe and effective in treating chronic ulcers of lower limb. This study was conducted only for two weeks and complete epithelialization and wound reduction of the wound was not awaited, hence it was not

possible to comment on hypertrophic scar or keloid formation during the course of the study. However review of literature has not shown any significant increase in the incidence of hypertrophic scar or keloid formation with use of PDGF.

Limitations of our study:

1) Follow up is short to derive conclusion on long term healing of ulcers.

2) The long term compliance of the patient for withdrawal of blood in preparing autologous platelet gel for complete epithelialization of the wounds has not been analyzed.

3) Storage of platelet gel has not been analyzed, since no literature is available for storage of freshly prepared platelet gel.

CONCLUSION

With the use of autologous Platelet Derived Growth Factor (PDGF) dressings in comparison with the control group (normal saline group) for the treatment of chronic ulcers of the lower limb, the following conclusions were derived,

1. PDGF showed faster and better healing rates among the study group.
2. Area reduction and percentage reduction was better in PDGF group.
3. In individual subgroups like ulcers due to diabetes mellitus, ulcers due to varicose vein, and pressure ulcers showed healing rates of >10% compared to the conventional dressing except for ulcers secondary to peripheral vascular disease.
4. Ulcers of peripheral vascular disease etiology did not show significant decrease in the area among the two groups studied.
5. There were no adverse effects or reactions seen when autologous PDGF was applied over the ulcer.

SUMMARY

The present study was conducted in KLES' Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum on 40 patients with chronic ulcers of lower limb.

The objectives of the present study was to know the efficacy of autologous platelet gel in epithelialization and wound reduction in chronic wounds of the lower limb in comparison with conventional method of treatment**.**

The two groups were randomized into study (PDGF) and conventional (NS) group. One group received treatment in the form of autologous platelet gel and other received saline dressing.

There was no statistical difference between the baseline characteristics like age, sex, and the location of the ulcer. The total platelet count was also not statistically significant among the groups

The final area reduced and the percentage of area reduced was statistically significant in the study as compared to the control group. In patients with PVD, there was no significant reduction in size between the two groups.

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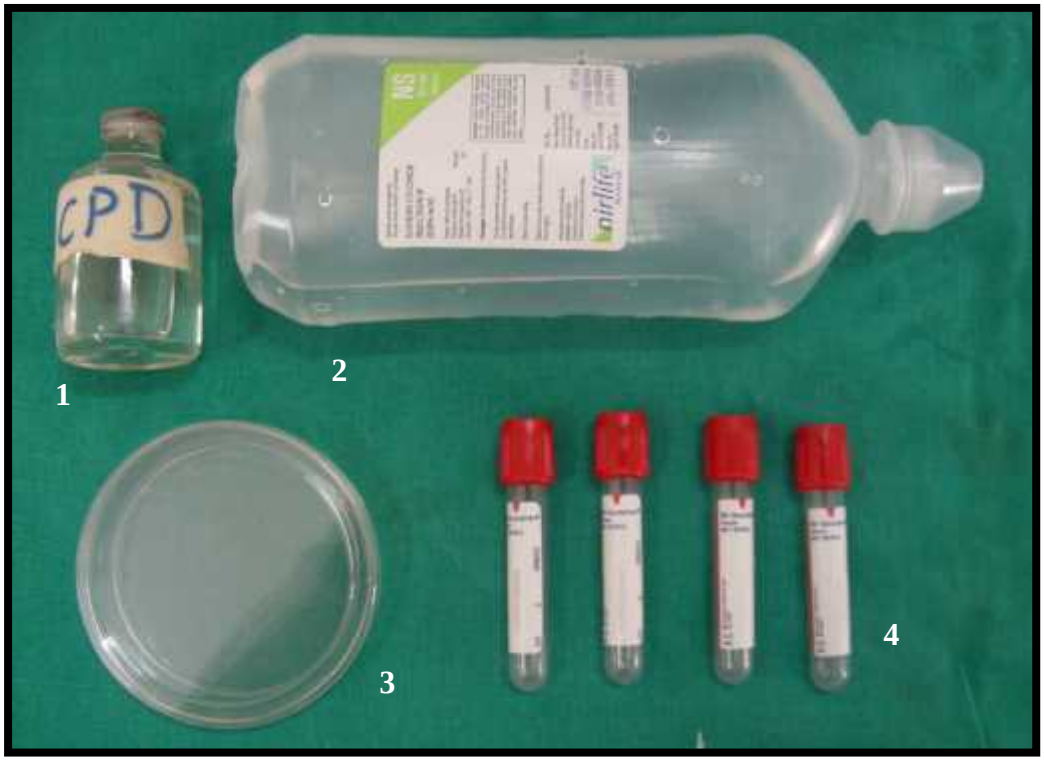
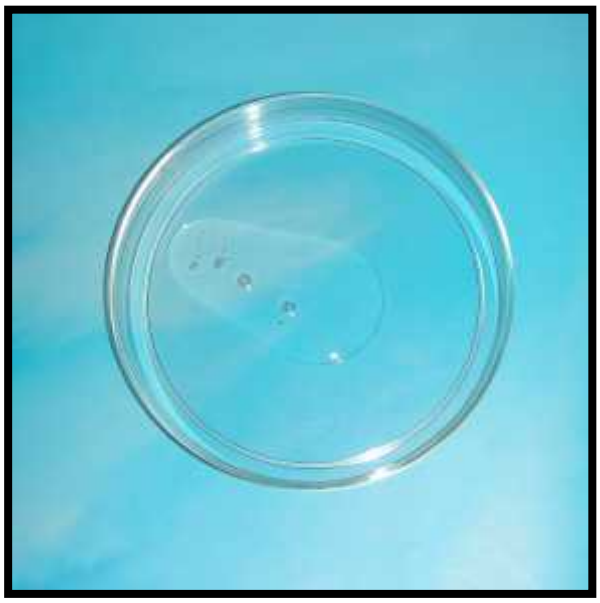
CENTRIFUGE

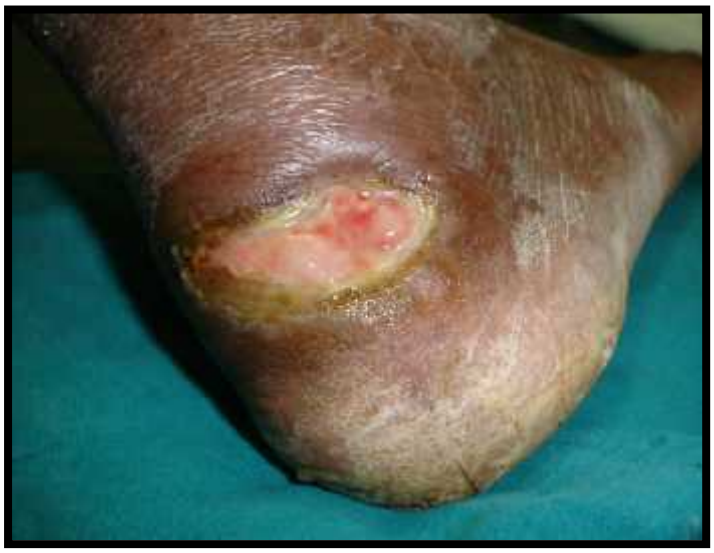
THAWING BATH



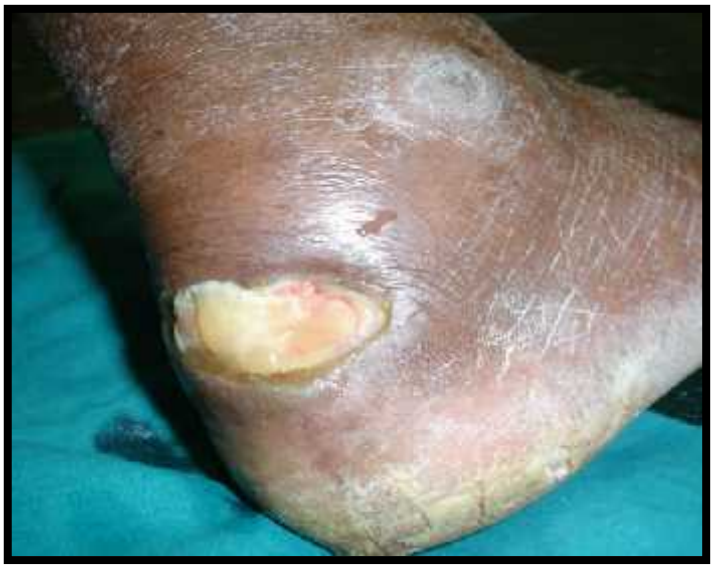
ARMAMENTARIUM
1.CPD 2. Normal saline 3. Petri dish 4. Vacutainer



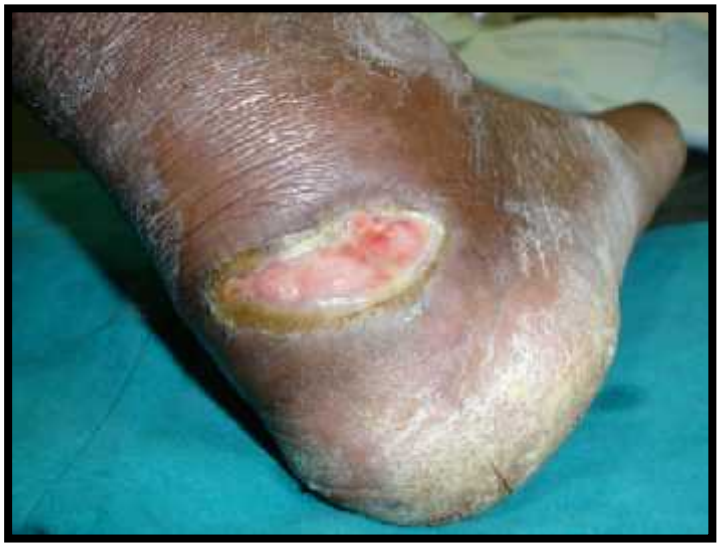
PRP GEL



BEFORE APPLICATION OF PRP GEL



PRP GEL APPLIED OVER THE ULCER



ULCER SHOWING WOUND REDUCTION

CONSENT FOR PARTICIPATION IN RESEARCH

Mr./Mrs._____we are requesting you to enroll yourself in study titled **"EFFICACY OF AUTOLOGOUS PLATELET GEL VERSUS THE REGULAR DRESSING IN EPITHELIALIZATION AND WOUND REDUCTION IN CHRONIC ULCERS OF THE LOWER LIMB"- A RANDOMIZED CONTROL TRIAL; AT KLES' Dr PRABHAKAR KORE HOSPITAL AND MRC, BELGAUM** conducted by Dr. Tejas Chiranjeevi, postgraduate student in M.S. (General Surgery) under guidance of Dr.V. M. Uppin, Proffesor, Dept of General surgery, at J.N.M.C., Belgaum under KLE University, Belgaum.

Respected Sir/ Madam we request you to enroll yourself to participate in our study as you meet the eligible criteria for participating in the study. During the study you will be asked some questions regarding your present, past medical history and you are supposed to answer to the best of your knowledge.

Your participation in research is voluntary. Your decision whether to participate or not, will not affect your relationship with the J.N.M.C. If you decide to participate you are free to withdraw at any time without affecting the relationship.

The purpose of research is to evaluate the effectiveness of autologous platelet gel in comparison with conventional treatment for the management of chronic ulcers of lower limb.

Procedure involved: The wound dressing will be done randomly either with Autologous platelet gel or Conventional (normal saline) dressing.

Risks and benefits: Since the study involves intervention, exposure to the blood and blood products and hence universal precaution will be taken for all patients. The patients will be benefited in terms of faster healing rate and shorter hospital stay.

Alternatives: even if you decline the participation in the study, you will get the routine line of management.

Privacy and confidentiality: The only people who will know that you are a research subject are members of the research team. No information about you or provided by you during research will be disclosed to others without your written permission except:

1. In emergency to protect your rights and welfare.
2. If required by LAW.

Authorization to publish reports: When the results of research are published or discussed, in a conference, no information will be displayed that would disclose your identity. Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed with your permission, or if required by law.

Compensation: In the event injury related to this research study, treatment will be made available through KLESH & MRC, Belgaum. There is not compensation or payment for such medical treatment from KLESH & MRC by law.

You will not be paid/ offered any free gifts for participating in the research. There will not be any remuneration for participating in the research. You will not be reimbursed for expenses.

Questions: In case you have any questions related to the stud, you can contact Dr Tejas. C (Phone No 9845428903). In case you have any questions about your rights as a study participant, you can contact Dr V. D. Patil (0831-2471350), principal, J. N. M. C, Belgaum.

Consent For Participation In Research Trial:

I, Mr/ Mrs/ Miss _____voluntarily agree for participation as as ubject of study. By signing this consent form I am not giving up any of my legal rights, I may withdraw from the study anytime. I am signing the consent form after having read or been read for me in vernacular language, including the risks and the benefits and having all my questions answered.

Subjects name _____

Signature or the Left Thumb print of the subject _____

Witness name _____ Signature _____

Name of Researcher _____ Signature _____

Date _____ Place _____

PROFORMA

"EFFICACY OF AUTOLOGOUS PLATELET GEL VERSUS THE REGULAR DRESSING IN EPITHELIALIZATION AND WOUND REDUCTION IN CHRONIC ULCERS OF THE LOWER LIMB"- A RANDOMIZED CONTROL TRIAL.

INVESTIGATOR: Dr TEJAS C **GUIDE: Dr V. M. UPPIN**

Name of the patient: _____

Age of the patient: _____

Sex: Male/ Female **I. P. NO _____**

Address:

Chief Complaints: 1)

2)

3)

Present history:

Past history:

Family history:

General Physical Examination:

Pallor/ icterus/ clubbing/ lymphadenopathy/ edema

Vitals: PR _____ BP _____

Local examination:

Size-

Site-

Shape-

Edge-

Margin-

Floor-

Base-

Slough- Present/Absent

Surrounding skin-

Vascular Examination-

Systemic examination:

Respiratory system;

Cardiovascular;

Central nervous System:

Per abdomen:

Diagnosis

Investigations:

1) Hb%-
2) Platelet Count-
3) Total Count
4) HbA1c-

5) Sr Creatinine

6) Blood Urea

Method:

Inclusion Criteria: 1) Age 35-70

2) Ulcer $\geq$ 4 weeks duration

3) Hb%- > 10 gms

4) Platelet count > 1, 50,000

5) HbA1c: 7-9

Exclusion Criteria: Ulcers with evidence of malignancy, active pus discharge, slough

Procedure involved: The subjects enrolled in the study are randomly allocated into two groups, P group (PDGF group) and C group (control group). Patients receive either PDGF dressing or conventional dressing once in 3 days for 15 days and the wound is assessed by tracing on graph and noting the characteristics. Under all aseptic techniques, 12-16 ml of blood was drawn intravenously from the anticubital region of patients forearm using vacutainer needle and vacutainers containing CPDA (4ml each). The vacutainers were thoroughly shaken to ensure mixture of anti coagulant with the drawn blood. The whole blood is then centrifuged at 1000 r.p.m. for 10 mins. PRP, Buffy coat and upper1-2 mm of R.B.C. layer is collected in a fresh vacutainer and again centrifuged at 1000 r.p.m. for 10 mins. The upper half of the supernatant is discarded and the lower half is mixed thoroughly to yield cPRP. 2.5 ml of PRP thoroughly mixed with 0.08 ml of 10% $Cacl_2$ / Calcium gluconate. This resulted in a

clot formation and thrombin formation. The clot is discarded and the thrombin is used for the preparation of concentrated Platelet rich Plasma (cPRP)

Wound characteristics

	YES	**NO**
Periwound edema		
Periwound erythema		
Wound purulence		
Limb pitting edema		
Limb brawny edema		
Wound granulation		
Wound Size		
Peripheral temp		

KEY TO MASTER CHART

1. SRF : Sole of Right Foot
2. SLF : Sole of Left Foot
3. DRF : Dorsum of Right Foot
4. GLL : Gaiters area Left Limb
5. GRL : Gaiters area Right Limb
6. LMLL : Lateral Malleolus Left Limb
7. LMRL : Lateral Malleolus Right Limb
8. DLF : Dorsum of Left Foot
9. DRF : Dorsum of Right Foot
10. MMRL : Medial malleolus Right Limb
11. HRF : Heel Right Foot
12. SRL : Shin Right Limb
13. ATRL : Achiles Tendon Right Limb
14. D & T : Diabetes and Trauma
15. D & S : Diabetes and spontaneous
16. Vv : Varicose vein
17. PVD : Peripheral Vascular Disease
18. RH : Right Hemiplegia
19. PS : Pressure Sore

CASES

S. NO	IP NO	NAME	AGE	SEX	SITE	ETIOLOGY	PLATELET COUNT	INITIAL AREA IN MM2	FINAL AREA IN MM2	AREA REDUCED IN	% AREA REDUCED
3	183613	SNP	60	M	GLL	Vv	185000	838	580	258	30.79
5	193563	NAM	56	M	SRF	D & S	275000	1166	790	376	32.25
7	196425	SD	50	F	LMRL	V & D	165000	642	420	222	34.58
8	199698	NMH	58	M	PLLL	D & PN	150000	1228	890	338	27.52
10	209724	SYM	55	M	ATRL	D &S	200000	696	480	216	31.03
11	209058	SKG	45	F	SRF	D & T	175000	1548	1090	458	29.59
13	210162	SKK	55	M	DRF	D & S	200000	874	600	274	31.35
17	210166	JT	60	M	SRF	D & T	215000	350	230	120	34.29
18	216959	AR	50	M	CRL	D & S	190000	1248	800	448	35.90
21	216998	NAH	52	M	PRF	D & T	220000	502	322	180	35.86
23	222824	PY	55	M	DRL	D & T	195000	459	303	156	33.99
24	248204	MYT	35	M	SRL	D & S	235000	550	360	190	34.55
28	239557	NMH	40	M	DRF	PVD	225000	452	395	57	12.61
29	239182	MSM	48	F	1ST WRF	D & T	190000	390	260	130	33.33
31	239199	SMD	60	F	LMLL	RH	175000	335	230	105	31.34
32	246209	VKG	50	M	GLL	Vv	165000	500	350	150	30.00
36	247923	RYS	58	M	HLF	TU	168000	417	280	137	32.85
37	256391	BKB	55	M	SRF	PVD	160000	396	350	46	11.62
38	259398	BRM	58	M	HRF	PS	190000	320	210	110	34.38
39	258566	SSS	66	M	SRF	D & S	200000	495	330	165	33.33
MEAN			53.30	3			193900.00	670.30	463.50	206.80	30.56
S.D.			7.34	17			29673.49	361.46	249.40	119.09	6.67

CONTROL

S. NO	IP NO	NAME	AGE	SEX	SITE	ETIOLOGY	PLATELET COUNT	INITIAL AREA IN	FINAL AREA IN MM2	AREA REDUCED IN	% AREA REDUCED
1	176969	PKG	64	M	SRF	D & T	225000	931	750	181	19.44
2	180854	RNH	70	M	DRF	D & S	180000	1024	900	124	12.11
4	187924	SBM	45	M	GRL	Vv	200000	905	735	170	18.78
6	194120	SRM	48	M	LMLL	D & T	190000	1493	1324	169	11.32
9	196425	SD	50	F	DLF	V & D	180000	753	667	86	11.42
12	206181	SN	58	M	DRF	PVD	250000	1590	1286	304	19.12
14	209559	RRP	55	F	MMRL	D & S	200000	1475	1189	286	19.39
15	210650	BIS	50	M	SRF	D & S	155000	714	620	94	13.17
16	212162	VRG	60	M	DRF	D & T	200000	498	395	103	20.68
19	216391	BKB	57	M	LMRL	RH	255000	300	240	60	20.00
20	222426	SSP	63	M	DLF	PVD	175000	695	600	95	13.67
22	225342	RH	70	M	SLF	D & S	215000	713	570	143	20.06
25	248861	DMT	62	M	SRF	D & S	200000	575	496	79	13.74
26	249318	LRT	60	F	MMLL	D & T	250000	512	410	102	19.92
27	251652	YSP	58	M	SRF	D & S	200000	450	400	50	11.11
30	231785	SYP	55	m	1ST WRF	D & T	185000	386	320	66	17.10
33	249737	MND	57	M	HRF	TU	170000	430	350	80	18.60
34	246209	GRS	55	F	DLF	D & T	140000	375	300	75	20.00
35	251254	IMB	60	M	DRF	D & S	145000	356	290	66	18.54
40	265929	SMP	40	M	GRL	Vv	180000	410	340	70	17.07
MEAN			56.85	4			194750.00	729.25	609.10	120.15	16.76
S.D.			7.61	16			32463.54	397.48	334.80	71.00	3.49