"AUTOLOGOUS PLATELET RICH PLASMA V/S CONVENTIONAL SUTURES - A RANDOMISED CONTROL TRIAL TO COMPARE THEIR EFFICACY IN ANCHORING SPLIT SKIN GRAFT ON WOUNDS"

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Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"AUTOLOGOUS PLATELET RICH PLASMA V/S CONVENTIONAL SUTURES – A RANDOMISED CONTROL TRIAL TO COMPARE THEIR EFFICACY IN ANCHORING SPLIT SKIN GRAFT ON WOUNDS ",** is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

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LIST OF ABBREVIATIONS

ACD	-	Anti-coagulant Citrate Dextrose
Alb	-	Albumin
BMI	-	Basal Metabolic Index
CaCl	-	Calcium chloride
CPDA	-	Citrate Phosphate Dextrose Adenine
DM	-	Diabetes mellitus
EDTA	-	Ethylenediaminetetraacetic Acid
EGF	-	Epithelial Growth Factor
FFP	-	Fresh Frozen Plasma
FTSG	-	Full Thickness Skin Graft
Hb	-	Haemoglobin
HIV	-	Human Immunodeficiency Virus
INR	-	Indian National Rupee
KLES	-	Karnataka Lingayat Education Society
PDGF	-	Platelet Derived Growth Factor
PLT	-	Platelet
POD	-	Post-operative Day
PRP	-	Platelet Rich Plasma

PVD - Peripheral Vascular Disease

RCT - Randomised Controlled Trial

RPM - Rotations per Minute

SC - Sodium Citrate

STSG - Split Thickness Skin Graft

TGF - Tumour Growth Factor

TLC - Total Leukocyte Count VEGF- Vascular endothelial growth factor

TP - Total Protein

WBC - White Blood Cells

Yrs - Years

ABSTRACT

AUTOLOGOUS PLATELET RICH PLASMA V/S CONVENTIONAL SUTURES - A RANDOMISED CONTROL TRIAL TO COMPARE THEIR EFFICACY IN ANCHORING SPLIT SKIN GRAFT ON WOUNDS.

Introduction: Split thickness skin grafting (STSG) restores cutaneous cover over wounds thus protecting the underlying surface from contamination, fluid loss and stimulates healing. Autologous platelet rich plasma (PRP) provides a large number of platelets and high concentration of growth factors which promote prompt adherence of skin graft to wound beds, angiogenesis and healing of the wound as well.

AIM: The aim of this study was to assess the immediate and subsequent uptake of STSG with application of PRP over recipient site.

METHODS: 80 wounds of various aetiologies were randomised into intervention group (n=40) which received PRP before placing STSG on recipient site and control group (n=40) in which the graft was fixed in place with sutures alone. Immediate graft adhesion and subsequent graft uptake were compared between the two groups.

RESULTS: The 80 wounds were distributed among 54 patients, 17 of them (31.48%) having multiple wounds. Irrespective of aetiology, and size, 87.5% grafts were adhered by 1st minute of application in the intervention group compared to nil in control group ($p< 0.0001$). Graft uptake was assessed on first three consecutive dressings. There was significantly better graft uptake in intervention group compared to control group [third dressing uptake (98.29%, 93%, $p< 0.0001$) respectively]. Difference in seroma and haematoma formation were also compared between the two groups and found to be not significant. ($p>0.05$)

CONCLUSION: Application of topical PRP facilitates STSG uptake. This study recommends its use especially in patients with a paucity of available area for donor skin harvesting.

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INTRODUCTION

Restoring the cutaneous covering of any wound is essential to protect the underlying surface from contamination, loss of excess fluid and stimulate healing. Split thickness skin graft allows for a large area of wound surface to be covered. The successful uptake of skin graft depends on local vascularity, wound microbiology, haemostasis, and adhesion. [1]

Healing of split skin graft occurs in the steps of anchorage, inosculation and maturation. [2] During wound healing, platelets are activated by contact with collagen. Platelets secrete stored intracellular mediators and cytokines from the cytoplasmic pool and release their α-granule content after aggregation. PRP also stimulates the natural process of fibrin clot formation as it contains Fibrin, fibronectin and vitronectin. The fibrin clot thus formed plays an important role in stimulating angiogenesis as well as serves as a matrix for further cellular proliferation. [3, 4] Also, cell proliferation, angiogenesis and cell migration are stimulated resulting in tissue regeneration. [2]

Anchoring of skin graft on wound bed has been done conventionally using staples or by suturing the margin of the graft to that of the wound or with cyano-acrylate glue or fibrin glue which have been found to be expensive and contribute to an increase in operating time. [1, 5]

The word “autologous” implies that this platelet rich plasma is obtained from the patient himself. It is essential that PRP is thus obtained because homologous plasma can prove to be immunogenic, resulting in adverse inflammatory reaction at

the graft site and pooled or single donor platelet concentrates lose their growth factor content due to the storage. [3]

Platelet Rich Plasma has a platelet concentration of 1,000,000/µL in 5ml of plasma. This not only provides a large number of platelets which undergo activation and release growth factors, but also provide growth factors present in the plasma in a highly concentrated form which promotes prompt adherence of skin graft to wound beds, angiogenesis and healing of the wound as well. There is evidence supporting that PRP reduces pain at the wound site and reduces scarring. [6]

Recent studies have investigated the use of the entity called Autologous Platelet Rich Plasma (PRP) as an anchoring agent for skin grafts over a variety of wounds ranging from burns to chronic non-healing ulcers. [1, 2, 3]

PRP has been proven to be a cost-effective method of anchoring skin grafts that has better long-term outcomes in the form of less scar hypertrophy and better cosmesis.

In this study, the healing properties of platelet rich plasma were employed and applied to study its efficacy in the anchoring of skin graft on wound beds and its contribution to the overall take of the graft. This study was a randomised controlled trial and attempted to further add to the body of knowledge being developed with regard to the use of PRP in skin grafting.

AIMS AND OBJECTIVES

AIM: To compare the efficacy of autologous platelet rich plasma with that of conventional sutures in anchoring split thickness skin grafts on wounds.

OBJECTIVES:

1) To study the immediate uptake of split thickness skin graft when anchored with PRP and conventional mechanical fixation with sutures.

2) To study uptake of skin graft post grafting with PRP application and conventional sutures.

REVIEW OF LITERATURE

A. Wound healing

Wound healing is the process by which the body restores homeostasis. [7]

A priori research posits that wound healing occurs in four phases which are haemostasis, inflammation, proliferation and maturation [8]. The timely initiation and end of these phases determine the success of the process of wound healing. [7, 9]

Upon injury, wounded cell membranes release vasoconstrictors, thromboxane A2. Resultant vasoconstriction exposes collagen to platelets and clotting factors. Platelet degranulation, activation and aggregation form the platelet plug while coagulation cascade gets activated to form fibrin mesh of the platelet plug thus achieving haemostasis. [8] This mesh serves both as a temporary extracellular matrix as well as a host defence against pathogens [7]

The subsequent 48 to 72 hours after injury constitute the inflammatory phase. Primarily, polymorphic nuclear cells begin this process within the first few hours of injury by removing pathogens and debris and subsequently attract macrophages by means of interleukins and tumour necrosis factors. Macrophage recruitment into wound microenvironment occurs in 24- 48 hours. These differentiate into M1, pro-inflammatory and subsequently M2, reparative phenotypes. The latter plays a key role in wound contraction via the myofibroblast proliferation. [7]

Neoangiogenesis, granulation and epithelialisation, are the key events in the subsequent proliferation phase. In 48-96 hours, growth factors and enzymes which are released by macrophages promote the formation of granulation tissue. [8]

Fibroblast growth factor and VEGF produced by damaged macrophages and keratinocytes respectively induce neoangiogenesis. Capillaries sprout from the existing blood vessels. [7] An environment which is high in lactic acid and hypoxic is conducive to angiogenesis. The re-establishment of vascular connections increases local oxygen vessels and facilitates leucocytes in clearing pathogens and slough. [8]

Neoangiogenesis goes hand in hand with re-epithelialisation which takes place under the influence of keratinocyte growth factor secreted by fibroblasts which stimulates the growth and migration of keratinocytes from the edge to the base of the wound, thus forming an early barrier. [8] The migration of keratinocytes from the periphery to the centre of the wound requires the cells to pass through the fibrin clot by dissolving it via plasmin. [7, 10]

Day 8 to 1 year comprises the remodelling phase wherein the type III collagen fibres are replaced by Type I collagen fibres, laid down by fibroblasts. The scar thus formed only regains 80% of initial strength of the skin. During the remodelling phase, the formation and degradation of collagen fibres occur in tandem and the balance between these two processes determines the outcome of the scar which includes hypertrophic scar or wound dehiscence. [8]

With a generalised understanding of wound healing, the specific role played by platelets and the coagulation cascade is summarised. Platelets play a poignant role in haemostasis by forming the platelet plug. Their activation and degranulation result in the coagulation cascade being set into motion and the formation of the fibrin mesh scaffold as well as the release of various growth factors responsible for subsequent stages of wound healing. In the inflammatory phase, neutrophil activity depends on their interaction with platelets and endothelial cells via the P and E selectins to

facilitate the recruitment of further inflammatory cells. Fibroblast growth factor released by platelets and macrophages stimulate the multiplication and migration of keratinocytes in the proliferation phase and is responsible for fibroblast migration into the wound which lays down the collagen rich matrix. [7]

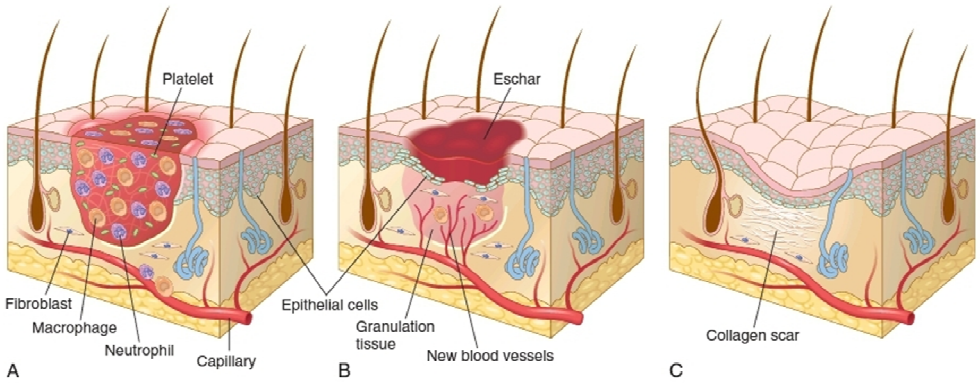


Figure 1 Steps of wound healing:
A: Haemostatic plug and inflammation, B: Proliferation of granulation tissue by vessel growth and proliferating fibroblasts, C: Remodelling to produce the fibrous scar.

Deviation from the timely commencement and cessation of these processes results in abnormal wound healing, causing chronicity. [7] These abnormalities in the sequence of events are brought about by various local and systemic factors including wound microenvironment oxygen levels, infection, remnant foreign body and age, gender, stress, arterial insufficiency, malnourishment and immunocompromised states. [11, 12]

Management of chronic wounds is essential to relieve the financial burden the condition poses on the economy. With an advanced understanding of the factors influencing wound healing, chronic wound management has become a targeted therapeutic process. For example, nutritional support, clearance of infection and restoration of mechanical cover and alteration of wound microenvironment by providing exogenous growth factors, modulating pH and oxygen levels. [7, 11, 13]

Out of the various options now available, our study was based primarily on two principles. Firstly, restoration of the mechanical cover over wounds by skin grafting and secondly, provision of a concentrate of growth factors by means of platelet rich plasma to the grafted wound which would aid in both healing of the wound as well as promote the uptake of the grafted skin over the recipient site. [7, 14]

B. Skin Grafting classification, uptake and loss.

Restoring cutaneous cover over a wound becomes essential in its management as skin not only protects but also maintains homeostasis. [7] The procedure of skin grafting is used to restore skin cover.

Skin grafting has been performed from ancient times and has its description in the Sushruta Samhita and the Ebert Papyrus. Skin grafting remained a common practice till the 2nd century as seen by the works of Celsus and Galen. Thereafter knowledge of this procedure was forgotten till Bunger performed nasal reconstruction by usage of free skin autografting in the early 1800s. [15]

With time various techniques evolved, starting with the “Pinch Graft " of Riverdin, and then the split thickness skin graft (STSG), developed by Ollier, Thiersch and Janzekovic. Classification of STSG is done on the basis of thickness as thin (0.15-0.3 mm), intermediate (0.3-0.45 mm) and thick (0.45-0.6 mm). [15]

Wolfe described the other common type of skin graft in 1875, the Full Thickness Skin Graft (FTSG), referring to the entire thickness of the dermis being included in the graft. These grafts have been found ideal in cases of burns scar deformity correction due to good plasticity, mobility, elasticity, colour matching and less scar formation, especially those over the face and hands. However, there are

disadvantages to this technique such as contractility and scar formation after graft take. [15]

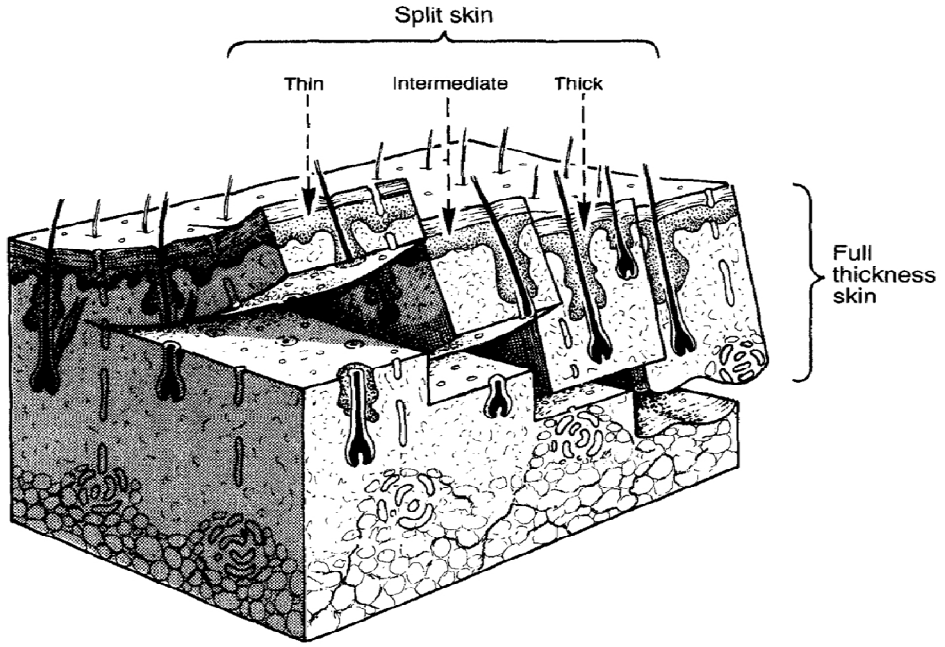


Figure 2: Thickness of various types of skin grafts and their constituents.

Grafts are tissues which are transferred from their original site sans their blood supply and must develop new blood vessels at the recipient site for their survival. [16]

Thus, understanding the blood supply of skin becomes imperative to perform a successful skin graft. (figure-3) portrays that direct subcutaneous arteries and branches of musculo-cutaneous and fascio-cutaneous artery run in the subcutaneous plane form where capillaries arise to supply the dermis and epidermis by forming deep subdermal, mid-dermal venous plexus and subpapillary plexus. [16] Depending on the thickness of the skin graft (vide infra), different levels of these plexuses get included in the grafted skin. [16]

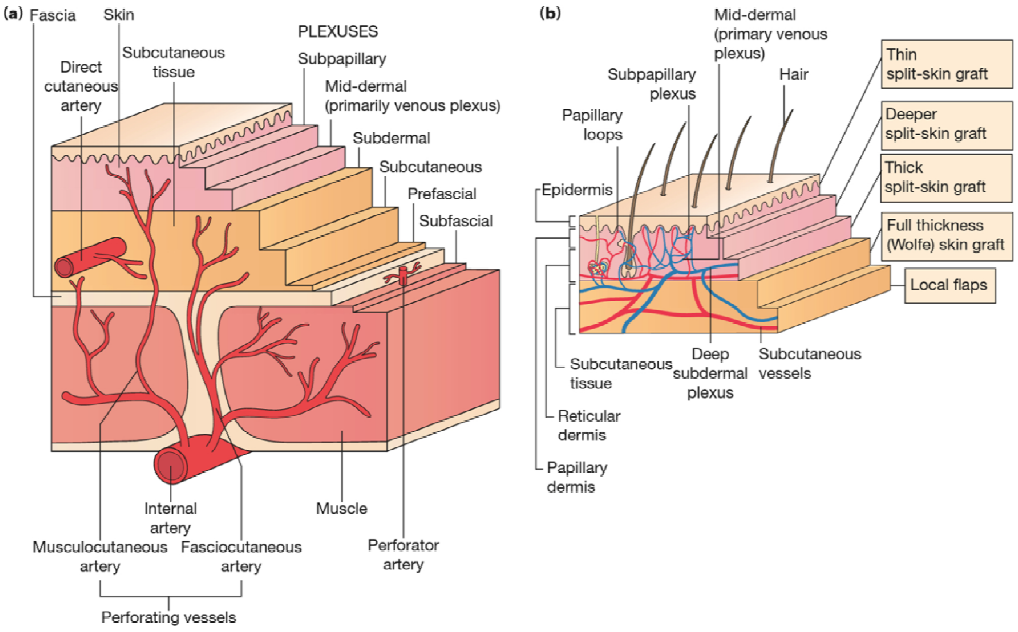


Figure 3: Anatomy of Skin and dermal vasculature

Once placed over the recipient ulcer, the skin graft adheres to it by fibrin strands. [17] During this phase, the graft receives nutrition by means of serum imbibition from the bed. [18] Fibrin strands formed initially break down within 48 hours, coinciding with revascularisation. This occurs by means of outgrowing capillary buds from the recipient site to the dermal surface of the graft. The fibrin strands get replaced by collagen, thereby strengthening the adhesion process in the next 4 days. [17]

These steps can be summarised into four stages of graft take namely adhesion, imbibition, inosculation and neo-angiogenesis. [16, 17, 18] Out of these, the paradigm role remains that of fibrin tissue fixation and vascularisation. [17]. A final stage of maturation is also described akin to the final stage of wound healing which lasts for several months to years after integration of graft into the wound bed. [18]

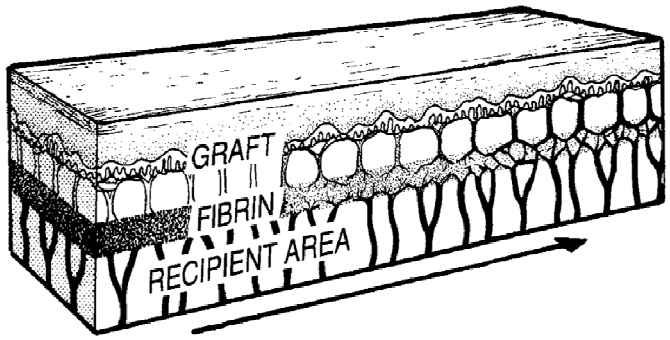


Figure 4: Skin graft adhesion and take over time with initial adhesion with fibrin strands and subsequent neoangiogenesis

The conditions ideal for graft uptake are rapid revascularisation and a short distance across which capillaries are required to grow. Thus any complication causing separation of graft from the bed like hematoma and seroma disrupt capillary connections between graft and bed. Also a mobile graft on recipient bed results in shearing forces displacing it thereby hampering graft take. [17]

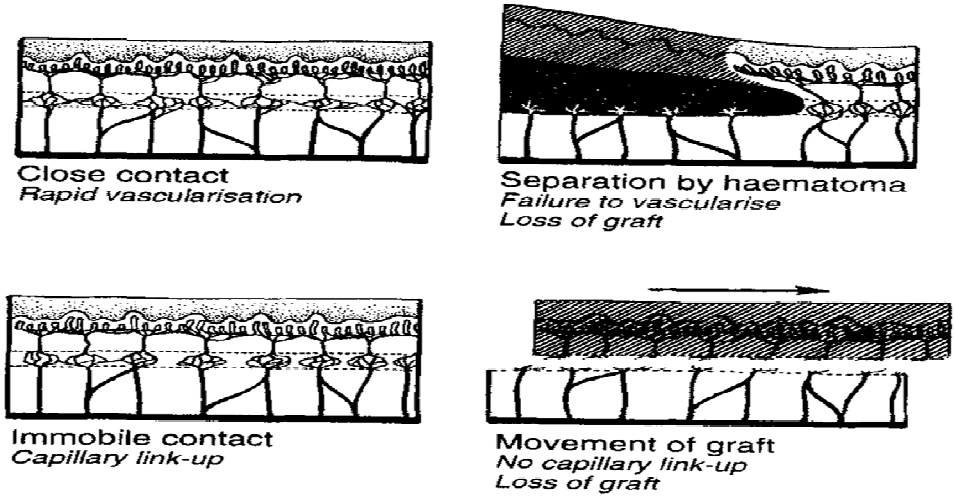


Figure 5: Effect of movement of graft on recipient site and need for immobile contact
Effect of underlying haematoma and seroma causing graft separation from the recipient bed.

In order to ensure the skin graft takes on the recipient site, it is essential that the local and systemic factors are favourable. Raised BMI, ischaemic heart disease, peripheral vascular disease, diabetes mellitus and immunocompromised states hamper skin graft uptake. [19]

Local factors that are required for graft uptake include a flat, vascular, red well-granulated wound bed which does not bleed unduly on touch and is free of biofilm. This surface should be free of pathogens which are known to cause a breakdown of skin like β haemolytic *Streptococcus.* Commensals such as *Staphylococcus aureus, S epidermidis* and *Pseudomonas* are expected to be present over the surface, but do not significantly change the uptake of the graft. [17]

Also, there must be complete haemostasis at the time of graft placement. Any blot clots present below the graft must be flushed out before the dressing is closed after placing and suturing graft in place. [17]

Skin grafts are usually sutured in place along the periphery, sometimes using the quilting method across the entire graft surface where immobilisation will be impossible. It is combined with fenestration of the graft to ensure that the bleeding that occurs from the blind insertion of the needle during quilting is drained. The fixation of graft plays an important role in ensuring its contact with the bed and eliminating the mobility of the graft on the bed. [17] Other methods of anchoring the graft include staples, fibrin glue, and cyano-acrylate glue. [2, 20]

Dressings applied over the graft in order to hold it in place include bolus dressing, pressure dressing, tie over dressing. These are suited for areas which are easy to immobilise. The bolus dressing serves to achieve haemostasis as well.

Pressure dressing applied with crepe bandage additionally brings down the surrounding oedema. Tie over dressing is more suitable over areas where localised pressure dressings are not suitable. [17]

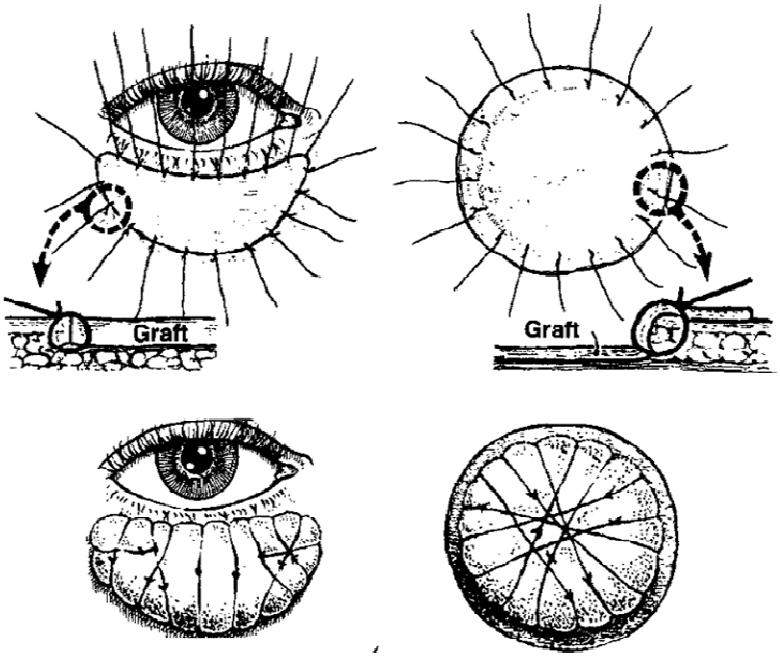


Figure 6: Tie over bolus dressing in the pressure method of skin grafting

Platelet Rich Plasma provides a large concentration of fibrin which facilitates native fibrin strand action in adhesion of graft. Also, the growth factors provided in the same, accelerate neoangiogenesis and collagen deposition and improves STSG uptake. [2, 17]

C. Platelet Rich Plasma- definition, preparation, preliminary work

Platelet Rich Plasma refers to a concentration of autologous human platelets in a small volume of plasma. This concentration is of the tune of 5 times the baseline value of platelet count in the therapeutic form of PRP. [3]

PRP supplies the growth factors and plasma proteins essential for initiation of wound healing at the site of action. These include- Platelet Derived Growth Factor (PDGFαα, PDGFββ, and PDGFαβ), Tumour Growth Factor (TGFβ1, TGFβ2), Vascular Endothelial Growth Factor, and Epithelial Growth Factor. Proteins released in the plasma include fibrinogen, fibronectin, and osteocalcin. [3, 21]

For PRP to remain effective, it has to be extracted in anticoagulated tubes, and centrifuged such that platelets remain viable. For this very reason, homogenous, lyophilised, stored platelet concentrates do not serve the purpose as the platelets are no longer viable and become antigenic thus causing more harm than good. [3]

The extracted PRP should be used within 10 minutes of clot initiation as the secretion of growth factors starts within 10 minutes of clot formation. Platelets have been seen to remain viable for up to 8 hours once developed and stay sterile if extracted and placed over a sterile table. [3]

Extraction of PRP follows various protocols. Most commonly, blood drawn in an anticoagulated bulb is centrifuged and separated into three layers viz blood and WBC, PRP and Platelet poor plasma from bottom up. [22]

Amaral et al studied the outcome of extracting blood in vacutainers with different anticoagulants [sodium citrate (SC), ethylenediaminetetraacetic acid (EDTA) and anticoagulant citrate dextrose (ACD)] for preparation of PRP. It has been found that sodium citrate had the maximum platelet extraction from PRP and nil changes in morphology of the platelets. [23]

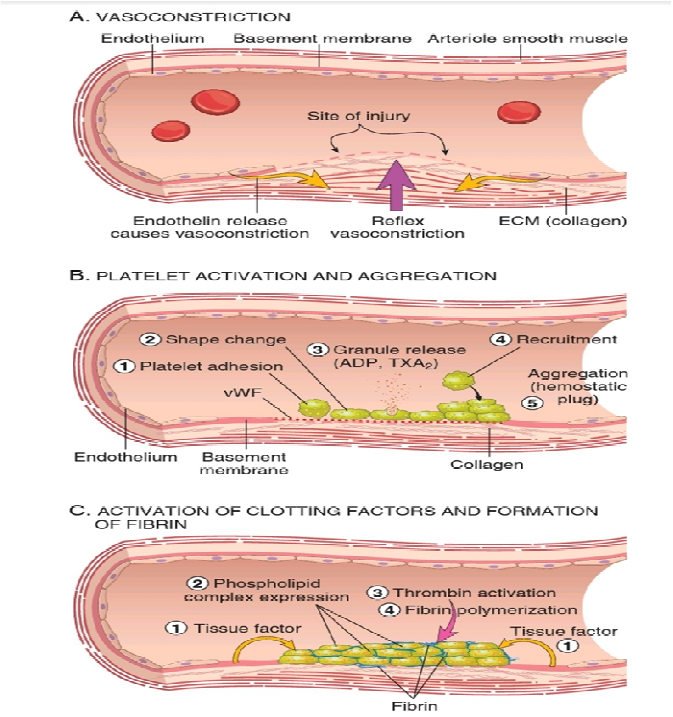
Mehta et al discussed the advantages and disadvantages of centrifugation of the blood sample used to extract PRP wherein, although commonly used, there remain the

drawbacks of platelet disintegration, premature release of growth factor and resultant loss of regenerative potential of the PRP. He also suggests that a cell sensitive filtration device is less traumatic, and has a 40% faster rate of extraction with similar quality of PRP. [24]

There exists great heterogeneity in methods of extraction, activation, dose, formulation and treatment regimens of PRP for example single and double spin centrifugation; CaCl, Thrombin, and photo-activated PRP; non-activated PRP, PRP gels, stored and fresh PRP. [22]

PRP has the following mechanism of action-

Tissue damage, surgical stimuli expose platelets to extracellular matrix proteins which set into motion the steps of platelet plug formation. The normal process of formation of fibrin strands from the fibrinogen content in PRP occurs like it does during in vivo haemostasis. During this process, Alpha granules, which contain the preformed growth factors, degranulate and release them. This release occurs actively from 10 minutes of clot formation and is 95% completed by 1 hour. [24] Cell membranes of the recipient surface bind to the growth factors via receptor proteins which unlock expression of genes responsible for cellular proliferation, osteoid production, matrix formation and collagen deposition. Such receptors are found in mesenchymal stem cells, fibroblasts, epidermal cells, osteoblasts and endothelial cells. [3]



A: Vasoconstriction caused by injury to vessel wall/ endothelium, ECM collagen exposed.

B: Platelet activation and aggregation- platelet adhesion, change in shape, release of granules and factors, recruitment of platelets, plug formation

C: Fibrin polymerisation and formation of fibrin strand mesh.

Figure 7: Steps in haemostatic platelet plug formation and fibrin activation

Hashemi et al found that PRP enhanced proliferation and migration of fibroblasts, without inducing morphological changes in them and was thus safe to be used on wounds for management. [25]

Carter et al performed a meta-analysis on PRP use in various wound management. Multiple studies found that PRP decreases the amount of exudate, and decreases formation of haematoma, which has an overall effect of decreasing the rate of infection. [26]

PRP is also a carrier of anti-inflammatory mediators, thus bringing down inflammation in surrounding tissue and reducing pain. [22]

This forms the basis of the diverse utility of PRP.

D. Platelet Rich Plasma Utility

PRP was first used in oro-maxillofacial surgery as addition to cancellous bone in mandibular reconstruction. [24, 27]

PRP's property of augmentation of the natural healing process has been used in orthopaedic conditions affecting muscles, tendons and bones, such as in lateral epicondylitis, osteoarthritis and plantar fasciitis. [28]

PRP injections for lateral epicondylitis, when compared to corticosteroid injections, have significant improvement in symptoms, as described in case series by Mishra et al. [24, 29]

Its osteo-promotive nature is the basis of its use in combination with autogenous bone graft in demineralised bone for doubling the rate of bone formation. The PDGF concentrate in PRP promotes angiogenesis, osteoblast differentiation and maturation and matrix production. This way PRP has found its use in orthopaedics. [24]

PRP is being extensively and successfully used in regenerative and aesthetic medicine. [28, 30] Use of PRP for rejuvenating the face and hands after ageing changes set in have been described by Hsu et al, Cameli, Charles-de- Sals. [28, 31, 32, 33]

PRP provides the growth factors which get depleted in the skin and promotes synthesis of extracellular matrix and adipose tissue. [28]. PRP combined with adipose tissue in breast augmentation has the added benefit of the former. Growth factors present in PRP negates the adverse effects caused by adipose tissue injection which include insensitivity of breast and nipple areolar complex, fat resorption. [28, 34, 35, 36]

Androgenic alopecia and alopecia areata treatment is based on PRP injections in various forms, with and without activators, as preservative solution for hair transplantation with great success. [22]

Therapeutic efficacy of PRP has been assessed and proven across various fields such as dermatology, orthopaedics, paediatric and cardiac surgery and urology along with chronic wound management, other than those described in this section. [28, 37]

In the subsequent section, a focused description on the role of PRP in skin grafting has been discussed.

E. PRP use in skin grafting

Platelet gel, a mixture of cryoprecipitate and platelet rich plasma, was used to fix graft over post burns raw area by Adly et al. a single raw area was divided equally and half of it was used as control where platelet gel was not applied before fixing the graft. 50 wounds were included in the study, and healing with platelet gel was significantly higher than the control group (0.94±0.08 and 0.89±0.08 respectively, $p< 0.001$) **(2011)** [38]

Waiker et al. performed an RCT on 200 patients with wounds of various aetiologies by applying PRP extracted in CDP-A lined vacutainers, spun at 1000 RPM for 5 min at recipient site in 100 intervention group patients, while fixing the graft by conventional sutures and staplers in the control group. Similar to previous studies they found significantly better outcomes in the PRP group in terms of graft oedema, haematoma, first dressing change, discharge, pain as well as scar hypertrophy as 3 months follow up. (**2015**) [1]

Dhua et al performed a RCT involving 40 patients with wounds of different aetiologies, including those with comorbidities like DM and hypertension. PRP was extracted in the immediate preoperative period in vacutainers containing citrate phosphate, dextrose-adenine as anticoagulant and underwent double centrifugation. First hard spin was at the rate of 3000 rpm for 20 minutes followed by a soft spin of the buffy coat and plasma layer at 1000 rpm for 10 minutes. Platelet poor supernatant was discarded and platelet rich plasma was used topically over the recipient site before placing the skin graft in the intervention group while the graft was sutured in place in the control group. Immediate graft uptake was assessed on table by movement of graft over the wound bed, % loss of graft, hematoma, graft oedema, frequency of change of dressing and discharge from recipient site were compared between the two groups and found to be significantly better in the PRP group. **(2017)** [2]

Similar study performed by Thimmanahalli et al included 60 patients with wounds of various aetiologies ready for graft uptake with 30 in intervention and control groups. Blood was drawn in CDPA lined vacutainers. Double spin technique was used to prepare PRP. Sutures were used where necessary in the intervention group as well. Immediate graft uptake of graft was observed in 100% of patients in PRP group. Only 2% of patients in the PRP group required re-grafting while 33.3% in the control group required re-grafting. They also found a significant haemostatic effect of PRP as seen by only 3.3 % of patients having haematoma in the PRP group as compared to 23.3% of patients in the control group. (**2019)** [39]

Fang et al performed a retrospective study studying the differences in mean wound healing time and scarring in two sets of 30 patients which were those that received PRP gel dressing at the donor site and those that received regular petrolatum dressing. They found significantly lower Vancouver Scar score in the PRP group compared to the control group along with a faster wound healing rate and post-operative pain. (**2019)** [6]

Vaheb et al studied the effect of PRP over the donor site of split thickness skin graft. The two groups of patients received petroleum gauze and wet dressing with PRP gel applied in the intervention group along with the same. Donor site in the intervention group was significantly faster in the intervention group (11.80, 16.30 days, $p<0.001$) as well as pain levels between the two groups. (**2020**) [40]

A randomised controlled trial carried out by Gupta et al divided 200 burn patients into two groups, one which received a thin layer of PRP topically just before placing the skin graft and the other where the graft was fixed with sutures. The former group also received sutures where necessary. PRP was extracted by drawing whole blood in ACD containing vacutainers and spinning it at 3500 RPM for 10 minutes. All patients in the intervention group showed significantly better graft uptake rate compared to the control group (88.86 v/s 42.5, $p<0.04$) along with lesser graft haematoma and graft loss. They also mention the difference in cost of treatment, PRP costing the patients 200- 300 INR versus 2000- 3000 INR for conventional staples and sutures. (**2020**) [41]

A meta-analysis compiling many similar works on PRP and skin grafting by Tyagi et al have drawn the conclusions that the pooled odds ratio for graft loss was 0.15 in the PRP group and that of haematoma formation was 0.21, both findings being statistically significant. They stress on the fact that the methods of preparation of PRP remain varied and stress on the need for uniform guidelines for preparing PRP.[42]

Chen et al performed another meta-analysis involving 11 studies and a total of 910 cases of skin grafts. PRP group was found to have significantly higher rate of skin graft take (mean difference= 5.47%, $p<0.0001$), lesser number of skin loss (risk ratio= 0.26, $p=0.0004$) and fewer haematoma formation (relative risk= 0.24, $p<0.0006$). They also found that there was no significant difference in the complication between the two groups thus signifying that PRP use does not add to harm that can be caused while skin grafting and can be used safely as an adjuvant. [43]

To summarise the previous studies, there are multiple ways of extracting PRP. Irrespective of methods of extraction, PRP increased the rate of graft uptake, reduced haematoma, graft oedema, discharge, pain at the site of application, and scar hypertrophy.

METHODS AND METHODOLOGY

a) Study Design: Randomised Control Trial

b) Study Period: January, 2021-December, 2021

c) Study Population: Patients with wounds requiring split thickness skin grafting who got admitted under General Surgery and Plastic Surgery Departments of KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi.

d) Inclusion Criteria-

- Patients above the age of 18 years with wounds prepared for skin grafting.
- Those patients who gave consent for the intervention.
- Patients on antiplatelet drugs were also included.

e) Exclusion Criteria-

- Patients with HIV, Hepatitis
- Malignancy,
- Coagulation disorders
- Diabetes mellitus
- Thrombocytopenia (<1.5L/cumm)

f) Sample Size: Total of 80 wounds divided as 40 in control and 40 in study groups.

g) Sampling Procedure:

Sample size calculation: The minimum sample size formula based on mean and standard deviation is

$$n = \frac{(z_\alpha + z_\beta)^2 (s_1^2 + s_2^2)}{(\bar{X}_1 - \bar{X}_2)^2}$$

Zα is linked with the level of significance and zβ is linked with the power of the test. For 5% level of the significance zα =1.96 and zβ =0.84 for 80% power of the test.

These values correspond to the mean percentage of significant graft loss in PRP group and control group in the study conducted by Osama et al. X_1 is the mean of the PRP group (0.94) and X_2 is the mean of the Control group (0.89).

s1 is the standard deviation of the PRP group (0.08) and s2 is the standard deviation of the second group (0.08). [38]

With these values the sample size obtained was 40. There were two groups with 40 cases in each group. Computer generated Random numbers by SPSS programme were used to assign the type of intervention chosen for the patient that is study group receiving PRP and control group sutures.

h) Instruments Used for Data Collection: Patients' demographic details were obtained in person and from case sheets. Dimensions of the ulcer and grafted area were calculated using flexible sterile scales and via mobile based application, Imito Measure.

Selection Criteria

In this study the wounds were considered as the population and not the patients per se. Patients with wounds of any aetiology that required split thickness skin grafting were resuscitated, and adequately prepared for receiving the graft. Patients having two or more non-contiguous wounds, of comparable dimensions were allocated to both control and intervention groups. In case of solitary ulcers, different patients will be recruited as study and control group.

Methodology:

Patients fitting into selection criteria were recruited into the study after Institutional Ethics Committee clearance and written informed consent from the patients was obtained.

Antiplatelet drugs (Aspirin) were stopped about 72- 48 hours before the surgery and resumed immediately after the surgery.

The amount of PRP required for every 100cm² area of wound is 5 ml as per prior studies. [1,2]

PRP Preparation: Size of the recipient site was determined prior to the operation. 3 ml of blood yields approximately 2.5ml of plasma after being centrifuged at 3500 rpm for 10 minutes. The quantity of blood thus determined was drawn from peripheral veins of the patients in immediate preoperative period or just before the start of procedure, under aseptic conditions and by closed technique into Sodium citrate bulbs. These were rotated at 3500 rpm for 20 minutes in REMI 720 Centrifuge at the Hospital blood bank. The buffy coat containing WBC and platelets and the top

layer of plasma were drawn into sterile syringes using 18 G needles and kept ready for application over the wound bed. [1,2]

Study Group

PRP was applied over the wound as a thin layer just before the skin graft was placed. Immediate uptake of the graft was assessed by moving the surgeon's finger over the graft. Sutures were applied where necessary to prevent lateral mobility of the graft over the wound. The wound was then covered with a non-adhesive mesh topped with betadine soaked cotton wool and then secured with compression dressing. Subsequent dressings were done as per protocol followed in the unit and the grafted area was assessed for % uptake of graft, haematoma and seroma formation at each dressing. [1,2]

Control Group

In the control group, graft was anchored over the wound with sutures. Assessment for immediate uptake was carried out by the same assistant in case of multiple wounds being grafted over the same patient, in the same manner as in the intervention group. The wound was covered by a non-adhesive mesh topped with betadine soaked cotton wool and then secured with compression dressing. Subsequent dressings were done as per protocol followed in the unit and the grafted area was assessed for % uptake of graft, haematoma and seroma formation at each dressing.

Outcome:

1. To assess immediate uptake of the graft an assistant moved a finger over the graft at 1 min, 2 min and 3 min after placing the graft. The significance of the difference of time taken for adhesion of the graft was measured and compared between the two groups.

2. To assess the uptake of graft on different post-op days the initial area of wound bed was measured, then the area of wound retaining the skin graft after dressing was measured on the first three days of dressing and percentage uptake of graft was compared between the groups. The pre and post procedure area grafted were measured both manually using sterile flexible scales and the mobile based application, Imito Measure.

i) Statistical Analysis:

For the continuous quantitative variables mean and standard deviation were calculated. The inter group continuous variables were compared using suitable tools of statistics like Unpaired Student's t Test. Two quantitative variables, within a group, were compared using Student's paired t test. The categorical data was expressed in terms of rates, ratios and percentages. The association between the outcome, clinical and demographic characteristics was tested using Chi-square test or Fisher's exact test. Discrete variables were represented by median. Nonparametric tests were used for comparing discrete variables. Suitable graphs have been used to depict the comparison. For all the tests the value of p less than 5% (0.05) was considered significant. Statistical Analysis was done using the SPSS Software version 20.0.

RESULTS

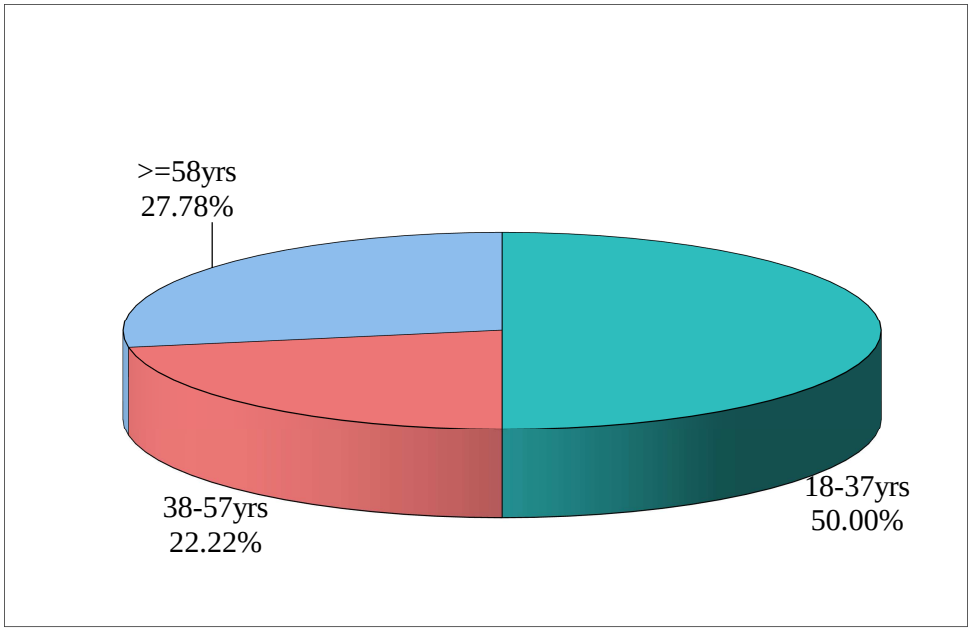
Total number of patients enrolled in the study was 54. 17 patients (31.48%) had more than one wound and were included in both case and control group. Total number of wounds grafted was 80 with 40 wounds in each group.

DEMOGRAPHIC DATA

Table 1: Age group wise distribution

Age groups	Number	Percentage
18-37years	27	50.00
38-57 years	12	22.22
>=58years	15	27.78
Total	54	100.00
Mean age	40.04	
SD age	17.51	

Graph 1: Age group wise distribution

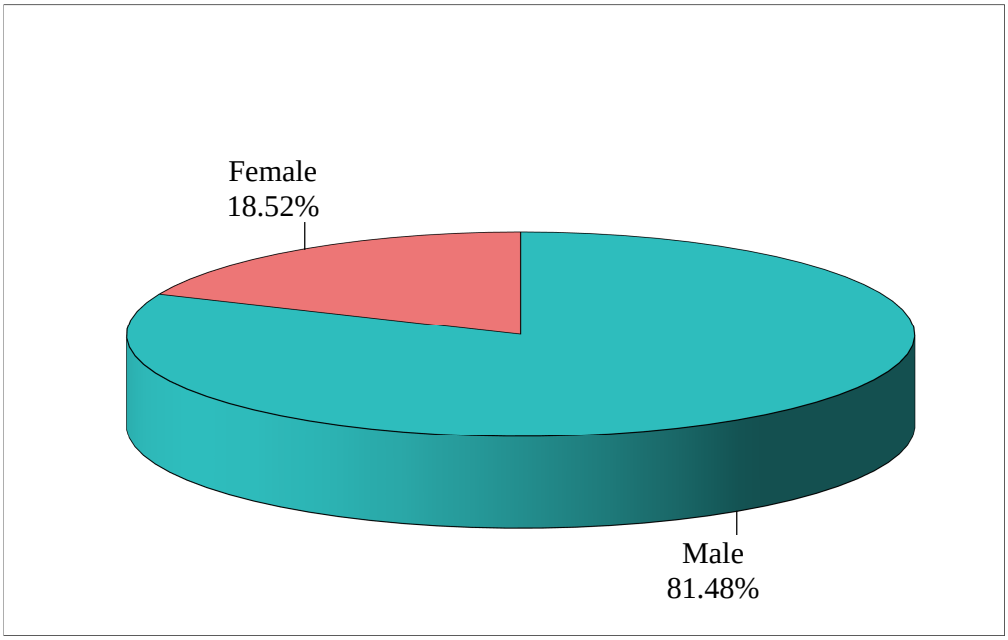


Mean Age (and Standard Deviation) of the population was 40.4 (17.51) years. Maximum number of patients were found to be in the age group of 18-37 years i.e. 27 (50%), with 22.22% in the 38-57 years age group and i.e. 12 patients and 27.78% in the age group of >58 years, 15%.

Table 2: Gender wise distribution

Gender	Number	Percentage
Male	44	81.48
Female	10	18.52
Total	54	100.00

Graph 2: Gender wise distribution

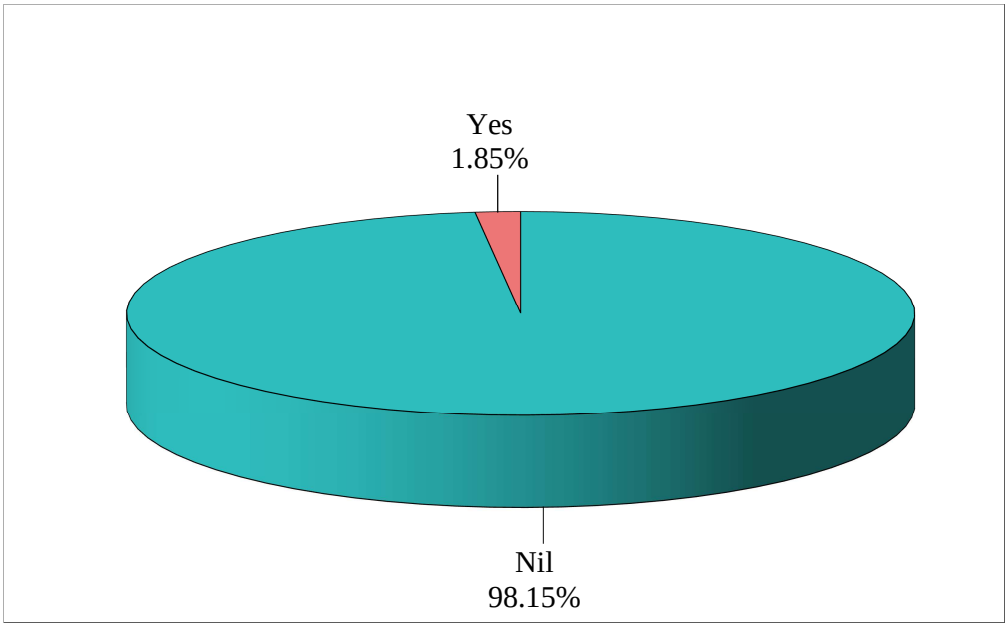


The total population comprised 44 (81.48%) male and 10 (18.52%) female.

Table 3: Comorbidities wise distribution

Comorbidities	Number	Percentage
Nil	53	98.15
Yes	1	1.85
Total	54	100.00

Graph 3: Comorbidities wise distribution

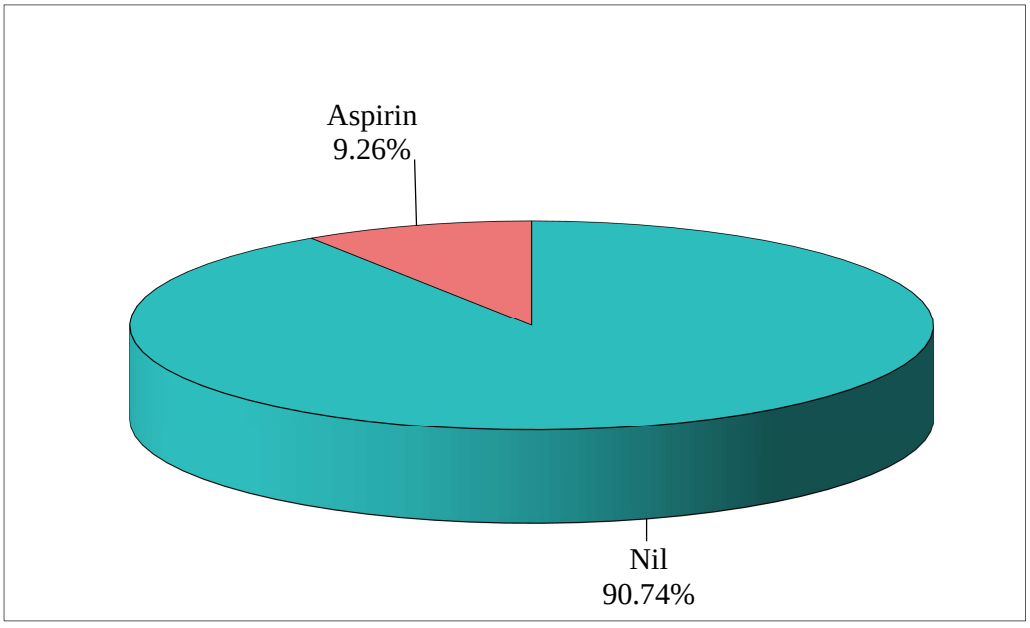


Only one patient had an underlying comorbidity other than those mentioned in the exclusion criteria.

Table 4: Antiplatelet drugs wise distribution

Antiplatelet drugs	Number	Percentage
Nil	49	90.74
Aspirin	5	9.26
Total	54	100.00

Graph 4: Antiplatelet drugs wise distribution

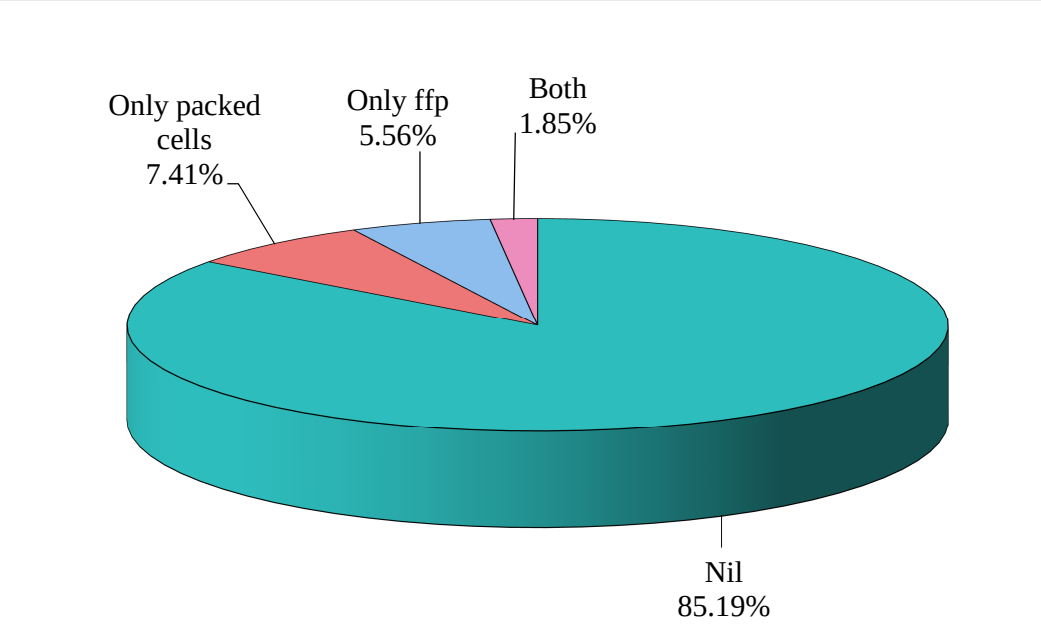


5 patients (9.26%) were on antiplatelet drugs preoperatively or post operatively, while 49 patients did not have any antiplatelet drug use.

Table 5: Pre-operative transfusion wise distribution

Pre-transfusion	Number	Percentage
Nil	46	85.19
Only packed cells	4	7.41
Only ffp	3	5.56
Both	1	1.85
Total	54	100.00

Graph 5: Preoperative transfusion wise distribution

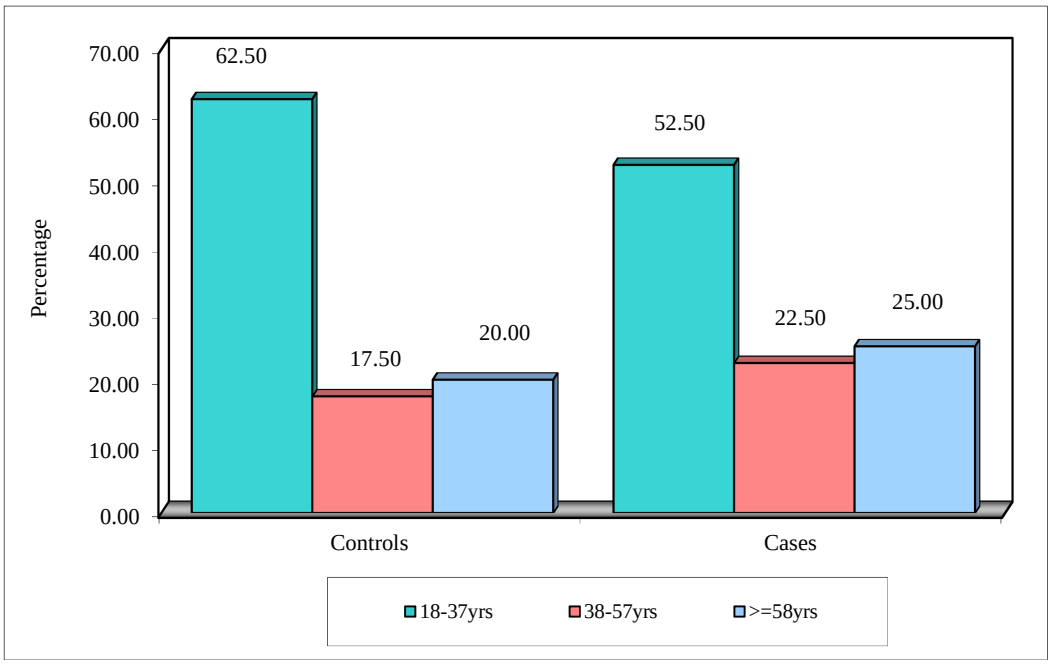


In the total population, 1 (1.85%) patient received both packed red cell transfusion and fresh frozen plasma transfusion, 3 (5.56%) patients received only FFP, 4 (7.41%) patients received PRBC transfusion alone and 46 (85.19%) patients did not receive any blood product transfusion.

Table 6: Comparison of controls and cases with age groups

Age groups	Controls	%	Cases	%	Total	%	Chi-square	p-value
18-37yrs	25	62.50	21	52.50	46	57.50	0.8200	0.6640
38-57yrs	7	17.50	9	22.50	16	20.00		
>=58 yrs	8	20.00	10	25.00	18	22.50		
Total	40	100.00	40	100.00	80	100.00		
Mean age	35.33		40.23		37.78			
SD age	17.06		16.72		16.96			

Graph 6: Comparison of controls and cases with age groups

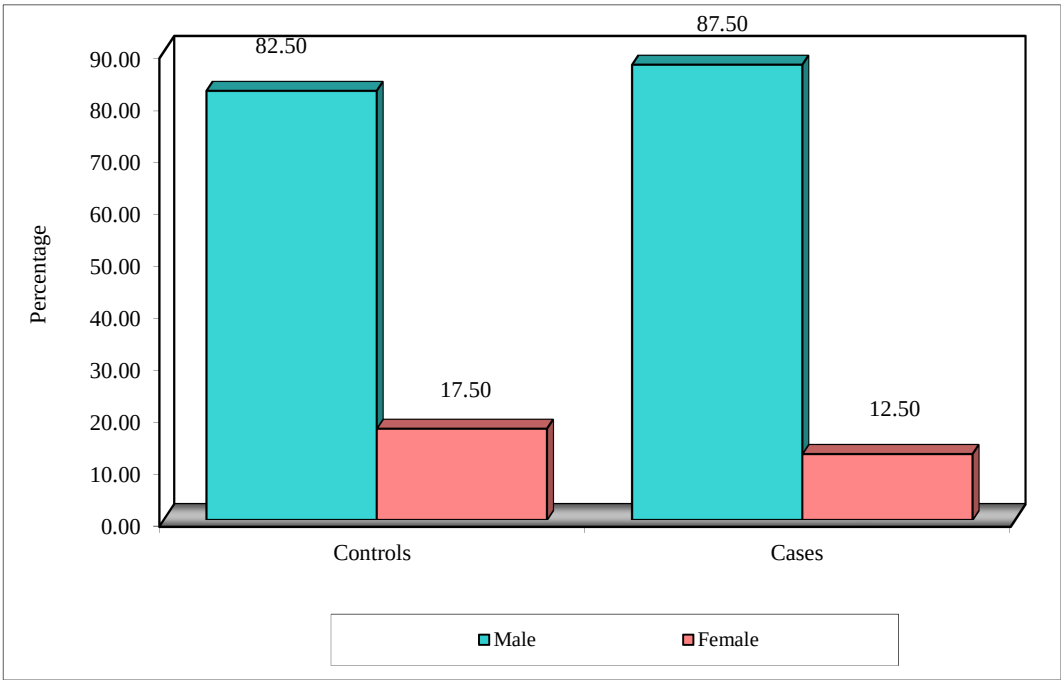


Maximum number of patients in both the control and intervention groups was in the 18-37 years category that is 25 (62.50%) and 21 (52.50%) respectively. 7 (17.50%) and 9 patients (22.50%) were in the 38-57 years age group in the control and intervention population. Least number of patients was above the age of 58 years in both the groups, that is 8 (20%) and 10 (25%) in control and intervention groups respectively. The comparison was performed by Chi-square test and no significant difference was found between the control and intervention groups. (p= 0.6640)

Table 7: Comparison of controls and cases with gender

Gender	Controls	%	Cases	%	Total	%	Chi-square	p-value
Male	33	82.50	35	87.50	68	85.00	0.3920	0.5310
Female	7	17.50	5	12.50	12	15.00		
Total	40	100.00	40	100.00	80	100.00		

Graph 7: Comparison of controls and cases with gender

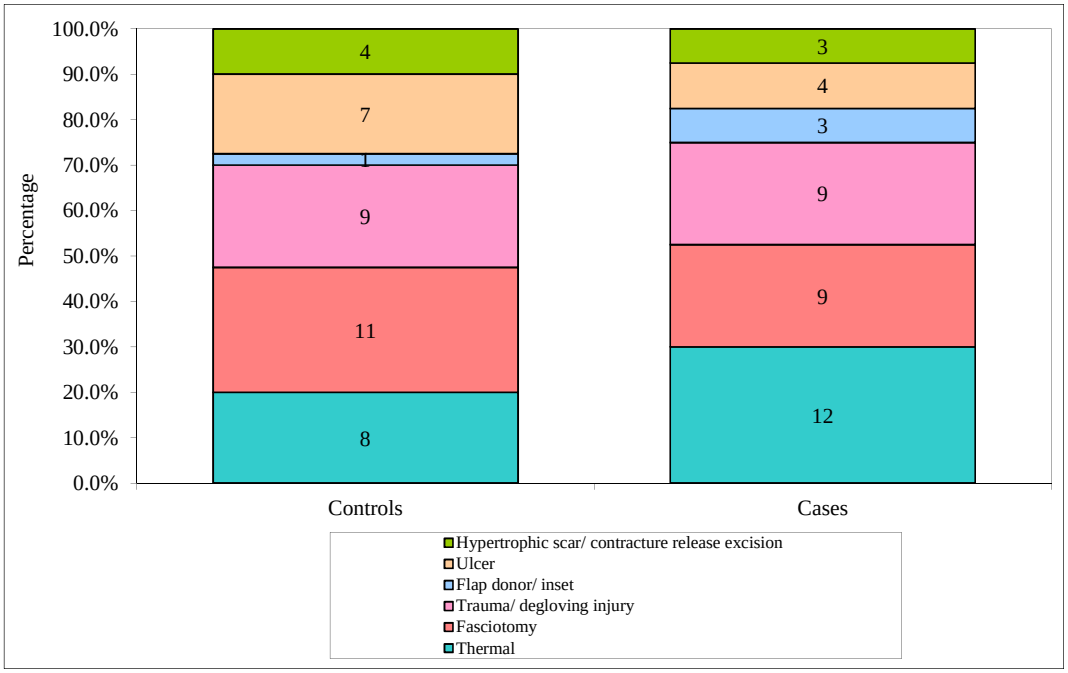


82.50% (33) of the population was male in the control group while 87.50% (35) was male in the intervention group, not statistically significant (p= 0.531).

Table 8: Comparison of controls and cases with Aetiology

Aetiology	Controls	%	Cases	%	Total	%	Chi-square	p-value
Thermal	8	20.00	12	30.00	20	25.00	2.9610	0.7060
Fasciotomy	11	27.50	9	22.50	20	25.00		
Trauma/ degloving injury	9	22.50	9	22.50	18	22.50		
Flap donor/ inset	1	2.50	3	7.50	4	5.00		
Ulcer	7	17.50	4	10.00	11	13.75		
Hypertrophic scar/ keloid/ contracture release excision	4	10.00	3	7.50	7	8.75		
Total	40	100.00	40	100.00	80	100.00		

Graph 8: Comparison of controls and cases with Aetiology



In the control group, 11 (27.50%) wounds were post fasciotomy ulcers, 8 (20.00%) of the wounds grafted were thermal burns, 9 (22.50%) wounds were sustained from degloving injuries/ traumatic ulcers, 7 (17.50%) ulcers were due to venous/ arterial diseases, 4 (10.00%) wounds were post keloid excision/ hypertrophic scar excision and 1 (2.50%) grafted site was a flap donor site.

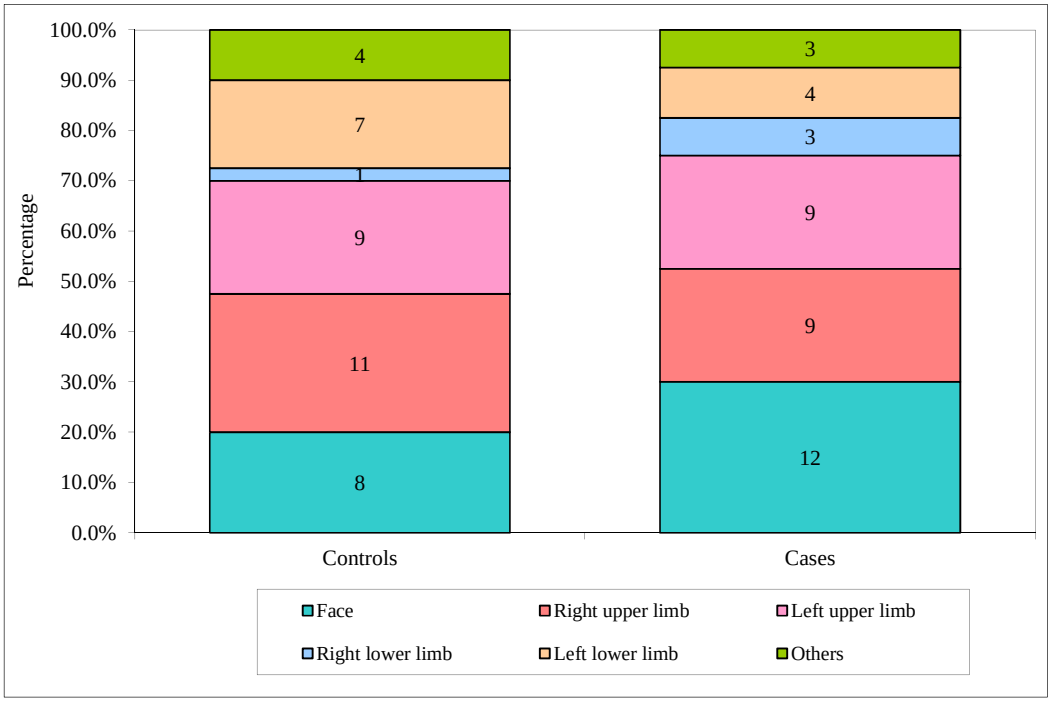
In the intervention group, 12 (30.00%) of the wounds grafted were thermal burns, 9 (22.50%) wounds were post fasciotomy ulcers, 9 (22.50%) wounds were sustained from degloving injuries/ traumatic ulcers, 4 (10.00%) ulcers were due to venous/ arterial diseases, 3 (7.50%) wounds were post excision/ hypertrophic scar excision/ contracture release and 3 (7.50%) grafted site was a flap donor site.

Chi-square test was performed and there was no significant difference in aetiology of wounds between the groups (p= 0.7060)

Table 9: Comparison of controls and cases with locations

Locations	Controls	%	Cases	%	Total	%	Chi-square	p-value
Face	8	20.00	12	30.00	20	25.00	4.3470	0.5010
Right upper limb	11	27.50	9	22.50	20	25.00		
Left upper limb	9	22.50	9	22.50	18	22.50		
Right lower limb	1	2.50	3	7.50	4	5.00		
Left lower limb	7	17.50	4	10.00	11	13.75		
Others	4	10.00	3	7.50	7	8.75		
Total	40	100.00	40	100.00	80	100.00		

Graph 9: Comparison of controls and cases with locations

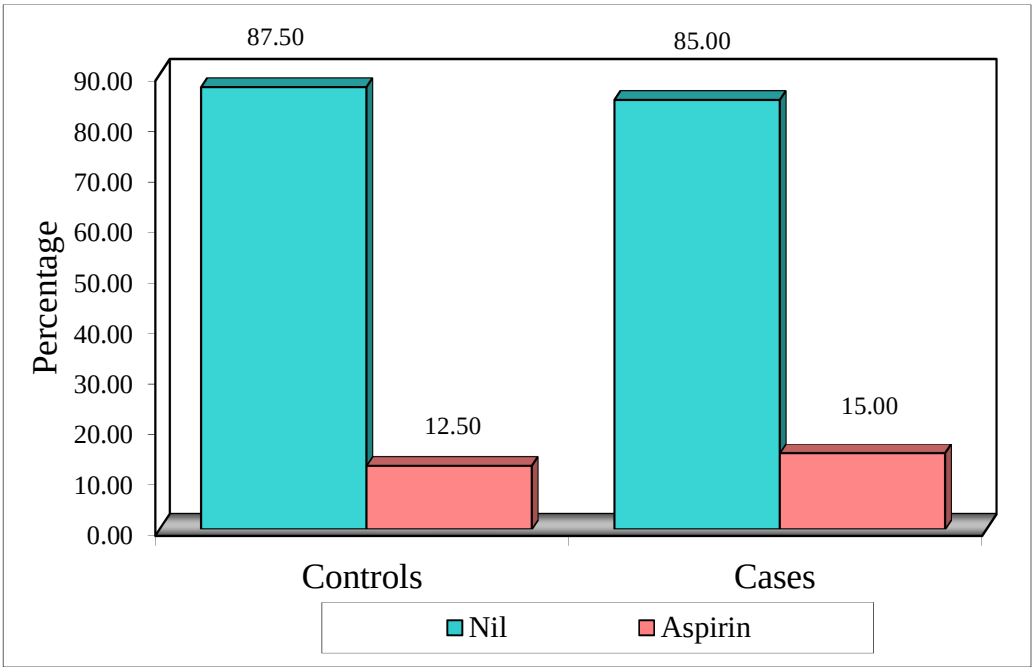


The commonest site of wounds grafted in the control group was the right upper limb (27.5%) while in the intervention group, the maximum number of wounds were located over the face (30.0%). There was no statistically significant difference in the location of wounds between the two groups (p= 0.51).

Table 10: Comparison of controls and cases with Antiplatelet drugs

Antiplatelet drugs	Controls	%	Cases	%	Total	%	Chi-square	p-value
Nil	35	87.50	34	85.00	69	86.25	0.1050	0.7450
Aspirin	5	12.50	6	15.00	11	13.75		
Total	40	100.00	40	100.00	80	100.00		

Graph 10: Comparison of controls and cases with Antiplatelet drugs

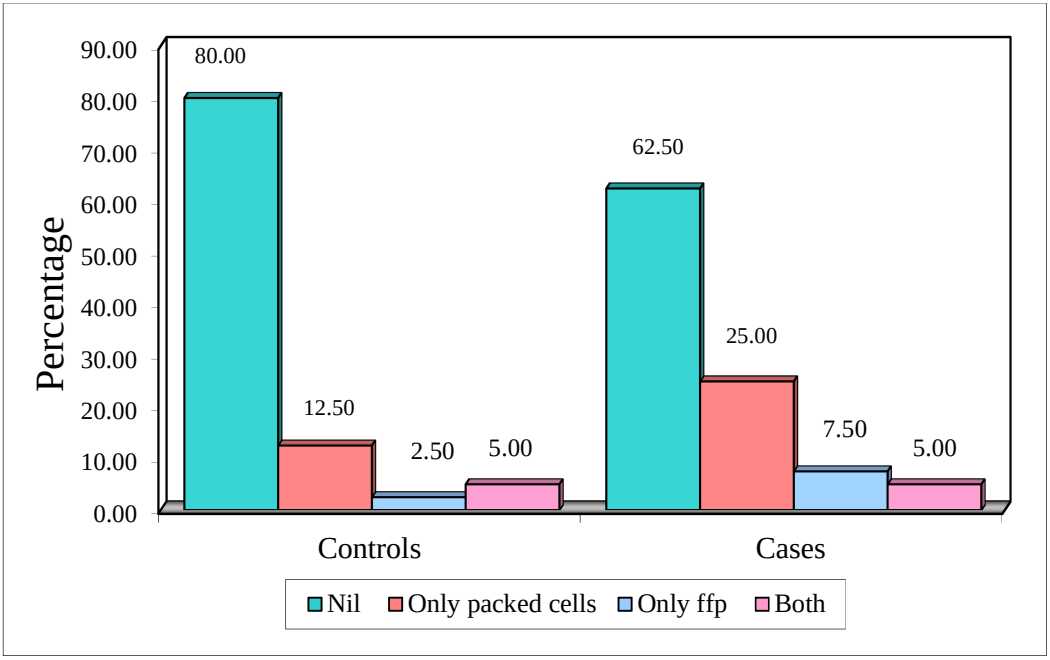


5 (12.50%) and 6 (15.00%) wounds in control and intervention groups belonged to patients who were receiving antiplatelet drugs at the time of grafting. This was not statistically significant (p= 0.745).

Table 11: Comparison of controls and cases with pre-transfusion

Pre-transfusion	Controls	%	Cases	%	Total	%	Chi-square	p-value
Nil	32	80.00	25	62.50	57	71.25	3.5260	0.3170
Only packed cells	5	12.50	10	25.00	15	18.75		
Only ffp	1	2.50	3	7.50	4	5.00		
Both	2	5.00	2	5.00	4	5.00		
Total	40	100.00	40	100.00	80	100.00		

Graph 11: Comparison of controls and cases with pre-transfusion



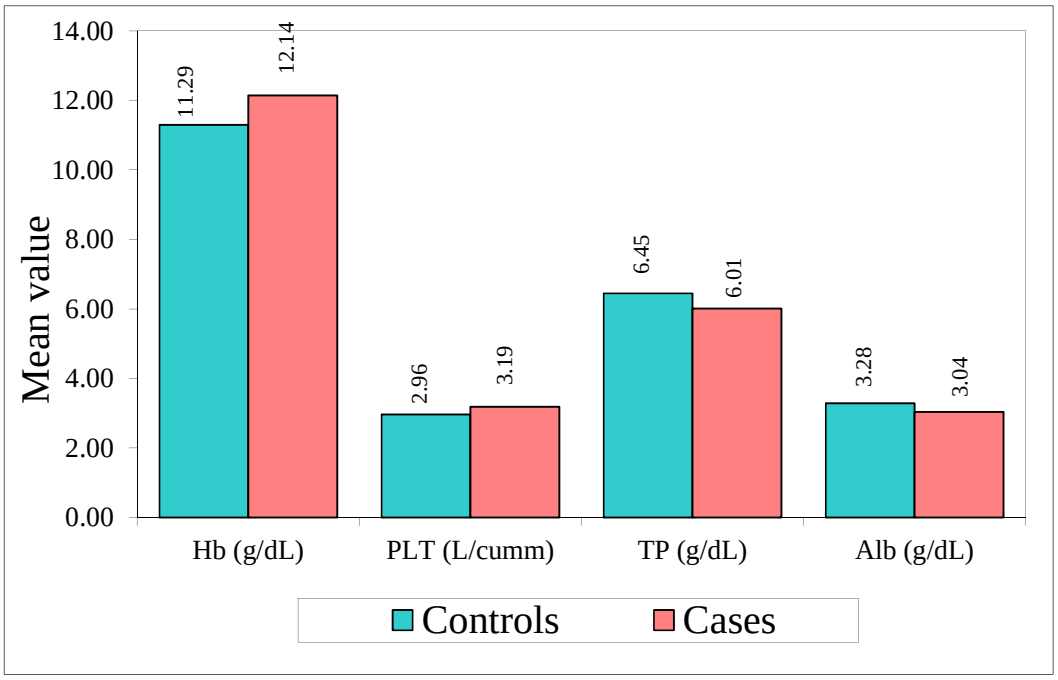
Majority of patients did not receive any transfusion (80.00% and 62.50% in control and case groups respectively). Among patients in control group, 12.50%, 2.50%, 5.00% patients received only packed red cells, only FFP and both blood products pre-operatively. 25.00%, 7.50%, 5.00% of patients in PRP group only packed red cells, only FFP and both blood products pre-operatively and this difference was not statistically significant.

Table 12: Comparison of controls and cases with clinical investigations by t test

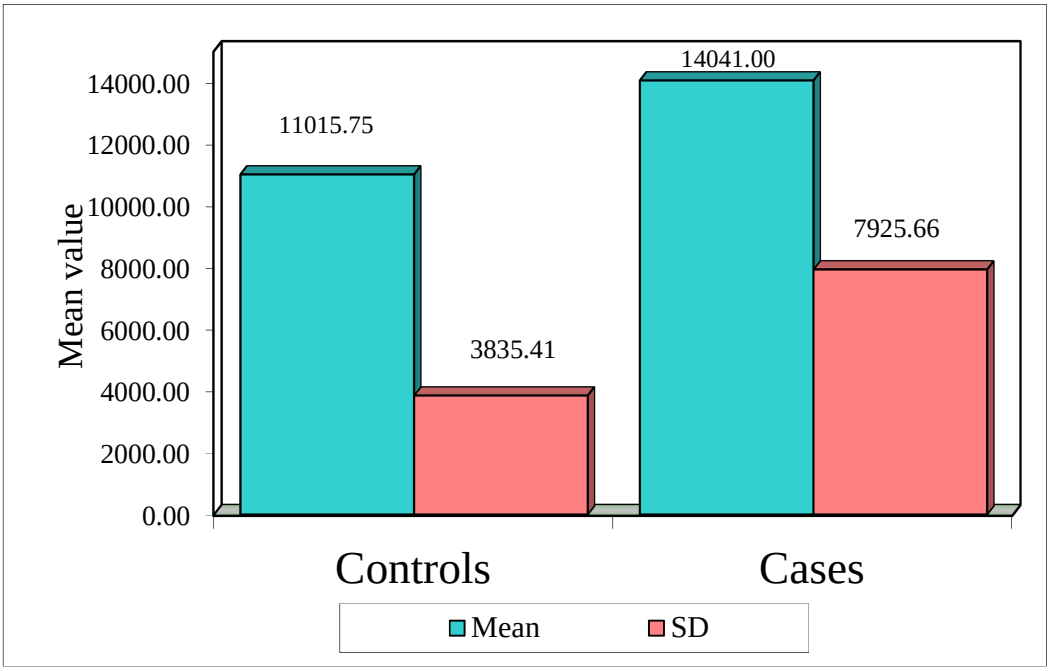
Parameters	Controls		Cases		t-value	p-value
	Mean	Std.Dev.	Mean	Std.Dev.		
Hb (g/dL)	11.29	1.90	12.14	2.20	-1.8496	0.0682
TLC (/cumm)	11015.75	3835.41	14041.00	7925.66	-2.1730	0.0328*
PLT (L/cumm)	2.96	1.45	3.19	1.36	-0.7151	0.4767
TP (g/dL)	6.45	0.90	6.01	1.02	2.0196	0.0469*
Alb (g/dL)	3.28	0.86	3.04	0.69	1.3326	0.1865

*p<0.05

Graph 12: Comparison of controls and cases with clinical investigations



Graph 13: Comparison of controls and cases with TLC scores by t test



Mean haemoglobin of patients in the control and PRP group were 11.29 g/dL and 12.14g/dL, which was not statistically significant (p= 0.0682).

Total leukocyte count in the control and PRP group was 11,015. 75 and 14,041.00 respectively and statistically significant (p= 0.0328).

Mean platelet count in the control and PRP group were 2.96 L/cumm and 3.19 L/cumm, not statistically significant (p= 0.4767).

Mean total protein of patients in control and PRP group were 6.45 g/dL and 6.01 g/dL which was statistically significant (p=0.0469).

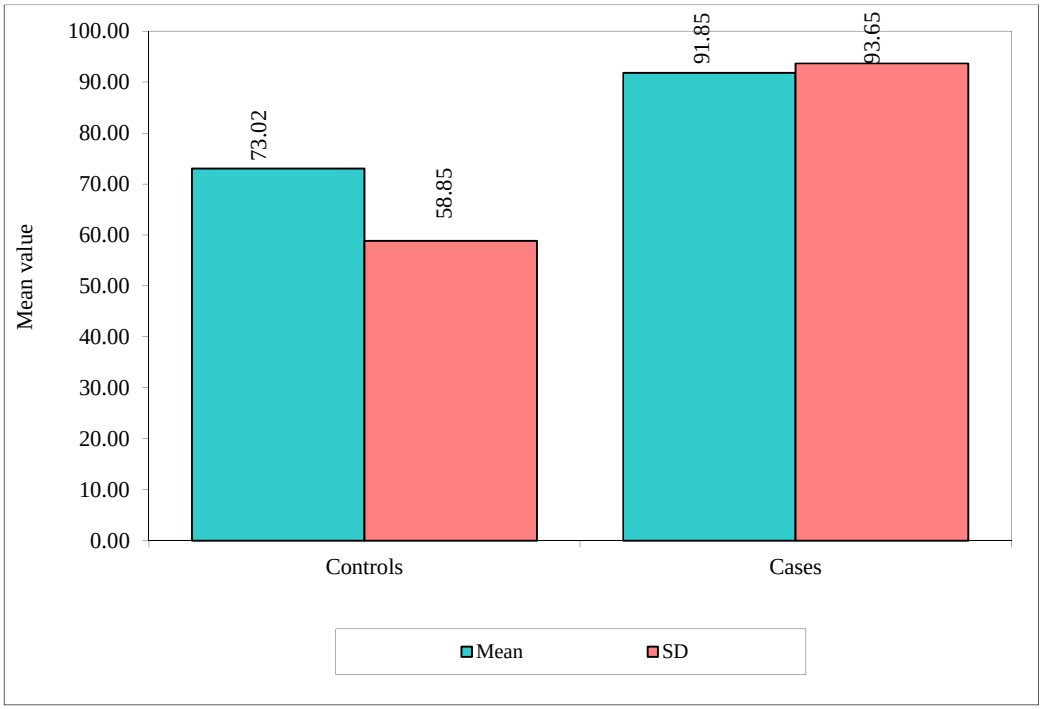
Mean albumin of patients in control and PRP group were 3.28 g/dL and 3.04 g/dL which was not significant (p=0.1865)

Table 13: Comparison of controls and cases with Initial area (cm^2) by t test

Parameters	Controls		Cases		t-value	p-value
	Mean	Std.Dev.	Mean	Std.Dev.		
Initial area (cm^2)	73.02	58.85	91.85	93.65	-1.0765	0.2850

*p<0.05

Graph 14: Comparison of controls and cases with initial area



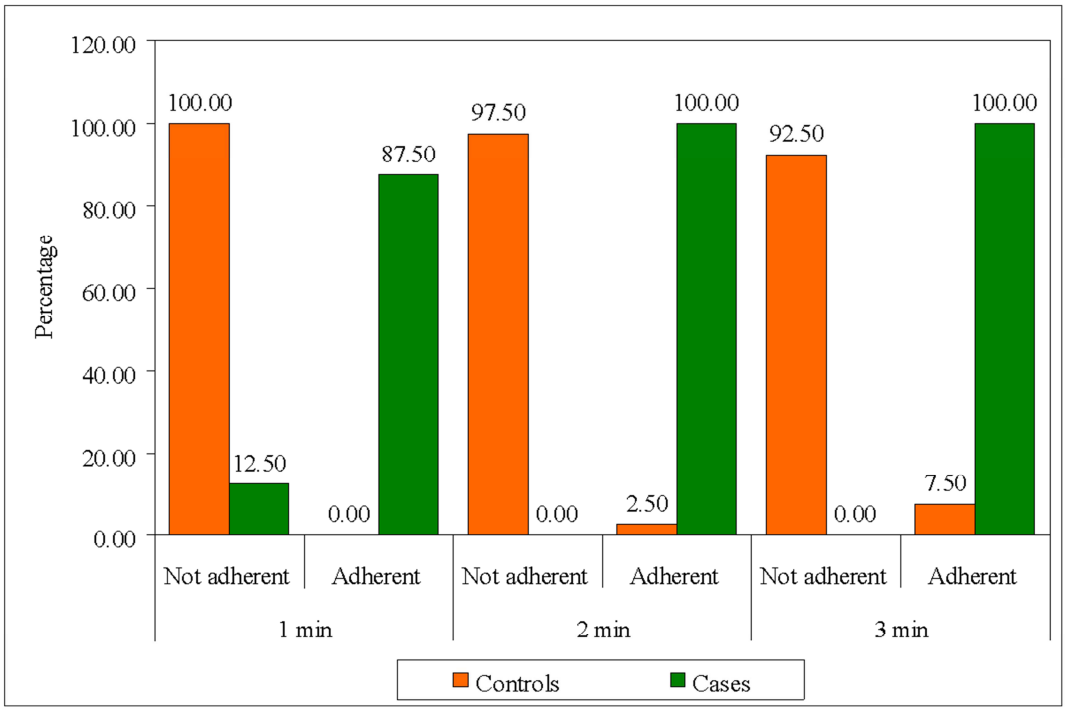
Mean initial area of the recipient wound in control and PRP group are 73.02 cm^2 and 91.89 cm^2 respectively which was not statistically significant (p= 0.285).

Table 14: Comparison of controls and cases with status of adhesion at different time points

Status of adhesion	Controls	%	Cases	%	Total	%	Chi-square	p-value
1 min								
Not adherent	40	100.00	5	12.50	45	56.25	62.2222	0.0001*
Adherent	0	0.00	35	87.50	35	43.75		
2min								
Not adherent	39	97.50	0	0.00	39	48.75	76.0980	0.0001*
Adherent	1	2.50	40	100.00	41	51.25		
3min								
Not adherent	37	92.50	0	0.00	37	46.25	68.8370	0.0001*
Adherent	3	7.50	40	100.00	43	53.75		
Total	40	100.00	40	100.00	80	100.00		

*$p<0.05$

Graph 15: Comparison of controls and cases with status of adhesion at different time points

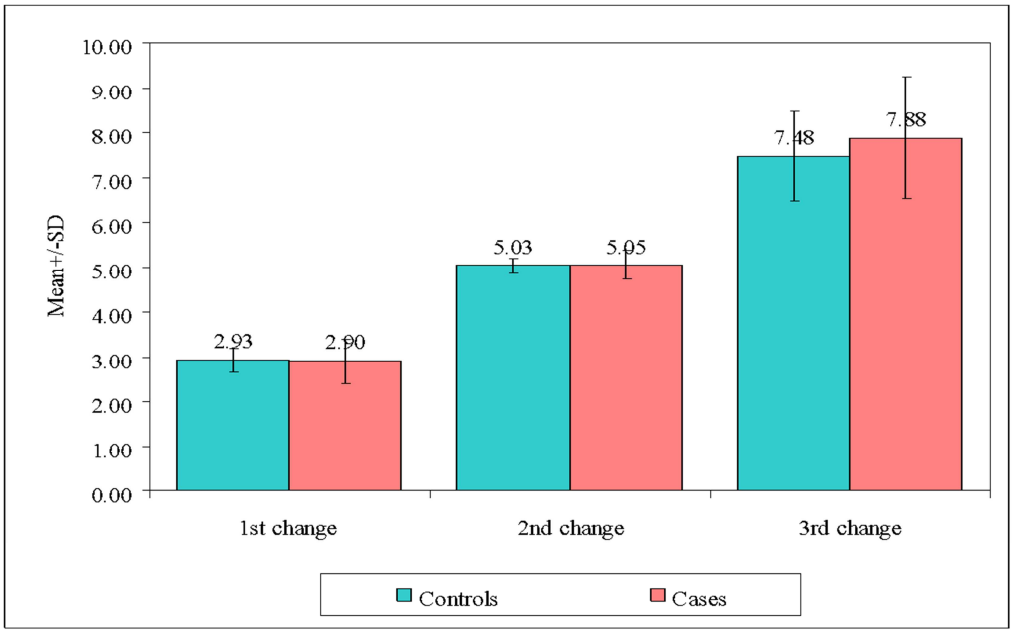


Immediate adhesion at 1 minute, 2 minutes and 3 minutes of placing the graft on wound bed was 0, 1 (2.5%) and 3 (7.50%) in control group and 35 (87.50%), 40 (100.00%), 40 (100.00%) in PRP group, all three readings being statistically significant ($p<0.0001$).

Table 15: Comparison of controls and cases with POD scores at different time points by independent t test

Changes in POD	Controls			Cases			t-value	p-value
	Mean	Std.Dev.	Median	Mean	Std.Dev.	Median		
1st change	2.93	0.27	3.00	2.90	0.50	3.00	0.2807	0.7797
2nd change	5.03	0.16	5.00	5.05	0.32	5.00	-0.4472	0.6560
3rd change	7.48	1.01	7.00	7.88	1.36	7.00	-1.4905	0.1401

Graph 16: Comparison of controls and cases with POD scores at different time points



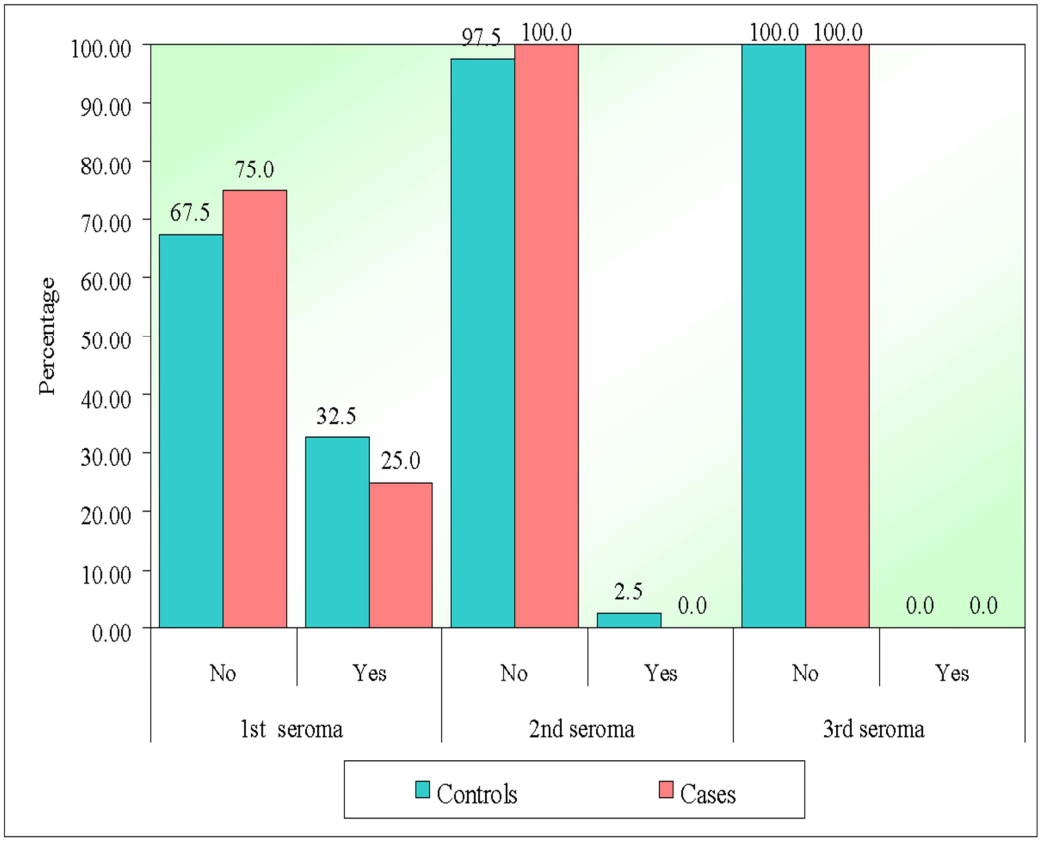
Median of post op day of change of 1st, 2nd, 3rd dressings after STSG was 3rd day, 5th day and 7th day in both groups. (p= 0.7797, 0.6560, 0.1401)

Table 16: Comparison of controls and cases with status of seroma at different time points

Status of seroma	Controls	%	Cases	%	Total	%	Chi-square	p-value
Seroma at 1st dressing								
No	27	67.50	30	75.00	57	71.25	0.5490	0.4590
Yes	13	32.50	10	25.00	23	28.75		
Seroma at 2nd dressing								
No	39	97.50	40	100.00	79	98.75	-	-
Yes	1	2.50	0	0.00	1	1.25		
Seroma at 3rd dressing								
No	40	100.0	40	100.00	80	100.00	-	-
Yes	0	0.00	0	0.00	0	0.00		
Total	40	100.0	40	100.00	80	100.00		

*$p<0.05$

Graph 17: Comparison of controls and cases with status of seroma at different time points



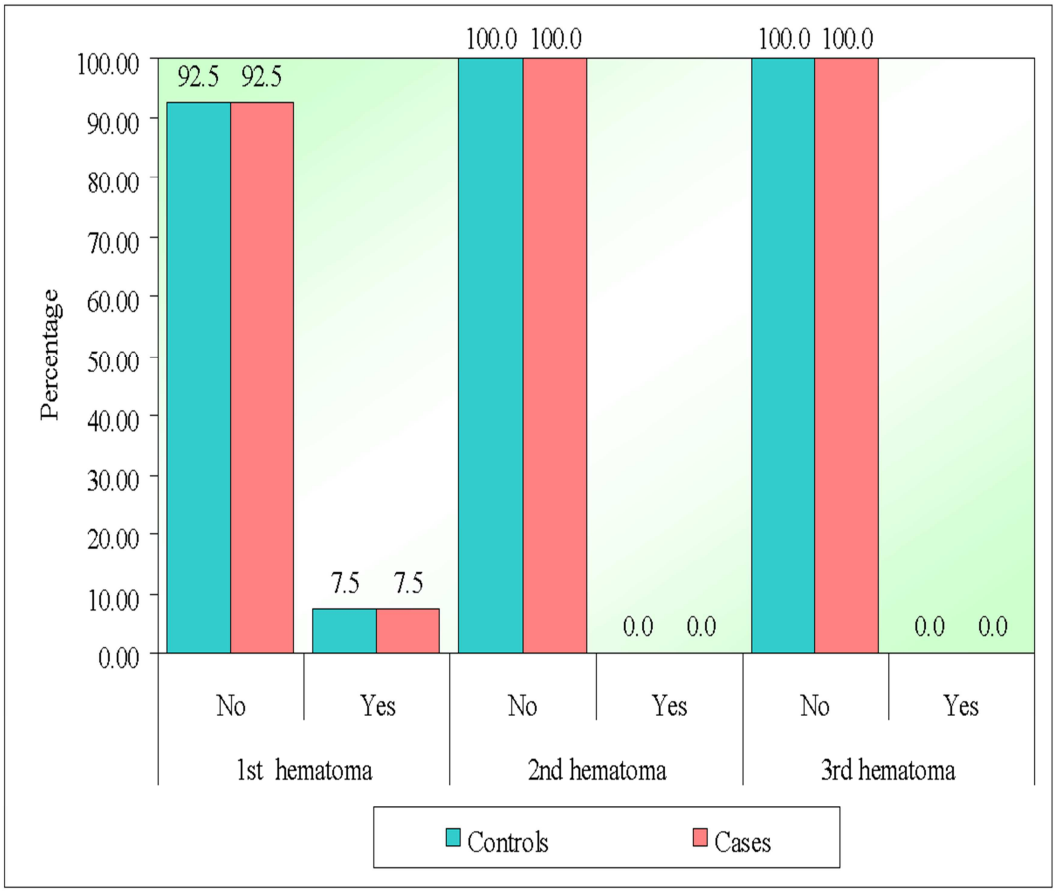
Seroma formation at the time of first dressing was seen in 28.75% of cases and 32.5% of controls and this difference was not statistically significant. (p=0.4590). 2.50% controls had seroma at the time of second dressing but none of the intervention group had seroma after the first day of change of dressing.

Table 17: Comparison of controls and cases with status of hematoma at different time points

Status of hematoma	Controls	%	Cases	%	Total	%	Chi-square	p-value
Haematoma at 1st dressing								
No	37	92.50	37	92.50	74	92.50	0.0000	1.0000
Yes	3	7.50	3	7.50	6	7.50		
Haematoma at 2nd dressing								
No	40	100.00	40	100.00	80	100.00	-	-
Yes	0	0.00	0	0.00	0	0.00		
Haematoma at 3rd dressing								
No	40	100.00	40	100.00	80	100.00	-	-
Yes	0	0.00	0	0.00	0	0.00		
Total	40	100.00	40	100.00	80	100.00		

*$p<0.05$

Graph 18: Comparison of controls and cases with status of hematoma at different time points



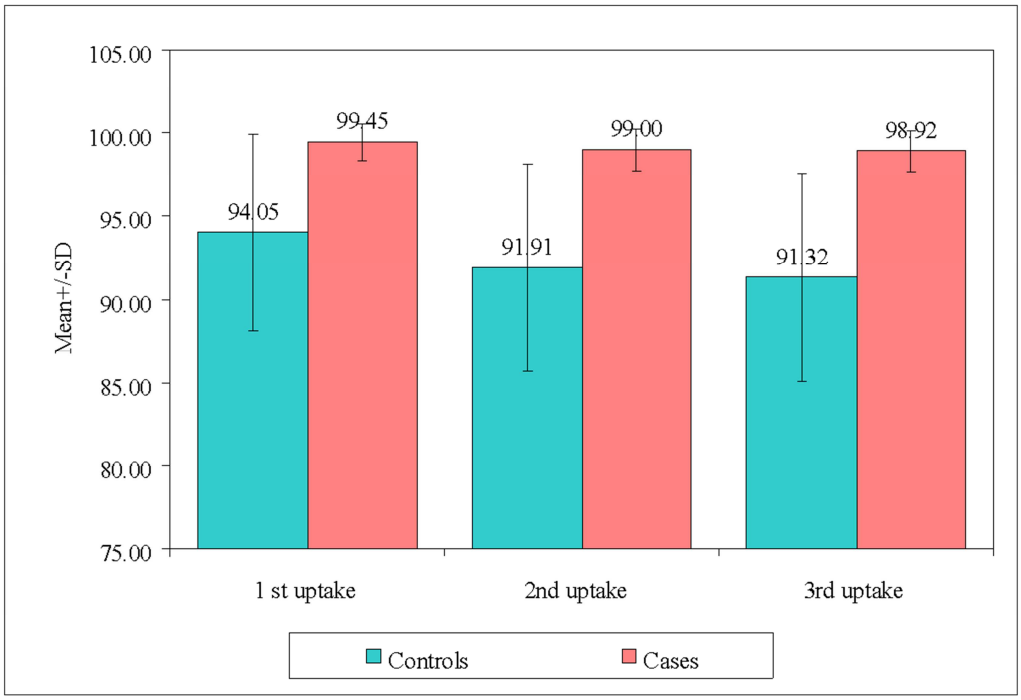
Haematoma formation was seen in 3 of the control group and 3 of the intervention group wounds that is in 7.5 % of the population with p value= 1.00

Table 18: Comparison of controls and cases with uptake (%) scores at different time points by independent t test

Uptake(%)at	Controls			Cases			t-value	p-value
	Mean	Std.Dev.	Median	Mean	Std.Dev.	Median		
Uptake (%) at 1st dressing	94.05	5.88	93.00	99.45	1.09	99.00	-5.7129	0.0001*
Uptake (%) at 2nd dressing	91.91	6.23	95.00	99.00	1.24	100.00	-7.0484	0.0001*
Uptake (%) 3rd dressing	91.32	6.30	93.00	98.92	1.27	99.70	-7.4827	0.0001*

*p<0.05

Graph 19: Comparison of controls and cases with uptake (%) scores at different time points



There was a significant difference in % uptake of graft between the Control and PRP group on all three dressing days, with the PRP group having a greater % uptake. On 1st day of dressing % uptake of graft was 94.50% and 99.45% in control and PRP groups (p=0.0001), on 2nd day uptake was 91.91% and 99.0% respectively (p= 0.0001) and 3rd day of dressing was 91.32% and 98.92% (p=0.0001).

DISCUSSION

In this study, 17 patients of the total 54 overlapped the case and control groups as the patients presented with multiple non-contiguous wounds which were divided among the two study groups. This provided the added benefit of making all conditions uniform among the two groups except for the intervention. In order to randomize the population, some of these patients with multiple wounds were allocated to only the case or control group as well. This was similar to the population chosen by Osama et al where the same wound was divided equally to serve a case and control both. Their study used platelet gel, while in the present study PRP was used in liquid form making its use on a single wound divided into case and control difficult. [38]

Platelet Rich plasma was extracted with a single spin of 3500 RPM for 20 minutes which yielded 2.5-2.7ml of plasma and PRP. PRP extraction has been done in several different ways in the previous studies. Although the method adopted in this study used a mixture of Platelet rich and platelet poor plasma, the overall results were comparable to those studies that used the double spin method of PRP extraction. It also gave the advantage of giving enough volume of PRP from a single prick thereby reducing the number of times the patient had to be pricked and the amount of blood drawn from the patient. [1, 2, 38]

The mean age of population was 40.4 years with maximum number of patients in the 18-37 years group. [27] Between the two groups, the age distribution was not found to be significant, with the maximum number of patients belonging to the 18-37 years group. Age distribution of population of studies performed by Gupta et al (45.1

years in case and 48.2 years in control) and Thimmannahalli et al (49.10±15.36 in PRP and 55.87±11.16 in control group) were similar. [39, 41]

Gender distribution in the total population was 81.48% (44) male and 18.52% (10) female. Group specific gender distribution was 82.50% (33) male in the control group while 87.50% (35) was male in the intervention group. There were 59 and 48 cases and controls for males in the study population of Gupta et al. [41]

Patients with diabetes mellitus had been excluded from this study in order to maintain uniformity between the case and control groups as well as adhere to randomisation. Single patient had underlying PVD and was allotted in the case group. Patients had immediate adhesion of graft within 1 minute of placing on the recipient site after application of PRP and 100% graft uptake at the third dressing. Previous articles have not specified their results with respect to patients with underlying peripheral vascular disease.

5 patients (9.26%) were on antiplatelet drugs pre-operatively or post operatively, while 49 patients did not have any antiplatelet drug use. Out of these five patients, one patient was a case of degloving injury of right foot for which she had undergone reverse soleal flap procedure and was started on antiplatelet drugs post-operatively. 4 other patients had underlying vascular injuries and thrombectomy or were prophylactically started on aspirin along with fasciotomy and debridement of degloving injuries. These 5 patients made up 8 wounds, with equal distribution in each group. Among the wounds in PPR group only one graft was not adherent on the wound bed at the first minute of placing it on the recipient site, while grafts in the wounds in control group were not adherent even at the end of 3 minutes of placing the graft on the recipient wound bed. These five patients had normal platelet count before

skin grafting was undertaken. Aspirin was continued till 48 hours before surgery. In the study performed by Waiker et al where Aspirin was withdrawn 72 hours before surgery and restarted 48 hours after the procedure. As per recent guidelines, aspirin, when indicated for peripheral vascular diseases, underlying vascular injury/ repair can be continued in peri-operative period [44]. However this poses the disadvantage of decreased platelet aggregation due to the action of aspirin and a decrease in healing properties of PRP. [45] Thus, in our study, antiplatelet drugs were withheld before the surgery.

Commonest aetiology of the wound in the PRP group was thermal burns and that in the control group degloving injuries. Irrespective of aetiology of the wound, the immediate adhesion of graft on the wound bed in PRP was significantly better in PRP group. The aetiologies of different wounds between the two groups were made uniform by keeping the same patient in both the PRP and Control groups. Similar aetiology profile was seen in the population in the study performed by Dhua et al, with no significant difference in PRP action with respect to aetiology. [2]

The commonest site of wounds grafted in the control group was the right upper limb (27.5%) while in the intervention group, the maximum number of wounds were located over the face (30.0%). The use of PRP on wounds situated over joints and those with uneven surfaces provided the added benefit of improved immediate adhesion of graft and better overall uptake.

Haemoglobin, Platelet count and total count were greater in the PRP group while total protein and albumin were higher in the control group. There was no significant difference in the pre-operative blood tests between the PRP and control groups.

Mean initial area of the recipient wound in control and PRP group are 73.02 cm^2 and 91.89 cm^2 respectively which was not statistically significant. Thus size of the wound did not play a role in effectiveness of PRP. The instant adhesion provided by the PRP helped with adhesion of grafts of larger area. Prior studies also have not studied graft size specific outcomes with respect to use of PRP.

Immediate adhesion at 1 minute, 2 minutes and 3 minutes of placing the graft on wound bed was 0, 1 (2.5%) and 3 (7.50%) in control group and 35 (87.50%), 40 (100.00%), 40 (100.00%) in PRP group, all three readings being statistically significant ($p<0.0001$). Previous studies performed by Gupta, Dhua, Thimmannahalli have found the same result with respect to immediate adhesion of graft on wound. However they have not specified the time between application of PRP and placing the skin graft on the recipient site. [2, 39, 41]

Immediate adhesion of graft occurred because the action of PRP mimics the natural process of fibrin strand formation and adhesion that occurs in the body in response to the skin graft. This is the first step of graft uptake and PRP provides a higher concentration of clotting factors, platelets and growth factors which accelerates fibrin strand formation. [17, 3]

There was no significant difference between the two groups in terms of which post-operative day the dressings were changed. Mean POD of change of dressing was 2.93 and 2.90 ($p= 0.7797$) in control and PRP group for the first change of dressing followed by 5.03 and 5.05 ($p=0.6560$) and 7.48 and 7.88 ($p=0.1401$) respectively for 2^{nd} and 3^{rd} dressing changes. In previous studies, dressing was done within one week of grafting either as per standard protocol or wetness of the dressing. [1, 2, 39, 41] According to the chronological order of steps of graft uptake, serum neo-angiogenesis

between the graft and the wound bed occurs by 72 hours of grafting. Any form of mechanical barrier between the graft and the wound bed disrupts the take of the graft by preventing neoangiogenesis. Therefore, first dressing within 2 to 3 days of grafting allows early evacuation of hematoma and seroma thereby improving graft uptake. In our study both the PRP and control group underwent standard protocol dressing on 3^{rd}, 5^{th} and 7^{th} post-op days.[17, 46]

Seroma formation was assessed at the time of dressing and compared between the two groups. Seroma formation at the time of first dressing was seen in 28.75% of cases and 32.5% of controls and this difference was not statistically significant. (p=0.4590).

2.50% controls had seroma at the time of second dressing but none of the intervention group had seroma after the first day of change of dressing.

Seroma formation was found in both the groups; however there was no statistical significance between the differences. Seroma formation occurs as a result of serum accumulating under a tented graft which has been placed over a crevice over an uneven recipient wound surface, after it has vascularised. The seroma thus formed prevents attachment of that area of graft to the recipient and needs to be drained and not just aspirated as the latter leads to prompt recurrence. With PRP application, adhesion of the graft over crevices on the wound bed is more effective and does not cause tenting of graft after removal of dressing. The use of sutures in both the groups also contributed to minimising the chances of seroma formation as these sutures prevent the movement of graft tangentially over the ulcer thereby preventing its displacement from graft bed and tenting. [17]

Hematoma was found in both groups only on the first day of dressing which was in 3 wounds in each group (7.5 %). PRP contributes to haemostasis at the recipient site thereby preventing formation of haematoma. [24]

The PRP group in our study showed a significantly greater % uptake of graft on all three days of dressing change that is % uptake of graft was 94.50% and 99.45% in control and PRP groups (p=0.0001) on 1st day of dressing, uptake was 91.91% and 99.0% respectively (p= 0.0001) on 2nd day and dressing was 91.32% and 98.92% (p=0.0001) on 3rd day of dressing.

This was comparable to the findings in previous studies performed by Gupta, Waiker, Thimmanahalli and Dhua, to name a few. [1, 2, 39, 41]

PRP contributes to every chronological step in uptake of a skin graft on the recipient site. At the outset, it contributes to haemostasis by providing a higher concentration of platelets which in turn sets the coagulation cascade into motion. With higher concentration of growth factors from plasma as well as those released by de-granulation of the platelets, action of macrophages and fibroblasts is accelerated. This contributes to neoangiogenesis. With re-establishment of blood supply to the graft, leukocytes reach the recipient site and facilitate formation of collagen via action of fibroblasts as well as remove pathogens which may have contaminated the graft or wound. PRP has been found to have anti-inflammatory properties which contribute to the same.

STRENGTHS

This study was a randomised controlled trial which investigated the use of PRP in a novel way in the institution for the first time. Patients were kept common between the two groups as far as possible to ensure that patient factor variability was negated. This allowed us to compare wounds on similar sites as well, for example in patients who sustained burns to both the hands, one hand served as control and the other served as intervention. The use of sutures here-ever necessary limited translational movement of the graft over the wound and this step was kept uniform between both groups.

LIMITATIONS

This study did not analyse the effect of PRP on graft site pain, and its effect on scar formation. The duration of hospital stay was not analysed, as many patients remained longer not necessarily for the sole purpose of skin graft care, for example burns patients. Although attempted, the entire population could not be composed of patients with multiple wounds serving as both control and intervention group. Further studies are required to assess whether PRP alone is enough to prevent tangential movement of graft on wound bed to determine a possibility of eliminating use of sutures to anchor the graft in place.

CONCLUSION

We attempted to study the effect of topical Platelet Rich Plasma over wound bed on anchorage of skin graft on wound measured in terms immediate adhesion of the graft on wound and subsequent percentage uptake of the graft on the recipient site as compared to using sutures alone to anchor the graft in place. We performed the study on 54 patients, who made up the 80 wounds with 40 in each limb of the study. It was found that the use of topical PRP just before placing the graft on the recipient site significantly improves graft adhesion, improves ease of suturing the graft in place, its uptake over joints and uneven wound surfaces which ultimately results in a better % uptake of graft. This ensures that the patient does not go in for re-grafting and can return home after a shorter duration of stay in the hospital. Therefore this study recommends the use of PRP in routine split thickness skin grafting especially in cases with dearth of skin graft donor sites or large areas that require to be grafted.

SUMMARY

Split thickness skin grafting (STSG) restores cutaneous cover over wounds thus protecting the underlying surface from contamination, fluid loss and stimulates healing. Autologous platelet rich plasma (PRP) provides a large number of platelets and high concentration of growth factors which promote prompt adherence of skin graft to wound beds, angiogenesis and healing of the wound as well.

PRP is used in various fields for its role in promoting growth, analgesia and anti-inflammatory process. A priori research has studied the use of PRP as a means of anchoring skin grafts on wounds.

The aim of this study was to assess the immediate adhesion and subsequent uptake of STSG with application of PRP over recipient site.

80 wounds of various aetiologies were randomised into intervention group (n=40) which received PRP before placing STSG on recipient site and control group (n=40) in which the graft was fixed in place with sutures alone. Immediate graft adhesion and subsequent graft uptake were compared between the two groups.

The 80 wounds were distributed among 54 patients, 17 of them (31.48%) having multiple wounds. Irrespective of aetiology, and size, 87.5% grafts were adhered by 1st minute of application in the intervention group compared to nil in control group ($p< 0.0001$). Graft uptake was assessed on first three consecutive dressings. There was significantly better graft uptake in intervention group compared to control group [third dressing uptake (98.29%, 93%, $p< 0.0001$) respectively]. Difference in seroma and haematoma formation were also compared between the two

groups and found to be not significant. (Haematoma formation= 7.5 % in both PRP and control groups with p value= 1.00 and Seroma on POD 3= 28.75% of cases and 32.5% of controls, p=0.4590.

Application of topical PRP facilitates STSG uptake. It decreases operative time by decreasing mobility of graft over the wound bed. Thus, use of PRP improves outcome of split thickness skin graft in wounds of various aetiologies and we recommend use of the same at recipient site of STSG.

BIBLIOGRAPHY

1. Waiker VP, Shivalingappa S. Comparison between Conventional Mechanical Fixation and use of Autologous Platelet Rich Plasma (PRP) in Wound Beds Prior to Resurfacing with Split thickness Skin Graft. World J Plast Surg 2015;4(1):50-59.
2. Dhua S, Suhas TR, Tilak BG. The Effectiveness of Autologous Platelet Rich Plasma Application in the Wound Bed Prior to Resurfacing with Split Thickness Skin Graft vs. Conventional Mechanical Fixation Using Sutures and Staples. World J Plast Surg 2019;8(2):185-194. doi: 10.29252/wjps.8.2.185.
3. Marx RE. Platelet-rich plasma (PRP): what is PRP and what is not PRP? Implant dentistry. 2001 Dec 1;10 (4):225-8.
4. Saluja H, Dehane V, Mahindra U. Platelet-Rich fibrin: A second generation platelet concentrate and a new friend of oral and maxillofacial surgeons. Annals of maxillofacial surgery. 2011 Jan;1(1):53.
5. Yen YH, Lin CM, Hsu H, Chen YC, Chen YW, Li WY, Hsieh CN, Huang CC. Skin graft fixation using hydrofiber (Aquacel® Extra). Annals of Plastic Surgery. 2018 Jun 1;80(6):616-21.
6. Fang Z, Yang X, Wu G, Liu M, Han J, Tao K, Hu D. The use of autologous platelet-rich plasma gel increases wound healing and reduces scar development in split-thickness skin graft donor sites. Journal of plastic surgery and hand surgery. 2019 Nov 2;53(6):356-60.
7. Opneja A, Kapoor S, Stavrou EX. Contribution of platelets, the coagulation and fibrinolytic systems to cutaneous wound healing. Thrombosis research. 2019 Jul 1;179:56-63.

8. Dryden SV, Shoemaker WG, Kim JH. Wound management and nutrition for optimal wound healing. Atlas of the oral and maxillofacial surgery clinics of North America. 2013 Mar 1;21(1):37-47.
9. Rodrigues M, Kosaric N, Bonham CA, Gurtner GC. Wound healing: a cellular perspective. Physiological reviews. 2019 Jan 1;99(1):665-706.
10. Rittié L. Cellular mechanisms of skin repair in humans and other mammals. Journal of cell communication and signaling. 2016 Jun;10(2):103-20.
11. Guo SA, DiPietro LA. Factors affecting wound healing. Journal of dental research. 2010 Mar;89(3):219-29.
12. Earley MJ. Wounds, healing and tissue repair. In: O'Connell PR, McCaskie AW, Williams NS, editors. Bailey & Love's Short Practice of Surgery. 27th ed. Boca Raton (FL): CRC Press; 2018. p. 24-33. ISBN 9781351617994
13. Schultz GS, Davidson JM, Kirsner RS, Bornstein P, Herman IM. Dynamic reciprocity in the wound microenvironment. Wound Repair and Regeneration. 2011 Mar;19(2):134-48.
14. Kanji S, Das H. Advances of stem cell therapeutics in cutaneous wound healing and regeneration. Mediators of inflammation. 2017 Oct 29;2017.
15. Kohlhauser M, Luze H, Nischwitz SP, Kamolz LP. Historical evolution of skin grafting—a journey through time. Medicina. 2021 Apr 5;57(4):348.
16. Goodacre T. Plastic and reconstructive surgery. In: O'Connell PR, McCaskie AW, Williams NS, editors. Bailey & Love's Short Practice of Surgery. 27th ed. Boca Raton (FL): CRC Press; 2018. p. 24-33. ISBN 9781351617994
17. McGregor AD. Free skin grafts. In: Fundamental techniques of plastic surgery. 10th ed. China: Churchill Livingstone; 2000. p. 35–60. ISBN 9780443063725

18. Scherer-Pietramaggiori SS, Pietramaggiori G, Orgill DP. Skin graft. In: Neligan PC, Gurtner GC, editor. Plastic surgery: Volume 1: Principles. 4th ed. Canada: Elsevier Science; 2018. p. 213–30. ISBN 9780323356947
19. Reddy S, El-Haddawi F, Fancourt M, Farrant G, Gilkison W, Henderson N, Kyle S, Mosquera D. The incidence and risk factors for lower limb skin graft failure. Dermatology research and practice. 2014 Jul 15;2014.
20. Kim Y, Kym D, Cho YS, Yoon J, Yim H, Hur J, Chun W. Use of fibrin sealant for split-thickness skin grafts in patients with hand burns: a prospective cohort study. Advances in Skin & Wound Care. 2018 Dec;31(12):551.
21. Fréchette JP, Martineau I, Gagnon G. Platelet-rich plasmas: growth factor content and roles in wound healing. Journal of dental research. 2005 May;84(5):434-9.
22. Hasiba-Pappas SK, Tuca AC, Luze H, Nischwitz SP, Zrim R, Geißler JC, Lumenta DB, Kamolz LP, Winter R. Platelet-rich plasma in plastic surgery: a systematic review. Transfusion Medicine and Hemotherapy. 2022;49(3):129-42.
23. Amaral RJ, da Silva NP, Haddad NF, Lopes LS, Ferreira FD, Cappelletti PA, de Mello W, Cordeiro-Spinetti E, Balduino A. Platelet-rich plasma obtained with different anticoagulants and their effect on platelet numbers and mesenchymal stromal cells behavior in vitro. Stem cells international. 2016 Oct;2016.
24. Mehta S, Watson JT. Platelet rich concentrate: basic science and current clinical applications. Journal of orthopaedic trauma. 2008 Jul 1;22(6):432-8.
25. Hashemi SS, Mahmoodi M, Rafati AR, Manafi F, Mehrabani D. The role of human adult peripheral and umbilical cord blood platelet-rich plasma on

proliferation and migration of human skin fibroblasts. World journal of plastic surgery. 2017 May;6(2):198.

26. Carter MJ, Fylling CP, Parnell LK. Use of platelet rich plasma gel on wound healing: a systematic review and meta-analysis. Eplasty. 2011;11.
27. Tayapongsak P, O'Brien DA, Monteiro CB, et al. Autologous fibrin adhesive in mandibular reconstruction with particulate cancellous bone and marrow. J Oral Maxillofac Surg. 1994;52:161–165.
28. Samadi P, Sheykhhasan M, Khoshinani HM. The use of platelet-rich plasma in aesthetic and regenerative medicine: a comprehensive review. Aesthetic plastic surgery. 2019 Jun;43(3):803-14.
29. Mishra A, Pavelko T. Treatment of chronic elbow tendinosis with buffered platelet-rich plasma. The American journal of sports medicine. 2006 Nov;34(11):1774-8.
30. Anitua E, Andia I, Ardanza B, Nurden P, Nurden AT. Autologous platelets as a source of proteins for healing and tissue regeneration. Thrombosis and haemostasis. 2004;91(01):4-15.
31. Hsu YC, Li L, Fuchs E. Emerging interactions between skin stem cells and their niches. Nature medicine. 2014 Aug;20(8):847-56.
32. Cameli N, Mariano M, Cordone I, Abril E, Masi S, Foddai ML. Autologous pure platelet-rich plasma dermal injections for facial skin rejuvenation: clinical, instrumental, and flow cytometry assessment. Dermatologic Surgery. 2017 Jun 1;43(6):826-35.
33. Charles-de-Sa´ L et al (2017) Effect of use of platelet-rich plasma (PRP) in skin with intrinsic aging process. Aesthetic Surg J38(3):321–328

34. Cervelli V, Gentile P, De Angelis B, Calabrese C, Di Stefani A, Scioli MG, Curcio BC, Felici M, Orlandi A. Application of enhanced stromal vascular fraction and fat grafting mixed with PRP in post-traumatic lower extremity ulcers. Stem cell research. 2011 Mar 1;6(2):103-11.

35. Serra-Mestre JM, Serra-Renom JM, Martinez L, Almadori A, D'Andrea F. Platelet-rich plasma mixed-fat grafting: a reasonable prosurvival strategy for fat grafts?. Aesthetic plastic surgery. 2014 Oct;38(5):1041-9.

36. Parrish JN, Metzinger SE. Autogenous fat grafting and breast augmentation: a review of the literature. Aesthetic surgery journal. 2010 Jul 1;30(4):549-56.

37. Lacci KM, Dardik A. Platelet-rich plasma: support for its use in wound healing. The Yale journal of biology and medicine. 2010 Mar;83(1):1.

38. Adly OA, Ahmad AS. Evaluation of topical application of platelet gel in skin grafted burn wounds. Egypt J Plast Reconstr Surg. 2011 Jul;35(2):233-7.

39. Thimmanahalli GU, Kumar M. Efficacy of autologous platelet rich plasma over conventional mechanical fixation methods in split thickness skin grafting. International Surgery Journal. 2018 Dec 27;6(1):108-13.

40. Vaheb M, Karrabi M, Khajeh M, Asadi A, Shahrestanaki E, Sahebkar M. Evaluation of the effect of platelet-rich fibrin on wound healing at split-thickness skin graft donor sites: a randomized, placebo-controlled, triple-blind study. The International Journal of Lower Extremity Wounds. 2021 Mar;20(1):29-36.

41. Gupta S, Goil P, Thakurani S. Autologous Platelet Rich Plasma As A Preparative for resurfacing burn Wounds with Split Thickness Skin Grafts. World J Plast Surg 2020;9(1):29-32. doi: 10.29252/wjps.9.1.29.

42. Tyagi A, Gupta A, Martires III VI, Romo M, Garg I, Tapia D, Gudino P, Lam S. Efficacy of Platelet-Rich Plasma in Reduction of Post-Operative Split-Thickness Skin Graft Loss and Hematoma Formation: A Meta-Analysis. Cureus. 2021 May 21;13(5).

43. Chen J, Wan Y, Lin Y, Jiang H. The application of platelet-rich plasma for skin graft enrichment: a meta-analysis. International Wound Journal. 2020 Dec; 17(6):1650-8.

44. Moster M, Bolliger D. Perioperative Guidelines on Antiplatelet and Anticoagulant Agents: 2022 Update. Current Anesthesiology Reports. 2022 Jan 19:1-1.

45. Kao DS, Zhang SW, Vap AR. A Systematic Review on the Effect of Common Medications on Platelet Count and Function: Which Medications Should Be Stopped Before Getting a Platelet-Rich Plasma Injection?. Orthopaedic Journal of Sports Medicine. 2022 Apr 12; 10(4):23259671221088820.

46. Yalagachin G, Hiregoudar AD, Mashal SB, Bagur A, Shivaramu NG. A comparative study of graft uptake in split skin grafting between the first postoperative dressing done on day 3 versus day 5. International Surgery Journal. 2021 Nov 26; 8(12):3615-21.

ANNEXURE I – CONSENT FORM

INFORMED CONSENT FOR PARTICIPATION IN RESEARCH STUDY

Mr/Mrs _____ we are requesting you to enroll yourself in study titled "**AUTOLOGOUS PLATELET RICH PLASMA AND CONVENTIONAL SUTURE/ STAPLES- A RANDOMISED CONTROL TRIAL TO COMPARE THEIR EFFICACY IN ANCHORING SPLIT SKIN GRAFT ON WOUNDS**." conducted by **_____,** Postgraduate in M.S. General Surgery under the guidance of **_____,** Professor in Department of General Surgery, J.N. Medical College, Belagavi under KLE University, Belagavi and **_____,** Professor and HOD, Plastic Surgery.

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 decide not to participate, you are free to withdraw at any time.

Purpose of study:

The purpose of the study is to compare the effectiveness of using Platelet Rich Plasma as an anchoring agent for split skin thickness graft on wound beds with that of conventional method of anchoring skin graft i.e. by sutures or staples.

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 Haemoglobin, Total Count, Differential Count, Platelet Count, RBS, Blood Urea, Serum Creatinine, Blood Grouping and USG Doppler will be done. 3ml blood will be collected from the patient using sterile technique while under anaesthesia just before the start of procedure of skin grafting. The blood collected in vial containing Na Citrate as anticoagulant and medium. This blood sample will then be centrifuged at 3500 rpm using centrifuge machine separating it into 3 layers. The top layer which is rich in platelets will be extracted into syringes with sterile 18 Gauge needle and kept ready for use. PRP will be applied over the wound as a thin layer just before the skin graft is placed. It will then be assessed for immediate uptake of the graft by moving the surgeon's finger over the graft. The wound will be covered by a non-adhesive mesh topped with betadine soaked cotton wool and then secured with compression dressing. Subsequent dressings will be done as per protocol followed in the unit and the grafted area shall be assessed for % uptake of the graft.

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
- Failure of uptake of significant area of the graft, requiring second surgery.
- Pain at recipient and donor site of skin graft.

Type of Study

This study is an interventional study. It involves using PRP as an anchoring agent for split skin thickness graft.

Participant selection

It includes all patients with wounds requiring split skin thickness grafting for treatment, as per the exclusion and inclusion criteria's.

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 that is given at our hospital which is split skin grafting over your wound and anchorage of the same with staples or sutures,. 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 will 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, as per Hospital policy. Patient will not incur any extra expenditure during the study/ as a result of unintended outcome of study. There is no compensation or payment for treatment in case of unintentional effect of the study by law.

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

If they have any queries about their rights as a study subject, they may call **DR. HARSHA HEGDE.**, Chairman, and Ethical Committee for Human Subjects Research Scientist D, ICMR, National Institute of Traditional Medicine, Belagavi. Phone number-9480422500.

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 II – PROFORMA

Sl No.	CRITERIA	DETAILS		
1.	Patient name/ IP NO/ DOA/ Date of operation			
2.	Age/ SEX			
3.	Etiology of wounds			
4.	HOPI			
5.	Comorbidities, and medications			
6.	Antiplatelet drugs			
7.	Initial Dimension of wound			
8.	Baseline Investigations			
9.	Immediate Pre-op investigations			
10.	Pre-op blood product transfusion			
11.	Pre-op antibiotics			
12.	Pre-op Dressings			
13.	Initial area of wound			
1.	Adherence of graft @1min @2min @3min			
2.	POD of change of dressing	POD	Seroma	Hematoma
	1st- change			
	2nd change			
	3rd change			
3.	% uptake of graft At 1st change POD___ At 2nd change POD____ At 3rd change POD_____			
4.	DATE of DISCHARGE			

ANNEXURE III: PHOTOGRAPHS

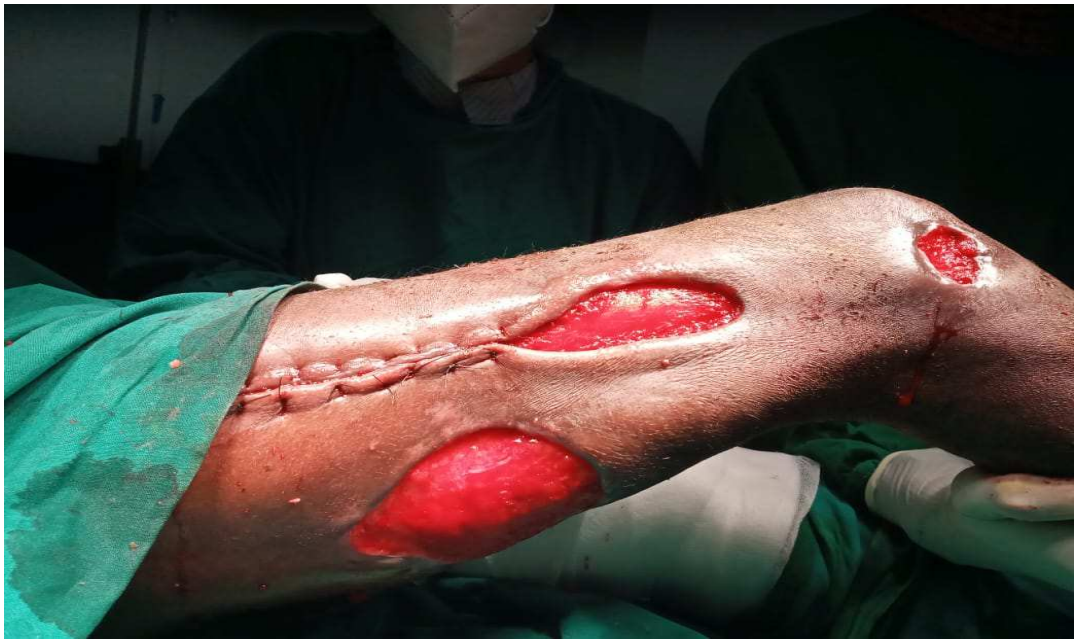


Photo 1: Multiple non- contiguous wound over lateral aspect of Right thigh post fasciotomy for compartment syndrome secondary to trauma.
Wound A: Control Wound B: PRP group

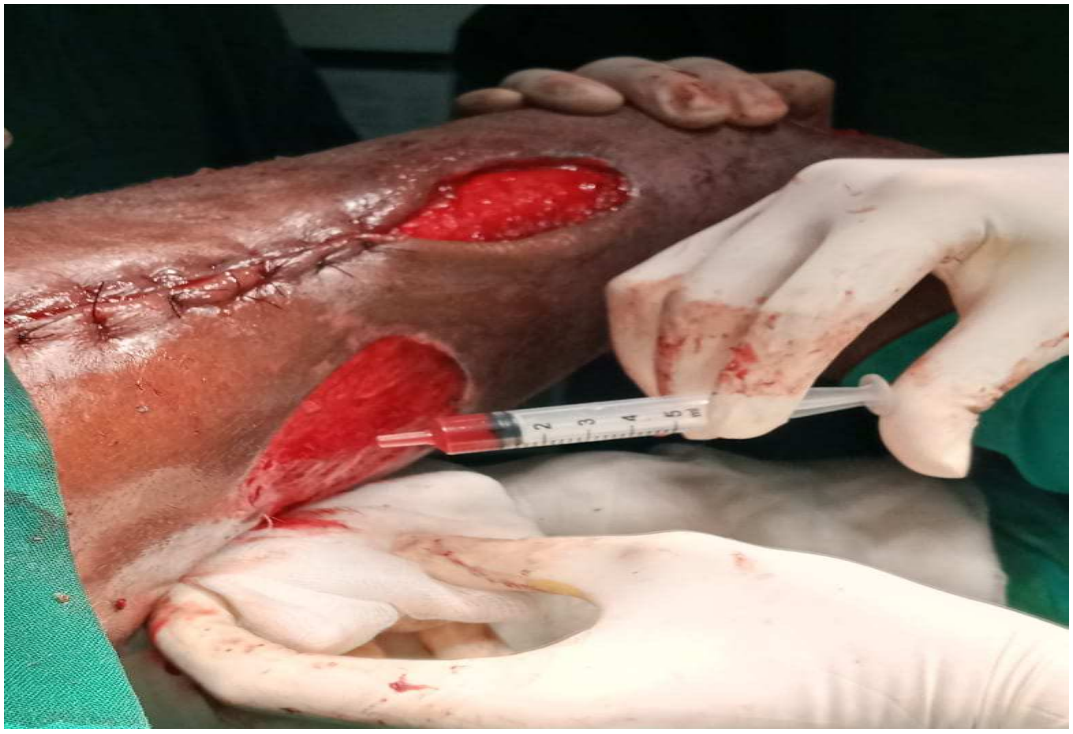


Photo 2: Application of PRP over study wound in sterile technique.

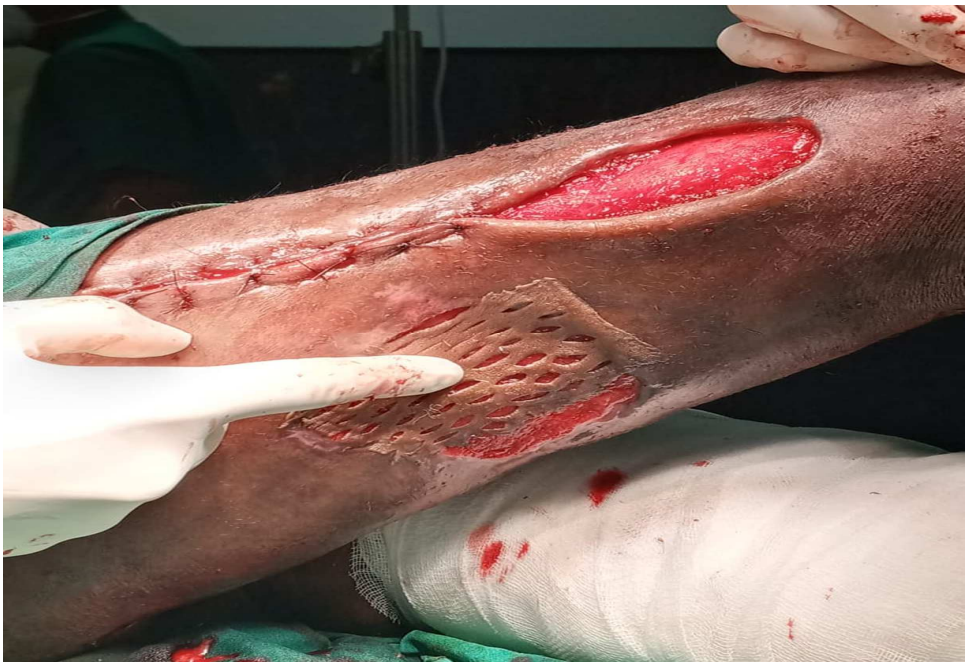


Photo 3: Assessment of STSG mobility on recipient site after application of PRP

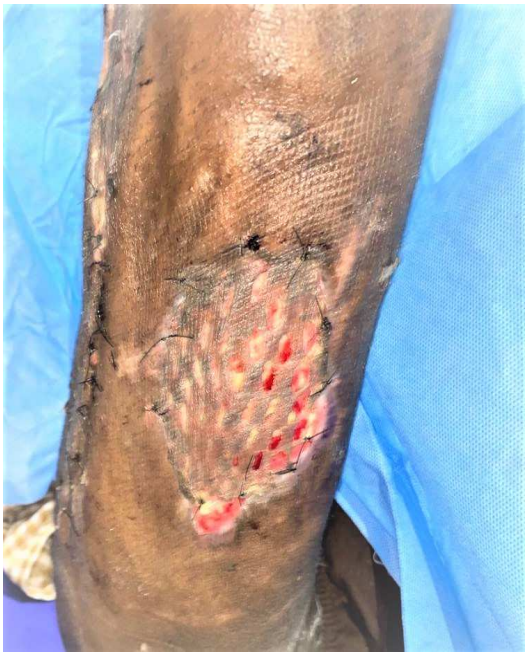


Photo 4: POD 3 first post-operative dressing of intervention group wound

Photo 5: POD 7 final take of graft in PRP group

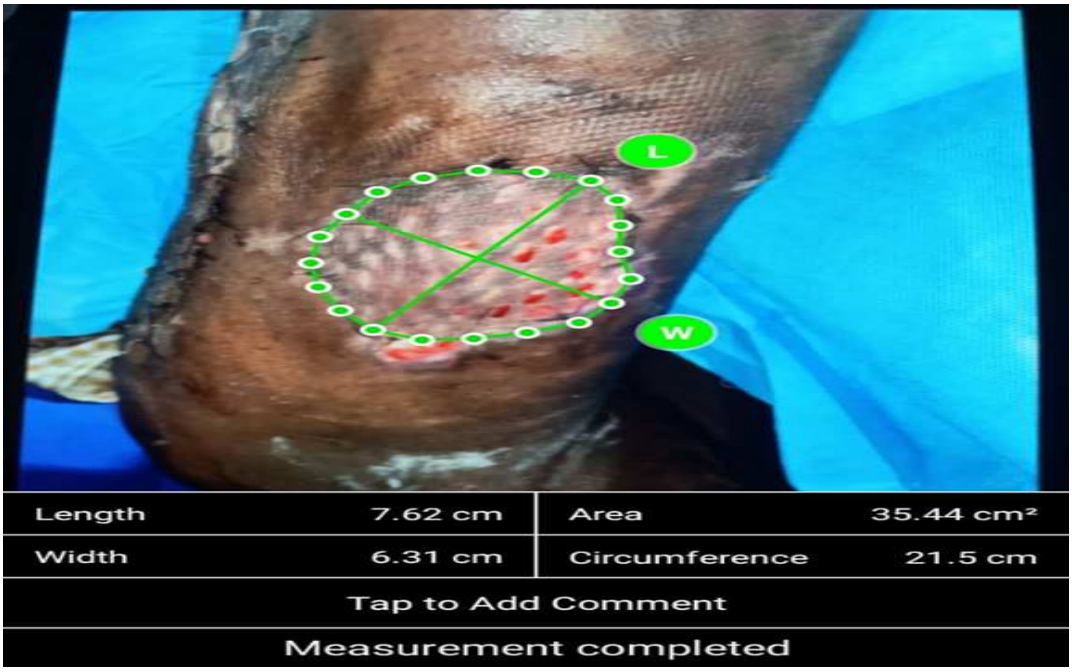


Photo 6: Assessment of Post- op Area of graft retained by using ImitoMeasure App

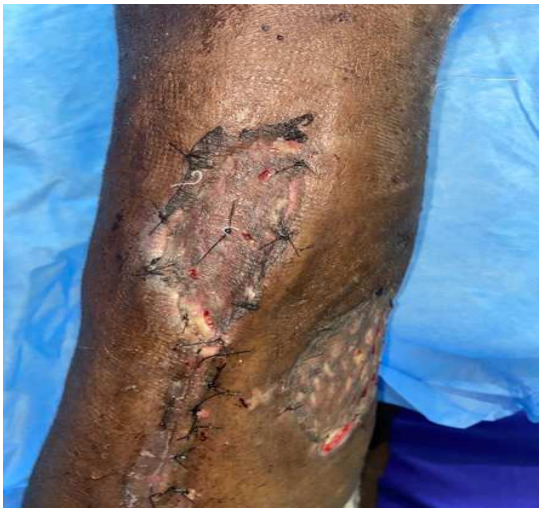


Photo 7: POD 3 of control group STSG site.

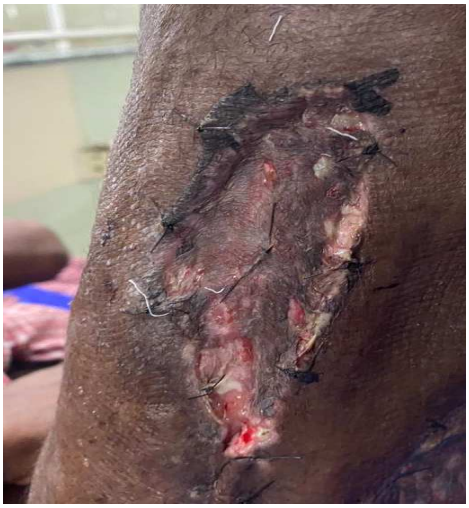


Photo 8: POD 7 of control group STSG site.

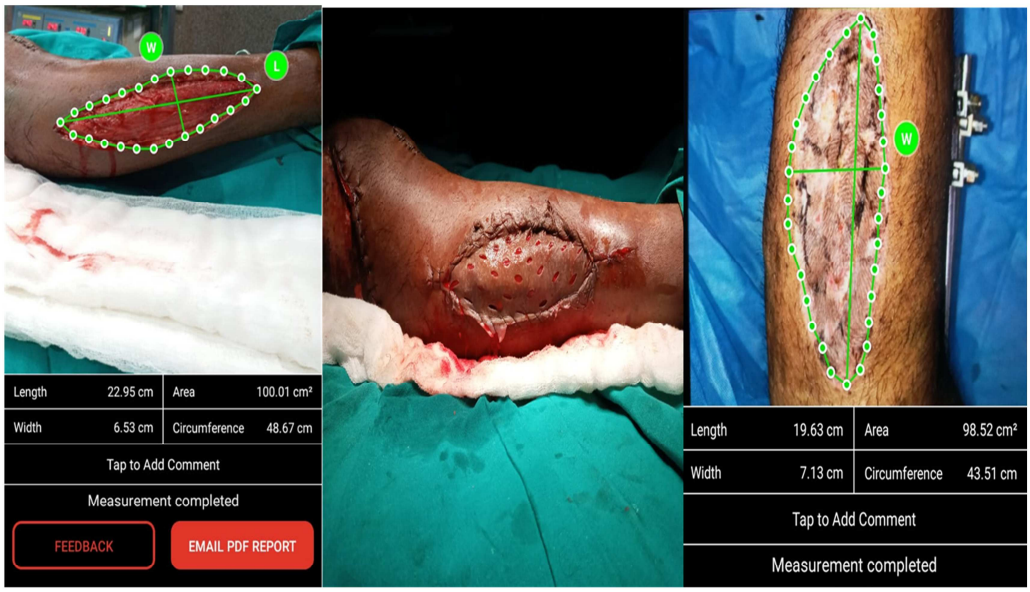


Photo 9: Post fasciotomy ulcer in PRP group grafted and final take of the graft

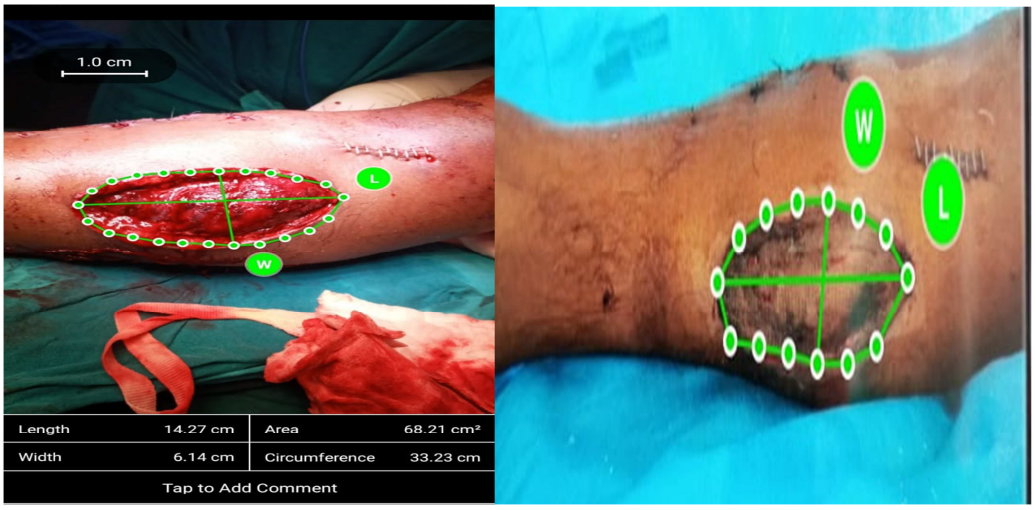


Photo 10: Control Group grafted area- with Post op- epidermal necrosis

ANNEXURE IV– KEY TO MASTER CHART

IP No	Same
Date of Admission	Same
Date of Procedure	Same
Date of Discharge	Same
Age	Same
Sex	Male 1 Female 2
Aetiology	Thermal 1 Fasciotomy 2 Trauma/ degloving injury 3 Flap donor/ inset 4 Ulcer 5 Hypertrophic scar/ contracture release excision 6
Location	Face 1 Right upper limb 2 Left upper limb 3 Right lower limb 4 Left lower limb 5 Back 6 Abdomen 7 Axilla 8 L inguinal region 9 R inguinal region 10 Others
Comorbidities	nil 1 PVD 2 AKI 3
Antiplatelet drugs	Nil 1 Aspirin 2
initial dimension (cm)	no code
pre-transfusion	nil 1 only packed cells 2 only ffp 3 both 4
Hb (g/dL)	no code
PLT (L/cm3)	no code
TC (/cm3)	no code
TP (g/dL)	no code
Alb (g/dL)	no code
initial area (cm2)	No code
1 min adhesion	Adherent 1 Not adherent 2
2 min adhesion	adherent 1 Not adherent 2

3 min adhesion	adherent 1 Not adherent 2
1st change Post op day	no code
2nd change Post op day	no code
3rd change Post op day	no code
Seroma on 1st dressing	Nil 1 present 2
Seroma on 2nd dressing	nil 1 present 2
Seroma on 3rd dressing (ml)	nil 1 present 2
Haematoma on 1st dressing	nil 1 present 2
Haematoma on 2nd dressing	nil 1 present 2
Haematoma on 3rd dressing	nil 1 present 2
Uptake (%) on 1st dressing	no code
Uptake (%) on 2nd dressing	no code
Uptake (%) on 3rd dressing	no code
Case/control	Case 1 control 2

ANNEXURE V–MASTER CHART

CASES

sl no	IP No	doa	dop	dod	Age (years)	sex	etiology	location	Comorbidities	Antiplatelet drugs	initial dimension (cm)	pre-transfusion	Hb (g/dL)	PLT (l/cumm)	TC (/cumm)	Serology	TP (g/dL)	Alb (g/dL0	initial area (cm2)	1 min adhesion	2 min adhesion	3 min adhesion	1st change (POD)	2nd change (POD)	3rd change (POD)	1 st seroma (yes/no)	2nd seroma (yes/no)	3rd seroma (yes/ no)	1st hematoma (yes/ no	2nd hemaoma (yes/ no	3rd hematoma (yes/ no	1 uptake(%)	2nd uptake (%)	3rd uptake (%)	Case/control
1	1080152	13-11-2021	21-12-2021	03-01-2022	35	1	1	4	1	1	14.97 x 12.35	1	10.3	3.6	18,400	1	7	2.9	123.85	1	1	1	3	5	10	1	1	1	1	1	1	100.00	96.10	96.10	1
2	1083746	03-12-2021		31-12-2021	30	2	3	4	1	2	18.53 x 8.24	2	9.4	7.16	12,300	1	6.5	3	104	2	1	1	3	5	10	1	1	1	1	1	1	100.00	100.00	100.00	1
3	1086849	20-11-2021	29-12-2021	10-01-2022	24	1	2	4	1	1	14.27 x 6.14	1	11.3	2.92	11,100	1	6.4	4.6	68.21	1	1	1	3	5	7	1	1	1	1	1	1	100.00	100.00	100.00	1
4	1084025	04-12-2021	29-12-2021	08-01-2022	58	1	5	4	1	1	10 x 8 cm	1	10.2	2.28	12,000	1	6	3	115.62	2	1	1	3	5	7	2	1	1	2	1	1	100.00	100.00	100.00	1
5	1080153	13-11-2021	.	03-01-2022	34	1	1	3	1	1	18.84 x 11.7	3	11.8	3.42	12,100	1	6.5	2.9	126.76	1	1	1	3	5	7	2	1	1	1	1	1	98.00	98.00	97.80	1
6	1079149	08-11-2021	13-11-2021	30-11-2021	23	1	1	2	1	1	15 x 8	4	9.5	4.89	35,000	1	4	2.2	320	1	1	1	2	5	7	2	1	1	1	1	1	100.00	100.00	100.00	1
7	1079149	08-11-2021	13-11-2021	30-11-2021	23	1	1	5	1	1	10 x 20	4	9.5	4.89	35,000	1	4	2.2	200	1	1	1	2	5	7	1	1	1	1	1	1	100.00	99.70	99.70	1
8	1079093	08-11-2021	20-11-2021	06-12-2021	40	1	2	4	1	1	7.51 x 6.45	2	8.8	5.49	12	1	6.7	2.7	32.35	1	1	1	3	5	10	1	1	1	1	1	1	100.00	100.00	100.00	1
9	1085229	11-02-2021	17-12-2021	23-12-2021	20	1	2	4	1	2	10 x 10	2	9.8	3.15	12,300	1	6.9	3.1	100	1	1	1	3	5	10	1	1	1	1	1	1	100.00	99.70	99.70	1
10	1084643	08-12-2021	21-12-2021	29-12-2021	20	2	5	7	1	1	20x 6	2	10	2	16,600	1	5.6	2.8	120	1	1	1	2	5	10	2	1	1	2	1	1	100.00	99.80	99.80	1
11	1082402	25-11-2021	26-11-2021	16-12-2021	52	1	4	2	1	1	5 x3	1	13.2	2.04	14,980	1	6.4	2.4	15	1	1	1	2	5	10	2	1	1	1	1	1	99.00	99.00	99.00	1
12	1082402	25-11-2021	26-11-2021	16-12-2021	52	1	4	2	1	1	5 x 4	1	13.2	2.04	14,980	1	6.4	2.4	20	1	1	1	2	5	10	2	1	1	1	1	1	99.00	99.00	99.00	1
13	1082402	25-11-2021	26-11-2021	16-12-2021	52	1	4	2	1	1	5x 5	1	13.2	2.04	14,980	1	6.4	2.4	25	1	1	1	2	5	10	2	1	1	1	1	1	99.00	99.00	99.00	1
14	1061417	26-07-2021			50	1	2	4	1	1	15 x 8	1	13.7	3.04	14,980	1	5.6	3	120	1	1	1	3	5	7	1	1	1	1	1	1	100.00	100.00	100.00	1
15	1066526	29-08-2021	05-10-2021	14-10-2021	30	1	1	6	1	1	7 x 20	3	14.1	2.76	7,200	1	6.6	4.3	140	1	1	1	3	5	7	1	1	1	1	1	1	100.00	100.00	100.00	1
16	1066526	29-08-2021	05-10-2021	14-10-2021	30	1	1	6	1	1	1 x 10	3	14.1	2.76	7,200	1	6.6	4.3	70	1	1	1	3	5	7	1	1	1	1	1	1	100.00	100.00	100.00	1
17	1075785	16-10-2021			18	2	6	4	1	1	45 x10	1	12.2	2.53	12,900	1	5.4	2.5	450	2	1	1	5	7	9	2	1	1	2	1	1	95.56	95.65	95.56	1
18	1075329	17-10-2021	25-10-2021	17-12-2021	37	1	2	4	1	2	4 x 20	2	10	2.35	14,000	1	7	4.5	80	1	1	1	3	5	7	1	1	1	1	1	1	95.56	96.75	96.75	1
19	1075329	17-10-2021	25-10-2021	17-12-2021	37	1	2	4	1	2	5 x 9	2	10	2.35	14,000	1	7	4.5	45	1	1	1	3	5	7	1	1	1	1	1	1	100.00	96.30	96.30	1
20	1030840				41	1	2	4	1	1	7 x 30	1	9.5	4	8,000	1	6.5	3	210	2	1	1	3	5	10	2	1	1	1	1	1	100.00	100.00	100.00	1
21	1046645	27-03-2021	05-04-2021	30-04-2021	18	1	2	4	1	1	5 x 7	1	11.6	2.86	14,700	1	6.8	3.9	35	2	1	1	3	5	10	2	1	1	1	1	1	100.00	100.00	100.00	1
22	1092962	26-01-22	15-02-22	23-02-22	22	1	2	3	1	1	10 x 4	1	8.2	1.27	8,600	1	6.9	3.1	40	1	1	1	3	5	7	1	1	1	1	1	1	98%	98%	98%	1
23	1106334	11-04-22	13-04-22	25-04-22	18	1	1	2	1	1	15 X 7	2	10.4	1.84	18,300	1	6.5	3	105	1	1	1	3	5	7	1	1	1	1	1	1	98%	98%	97.80%	1
24	1098188	24-02-22	04-03-22	14-03-22	60	1	1	5	1	1	12 x 8	1	10.7	0.97	8,800	1	6.4	4.6	96	1	1	1	3	5	7	1	1	1	1	1	1	99%	98%	98%	1
25	1097258	19-02-22	22-02-22	07-03-22	28	1	1	2	1	1	10 x 5	1	14.1	3.27	14,800	1	6	3	50	1	1	1	3	5	7	1	1	1	1	1	1	99%	98%	98%	1
26	1106334	11-04-22	13-04-22	25-04-22	18	1	1	3	1	1	10 X 4	2	10.4	1.84	18,300	1	6.5	3	40	1	1	1	3	5	7	1	1	1	1	1	1	100%	98%	97.70%	1
27	1106345	11-04-22	12-04-22	21-04-22	59	1	3	4	1	1	3.5 x 3	1	12.3	2.97	9,000	1	4	2.2	10.5	1	1	1	3	5	7	1	1	1	1	1	1	100%	100%	100%	1
28	1105381	06-04-22	07-04-22	16-04-22	43	1	3	5	1	1	3 x 2	1	13.5	1.95	10,000	1	4	2.2	6	1	1	1	3	5	10	1	1	1	1	1	1	100%	1005%	100%	1
29	1106354	11-04-22	27-04-22	07-05-22	64	1	5	4	1	1	4 x 4	1	14	2.7	5,800	1	6.7	2.7	16	1	1	1	3	5	7	1	1	1	1	1	1	100%	99%	99%	1
30	1098188	24-02-22	04-03-22	14-03-22	60	2	1	5	1	1	10 x 8	1	10.7	0.97	8,800	1	6.4	4.6	80	1	1	1	3	5	7	1	1	1	1	1	1	100%	99.80%	98%	1
31	1097258	19-02-22	22-02-22	07-03-22	28	1	1	3	1	1	10 x 8	1	14.1	3.27	14,800	1	6	3	80	1	1	1	3	5	7	1	1	1	1	1	1	100%	98%	98%	1
32	1113898	18-05-22	20-05-22	27-05-22	31	1	6	8	1	1	7 x 8	1	13.4	3.25	6,900	1	6.4	2.4	56	1	1	1	3	5	7	1	1	1	1	1	1	99%	99%	98.70%	1
33	1109914	29-04-22	21-05-22	31-05-22	70	1	5	5	1	1	7 x 4	1	12.1	1.93	6,870	1	8.2	3.6	28	1	1	1	3	5	7	1	1	1	1	1	1	100%	100%	100%	1
34	1122935	27-06-22	29-06-22	09-07-22	65	1	3	4	1	1	7 x 4		9.7	7.58	9,200	1	7	3.8	28	1	1	1	3	5	7	1	1	1	1	1	1	99%	98%	98%	1
35	1110453	02-05-22	21-05-22	01-06-22	67	1	3	4	1	1	7 x 5	1	10.5	1.72	18,670	1	6.4	2.8	35	1	1	1	3	5	7	1	1	1	1	1	1	100%	100%	100%	1
36	1110669	26-11-40	09-05-22	16-05-22	36	2	6	1	1	1	10 x 7	1	12.5	2.45	5,600	1	7.2	3	70	1	1	1	3	5	7	1	1	1	1	1	1	100%	100%	100%	1
37	1118629	09-06-22	10-06-22	25-06-22	62	1	3	4	1	1	10 x 8	1	13.3	4.78	8,400	1	6.5	2.6	80	1	1	1	3	5	7	1	1	1	1	1	1	100%	98%	98%	1
38	1120469	17-06-22	25-06-22	01-07-22	70	1	3	4	1	1	10 x 8	1	12.1	1.59	6,700	1	6.9	2.72	80	1	1	1	3	5	7	1	1	1	1	1	1	100%	99%	99%	1
39	1101992	18-03-22	25-06-22	09-05-22	42	1	3	4	2	2	10 x 16	2	7.2	2.14	9,800	1	5.8	2.4	160	1	1	1	3	5	7	1	1	1	1	1	1	100%	100%	100%	1
40	1086849	20-11-2021	29-12-2021	10-01-2022	24	1	2	4	1	1	14.27 x 6.14	1	11.3	2.92	11,100	1	6.4	4.6	68.21	1	1	1	3	5	7	1	1	1	1	1	1	100.00	100.00	100.00	1

CONTROL

sl no	IP No	doa	dop	dod	Age (years)	sex	etiology	location	Comorbidities	Antiplatelet drugs	initial dimension (cm)	pre-transfusion	Hb (g/dL)	PLT (L/cumm)	TC (/cumm)	Serology	TP (g/dL)	Alb (g/dL0	initial area (cm2)	1 min adhesion	2 min adhesion	3 min adhesion	1st change (POD)	2nd change (POD)	3rd change (POD)	1 st seroma (yes/no)	2nd seroma (yes/no)	3rd seroma (yes/ no)	1st hematoma (yes/ no)	2nd hemaoma (yes/ no)	3rd hematoma (yes/ no	1 uptake(%)	2nd uptake (%)	3rd uptake (%)	Case/control
41	1079093	08-11-2021	20-11-2021	06-12-2021	40	1	2	4	1	1	9.01 x 3.21	2	8.8	5.49	12	1	6.7	2.7	20.53	2	2	2	3	5	10	2	1	1	1	1	1	69.00	69.00	69.00	2
42	1079149	08-11-2021	13-11-2021	30-11-2021	23	1	1	4	1	1	10 x 30	4	9.5	4.89	35,000	1	4	2.2	300	2	2	2	2	5	7	2	1	1	1	1	1	95.00	93.00	93.00	2
43	1079149	08-11-2021	13-11-2021	30-11-2021	23	1	1	5	1	1	15 x 10, 12 x 7	4	9.5	4.89	35,000	1	4	2.2	234	2	2	2	2	5	7	2	1	1	1	1	1	95.00	90.00	90.00	2
44	1078805	17-11-2021			60	1	2	4	1	2	20 x 6	2	9.6	5.24	16,000	1	5.8	2.1	120	2	2	1	3	5	10	1	1	1	1	1	1	97.00	96.30	96.30	2
45	1080153	13-11-2021	.	03-01-2022	34	1	1	2	1	1	17.8 x 12.58	3	11.8	3.42	12,100	1	6.5	2.9	119.91	2	2	2	3	5	7	1	1	1	1	1	1	89.00	88.00	88.00	2
46	1075329	17-10-2021	25-10-2021	17-12-2021	37	1	2	4	1	2	7 x 10	2	10	2.35	14,000	1	7	4.5	70	2	2	2	3	5	7	2	2	1	2	1	1	92.85	92.85	92.85	2
47	10334046	06-01-2021	07-01-2021	15-01-2021	23	1	6	2	1	1	15 x 7	1	16.2	2.27	8,200	1	6.9	3.5	105	2	2	2	3	5	7	1	1	1	1	1	1	100.00	100.00	100.00	2
48	10334046	06-01-2021	07-01-2021	15-01-2021	23	1	6	1	1	1	12 x 7	1	16.2	2.27	8,200	1	6.9	3.5	84	2	2	2	3	5	7	1	1	1	1	1	1	100.00	100.00	100.00	2
49	1033699	04-01-2021	05-01-2021	13-01-2021	24	2	1	8	1	1	15 x 7	1	13.07	2.9	7,000	1	6	2.8	105	2	2	2	3	5	7	1	1	1	1	1	1	98.00	98.00	98.00	2
50	1034802	13-01-2021	.	23-01-2021	40	1	2	3	1	1	10 x 7	1	14.1	2.45	8,900	1	7	3.7	70	2	2	2	3	5	7	1	1	1	1	1	1	94.00	93.50	93.00	2
51	1035633	18-01-2021	20-01-2021	01-02-2021	38	1	4	5	1	1	7 x 3	1	12.9	2.62	7,900	1	6.8	3.8	21	2	2	2	2	5	7	1	1	1	1	1	1	100.00	100.00	100.00	2
52	1036637	26-01-2021	27-01-2021	04-02-2021	38	2	5	5	1	1	12 x 7	1	11.7	2.76	15,000	1	5.9	2.4	84	2	2	2	3	6	8	1	1	1	1	1	1	100.00	90.00	90.00	2
53	1046645	27-03-2021	05-04-2021	30-04-2021	18	1	2	4	1	1	8 x 4	1	11.6	2.86	14,700	1	6.8	3.9	32	2	2	2	3	5	10	2	1	1	1	1	1	100.00	98.00	90.00	2
54	1032645	26-12-2021	04-01-2021	31-01-2021	60	1	5	4	1	2	13 x 8	1	11.1	2.45	10,000	1	7	3.9	104	2	2	2	3	5	7	2	1	1	1	1	1	97.00	90.00	90.00	2
55	1075329	17-10-2021	25-10-2021	17-12-2021	37	1	2	4	1	1	7 x 10	2	10	2.35	14,000	1	7	4.5	70	2	2	2	3	5	7	2	1	1	2	1	1	92.85	92.85	92.85	2
56	1091818	18-01-22	26-01-22	07-02-22	25	1	5	8	1	2	10 x 8	1	14.1	2.78	8,600	1	6.7	2.7	80	2	2	2	3	5	8	2	1	1	1	1	1	95.00%	90.00%	87.70%	2
57	1101963	17-03-22	18-03-22	28-03-22	23	1	3	4	1	1	10 x 3	1	14.8	2.6	12,400	1	4	2.2	30	2	2	2	3	5	8	1	1	1	1	1	1	98.00%	90.00%	90.00%	2
58	1098722	28-02-22	03-02-22	11-03-22	68	1	2	2	1	1	30 x 9	1	10.9	4.25	20,200	1	4	2.2	270	2	2	2	3	5	7	2	1	1	1	1	1	95.00%	92.00%	90.00%	2
59	1097258	19-02-22	22-02-22	07-03-22	28	1	1	3	1	1	10 x 3	1	14.1	3.27	14,800	1	5.8	2.1	30	2	2	2	3	5	7	1	1	1	1	1	1	95.00%	90.00%	89.90%	2
60	1098188	24-02-22	04-03-22	14-03-22	60	2	1	4	1	1	9 x 13	1	10.7	0.97	8,800	1	6.4	4.6	80	2	2	2	3	5	7	2	1	1	1	1	1	80.00%	78.00%	77.00%	2
61	1097466	22-02-22	22-02-22	24-02-22	56	2	2	2	1	1	5 x 2	1	11.6	5.99	13,100	1	7	4.5	10	2	2	2	3	5	7	1	1	1	1	1	1	95.00%	93.00%	93.00%	2
62	1093339	29-01-22	15-02-22	08-03-22	8	1	3	4	1	1	12 x 4	1	12.5	2.76	14,800	1	6.9	3.5	48	2	2	2	3	5	10	1	1	1	1	1	1	80.00%	75.00%	73.20%	2
63	1093339	29-01-22	15-02-22	08-03-22	8	1	3	4	1	1	5 x 7	1	12.5	2.76	14,800	1	6.9	3.5	48	2	2	2	3	5	10	1	1	1	1	1	1	95.00%	95.00%	95.00%	2
64	1097715	22-02-22	23-02-22	28-02-22	9	1	1	9	1	1	15 x 7	1	11.7	4.76	8,600	1	6	2.8	105	2	2	2	3	5	7	1	1	1	1	1	1	92.00%	88.00%	87.60%	2
65	1091342	15-01-22	07-02-22	14-02-22	8	2	3	9	1	1	4 x 6	1	11	2.04	13,800	1	7	3.7	24	2	2	2	3	5	7	1	1	1	1	1	1	94.00%	93.00%	92.80%	2
66	1091342	15-01-22	07-02-22	14-02-22	8	2	3	10	1	1	7 x 6	1	11	2.04	13,800	1	7	3.7	24	2	2	1	3	5	7	1	1	1	1	1	1	95.00%	93.00%	93.00%	2
67	1097984	23-02-22	24-02-22	02-03-22	32	1	5	1	1	1	3 x 2	1	15.4	3.73	6,900	1	5.9	2.4	6	2	2	2	3	5	7	1	1	1	1	1	1	98.00%	98.00%	95.00%	2
68	1097984	23-02-22	24-02-22	02-03-22	32	1	5	1	1	1	4 x 5	1	15.4	3.73	6,900	1	5.9	2.4	6	2	2	2	3	5	7	1	1	1	1	1	1	95.00%	95.00%	95.00%	2
69	1097984	23-02-22	24-02-22	02-03-22	32	1	5	1	1	1	2 x 2	1	15.4	3.73	6,900	1	5.9	2.4	6	2	2	2	3	5	7	1	1	1	1	1	1	98.00%	97.00%	96.50%	2
70	1097398	21-02-22	23-02-22	03-03-22	56	1	3	4	1	1	5 x 4	1	10.7	3.03	8,900	1	7	4.5	20	2	2	2	3	5	7	1	1	1	1	1	1	93.00%	90.00%	89.70%	2
71	1092962	26-01-22	15-02-22	23-02-22	22	1	2	5	1	3	10 x 4	1	8.2	1.27	8,600	1	6.7	2.1	40	2	2	2	3	5	7	1	1	1	1	1	1	95.00%	90.00%	90.00%	2
72	1109340	04-03-22	05-03-22	08-03-22	8	1	2	4	1	1	5 x 3	1	12.8	7.38	5,900	1	4	2.9	15	2	2	2	3	5	7	2	1	1	1	1	1	94.00%	93.00%	90.00%	2
73	1099399	04-03-22	05-03-22	12-03-22	8	1	6	2	1	1	7 x 3	1	13	3.83	13,240	1	4	4.5	21	2	2	2	3	5	7	1	1	1	1	1	1	92.00%	90.00%	90.00%	2
74	1104122	30-03-22	31-03-22	12-04-22	43	1	3	5	1	1	3 x5	1	16.5	2,89	7,800	1	5.8	3.5	15	2	2	2	3	5	7	2	1	1	1	1	1	93.00%	90.00%	90.00%	2
75	1101263	14-03-22	22-03-22	26-03-22	9	1	6	3	1	1	2 x3	1	11.4	01-01-00	15000	1	6.5	3.5	6	2	2	2	3	5	7	1	1	1	1	1	1	93.00%	90.00%	90.00%	2
76	1106334	11-04-22	13-04-22	25-04-22	8	1	1	5	1	1	20 x 5	2	10.4	1.84	18,300	1	6.5	3	100	2	2	2	3	5	7	1	1	1	1	1	1	95.00%	94.00%	93.30%	2
77	1109914	29-04-22	21-05-22	31-05-22	70	1	5	5	1	1	6 x 5	1	12.1	1.93	6,870	1	8.2	3.6	28	2	1	1	3	5	7	1	1	1	1	1	1	98.00%	97.00%	97.00%	2
78	1120770	18-06-22	20-06-22	05-07-22	58	1	2	5	1	1	30 x 5	1	11.1	3.93	7,400	1	6.9	3.8	150	2	2	2	3	5	8	2	1	1	2	1	1	95.00%	95.00%	95.00%	2
79	1121231	20-06-22	21-06-22	29-06-22	59	2	3	5	1	1	10 x 8	1	11.2	3.8	5,800	1	6	2.4	80	2	2	2	3	5	7	1	1	1	1	1	1	96.00%	96.00%	96.00%	2
80	1113689	06-06-22	10-06-22	10-07-22	67	1	3	4	1	1	12 x 6	1	10.5	2.88	8,200	1	7	3.9	72	2	2	2	3	5	7	1	1	1	1	1	1	93.20%	93.00%	93.00%	2