"COMPARISON OF PLATELET RICH PLASMA INJECTION WITH NORMAL SALINE DRESSING IN RATE OF CHRONIC ULCER HEALING, A ONE YEAR RANDOMISED CONTROL TRIAL"

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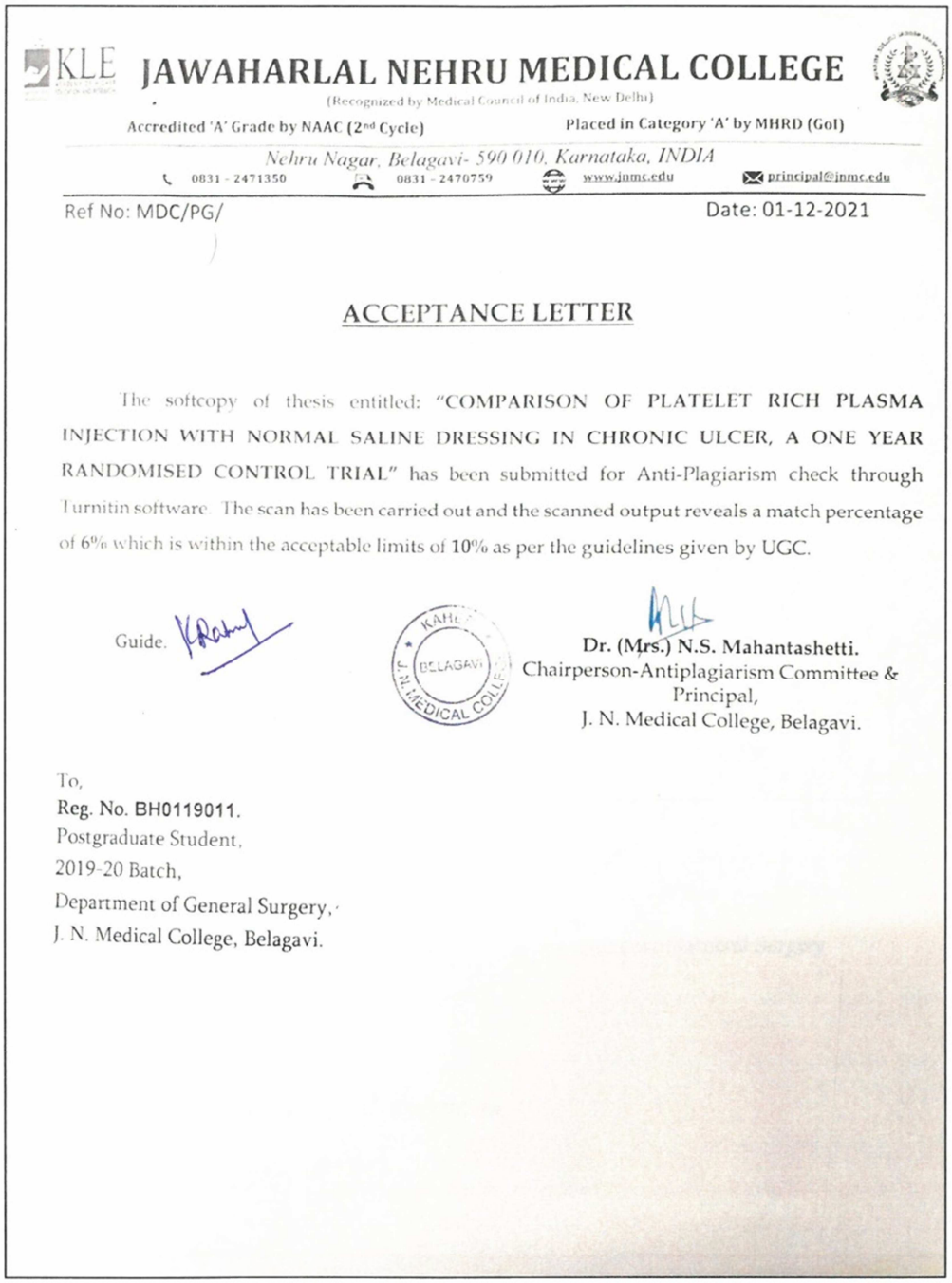

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Hereby requesting you to give us the permission to reduce our sample size in our dissertation, due to inevitable situation of COVID – 19 pandemic , which lead to reduction in the number of admitted cases for the year of 2020.

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ABSTRACT

“COMPARISON OF PLATELET RICH PLASMA INJECTION WITH NORMAL SALINE DRESSING IN RATE OF CHRONIC ULCER HEALING, A ONE YEAR RANDOMISED CONTROL TRIAL”

INTRODUCTION

Chronic wounds are critical health challenge in India. Our goal is to establish fixed protocols to simplify wound healing. Chronic wound are those which donot heal within a time period of 3 months.

Various different treatment strategies are available for promoting wound healing but no specific protocols are present for the same. Platelet rich plasma is one of the upcoming treatment modality available for chronic wound healing.

PRP acts by releasing various growth factors such as platelet derived growth factor, fibroblast growth factor, Epidermal Growth Factor (EGF), platelet derived angiogenesis factor and platelet factor-4. These factors are released in the local wound environment which leads to increase wound healing.

Chronic wound has a great financial burden and mental burden over the patient. So proper wound care should be given and usage of platelet rich plasma can enhance wound healing.

AIM

To compare injection platelet rich plasma dressing versus normal saline dressing in healing of chronic ulcers in terms of mean percentage reduction in ulcer area.

MATERIALS AND METHODS

This will be a randomized control trial done in the department of surgery in KLE Dr. PRABHAKAR KORE CHARITABLE HOSPITAL AND MEDICAL RESEARCH CENTRE from 1st January, 2020 to 31st December, 2020. All consecutive patients fulfilling the criteria and who give informed consent during the period of study will be the sample of this study. Wound healing in terms of percent reduction in ulcer size over 6 weeks were compared between the patients undergoing injection platelet rich plasma dressing and normal saline dressing

RESULTS

During this study, 60 patients who had chronic non healing ulcers were randomly distributed into study (PRP) and control (normal saline dressing) group with 30 patients in each group. These groups were studied for the effect of Regular versus PRP dressing on reduction of wound size and total healing of the chronic nonhealing ulcer.

Patients ranging from age group 30 to 80 years out of which 47 were male and 13 were females. Reduction in size were compared on day 1 and day 42.

This study showed that there was no significant difference in reduction of wound area between both the groups. This suggests that platelet rich plasma dressing is non inferior to normal saline dressing , thus can be used as an alternative chronic wound healing.

CONCLUSION

Chronic wounds ulcers are becoming a global socioeconomic problem. All of the traditional treatments for chronic wound healing/ulcer are time consuming and

costly. PRP application requires minimum setup and low cost as compared to the costly preformed preparations available in the market.

If required this can also be used on OPD basis, thus reducing the hospital stay for the patient. Platelet rich plasma dressing can be used in the treatment of chronic ulcers.

KEYWORDS – PLATELET RICH PLASMA, CHRONIC WOUNDS, PLATELET DERIVED GROWTH FACTOR

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INTRODUCTION

Chronic wounds are outlined as wounds, that didn't undergo proper process of healing to supply anatomic and useful integrity within a period of three months as outlined by numerous authors. A better definition of a non-healing wound is that the wounds which are unable to heal within time period of 3 months, which may lead to fibrous scar.

Cutaneous ulcers are identified by the loss of epidermis, dermis or ever deeper structures such as muscle and fascia.

Apart from classical strategies such as normal saline dressing, numerous newer modalities are coming up, one of them being platelet rich plasma dressing. This help us to establish a standardised and improved protocol for dressing[1] .

Growth factors are released via alpha granules in platelets that are situated at platelet cell membrane that favor platelet derived growth factor (PDGF), Epidermal Growth Factor (EGF), platelet derived angiogenesis factor and platelet factor-4. They are responsible for increase of rate of healing process. Several studies have been done regarding plasma extract in wound healing which has shown tremendous results but very few studies are done comparing it with normal saline dressing.

Various different formulations of platelet rich plasma are available such as perilesional injection and local application in form of gel. It is a underutilised resource which is yet to find its place in wound healing.

1.1 Wound Dressings

Nature of wound determine the type of dressing that will be used. Purpose of our dressing is to create a healthy atmosphere for rapid healing which usually include maintaining humidity, clearing the infection via debridement, physical barrier to outside environment. Wound dressing are broadly classified into non adherent fabrics, absorptive, occlusive, cream, ointment and solutions.[1]

1.1.1 Vacuum Assisted Closure Therapy:

The goal of this treatment is to cover the area for 5 minutes and apply a negative pressure of 125mmHg for 2 minutes to clean the oedema fluid out of the area, enhance local perfusion and stimulate cellular proliferation. [2,8]

1.1.2 Amnion Liquid Derivative Stem Cell Therapy:

Proinflammatory mediators are controlled by mesenchymal cells in the amniotic fluid. hyaluronic acid is abundantly present. HGF, VEGF AND VEGF-A helps in stimulating factors that enhance endothelial cell proliferation with improvement of stability[3,25]. Furthermore keratinocyte proliferation promotes extracellular matrix formation and fibroblastic activity. It promotes inflammation in acute and chronic wounds, improves epithelisation, and promotes therapeutic / regeneration activities.[3]

1.1.3 Ozonated Oil Usage:

This is frequently used for disinfecting the wounds and aids in healing. TGF-beta and VEGF levels rise as a result of PDGF's active engagement in wound healing. [4] Antifungal, antibacterial, and antiseptic properties are all present in ozonated oils. Antimicrobial activity against bacteria such as Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis and E. coli. Olive oil has been utilised because of their high unsaturated fatty acid content.

1.1.4 Using Honey and Propolis:

Honey's wound healing properties have been empirically validated since long time. Debridement, anti-inflammatory, anti-amoebic, anti-fungal, antiviral, anti-oedematous, wound epithelialization, and granulation are all accelerating effects in addition to antimicrobial property. [4]

1.1.5 Nigella Sativa use:

For millennia, Nigella sativa has been used to treat a wide range of ailments throughout the Middle East, North Africa, and Asia. Antipyretic, antibacterial, antiparasitic, and anti-inflammatory properties are among the pharmacological effects. Its thymoquinone antioxidant activity protects against oxidative damage. [2,23]

1.1.6 USE OF HYPERICUM PERFORATUM

Flavonoids, carotene, etheric oils, phenolic acid, and vitamin E are all found in Hypericum Perforatum (HP). Each component has a distinct impact. Hyperforin, in particular phenolic acid products, possesses analgesic, antibacterial, anti-inflammatory, and antioxidant properties. Increases collagen production without increase in mitotic activity but by enhancing fibroblast migration and stimulation.[27]

1.1.7 Curcuma Longa Extract and Refined Sheep Combination:

Premature epithelization, improved neovascularization, augmented cell migration, including myofibroblasts, fibroblasts, and macrophages with immense collagen content are all benefits of Curcuma Longa plant with curcumin as active principle. It also aids in the healing process by promoting the generation of TGF- from growth factors. Saturated PUFA such as linoleic (n-6), linolenic (n-3) and oleic acid are found in refined sheep oil (n-9) which plays a vital part in healing of wound by proliferation of epithelial cell. [4,15]

1.1.8 Use of Silver Sulfadiazine:

For millennia, silver is being utilised to heal and protect people from numerous diseases. Silver ions in free form have a strong antibacterial effect. Neutropenia, erythema, crystalloid, and methemoglobinemia are some of the systemic problems caused by silver sulfadiazine, silver nitrate, and mafenide acetate. Scarring, kidney injury, and resistant to microbes are also more common in acute and chronic wounds than with traditional wound treatment. Platelets also have antibacterial action against some skin microorganisms and is used in burn wound locally. [11,13]

Due to low economic status patients donot purvey for commercially available PRP, extraction of platelet from patients blood has been exploited empirically on chronic non healing wounds which affects 1.9 to 13.1 percent of the world's population.[2,3] Because of the ageing population and increase in risk factors for arterial sclerosis like smoking, increased BMI, and type 2 DM, the number of new cases of chronic ulcers is likely to rise. Nearly 10% of the population is expected to suffer a chronic wound in their lifespan, with a wound associated mortality incidence of 2.5 percent. [3]

This research aims to see how PRP influences the course of healing in hospitalised patients with non-healing wounds. PRP's healing properties are evident that thrombocytes are a natural pool of different types of growth factors, including factors that play an important part in regeneration of tissues. [1] These ulcers have a great impact on patients in terms of economical burden as well as life quality and the health system.[4]

In meta-analysis done recently for the usage of PRP for skin ulcers, it was discovered PRP aids ulcer healing and, as a result, there is a significant improvement in non healing ulcers as compared to standard wound treatment. [5,6] Furthermore,

platelets have antibacterial action against some skin microorganisms, and clinical data shows that infection rates are lower in PRP-treated wounds.[6] As a result, PRP therapy has a number of advantages that could make it a useful therapeutic option for small, difficult-to-heal ulcers. [7] Hence, this study intends to validate the therapeutic role of PRP/allogenic platelet concentrate in healing of chronic non-healing ulcers.

OBJECTIVE

To compare injection platelet rich plasma dressing versus normal saline dressing in healing of chronic ulcers in terms of mean percentage reduction in ulcer area.

REVIEW OF LITERATURE

It is vital to understand the process of non-healing ulcers to relate it to usage of PRP in wound healing

3.1 Evolution of Wound Healing

Oldest descriptions about wound healing can be traced back to around 2000 B.C. The Sumerians used 2 methods to treat wounds: a non-secular methodology based on incantations, and a physical methodology based on smearing poultice-like substances on the wound. Difference between infected and non infected wounds were first recognised by Egyptians. A duplicate of an older record, the Edwin Smith Surgical Papyrus, lists at least 48 different types of wounds. The administration of potions comprising of honey with antibacterial activity, lint as absorbent, and grease as barrier for wound management is described in a later manuscript (Ebers Papyrus, 1550 B.C.).

The Greeks categorised wounds as acute or chronic based on the information provided by the Egyptians. Galen of Pergamum (12O–201 A.D.), who was allotted as the gladiator's doctor, stressed the need of water in wound healing. The function of moisture in a dry wound environment was only discovered after the nineteenth century.[8]

The invention of antiseptics and their relevance in minimising wound infections was the next important step in the history of wound healing. Polymeric dressings were first used in the 1960s and 1970s. Specific criteria, such as penetrability to gases, varied levels of peneterance, and completely diverse physical forms, will be specially developed for these compound dressings. Wound healing currently involves the influence of growth factors, cytokines, and biological

engineered material, among other things. The optimum wound healing is achieved by combining these methods.

3.2 Wound Healing:

Healing of wound includes complex biological and biochemical process that results in the recovery of structural and functional integrity and performance. Despite the fact that particular tissues may have different healing qualities, all tissues repair using the same principles.

Homeostasis, Inflammation, Proliferation, and Tissue Remodelling or Resolution are the four processes that make up the wound-healing procedure.[9]

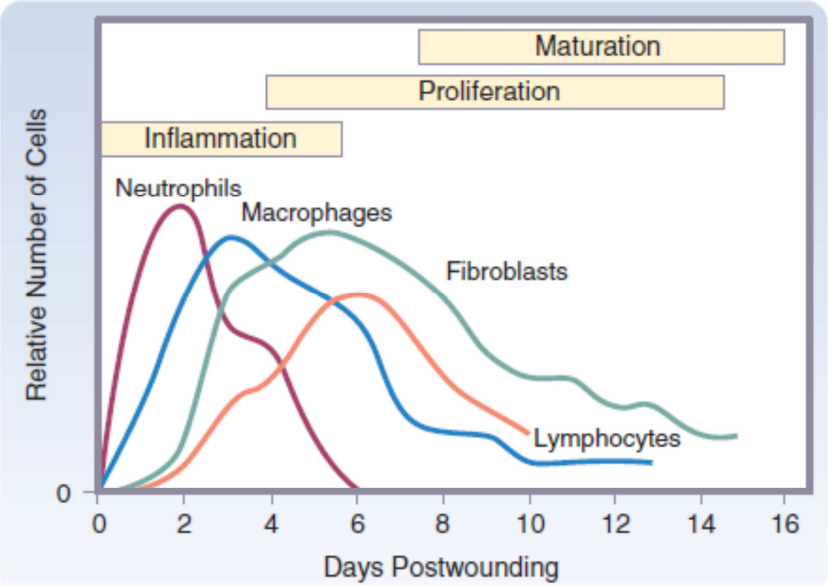
Native, systemic, and technological problems are all factors that obstruct traditional healing and should be considered by the surgeon.

Stages of Wound Healing:

1. Haemostasis and inflammation,

2. Proliferation

3. Maturation and remodelling



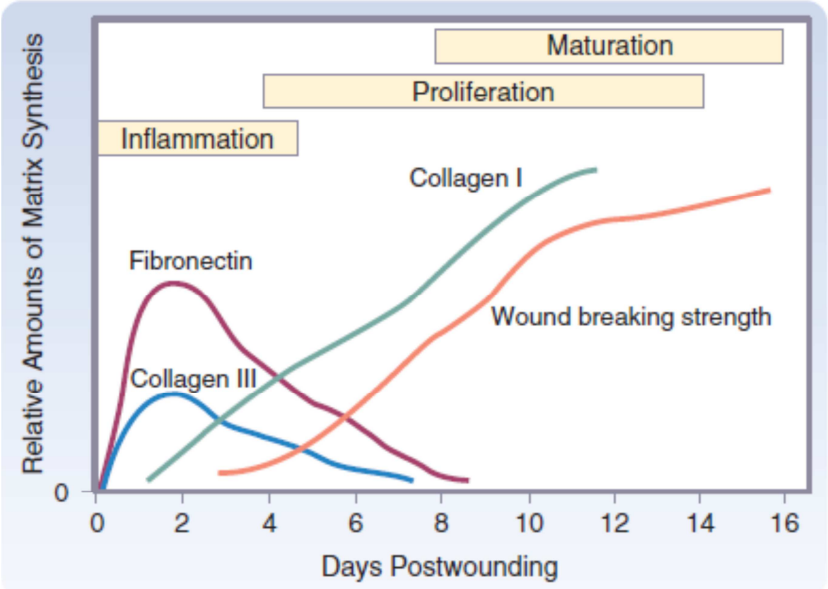


Figure 3.2.1 The cellular, biochemical, and mechanical phases of wound healing

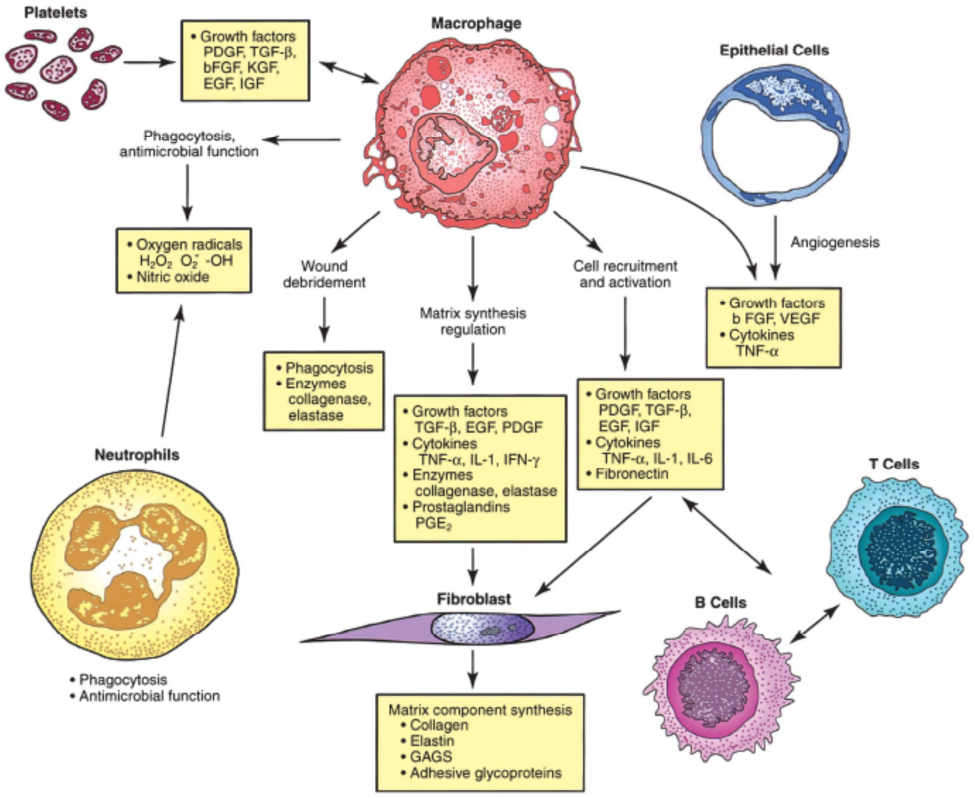


Figure 3.2.2 Interaction of cellular and humoral factors in wound healing

3.2.1 Phase of Haemostasis and Inflammation

Following chemotaxis, haemostasis precedes and initiates inflammation. Wounding, by definition, compromises tissue integrity, causing blood vessel division and disclosure of platelets to extracellular matrix. Aggregation of platelets, degranulation, and initiation of the coagulation pathway occur when sub endothelial collagen is exposed to platelets. Platelet granules release platelet-derived growth factor, platelet activating factor (PAF), transforming growth factor (TGF), fibronectin, and 5HT, among other wound-active chemicals. Furthermore providing haemostasis, the fibrin clot system act as framework for migrating cells.

Following an injury, cellular infiltration occurs in a predictable pattern. Leucocytic neutrophils are the initial cells to arrive at the wound site, reaching maximum in 24-48 hrs. Augmented permeability of vessels, production of prostaglandin, and hence the manifestation of chemicals with chemotactic property such as interleukin-1 (IL-1), complement factors, tumour necrosis factor (TNF), TGF, Platelet factor-4, or bacterial toxins all encourage neutrophil movement.

The phagocytosis of microorganisms and tissue debris is thought to be neutrophils' principal function. PMNs are also a foremost basis of cytokines in the initial stages of inflammation, particularly TNF-, which can have a considerable impact on subsequent angiogenesis and collagen formation. [10] In the early stages of wound healing, proteases like collagenases are released by PMNs, which aid in matrix with ground material disintegration. Apart from controlling infections, these cells don't seem to partake any role in deposition of collagen and mechanical wound strength procurement. Neutrophil factors, on the other hand, are involved in the delay of wound epithelial closure[11].

The 2nd type of inflammatory cell that occupies the site is macrophages, which are known to be critical for successful wound healing. [12] By 48-96 hours after damage, macrophages have accumulated in significant quantities inside the wound and will remain there until the lesion has healed completely.

3.2.2 Phase of Proliferation

This stage is the 2nd stage of wound healing, lasting from day 4th to day 12. The tissue continuity is reestablished at this period. Because 'fibroblasts and epithelial cells' are the final cells to invade during healing of wound, PDGF is the most potent chemotactic element for them. [13,14] Recruited fibroblasts proliferate after entering the wound environment, then are triggered to undertake their main task of matrix production & remodelling. The cytokines and growth factors released by wound macrophages are responsible for this activation Fibroblasts isolated from wounds manufacture more collagen, proliferate less, and actively execute matrix contraction than non-wound fibroblasts. Though it's apparent that the cytokine-rich wound environment is important for phenotypic change and activation, the precise mediators are incompletely understood.[15,16] Lactate, which collects in large amounts in the wound surroundings with time, could also be a strong supervisor of formation of collagen via a nucleotide (ADP)-ribosylation mechanism. [27,18]. During this stage of repair, endothelial cells also grow in large numbers. These cells are involved in angiogenesis, which is necessary for wound healing. Endothelial cells migrate from the wound's intact venules. TNF-, TGF-, and VEGF are cytokines and growth factors that regulate their migration, replication, and development of new capillary tubes. VEGF is produced by a variety of cells, although macrophages are a chief basis in the healing of wound, and receptors are only found on endothelium cells. [19,20]

Dermatan and chondroitin sulphate are the most common glycosaminoglycans in wounds, and they are generated by Fibroblasts, who increase their amount during the first three weeks of recovery. The relations between proteoglycans and collagen is currently being investigated; it is hypothesised that the lattice produced by sulfated proteoglycans is necessary for the gathering of collagen subunits into fibres and fibrils. The degree of sulfation appears to be critical in influencing the structure of collagen fibrils. Proteoglycans are integrated into the collagen framework as scar collagen is deposited. However, when scars mature and collagen remodels, the amount of proteoglycans decreases.

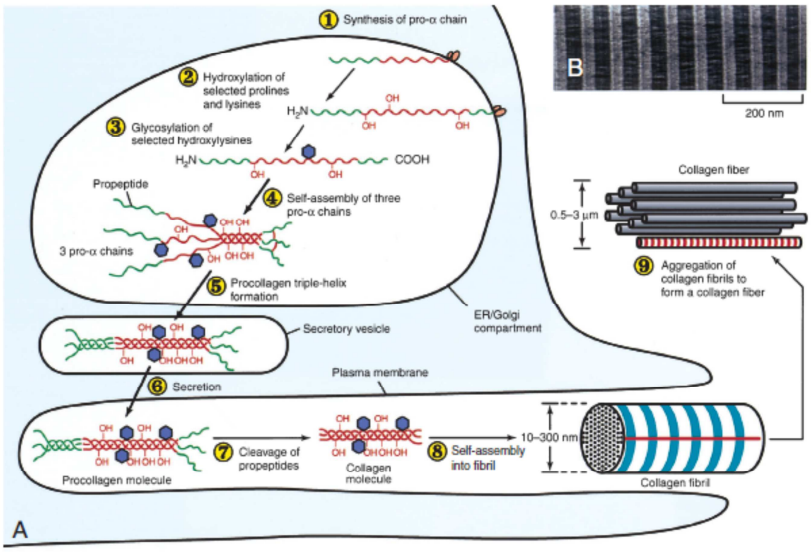


Figure 3.2.3 Intracellular and extracellular events in the formation of a collagen fibril

3.2.3 Phase of Maturation and Remodelling

During the fibroplastic phase, the scar matures and remodels, which is marked by a reorganisation of previously created collagen. Matrix metalloproteinases (MMPs) counteract collagen, therefore the net amount of collagen of wound is the consequence of a equilibrium between degradation and synthesis of collagen. There is increase in the synthesis of collagen which ultimately lead to formation of scar rich in collagen. The quantity and quality of newly deposited collagen determine wound strength and mechanical integrity within the recent wound. Early matrix scaffolding is represented by fibronectin and collagen type III; subsequent important matrix components are represented by glycosaminoglycans and proteoglycans; and the final matrix is represented by collagen type I. As a result, the matrix deposition at the ulcer site follows a predictable pattern.

The total collagen within the wound reaches a plateau 12 weeks after injury, although for several months the strength of the wound keeps on increasing but never reaching as the original. [21] Reduced collagen solubility, enhanced power, and high resistance to collagen matrix enzymatic degradation are all benefits of fibril production and cross-linking. Fibrillin is a glycoprotein released by fibroblasts that aids in the development of elastic fibres in connective tissue. After about half to one year, scar remodelling proceeds, slowly leading to a mature, vascular, and fibrous scar. Healed tissue will never have the same mechanical strength as normal tissue. Collagen is constantly being replaced in the extracellular matrix during healing process of wound, and during tissue homeostasis. Degradation of collagen is due to action of collagenase. Collagen production & collagenolysis are organized by growth factors and cytokines. Only a few elements have an impact on both collagen remodelling processes. TGF-, for example, stimulates the creation of tissue inhibitors

of metalloproteinase, which increases new collagen transcription while decreasing collagenolysis. [22] As a result, the balance between synthesis and lysis is the final determining element of wound strength and integrity.

3.3 GROWTH FACTORS ROLE IN NORMAL WOUND HEALING

Cytokines and Growth factors are polypeptides that increase cellular migration, proliferation, and function in healthy and injured tissue. They are named on the basis of the cells from where they were recovered (e.g. PDGF) or their first recognized function (e.g., Fibroblast growth factor, FGF). Many functions have been found in growth factors, therefore their titles are frequently misleading. The majority of growth factors are extremely powerful and have a considerable effect even at nanomolar concentrations. They can be autocrine (i.e growth factor influence the cell by which it was produced), paracrine (the growth factor is released into the extracellular environment and acts on nearby cells), or endocrine (it acts on cells farther away from the site of release). In determining the effectiveness of growth factors, the time of release may be just as important as the concentration. Because these polypeptides work by binding to cell-surface receptors, the suitable receptors should be present so that it can produce the required action. Growth factors have diverse effects on separate cells; for example, they may be chemo attractive to one type of cell whereas encouraging replication in another. Activation of second-messenger molecules by kinases or phosphatases, which result in the 'naturation or denaturation' of proteins in the target cell's cytoplasm or nucleus. Nuclear proteins Phosphorylation is trailed by the beginning of target gene transcription.[23]

When the non healing wounds supernatant was compared to that of acute wounds, it was discovered that the former had a significant reduction in growth factors, indicating that growth factors were rapidly metabolised by proteases present

inside the wound, bacteria, or cellular source. In ulcers secondary to diabetes and venous insufficiency growth factors are reduced due to a fibrin sequestration mechanism around the capillaries. [24]

3.4 CHRONIC NON HEALING ULCERS

Chronic non healing wounds are defined as wounds those did not heal in a timely and orderly manner over a three-month period, resulting in anatomic and functional integrity.

Due to a delayed, partial, or disorganised healing process, such wounds frequently develop pathologic inflammation. These may be extended as a consequence of an ageing population and increased rates of atherosclerotic disease risk factors like diabetes mellitus and smoking. These wounds provide a trial to healthcare providers and place a significant financial and economic strain on health-care systems. Patients also report a decrease in their life quality and a sense of isolation from society.

There are several kinds of non healing ulcers, including 'arterial, diabetic, pressure, and traumatic ulcers'. Inflammation, tissue creation, and tissue remodelling are the three steps of the traditional wound healing process. However, when the traditional healing mechanism is disturbed, an ulcer will develop into a chronic condition due to a shortage of cytokines and growth factors, which slow down the process of healing. [25]

Chronic ulcers, also known as non-healing ulcers, are non-healing lesions that develop spontaneously or traumatically in the lower limbs, donot respond to initial medical treatment, or persist in spite of appropriate treatment and don't heal in a distinct period of time, and have an intrinsic aetiology which may be associated

with systemic or local disorders. [27, 26]

"Foot ulcers precede more than 85% of lower limb amputations, and polygenic disease is one of the leading causes of non-traumatic amputations in the world." Around 15 to 25% of people with polygenic illness lead to development of a foot lesion, with 12% requiring amputation of the lower extremity. People having diabetic foot ulcers are at risk of infection, which can lead to chronic non-healing ulcers. Venous ulcers, diabetic ulcers, pressure sores, neuropathic ulcers, and other chronic lesions are common. [28]

Venous illness is responsible for the bulk of non healing ulcers in lower limbs, as venous hypertension damages vessel walls, resulting in skin breakdown. [30] In the general population, the venous nonhealing ulcers prevalence is between 1 to 2%, accounting for practically 75 to 80% of vascular ulcers. [26,29,30]

The objective of wound care therapy is to close the ulcer. Cleaning of wound with debridement of necrotic tissue, infection avoidance, diagnosis and treatment such as mechanical off-loading, blood glucose control, and local ulcer care with dressings are all critical for the healing of a ulcer which is not healing. [27.32.33] However, there are different risk factors those might influence and add to decrease in healing of wound , including the following:

A. Local causes like ulcer with infection, necrotic tissue, repeated trauma, and tissue hypoxia.

B. Systemic diseases like immunodeficiency, diabetes mellitus, or malnutrition.

C. Drugs like corticosteroids.[25]

Increasing data suggests that chronic ulcers are caused by an unfavourable mix of structural damage and the creation of a chronic biofilm infection, which triggers host reactions, more structural damage, and therefore creates a vicious cycle.[34,35,36,37]

These issues are addressed by regular available treatment modalities for non-healing ulcers, which include necrotic tissue debridement and providing moist environment for wound healing, pressure relief in the wound area, infection management with antibiotics, antiseptics, and topical antibacterial agents, management of ischaemia, and medical treatment of co morbidities.

Some of the most modern therapies for non-healing ulcers include skin grafting, VAC (vacuum aided closure), hyperbaric oxygen therapy, and surgical management such as reconstructive surgery.

Despite all of these efforts, ulcers that do not heal, linger for many months to years, or relapse post healing which require these innovative wound care procedures. In the field of vascular treatments, management of nonhealing ulcers by cellular therapy has been a big revolution. The components found in the blood and platelet concentrate, which contains numerous cytokines and growth factors, are used to treat wounds and ulcers using the patient's own body cells. These combined therapy approaches are both safe as well as effective, with no negative side effects. Developing cellular therapies, like PRP therapy, have gotten a lot of attention in the last two decades because of their potential use as the therapeutic agent in regenerative medicine for a variety of long term disorders, and they can play a supportive part in a standardised, high-quality management plan[40,41].

Nowadays PRP is progressively being employed as a novel replacement strategy in a variety of medical disciplines (i.e. cosmetic surgery, dentistry,

traumatology, dermatology, ophthalmology). Platelet growth factors stimulate biological processes such as chemo taxis, cellular proliferation, angiogenesis, and cellular differentiation, all of which are important in tissue healing and restoration. Several investigations on the role of PRP in the management of nonhealing ulcers have also yielded promising results.[42]

3.5.1 Platelet Rich Plasma (PRP)

PRP is a blood plasma that have been enhanced levels of platelets through centrifugation. PRP is thought to have an effect because it contains multiple different growth factors that promote soft tissue recovery.

3.5.2 Historical background

PRP was first described as having potentially positive effects by Schulte et al. in 1960s.[43,44] They didn't use pooled plasma and instead used regular plasma to treat wound problems. As a result, only autologous blood was utilised, with no centrifugation, and the work done didn't receive any international recognition. Yamamoto et al. reported the usage of platelet-rich plasma for hemostasis as well as following heart surgery in 1993.[45] In 1996 and 1997, a full description of PRP preparation was published, as was the first clinical trial in the field of oral surgery.[46,47] Coming that, PRP became popular among dental surgeons in the following years.

The expertise gathered from PRP was broadened on several areas of non-healing ulcers by this study group. While scientists agree that PRP has the potential to have a major impact on tissue recovery in musculoskeletal surgery, large randomized controlled trials are needed to confirm its effectiveness. Furthermore, a number of co-factors, for instance the technique of preparation as well the time at which it was administered, route of administration may have a major impact on the

patients.

PRP injections were initially utilised for open heart surgery in 1987, according to reports.[48] PRP was first used in the dental sector over 20 years ago to promote rapid healing of wound in patients suffering from cancer with jaw reconstruction. Platelet rich plasma has been utilised by doctors to help with healing of bone due to spinal injury and recovery of soft tissue after plastic surgery. Platelet rich plasma therapy got widespread attention in initial 2009, where it was revealed that 2 Pittsburgh Steelers players had had PRP treatment for ankle injuries just before their victory in Super Bowl.PRP developed an acceptable, although untested, management for injuries related to sports as a result of the media attention. PRP injections are currently employed in a variety of settings, including general surgery, orthopaedics, cardiology, cosmetic surgery, urology, and faciomaxillary surgery. As a consequence, a number of studies are currently being conducted for better understanding of PRP's mode of action, enhance the management, and formally establish efficacy in the randomised controlled trials.[49]

3.5.3 Platelet physiology and function:

A average blood sample consists of ninty three percent RBCs , six percent of thrombocytes, and one percent of WBCs.[50] In 1842, French surgeon Alfred Donné discovered platelets in the blood.[51] These are tiny discoid cells with a 7- to 10-day lifespan. Platelets are activated and clump together after an injury that produces bleeding, releasing granules which consist of growth factors those trigger the healing and inflammatory progression. Platelets are responsible for connective tissue formation with revascularization, haemostasis and they have been the subject of the majority of research during the last century. [49] Platelet activation result in the release of healing proteins called growth factors, which has only been discovered in

the last two decades. There are many different growth factors that have different functions, when they all work together, they can speed up tissue and wound healingThe PRP treatment philosophy aims to reverse the RBC to platelet ratio via reducing red blood cells to five percent and increase in concentration of platelets with a potent mixture of growth factors increasing to 94 percent. [53] A healthy person's platelet count should be between 150,000 and 450,000 cells per microliter of blood. Most investigations have revealed that rate of tissue healing with PRP can be predicted with atleast rise of 5 times the regular concentration of thrombocytes, however greater concentrations didn't show additional augmentation of healing of wound. The optimal concentration has yet to be determined. The wide range of equipments used for platelet concentration and methodologies employed in diverse research may impact platelet degranulation properties, which can alter clinical outcomes, thus making it difficult to interpret the results.[53]

3.5.4 Growth Factors Present in Platelet Rich Plasma

Platelets which get trapped become activated and release granular content during the time of normal wound healing. Platelet granules has been found to contain mitogenic as well as chemotactic growth factors such as PDGF, VEGF, TGF, IGF, which are vital in wound healing. These factors effects on the cell behaviour and healing of wound are being intensively investigated. PDGF is the growth factor that has received the most attention. Monocytes, mesenchymal stem cells, fibroblasts, neutrophils and osteoblasts can all be chemotactic when PDGF is released into a wound bed. PDGF act as a potent mitogen for smooth muscle cells and fibroblasts and it plays a role in very phase of wound healing i.e angiogenesis, creation of fibrous tissue, and re-epithelialization.

The three isoforms of PDGF are PDGF-AA, PDGF-AB, and PDGF-BB. Several recent studies have documented a direct increase in the growth factors PDGF-AB, VEGF ,EGF , and TGF with increase in platelet numbers, however this study was unable to establish this. Only for PDGF, TGF, and EGF was a direct relationship between number of cells and growth factor produced in another study by the same authors. There was no link discovered between VEGF and IGF.

These factors primarily serve to stabilise tissues damaged during the early phases of repair of tissue by instructing local epithelial and mesenchymal cells to migrate, divide , and enhance synthesis of matrix and collagen, which result in fibrous tissue with scar formation . FGF-2 and VEGF are necessary for encouraging the development of new blood vessels to bring nutrition and predecessor cells to the site of damage; however, neo-vascularization requires other substances. IGF-binding proteins transport IGF, which is a typical factor of plasma. Storage of IGF-1 in the platelets is uncertain, with some studies showing it to be lacking and the majority of literature identifying IGF-1 presence in platelets; yet, IGF-1 in PRP has been discovered in the majority of investigations[50]. PRP warrants fair contemplation as an additional treatment for certain uses due to its role in multiple healing pathways. These factors help tissue regeneration and healing of wound following trauma in physiological settings. The basic idea behind PRP is the combination of such elements in order to generate a greater-than-normal effect.

The decrease of growth factors in chronic wounds has been documented in several experimental clinical trials. Platelet aggregation is critical for skin healing because it releases adhesion molecules , growth factors, and lipids that regulate the migration as well as proliferation, and function of keratinocytes, endothelial cells , and fibroblasts.[54,55] The examination of chronic wound supernatant compared to

supernatant of acute wound demonstrated a significant reduction in growth factors in chronic wounds, with rapid growth factors metabolization secondary to action of proteases detected within the wound. PRP acts as a safe, biocompatible and effective surgical hemostatic agent. PRP improves the tissues hemostatic reaction to injury, speeds epithelial endothelium as well as epidermal regeneration, angiogenesis stimulation, increases synthesis of collagen, promotes healing of the soft tissues, reduces dermal scarring, and reverses the glucocorticoid-induced suppression of healing of wound. PRP has an antibacterial impact due of its high leukocyte concentration.

TABLE 3.1 Various Growth Factors and Their Function

Growth factor	Function
Transforming growth factor-ß (TGF- ß)	Stimulates undifferentiated mesenchymal cell proliferation Regulates endothelial, fibroblastic, and osteoblasticmitogenesis Regulates collagen synthesis and collagenase secretion Regulates mitogenic effects of other growth factors Stimulates endothelial chemotaxis and angiogenesis Inhibits macrophage and lymphocyte proliferation
Fibroblast growth factor (FGF)	Promotes growth and differentiation of chondrocytes and osteoblasts Mitogenic for mesenchymal cells, chondrocytes, and osteoblasts
Platelet-derived growth factor a and b (PDGF)	Mitogenic for mesenchymal cells and osteoblasts Stimulates chemotaxis and mitogenesis in fibroblast, glial, or smooth muscle cells Regulates collagenase secretion and collagen synthesis. Stimulates macrophage and neutrophil chemotaxis

Epidermal growth factor (EGF)	Stimulates endothelial chemotaxis or angiogenesis Regulates collagenase secretion Stimulates epithelial or mesenchymal mitogenesis
Vascular endothelial growth factor (VEGF)	Increases angiogenesis and vessel permeability Stimulates mitogenesis for endothelial cells
Connective tissue growth factor (CTGF)	• Promotes angiogenesis • Cartilage regeneration • Fibrosis and platelet adhesion
Insulin like growth factor (ILGF 1 and 2)	Chemotactic for fibroblasts and stimulates protein synthesis Enhances bone formation
Platelet factor 4 (PF-4)	Stimulate the initial influx of neutrophils into wounds Chemo-attractant for fibroblasts
Interleukin 8 (IL-8)	Pro-inflammatory mediator Recruitment of inflammatory cells Keratinocyte growth factor (KGF) Promote endothelial cell growth, migration, adhesion and survival Angiogenesis

MATERIALS AND METHODS

Source of data were diabetic foot ulcer patients admitted in the department of general surgery at KLES Dr.Prabhakar Kore Charitable Hospital and Medical Research Centre, Nehru Nagar, Belagavi, in the year 2020 between January to December.

In view of covid 19 pandemic and scarcity of patients in elective setting, KAHER extended the period of data collection for 3 months until March 2021.

a) **Study design**: A randomized control trial

b) **Duration of data collection**: 1 year 3 months

c) **Study Period**: January 2020 to March 2021

d) **Study Population**: Patients with chronic ulcers, admitted in general surgical wards

e) **Selection criteria**:

1) **Inclusion criteria**

- Patient in the age group of 18-75 years
- Patient with an ulcer of > 4 weeks duration
- Ulcer size should be less than 100cm^2

2) **Exclusion criteria**

- Patient suffering from cardiovascular disease or on anticoagulant therapy.
- Patients having wound with exposure of tendon or bone.
- Patient with any immunosuppressive disease or on immunosuppressant therapy.
- Ischemic limb

f) **Sampling procedure**:

Computer generated random numbers by SPSS programme are used to assign the type of intervention chosen for the patient that is, group A (PRP injection) and group b (normal saline dressing)

g) **Sample size**:

Total sample size of 60 cases. 30 in group A i.e case and the other 30 in group B i.e control.

Calculation of sample size

$$n = \frac{(Z_{\alpha/2} + Z_{\beta})^2 \ (Sd_1^2 + Sd_2^2)}{(\mu_1 - \mu_2)}$$

For $\alpha = 5\%$

$\beta = 20\%$

$Z_{\alpha/2} = 1.96$

$Z_{\beta} = 0.84$

$$n = \frac{[1.96 + 0.84]^2 \ [(6.88)^2 + (7.8)^2]}{[25.77 - 21.84]^2}$$

$$n = \frac{[2.8]^2 \ [47.33 + 60.84]}{[3.93]^2}$$

$$n = \frac{[7.84] \ [108.17]}{15.44}$$

$$n = \frac{215.78}{15.44} = 54.9 \approx 55$$

So Sample size: 110 (55 in each group)

s per the university guidelines and in view of covid 19 pandemic the sample size was decreased as the amount of patients coming to hospital had decreased resulting in total sample size of 60.

N = sample size

$\mathbf{z_{\alpha/2}}$ = Critical value in a normal distribution. For a 95% confidence interval, the standard value is 1.96. α is called the 'level of significance' or the probability of making a type 1 error

$\mathbf{z_{\beta}}$ = Standard value for a (1- β) of more than 90%. β is probability of making a type 2 error with (1- β) referred to as the ' power of study'

PROCEDURE

Clearance from institutional ethical committee was taken. Patient was explained about the procedure and informed consent was taken.

A 10ml blood was collected from the patient using sterile technique.

The blood collected in vial containing sodium citrate as anticoagulant.

This blood sample was then centrifuged at 1500rpm using centrifuge machine separating it into 2 layers.

The supernatant fluid (around 4-6 ml) is rich in platelets is extracted carefully with the help of pipette.

This autologous PRP is injected with 26 X 1/2 gauge needle syringe 1cm away from edge and 2mm deep of wound subcutaneously using aseptic measures.

The dressing was done using Vaseline gauge and sterile dressing material

This dressing is kept in place for 7 days after which 2nd dose of injection is repeated.

Patient follow up is done weekly for 6 weeks.

With each follow up following parameters are recorded i.e. length, width and depth of wound. This was taken as an indirect measure of rate of wound healing

Outcome was assessed on the basis of comparison of dimensions of ulcer with weekly followup and graphical representation of the same

INVESTIGATIONS

1) Complete blood picture
2) Mini renal profile
3) Liver function test
4) Fasting blood sugar
5) Colour Doppler of lower limb

Outcome

Patients in control and intervention group underwent once a week injection of PRP.They were followed up for a total of 6 weeks. Wound healing in terms of percent reduction in ulcer size over 6 weeks were compared.

Healing of ulcer was observed in terms of decrease in area of wound at the beginning(Day_0) and at the end of study(Day_{42}). During every dressing, any discharge from the ulcers were also noted.

Calculation of wound area:

The dimensions of the ulcer i.e. length, width and area were measured by outlining the ulcer over a sterile transparent film placed over it. This was followed by placing the film over graph paper and counting the number of squares also referred to as 'grid tracing'. The length of the smallest square is 1mm

The measurement of ulcer dimensions on day 0(x) = initial wound area and day 42(y) = final wound area. The reduction in area and percentage reduction in area are calculated as follows:

Wound area as on Day0 = x

Wound area as on Day42 = y

wound area reduction = x-y

% wound area reduction = $\frac{x-y}{x} x100$

RESULTS

This study was conducted at KLE Dr. Prabhakar Kore Hospital and MRC, Belagavi and the findings were tabulated.

During the study interval from, January 2020 - March 2021. 60 patients who had chronic non healing ulcers were randomly distributed into study (PRP) and control (normal saline dressing) group with 30 patients in each group. These groups were studied for the effect of Regular versus PRP dressing on reduction of wound size and total healing of the chronic nonhealing ulcer.

Students paired 't' test was used for analysis

Age Distribution:

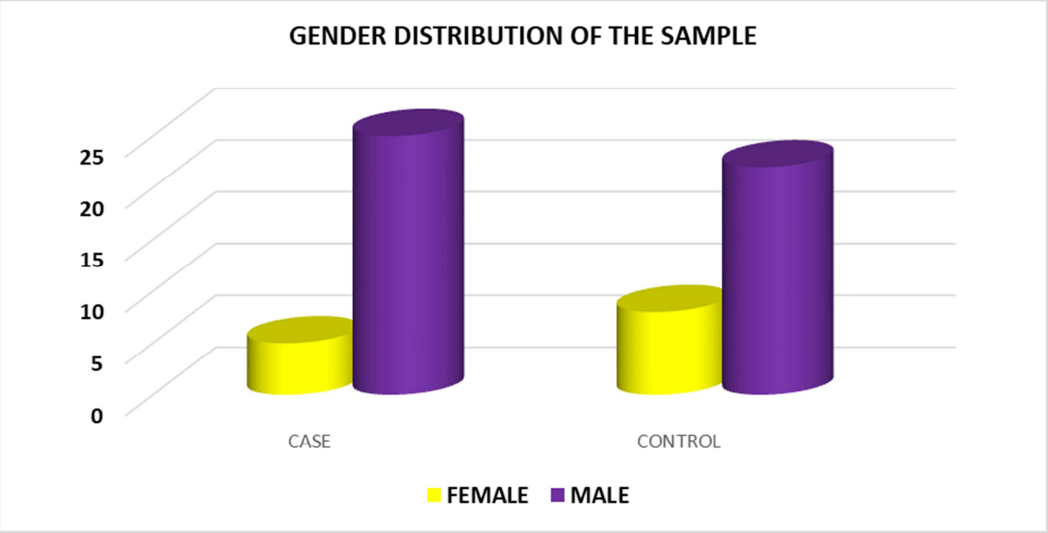
This study included of patients ranging from age group 30 to 80 years. Majority of patients had age between 50 and 60. This signify that the chronic ulcers are more common in older population

AGE	CASE	CONTROL
30 - 39	1	3
40 - 49	7	2
50 - 59	11	11
60 - 69	9	11
≥ 70	2	3
TOTAL	30	30

	CASE				CONTROL					
	MEAN	**S.D.**	**MINIMUM**	**MAXIMUM**	**MEAN**	**S.D.**	**MINIMUM**	**MAXIMUM**	**p VALUE**	**INFERENCE**
AGE	55.67	10.74	32	83	56.87	11.90	30	78	0.6832	NS

Gender Distribution

Out of 60 patients 47 were male and 13 were females which shows male predominance



GENDER	CASE	CONTROL	TOTAL
FEMALE	5	8	13
MALE	25	22	47
TOTAL	30	30	60

Etiology of Chronic Non-Healing Ulcer

In this study maximum patients had diabetic foot ulcers followed by traumatic ulcers and then other etiologies.

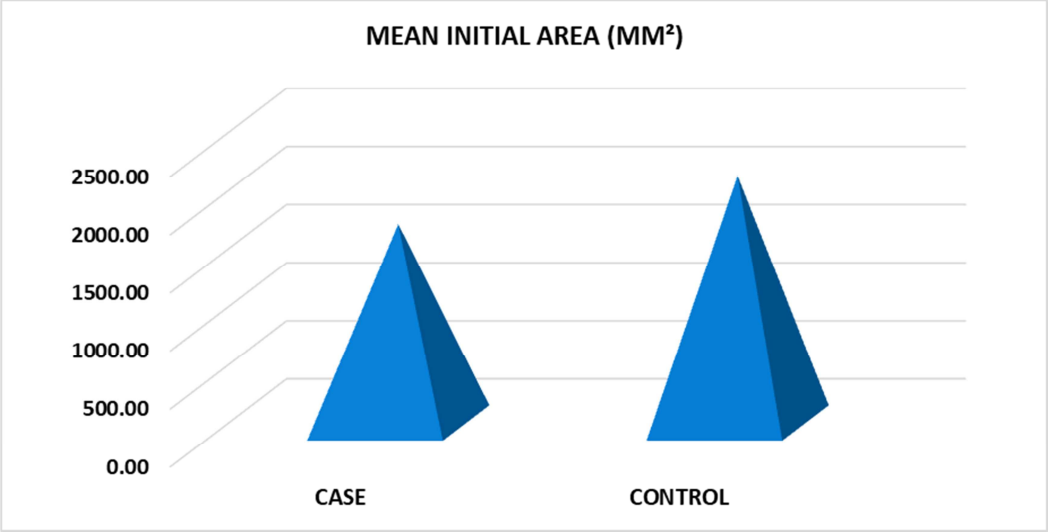
ETIOLOGY	CASE	CONTROL
Diabetic	1	0
Diabetic &PVD	0	1
PVD	2	3
Spontaneous	1	1
Spontaneous & Diabetic	10	11
Traumatic	9	7
Traumatic & Diabetic	6	5
Varicose Ulcer	1	2
TOTAL	30	30

Ulcer size

Ulcer size of all ulcers in case and control groups were compared on day 1 and day 42 with the reduction in the area size of the ulcer. This was tabulated as shown below

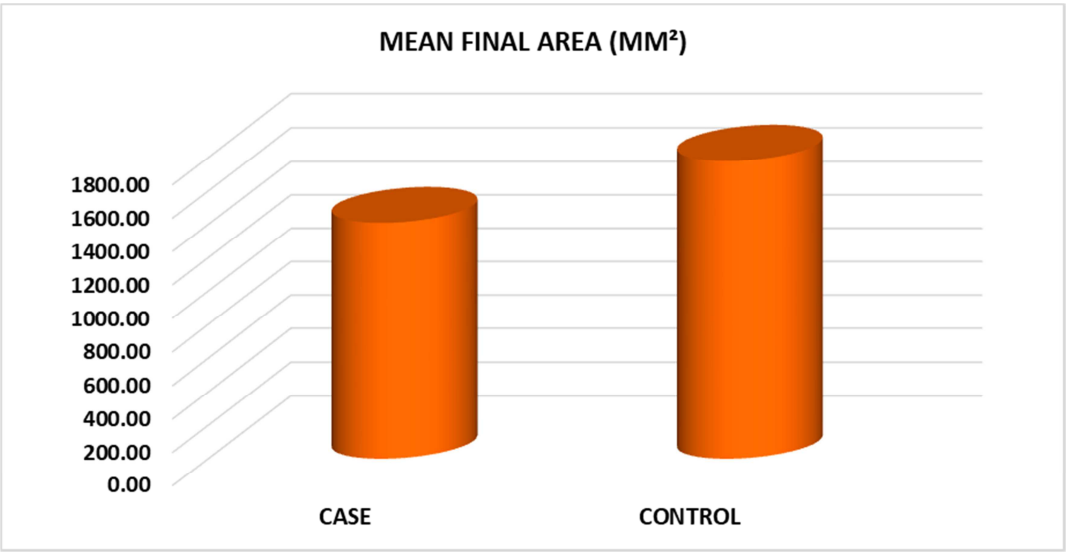
CASE				CONTROL					
MEAN	S.D.	MINIMUM	MAXIMUM	MEAN	S.D.	MINIMUM	MAXIMUM	p VALUE	INFERENCE
1710.93	1362.14	224	6476	2125.50	1656.19	326	7634	0.2940	NS

INITIAL AREA (MM)



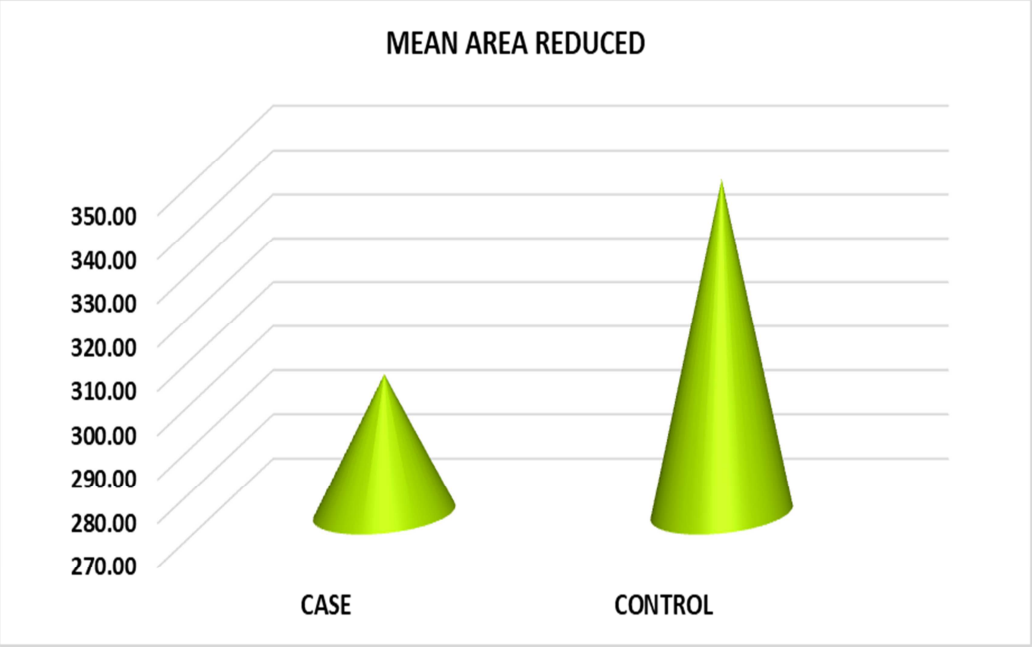
CASE				CONTROL					
MEAN	S.D.	MINIMUM	MAXIMUM	MEAN	S.D.	MINIMUM	MAXIMUM	p VALUE	INFERENCE
1409.50	1110.60	144	4934	1779.70	1398.29	280	6398	0.2608	NS

FINAL AREA (MM2)



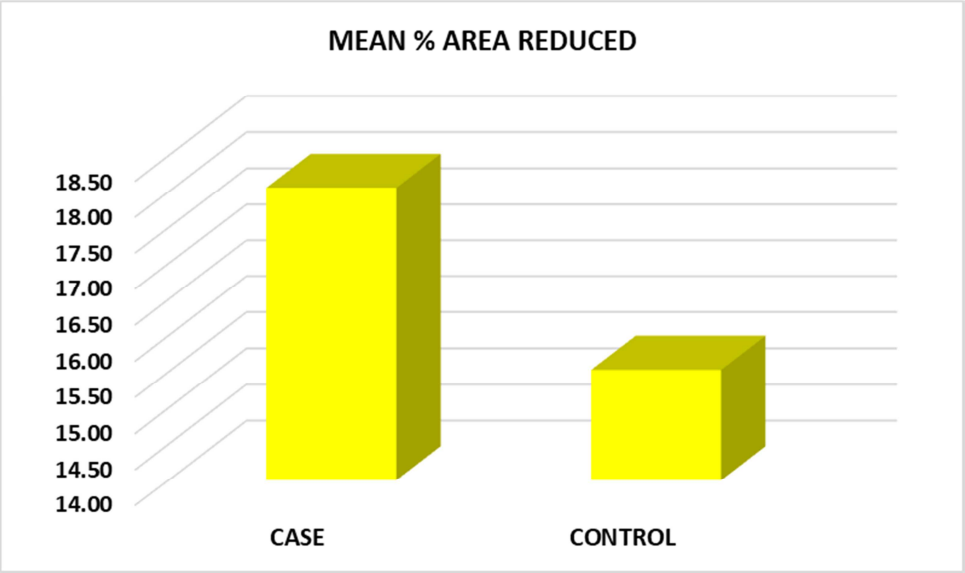
CASE				CONTROL					
MEAN	**S.D.**	**MINIMUM**	**MAXIMUM**	**MEAN**	**S.D.**	**MINIMUM**	**MAXIMUM**	**p VALUE**	**INFERENCE**
301.43	307.51	47	1542	345.80	292.18	46	1236	0.5689	NS

AREA REDUCED



CASE				CONTROL					
MEAN	**S.D.**	**MINIMUM**	**MAXIMUM**	**MEAN**	**S.D.**	**MINIMUM**	**MAXIMUM**	**p VALUE**	**INFERENCE**
18.05	6.91	2.9	35.7	15.53	5.17	6.7	28	0.1140	NS

PERCENTAGE AREA REDUCED



DISCUSSION

Chronic wounds act as a huge public health issue, particularly in impoverished countries such as India, these wounds generally lack the Growth Factors required for the healing process, making them difficult to cure and prone to infection.

Any treatment modality's main goal is to get the wound closed as soon as possible. Debridement, infection control, revascularization of ischemic tissues, and avoiding unwarranted pressure over the wound are all part of the standard treatment. Although skin grafting has demonstrated some success, it is not proficient in providing the essential growth factors which are required for healing process and has high cost. [56,57]

In Cochrane research, the authors evaluated foam dressings, hydrocolloids, low adherent dressings, alginates, and hydrogels under suitable compression bandages and found no significant differences in healing rates.[58] The use of epidermal growth factor (EGF) as a topical treatment for venous non-healing ulcers didn't result in re-epithelialization.[59]

Infected wounds of all kinds are treated with topical silver or silver dressings, however there is no proof that they work. [60] Topically applied platelet concentrate or PRP, a trial tool when used in conjunction with standard care of wound, can be used in enhancing chronic wounds into a condition of proliferation and increase healing by releasing numerous growth factors as well as cytokines in the wound, imitating conditions of natural healing[5,61] Pressure ulcers are currently treated with relief of pressure, surgical debridement, and preserving clean environment of wound, as well as systemic antibiotics. Nowadays Antimicrobial and pain-relieving dressings are now being developed for use beneath compression bandages. [62]

PRP contains a variety of growth factors that aid wound healing. Furthermore, the increased concentration of WBCs in PRP aids in the prevention of infections, and PRP has been utilised to heal wounds since 1985 [63, 64].

The goal of this trial was to see if usage of PRP can lead to treatement of chronic non-healing ulcers which may improve healing rates. Patients who got PRP dressing in the study group and patients who received control dressing had identical baseline features for instance age, sex, and ulcer aetiology.

Knighton et al. demonstrated in 1986 that using ones own platelet factors increased rate of granulation tissue epithelialisation, resulting in thorough healing of chronic nonhealing ulcers . This lead to the first clinical investigation to show that locally active components obtained through autologous blood can aid in the healing of persistent skin ulcers. [65]

During the healing process, the growth factors generated by PRP are important in influencing recruitment of mesenchymal cell, proliferation, and production of extracellular matrix. [66] In vitro, PDGF increases chemotaxis, proliferation, and expression of novel gene in monocytes, fibroblasts, and macrophages, all of which are important for tissue healing. Cell proliferation with protein and collagen synthesis are stimulated by transforming growth factor. They also result in the inhibition of tumor and fibroblastic cell lines. Platelet-derived angiogenesis factor stimulates the formation of new capillaries by causing endothelial cells to migrate. Platelet-derived epithelial cell growth factor is a stimulant for numerous cells, including epithelial cells and fibroblasts, and is partly responsible for the first entry of neutrophils into the wound region. This was recently proposed as the method due to which platelet factors impact the method of angiogenesis and revascularization, supporting the creation of

granulation tissue.[67] Leucocytes, in addition to releasing growth factors, aid wound healing by avoiding infections.

The cellular and molecular induction of a normal response in wound healing, analogous to platelet activation, is assumed to be PRP's mode of action. [68] PRP helps to speed up the healing process in all stages of a wound (most prominent in angiogenesis). [68]

The initial ulcer area (in mm2) was similar in both groups in this study. However, as compared to the control group, individuals treated with PRP had a considerably superior reduction in ulcer size and full closure, and this difference was found to be statistically non significant.

In our case study, 30 patients were assigned to the case group and 30 to the control group, each with one wound/ulcer that was managed with a repeated dose of PRP subcutaneous injections all around the ulcer boundary. Every patient had wound healing with a reduction in size of wound, with a mean reduction of 18.05 in ulcer size during the research period. The findings showed that autologous PRP is effective in treating chronic non-healing ulcers.

Frykberg et al.[69] studied "49 patients which had total of 65 non-healing ulcers and found that the 63 of 65 ulcers reacted with a decrease in area and volume in a mean of 2.8 weeks with 3.2 treatments".

Steenvoorde et al [70] studied "12 patients which had 13 wounds and discovered that seven of the 13 wounds needed more than one application, that lead to an average of 2.2 applications with treatment time of 4.2 weeks".

The result of our study shows that the application of PRP is non inferior to the normal saline dressing in healing of chronic wounds. This signifies that it can be used as an alternate for dressing in chronic ulcers.

Several studies has been done on the usage PRP for the management of non-healing ulcers, with promising results; however, there is currently a scarcity of important technical data regarding the benefits of PRP in therapeutic treatments.

CONCLUSION

In this study comparison between platelet rich plasma and normal saline dressing was done to look for the rate in healing of the chronic ulcer.

The rate of reduction in ulcer area in both control and case group was found out to be non significant which shows the both techniques are equally effective in chronic wound healing. This signifies that the platelet plasma dressing is non inferior to normal saline dressings and can be used as an alternate method.

Chronic wounds ulcers are becoming a global socioeconomic problem. All of the traditional treatments for chronic wound healing/ulcer are time consuming and costly. PRP application requires minimum setup and low cost as compared to the costly preformed preparations available in the market.

If required this can also be used on OPD basis, thus reducing the hospital stay for the patient.

Here we are trying to increase our knowledge on platelet rich plasma and its use in chronic wound healing. In conclusion we can say that it can be used as an alternate method for dressing, although further research and randomised control trial should be done on large patient group to validate the results.

SUMMARY

Our study was conducted from January 2020 to march 2021 in which 30 patients were taken in each case and control group

In this study which included total of 60 patients, age extended from 30 to 80 years out of which the maximum amount of patients were between 50 – 60 years which implies that old age is one of the risk factor for non healing of wound.

Among 60 patients, 47 were males and 13 were females

In our study majority of patients had diabetic foot ulcer followed by traumatic ulcer and other etiologies

In this study mean initial area in case group was 1710 mm^2 and in control group it was 2125 mm^2

In our study the reduction in final area in case group was 18% and in control group was 15.5 %which came out to be non significant. This shows that the PRP dressing was non inferior in terms of healing as compared to normal saline dressing.

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ANNEXURE - I - ETHICAL CLEARANCE

K.L.E. ACADEMY OF HIGHER EDUCATION AND RESEARCH
(Deemed – to- be- University)
Accredited 'A' Grade by NAAC (2nd Cycle) Placed in Category 'A' by MHRD (GoI)
JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)
Website: http//www.jnmc.edu
E-Mail : dome@jnmc.edu
Phone: (+ 91-(0)831 Office : 2472550
Principal: 2471701
Fax No. +91 (0)831 – 2470759

Ref: MDC/DOME/ 282 **Date: 24/12/2019**

To,
REG NO: BH0119011
PG student in Surgery,
J.N.Medical College,
BELAGAVI.

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"COMPARISON OF PLATELET RICH PLASMA INJECTION WITH NORMAL SALINE DRESSING IN RATE OF CHRONIC ULCER HEALING, A ONE YEAR RANDOMISED CONTROL TRIAL",** is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

(Dr. Anita Dalal)
Member Secretary
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

(Dr. Roopa M Bellad)
Chairman,
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

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ANNEXURE – II – CONSENT FORM

INFORMED CONSENT FOR PARTICIPATION IN RESEARCH STUDY

Mr./Mrs. _____ we are requesting you to enroll yourself in study titled "PRP injection vs normal saline dressing in rate of chronic ulcer healing, Randomized Control Trial" conducted by REG NO: BH0119011, Postgraduate in M.S.General Surgery under the guidance of DR._____ Associate professor in Department of General Surgery, J.N. MEDICAL COLLEGE, Belgaum under KLE university, Belagavi.

Respected Sir/ Madam,

We request you to participate in our study. Your participation in the research is voluntary. Your decision to participate in the study or otherwise will not affect the relationship with KLES Prabhakar Kore hospital. If you decided not to participate, you are free to withdraw at any time.

Purpose of study:

The purpose of the study is to find out the effectiveness of prp injection in healing chronic ulcer as compared to normal saline dressings.

Procedure involved:

If you agree to enroll yourself in this study, your detailed history will be taken and you will be clinically examined in detail. Investigations like Hemoglobin, Total Count, Differential Count, Platelet Count, RBS, Blood Urea, Serum Creatinine, Blood Grouping, USG Doppler will be done. The cost of investigations in the above mentioned study will be borne by the principal investigator of the research. A 10ml blood will be collected from the patient using sterile technique. The blood collected in vial containing sodium citrate as anticoagulant. This blood sample will then be centrifuged at 1500rpm using centrifuge machine separating it into

2 layers. The above layer which is rich in platelets is extracted carefully with the help of pipette. PRP is injected with 26 X 1/2 needle syringe 1cm away from edge and 2mm deep of wound subcutaneously using aseptic measures. The dressing is done using Vaseline gauge and sterile dressing material .This dressing is kept in place for 7 days after which 2nd dose of injection is repeated. Patient follow up is done weekly for 6 weeks. With each follow up comparing the rate of healing by dimensions i.e. length, width and depth of wound. Epithelisation of wound and granulation tissue formation will also be assessed.

Risks and Benefits:

There is no increased risk involved in being a part of this study and the complications are those which are normally anticipated as follows-

- Bleeding
- Infection
- Haematoma (rarely)
- Pain at injection site

Type of Study

This study is an interventional study. It involves injecting prp injection around the periphery of the wound.

Participant selection

It includes all patient with chronic ulcers meeting inclusion and exclusion criterias.

Voluntary Participation

Your participation in research is voluntary. It is your choice whether to participate or not. Your decision whether to participate in the study or not will not change present or future health care services offered to you and will not affect your relationship with J.N. Medical College. If you choose not to participate in this study, you will still be offered the routine treatment of chronic ulcer that is given at our hospital. You will continue to receive the routine care at our hospital even if you decline to participate in this study. If you decide to participate you are free to withdraw at any time.

Privacy and Confidentiality:

The only people who will know that you are the research subject will be the members of the research team. No information about you or information provided by you during the 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 Results:

When the results of the 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. Results of the study will be used to compare the two procedures on the points listed above.

Right to refuse or withdraw from study:

You do not have to participate in this research if you do not wish to.You can withdraw at any time from the study. There will be no penalty for withdrawal. Your treatment and care in this hospital will not change irrespective of whether you agree to participate or not. You can be removed from the study if necessary.

Alternative:

You are free to withdraw yourself from this study at any point of time. You will continue to receive the routine care even if you decline to participate in the study. You will be treated for the same even if you have declined from the study. You will be informed about any new information that may affect your decision to participate in the study.

Institutional/sponsor's policy:

In the event of any injury related to the study, treatment will be made available through KLE's Hospital & MRC, Belgaum. There is no compensation or payment for such medical treatment by law. If you are injured you may contact REG NO: BH0119011, Post graduate student, Department of General Surgery, KLE's Hospital& MRC

CONSENT STATEMENT

Mode of communication of consent form: Verbal / Written

Contents:

Self read /Read out by

Investigator

Participant's awareness regarding voluntary withdrawal from study: Yes / No

Investigators decision to remove participants from study: Yes / No

Awareness regarding voluntary participation: Yes/ No

Adequate time given to clarify any doubts about the study or rights to study participant: Yes/ No

In case they have any questions related to the study, in future or in case of study related injury or illness, they can contact REG NO: BH0119011, Department of General Surgery, KLES Hospital and MRC, Belagavi, **DR. _____**., Dept. Of General Surgery, KLES Hospital and MRC, Belagavi.

If they have any queries about their rights as a study subject, they may call **DR. ROOPA BELLAD$_{M.D.}$**, Chairman, and Ethical Committee for Human Subjects Research. Professor, Department of Paediatrics, J. N. Medical College, Belagavi, Phone number-9448113403.

Signature or left thumb print of participant or legally authorized representative

_____Participant's name. _____Participant's signature/thumb print

_____Experimenters' name _____Experimenters' signature

_____ Witness' name

_____Witness' signature

ANNEXURE - III - PROFORMA

PROFORMA OF INDIVIDUAL PATIENT

NAME: AGE:

SEX: RELIGION:

IP NO: DATE OF ADMISSION:

OCCUPATION: DATE OF DISCHARGE

HISTORY –

COMORBIDITIES -

VITALS -

LOCAL EXAMINATION –

CBC

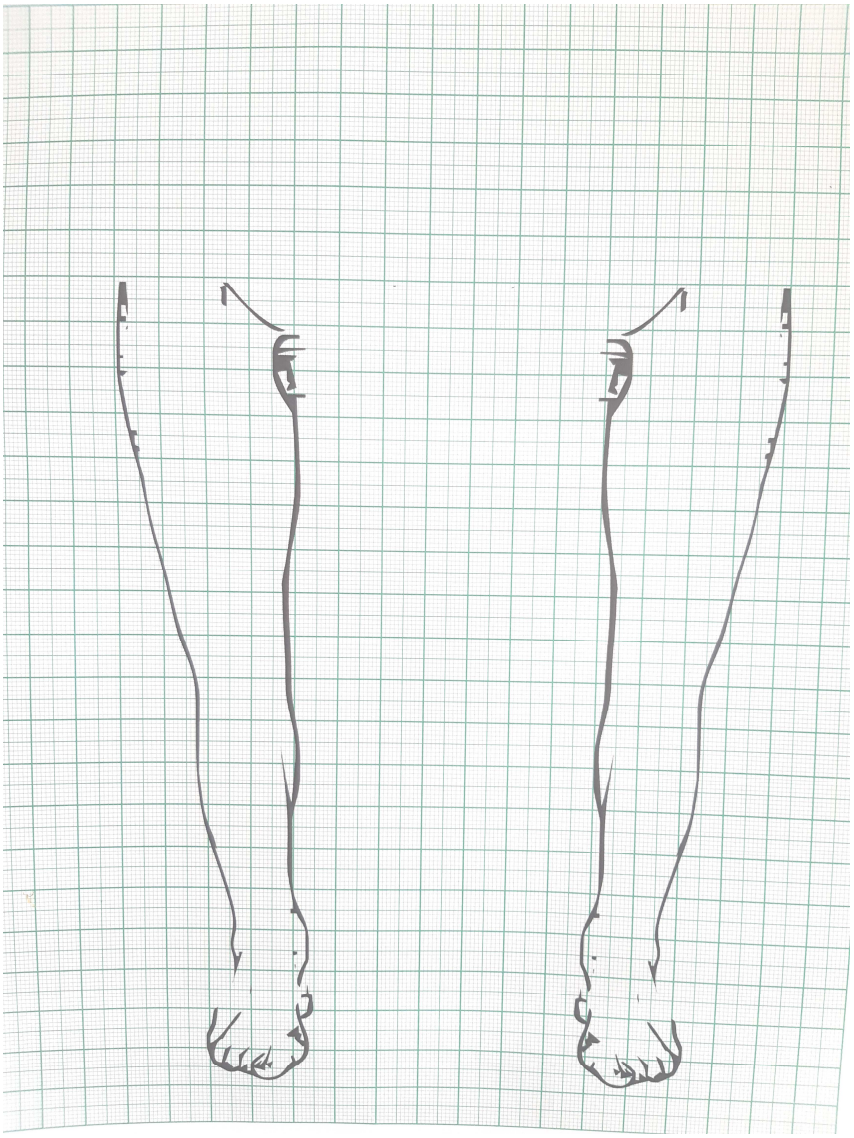
- HB -
- TLC –
- PLATELET COUNT –
- WBC COUNT –

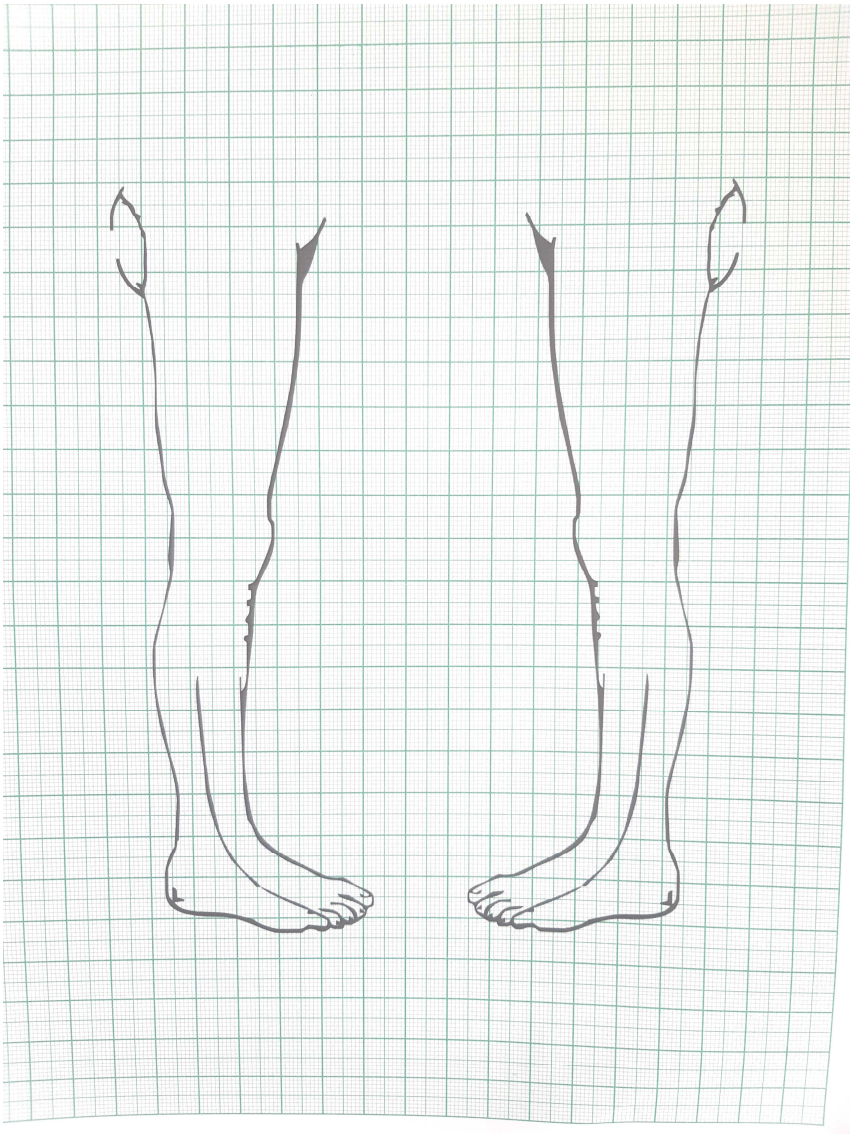
PTINR –

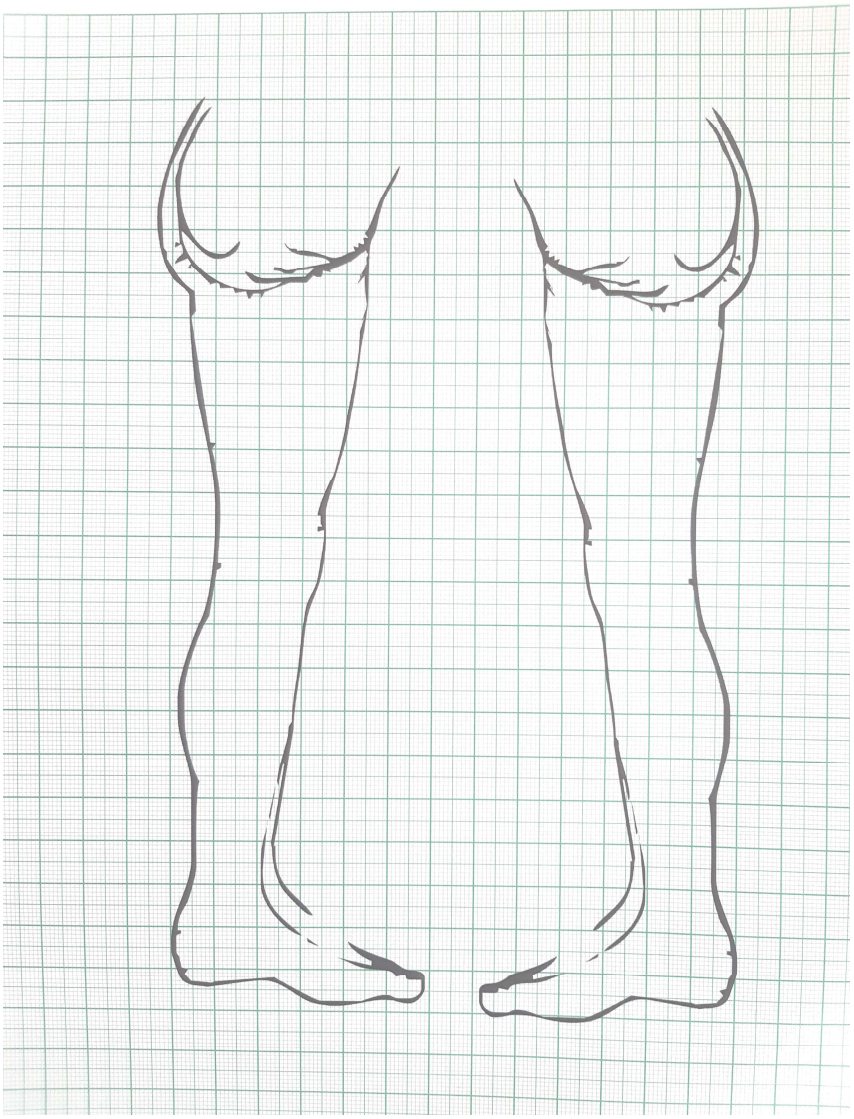
DOPPLER SCAN –

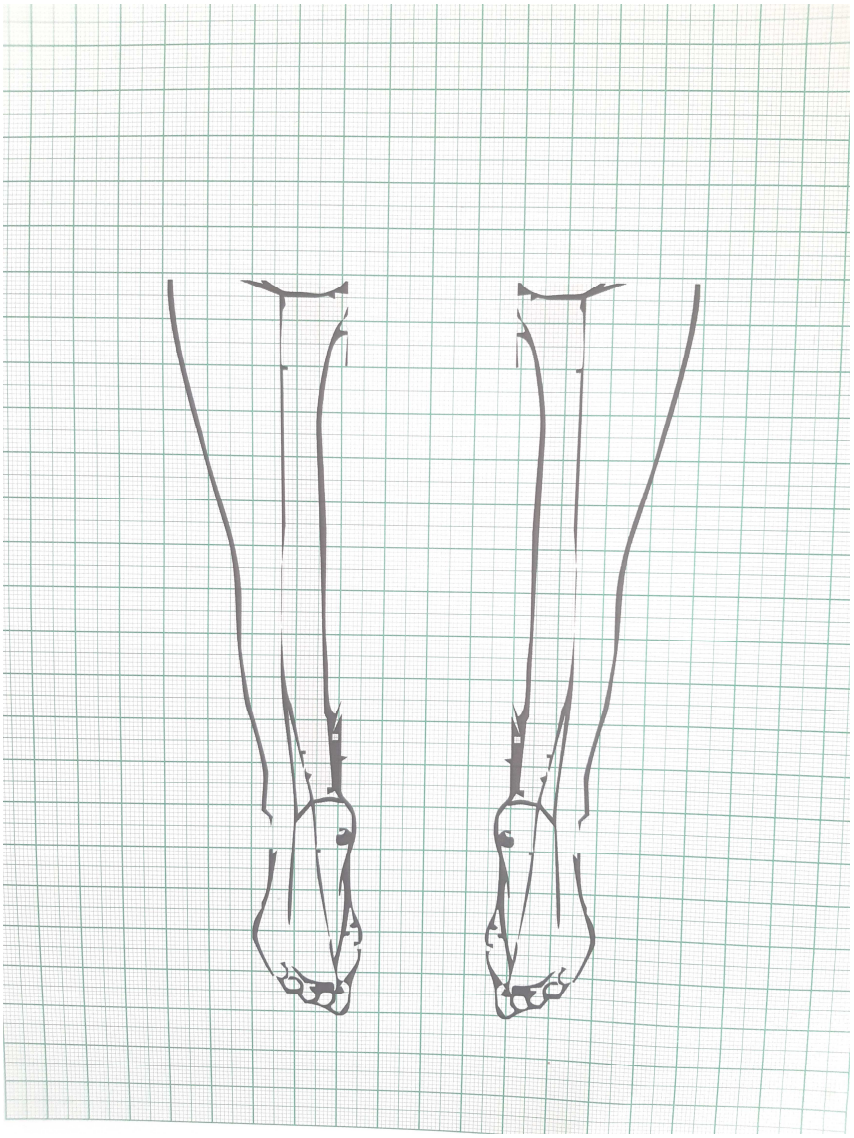
MR

LFT –



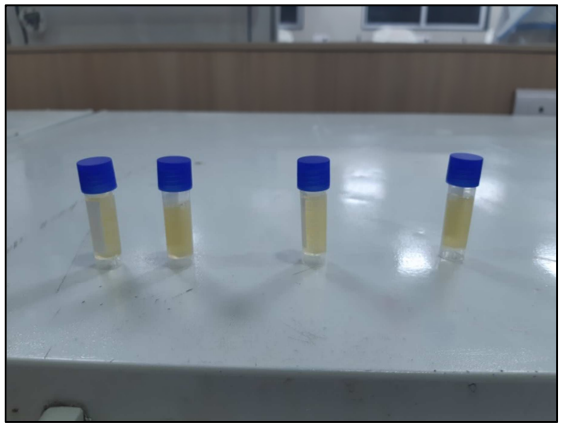




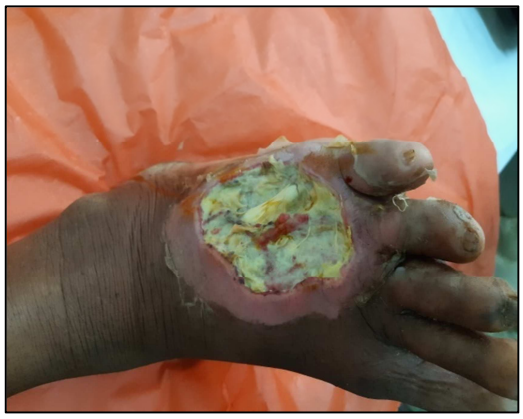


ANNEXURE – IV PHOTOGRAPHS

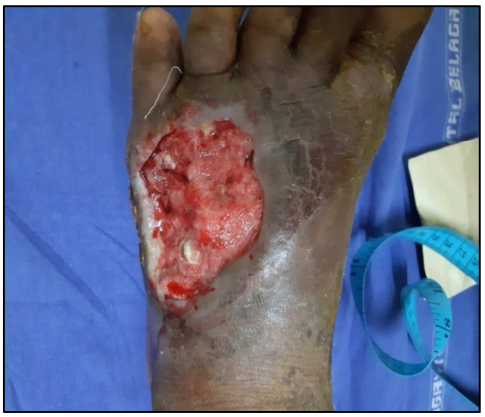
CENTRIFUGE MACHINE



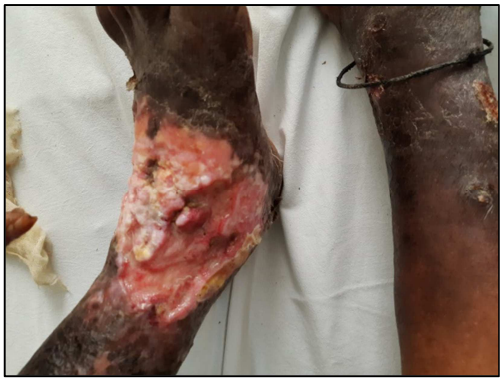
PLATELET RICH PLASMA



DAY 1



DAY 42



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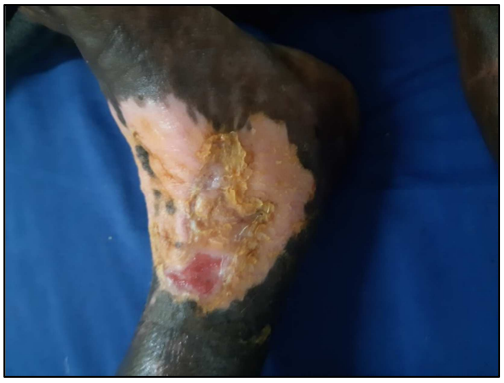

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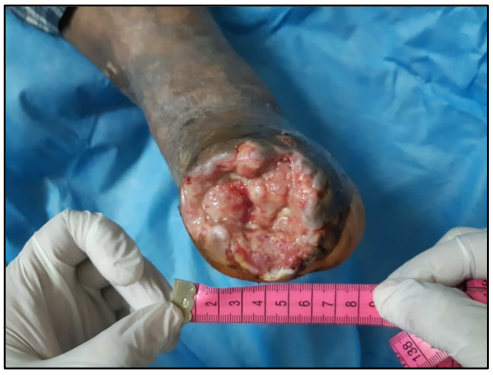

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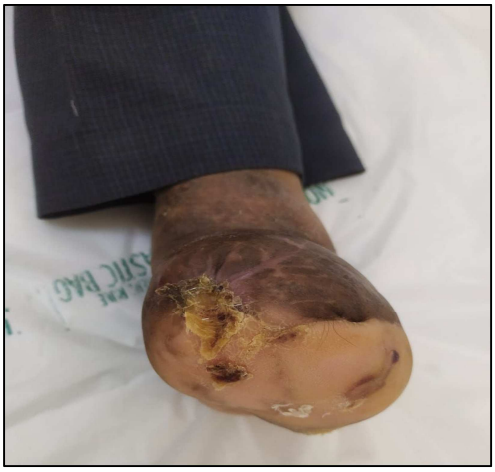

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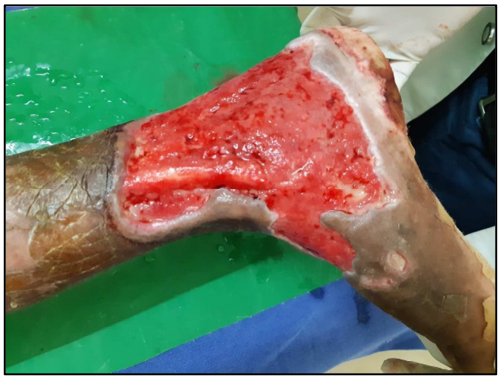

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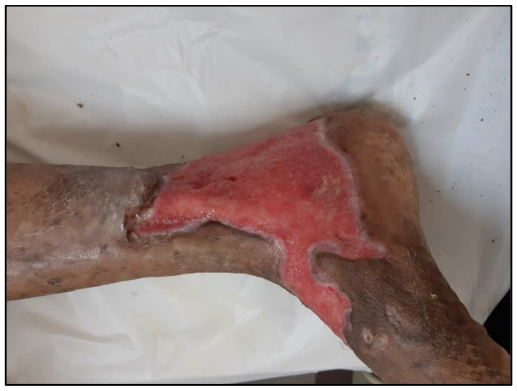

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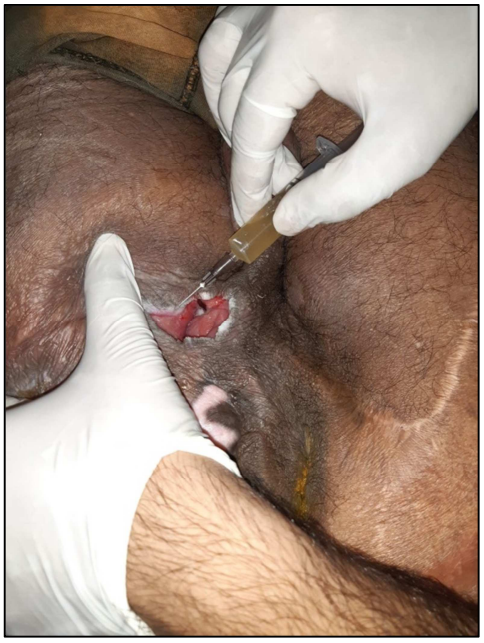

PRP APPLICATION

ANNEXURE – V KEY TO MASTER-CHART

S – Spontaneous

T – Traumatic

D – Diabetic

VU – Varicose ulcer

PVD – Peripheral Vascular Disease

RF – Right Foot

LF – Left Foot

LL – Left Leg

RL – Right Leg

RU – Right Upper Limb

RH – Right Hand

LG – Left Gluteal Region

VV – Varicose Veins

Group A

S. NO	IP NO.	AGE	SEX	SITE	ETIOLOGY	PLATELET COUNT	INITIAL AREA (MM^2)	FINAL AREA (MM^2)	AREA REDUCED	% AREA REDUCED	COLOR DOPPLER
1	996695	51	M	RF	S&D	251000	440	362	78	17.7	N
2	993025	61	M	RF	S&D	310000	3542	3260	282	7.9	N
3	1036595	56	M	LF	S&D	144000	224	144	80	35.7	N
4	1016510	83	M	RF	T&D	458000	2430	1842	588	24.1	N
5	994151	57	M	RL	T&D	420000	1264	986	278	21.9	N
6	1016310	65	M	RF	T&D	246000	1240	1092	148	11.9	N
7	992785	41	M	RF	S&D	384000	1234	964	270	21.8	N
8	1037546	53	M	RF	S&D	271000	640	526	114	17.8	N
9	1028434	64	M	RF	S&D	261000	1286	1142	144	11.1	N
10	1032588	65	M	LF	S&D	310000	460	366	94	20.4	D
11	1031678	50	M	RF	D	420000	626	548	78	12.4	N
12	1020632	44	M	LF	T&D	710000	1210	1056	154	12.7	N
13	1032837	55	F	RF	T	661000	940	748	192	25.6	N
14	1024735	61	F	RL	S&D	730000	1234	986	248	20	N
15	1036388	68	F	LF	T&D	407000	1276	1124	152	11.9	N
16	995781	45	M	RF	S&D	204000	680	579	101	14.8	N
17	1006782	45	M	LF	S&D	409000	3056	2476	580	18.9	N
18	1003412	52	M	RF	VU	156000	1580	1252	328	20.7	VV
19	1039178	47	M	RL	PVD	199000	2478	1769	709	28.6	PVD
20	1025525	55	M	RF	PVD	171000	234	187	47	20	PVD
21	1027289	68	F	RL	T	397000	2538	1798	740	29.1	N
22	1004372	32	F	RU	T	360000	614	532	82	13.3	N
23	992940	62	M	RF	T	328000	6476	4934	1542	23.8	N
24	996347	46	M	LL	T	412000	668	539	129	19.3	N
25	1003712	76	M	LT	T&D	263000	2034	1842	192	9.4	N
26	1033185	47	M	RF	T	377000	4292	3673	619	14.4	N
27	1021120	50	M	RL	T	260000	3026	2937	89	2.9	N
28	992292	53	M	RL	T	347000	2036	1749	287	14.1	N
29	1001691	62	M	LF	S	253000	2084	1698	386	18.5	N
30	1042346	56	M	LF	T	340000	1486	1174	312	20.9	N

Group B

S. NO	IP NO.	AGE	SEX	SITE	ETIOLOGY	PLATELET COUNT	INITIAL AREA (MM^2)	FINAL AREA (MM^2)	AREA REDUCED	% AREA REDUCED	COLOR DOPPLER
1	1031098	62	M	LF	S&D	361000	920	740	180	19.5	N
2	1012803	67	M	LF	S&D	408000	1526	1264	262	17.16	N
3	1042183	60	M	RF	T&D	311000	1246	1162	84	6.7	N
4	1033510	78	M	LF	S&D	223000	658	542	116	17.6	N
5	1031531	60	M	LF	T&D	228000	2564	2324	240	9.3	N
6	1033788	53	M	RF	S&D	218000	326	280	46	14.1	N
7	991633	50	M	LL	S&D	212000	2042	1732	310	15.1	N
8	992785	41	M	RF	S&D	384000	926	858	68	7.3	N
9	1007226	59	M	LF	D&PVD	196000	628	546	82	13	D
10	1004771	76	M	LF	S&D	311000	3624	2857	767	21	N
11	993018	55	M	RF	S&D	263000	3060	2176	884	28	N
12	1000198	34	M	LF	T&D	702000	2876	2368	508	17.6	N
13	1005226	65	M	LF	S&D	187000	946	875	71	7.5	N
14	1012042	53	F	RF	S&D	328000	1286	1053	233	18.1	N
15	994141	71	F	LF	S&D	377000	1640	1297	343	20.9	N
16	994151	57	M	RL	T&D	478000	1678	1347	331	19.7	N
17	990994	66	F	RF	T&D	315000	2586	2267	319	12.3	N
18	1005034	50	M	LF	VU	313000	2036	1726	310	15.2	VV
19	1029257	68	M	LF	PVD	454000	678	584	94	13.8	PVD
20	1018022	66	M	RF	PVD	215000	1286	1034	252	19.5	PVD
21	1002544	59	F	RF	T	313000	1236	1086	150	12.1	N
22	1007191	30	F	RH	T	169000	640	587	53	8.2	N
23	994514	67	M	RL	T	211000	6082	5421	661	10.8	N
24	998322	50	M	LL	T	370000	4286	3461	825	19.2	N
25	1032865	65	M	RL	T	133000	1620	1376	244	15	N
26	1015140	50	M	RL	T	167000	4046	3465	581	14.3	N
27	1015237	61	M	LF	T	320000	7634	6398	1236	16.1	N
28	1037240	54	F	LF	PVD	160000	2056	1764	292	14.2	PVD
29	1000174	34	F	LG	S	230000	1246	1043	203	16.2	N
30	1039353	45	F	RL	VU	355000	2387	1758	629	26.3	VV