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CONVENTIONAL MECHANICAL FIXATION AND USE OF AUTOLOGOUS PLATELET RICH PLASMA(PRP) IN WOUND BEDS PRIOR TO RESURFACING WITH SPLIT SKIN GRAFT

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INTRODUCTION

Platelet rich plasma is known for its hemostatic, adhesive and healing properties due to the many growth factors released from the platelets into the wound site. Autologous PRP helps to achieve stable hemostasis as it mimics the final stages of the clotting cascade. It adheres to the bed grafts immediately to prevent any collection under the graft or undue shear.(1,2)Decreased production and release, entrapment, degradation or combination of these mechanisms can lead to a decreased growth factors in chroniculcers that delay wound healing, which can be overcome by PRP.(3,4) Factors affecting graft take include shearing of graft, contaminated or poorly vascularised bed, seroma/ hematoma formation, co morbid conditions and smoking. Graft take is decreased in structures with decreased blood supply such as cartilage, bone and tendon. Wound bed should be vascular, free of streptococcal infection, pus. The success of skin graft depends on , haemostasis and adhesion of skin graft to wound bed, local vascularity, wound microbiology. Hemostasis can be achieved by applying epinephrine socks to the wound bed application to application of skin grafts, but with local and systemic side effects.(5) While normal platelets are approximately 1,50,000 to 4,50,000 / cubic mm in the blood, platelet rich plasma (PRP) has a higher platelet ratio than baseline compared to the same volume of whole blood. Growth factors in PRP promote angiogenesis, collagen synthesis and epithelisation, reduce dermal scarring and facilitate remodelling.(6) Skin graft is conventionally fixed to wound margins with sutures, staplers, cyano acrylate glue or fibrin glue and quilted to the wound bed in order to prevent shearing and seroma under the graft. However, these methods added to the operating time and cost.' (7,8)As it mimics the last steps of coagulation cascade Autologous PRP helps achieve stable haemostasis

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MATERIALS AND METHODS

Source Of data- patients having acute and chronic traumatic ulcers , infective wounds ,chronic ulcers admitted to ESIC MC &PGIMSR BANGALORE between November 2022 to November 2023 were included in the study.

Method of collection of data:

The present study is a prospective randomised comparative study.

Here patients will be divided into two
groups, A (PRP group) and B (Control group).

Inclusion criteria

Acute and chronic traumatic ulcers

infective wounds

chronic ulcers were

Patients with co-morbidities like diabetes and hypertension and those with aspirin analogues were also included in the study.

Exclusion criteria-

Patients who were positive for

HbsAg

HIV

HCV

malignancy

coagulation disorders

In group A, split skin graft (ssg) with application of autologous PRP on wound beds will be done. In group B, conventional methods like staples/sutures will be used to anchor the skin grafts. For randomization in procedure, all the subjects will be randomly allocated according to random number table into two equal arms of study by permuting the total sample size.

A) In the PRP group, PRP will be topically applied on wound beds through the cannula from the syringe and instant anchorage of skin graft to wound bed will be confirmed by moving the graft on the bed with the finger which will be done by assistants who will be blind to the study.

B) In the control group, sutures or staplers will be used to secure the graft to the wound margins and bed.

The graft will then covered with a non-adhesive mesh topped with betadine soaked cotton wool and secured with compression or tie over bolus dressings as indicated.

Conventionally, first graft inspection is done in the early post-operative period i.e. within one week, but the indications of early change of dressing in our study was wetness of outer dressing, odour and pain in both groups.

Objective parameters like discharge from graft site with significant graft loss, graft

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edema and hematoma, frequency of dressings , duration of stay in hospital were noted. Moisturisers, pressure garments and massage were advised to prevent lymphedema and scar hypertrophy in both groups. Most of the patients were followed up for a period of three months from the time of discharge to assess scar hypertrophy in the early post-operative period.

Sample size:

Considering graft edema as the primary outcome measure in two groups (Conventional fixation & difference between two proportions.

Based on previous literature, data indicate that the probability of graft edema with conventional mechanical fixation was 0.68 (68%) and with Autologous PRP i 0.10 (10%)

To be able to reject the null hypothesis that the probability of graft edema in both groups are equal with probability (power) 0.99 & type I error of 0.01 (1%) we require a minimum of 28 subjects in each group (Total of 56 subjects).

n1 = n2 = 2PQ(Z1 a/2 +Z1-B)2 / (P1-P2)2

z1-α/2 - table values for alpha error of 0.01 (1%) is 2.58

z1-β - - table values for power of 0.99 (99%) is 2.33

P - (Pooled proportion) P1+P2/2 = 0.68+ 0.1/2 =0.39

Q - 1-P = 1-0.39 = 0.61

Considering non response & loss to each group (total 100) will be included in the study.

Statistical Analysis :

The statistical analysis was done using Statistical Package for Social Sciences (SPSS) Version 20.0 Statistical Analysis Software. The values were represented in number (%)

Considering graft edema as the primary outcome measure in two groups (Conventional fixation & PRP), sample size was calculated to test the hypothesis for difference between two proportions.

Based on previous literature, data indicate that the probability of graft edema with conventional mechanical fixation was 0.68 (68%) and with Autologous PRP i

To be able to reject the null hypothesis that the probability of graft edema in both groups are equal with probability (power) 0.99 & type I error of 0.01 (1%) we require a minimum of 28 subjects in each group (Total of 56 subjects).

table values for alpha error of 0.01 (1%) is 2.58

table values for power of 0.99 (99%) is 2.33

(Pooled proportion) = 0.39

Q= 1-P=1-0.39=0.61

Final sample size of the study =100

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Statistical Analysis :

The statistical analysis was done using Statistical Package for Social Sciences (SPSS) Version 20.0 Statistical Analysis Software. The values were represented in number (%)

Figure 15. A and B. 3 months post-operative: well settled scar with PRP application.

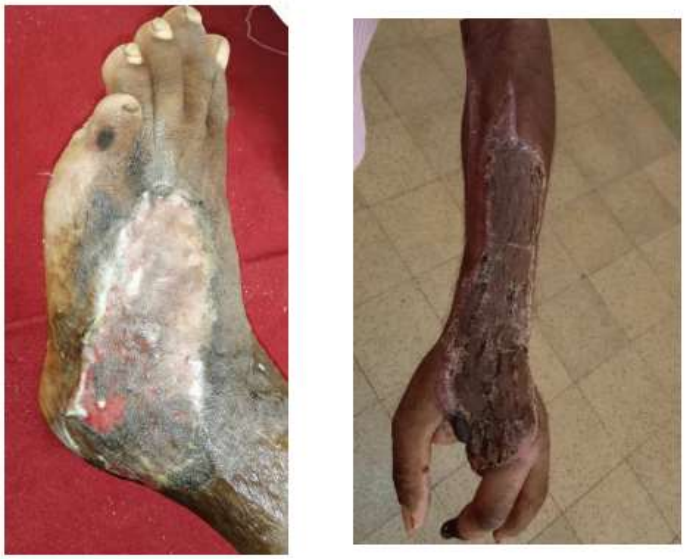

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Figure 6. Instruments required for the procedure

REMI Centrifuge

syringes with 16 gauge cannula and 10ml vacutainer

HUMBY'S KNIFE

OBSERVATION & RESULTS

Here patients will be divided into two groups, A (PRP group) and B (Control group) in a group of 50 each.

The proportion of patients revealing instant adhesion, graft edema, discharge from the graft site, hematoma with significant graft loss and scar hypertrophy were calculated for each of the groups.

Similar computations were done between control and PRP group with regard to day of first graft inspection, frequency of post-operative dressings and stay in hospital.

Difference in proportion between two groups was tested through Chi-square test. $P \leq 0.05$ was considered for statistical significance.

The present study is a prospective comparative study conducted from November 2018

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to May 2020. Here patients will be divided into two groups, A (PRP group) and B (Control group) in a group of 50 each.

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Difference in proportion between two groups was tested through Chi-square test. $P \leq 0.05$ was considered for statistical significance.

	Mean (SD)		P value
	PRP	Control	
Age in years	41.8 (16.0)	45.7 (15.6)	0.216

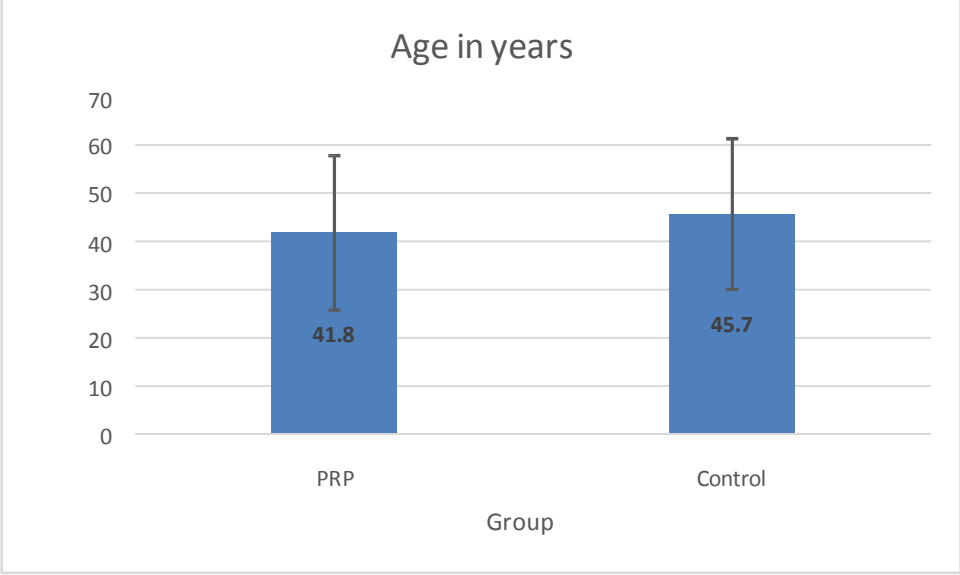

Fig 16. Comparison of age between PRP and control groups

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Gender	N (%)		P value
	PRP	Control	
Female	10 (20.0)	10 (20.0)	1
Male	40 (80.0)	40 (80.0)	
Total	**50 (100)**	**50 (100)**	

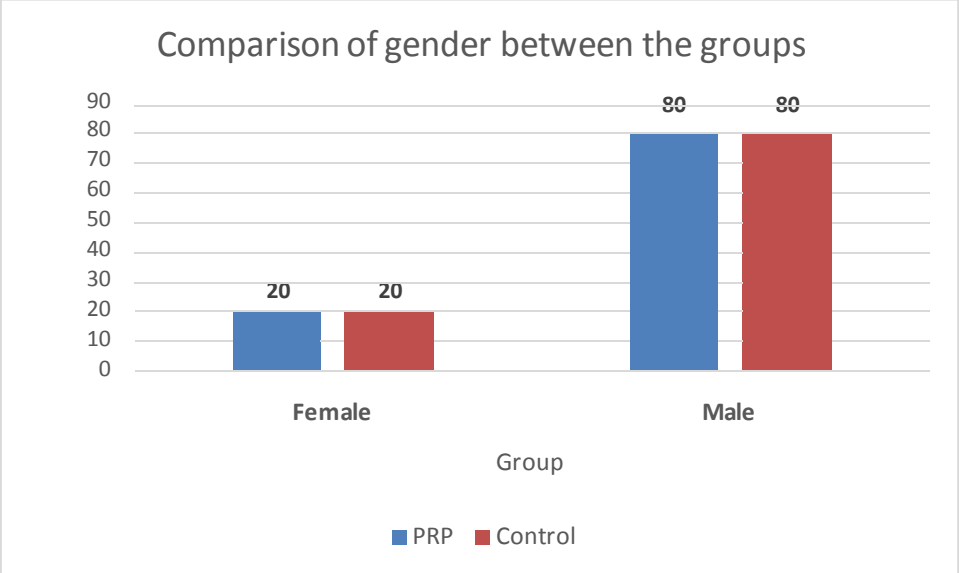

Fig 17. Comparison of gender between PRP and control groups

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Aetiology	N (%)		P value
	PRP	Control	
Chronic ulcer	14 (28.0)	20 (40.0)	0.112
Infection	13 (26.0)	17 (34.0)	
Trauma	23 (46.0)	13 (26.0)	
Total	**50 (100)**	**50 (100)**	

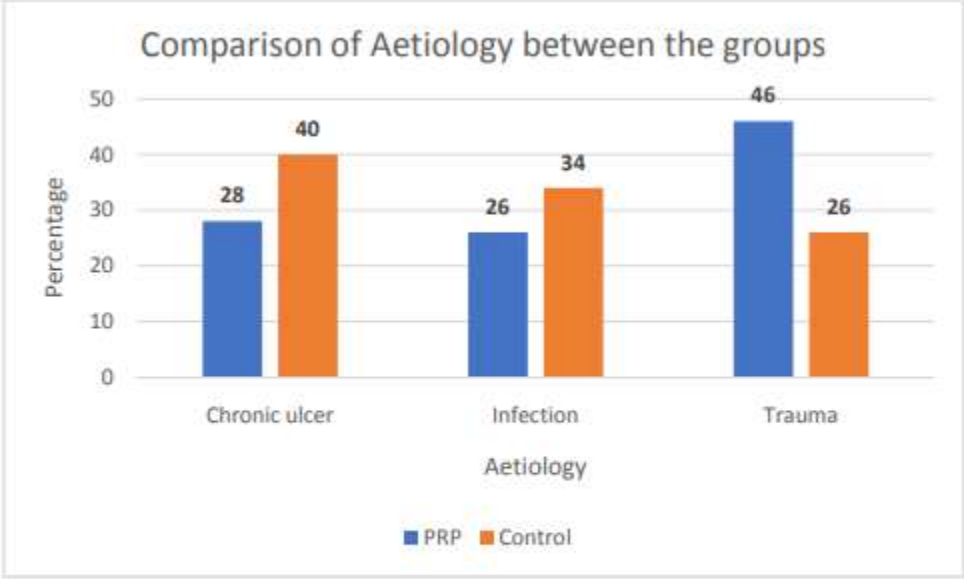

Fig 18. Comparison of Aetiology between PRP and control groups

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Anatomical distribution	**N (%)**		**P value**
	PRP	**Control**	
Lower limb	34 (68.0)	43 (86.0)	0.172
Upper limb	9 (18.0)	5 (10.0)	
Abdomen	4 (8.0)	1 (2.0)	
Chest	3 (6.0)	1 (2.00	
Total	**50 (100)**	**50 (100)**	

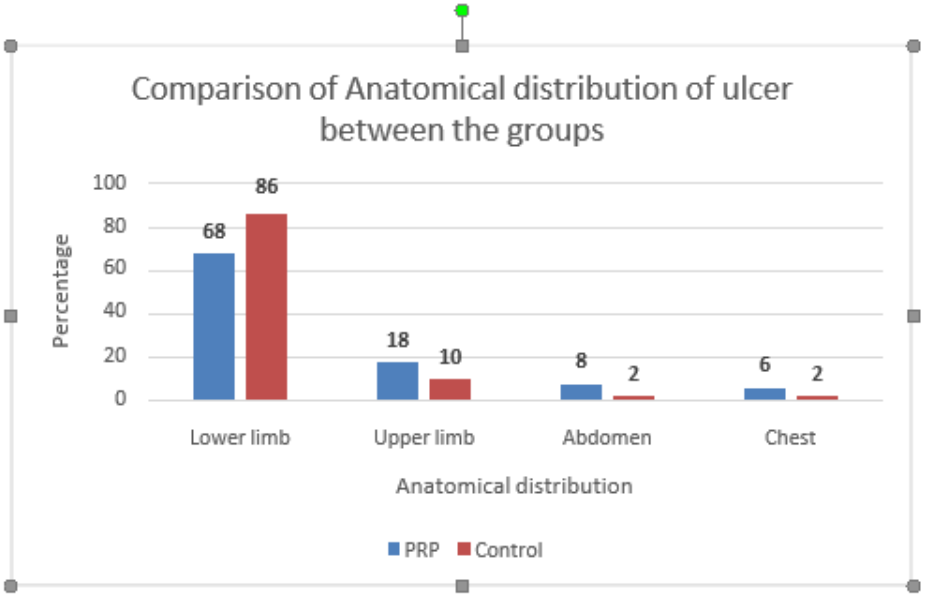

Fig 19. Comparison of anatomical distribution of ulcer between PRP and control groups

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Comorbidities	N (%)		P value
	PRP	Control	
Nil	26 (52.0)	23 (46.0)	0.456
Diabetes mellitus	14 (28.0)	21 (42.0)	
Hypertension	8 (16.0)	5 (10.0)	
Both DM and HTN	2 (4.0)	1 (2.0)	
Total	**50 (100)**	**50 (100)**	

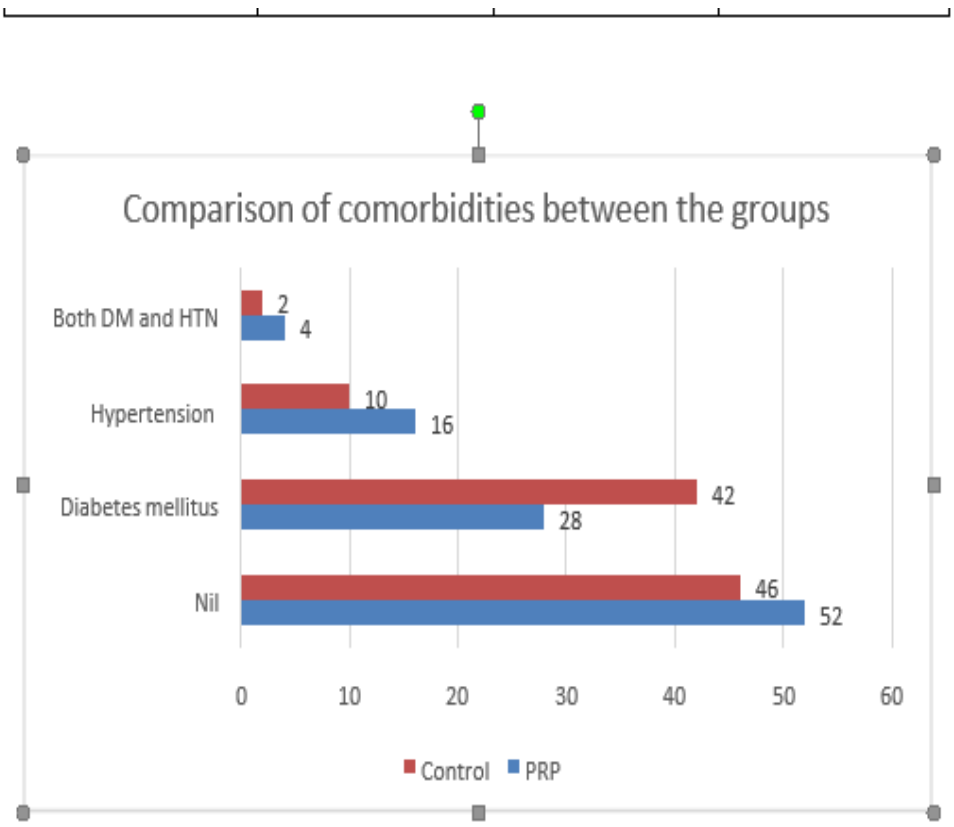

Fig 20. Comparison of comorbidities between PRP and control groups

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Aspirin analogue	N (%)		P value
	PRP	Control	
Yes	17 (34.0)	16 (32.0)	0.832
No	33 (66.0)	34 (68.0)	
Total	**50 (100)**	**50 (100)**	

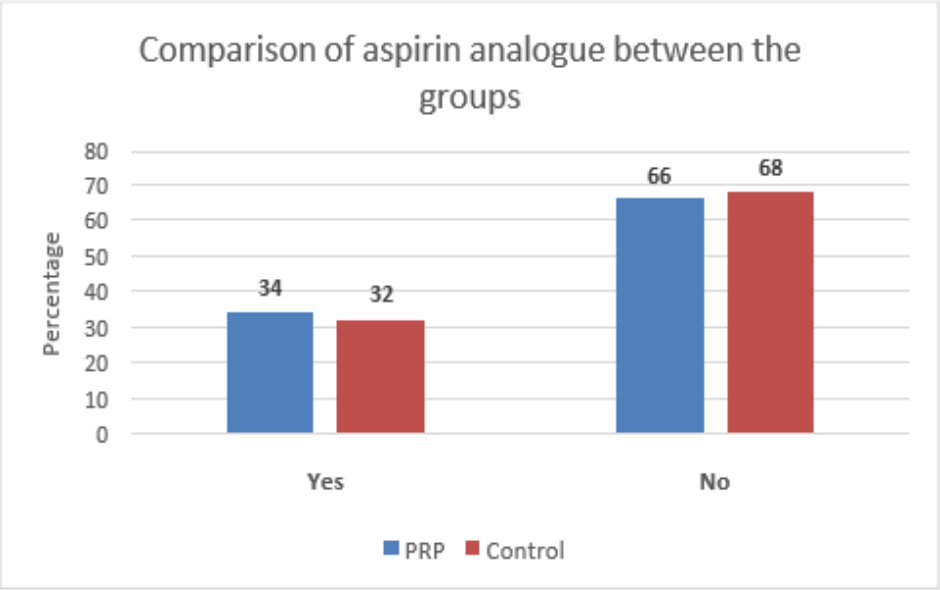

Fig 21. Comparison of aspirin analogue between PRP and control groups

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Instant adhesion	**N (%)**		**P value**
	PRP	**Control**	
Yes	50 (100)	0 (0)	<0.001
No	0 (0)	50 (100)	
Total	**50 (100)**	**50 (100)**	

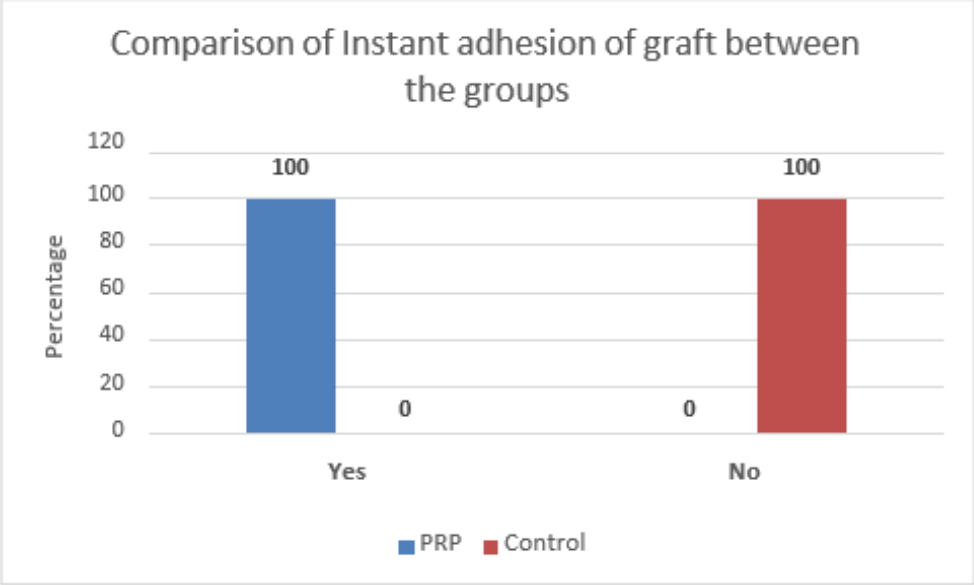

Fig 22. Comparison of instant adhesion of graft between PRP and control groups

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Graft edema	N (%)		P value
	PRP	Control	
Yes	5 (10.0)	32 (64.0)	<0.001
No	45 (90.0)	18 (36.0)	
Total	**50 (100)**	**50 (100)**	

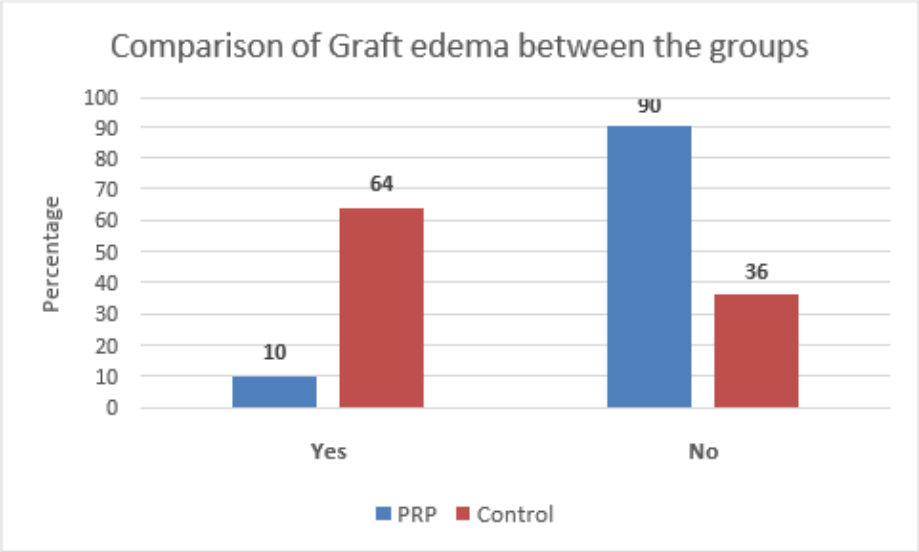

Fig 23. Comparison of graft edema between PRP and control groups

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Discharge from graft site	N (%)		P value
	PRP	Control	
Yes	1 (2.0)	10 (20.0)	0.008
No	49 (98.0)	40 (80.0)	
Total	**50 (100)**	**50 (100)**	

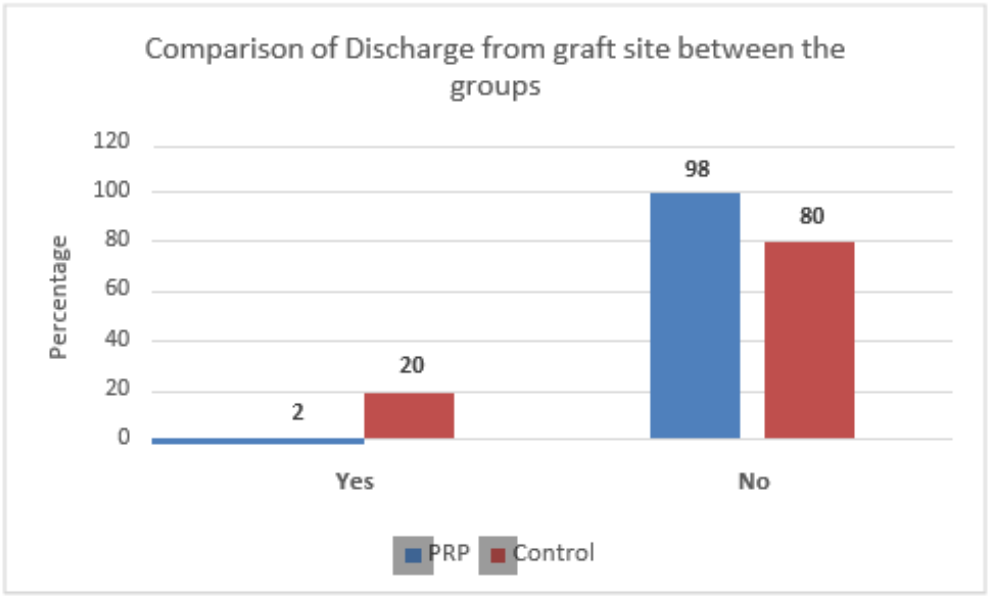

Fig 24. Comparison of Discharge from graft site between PRP and control groups

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Heamatoma with graft loss	N (%)		P value
	PRP	Control	
Yes	2 (4.0)	8 (16.0)	0.092
No	48 (96.0)	42 (84.0)	
Total	**50 (100)**	**50 (100)**	

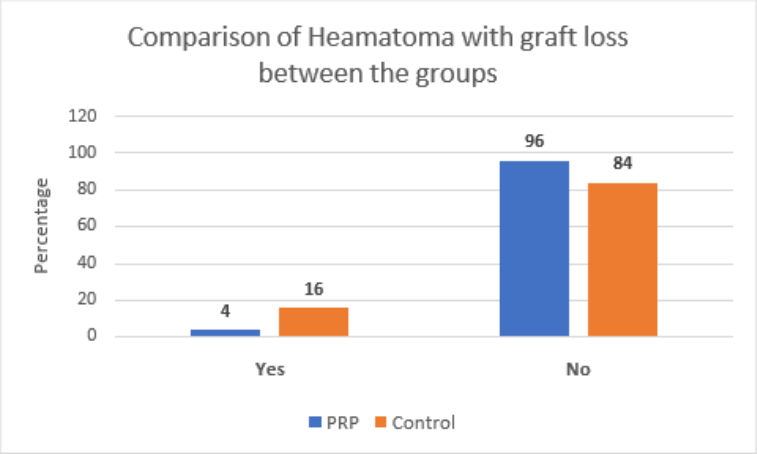

Fig 25. Comparison of hematoma with graft loss between PRP and control groups

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Day of first graft inspection	N (%)		P value
	PRP	Control	
<1 Week	3 (6.0)	43 (86.0)	<0.001
>1 Week	47 (94.0)	7 (14.0)	
Total	**50 (100)**	**50 (100)**	

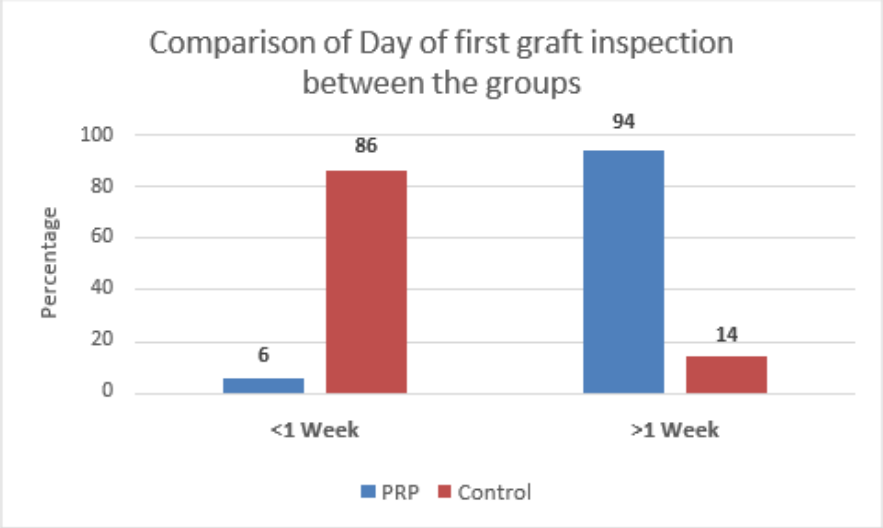

Fig 26. Comparison of Day of first graft inspection between PRP and control groups

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Frequency of dressings	**N (%)**		**P value**
	PRP	**Control**	
1-2 times	47 (94.0)	7 (14.0)	<0.001
3-5 times	3 (6.0)	43 (86.0)	
Total	**50 (100)**	**50 (100)**	

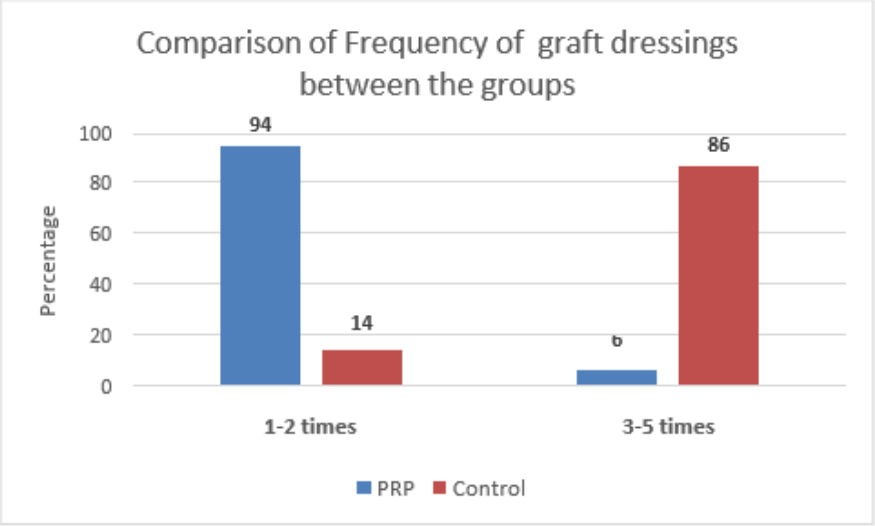

Fig 27. Comparison of frequency of graft dressings between PRP and control groups

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Table 15. Comparison of Stay at hospital between the groups

Stay at hospital	N (%)		P value
	PRP	Control	
Up to 10 days	46 (92.0)	18 (36.0)	<0.001
>10 days	4 (8.0)	32 (64.0)	
Total	**50 (100)**	**50 (100)**	

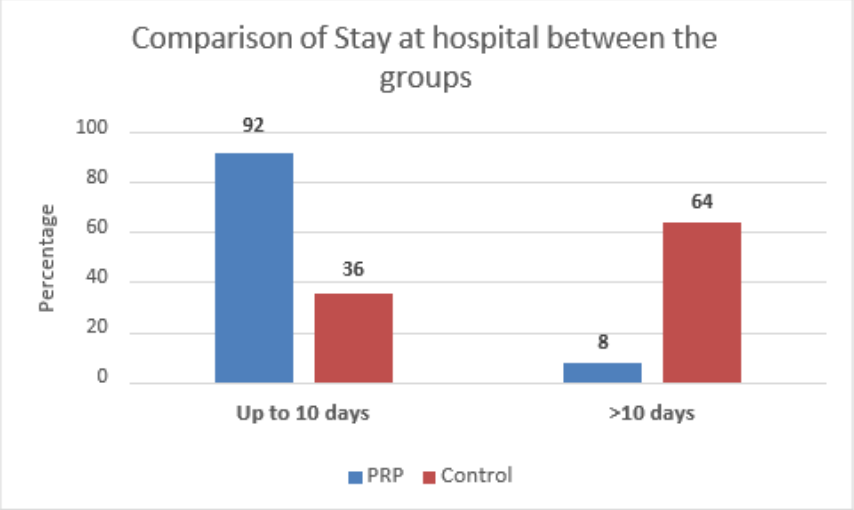

Fig 28. Comparison of stay at hospital between PRP and control groups

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Topic	**Topic**
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 PlastSurg 2015;4(1):50-59.[(1)]	A COMPARATIVE STUDY OF CONVENTIONAL GRAFT FIXATION VERSUS AUTOLOGOUS PLATELET RICH PLASMA (PRP) FIXATION IN WOUND BEDS PRIOR TO SPLIT SKIN GRAFTING.
Randomized controlled prospective study	A prospective randomised comparative study.
Sample size-100 in each group	**Sample size**-50 in each group

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Parameters	**Parameters**
Instantadhesion, graft edema, discharge from the graft site, hematoma with significant graft loss and scar hypertrophy. day of firstgraft inspection, frequency of post-operative dressings and stay in plastic surgery unit.	1)Instant adhesion, 2)Graft edema, 3)Discharge from graft site, 4)Hematoma with significant graft loss, 5)Day of first graft inspection. (<1 week, >1 week), 6) Frequency of dressings. (1-2 times, 3-5 times), 7) Stay in general surgery unit. (10 days, >10 days)

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Discussion- 95% of PRP group who underwent first graft inspection on the tenth day showed dry, non-oedematous firmly adherent graft. 86% in control group underwent frequent dressing within 15 days compared to 95% in the PRP group who underwent only two dressings within fifteen days	**Discussion-.** In the PRP group, 94% of patients underwent first post-graft dressing after one week, among them, 94% underwent first graft inspection between 10 and 12 post-graft days and the graft was found to be well adhered and dry. Seropurulent discharge was seen at the graft site in 10% of control patients which was insignificant in the PRP group. There was hematoma under the graft with significant graft loss necessitating secondary grafting in 16% of patients in the control group which was 4% in PRP group. 94% of patients in the PRP group underwent only two post-operative dressings, one on the tenth and the second on the fifteenth day, whereas 86% in the control group required frequently (at least 3-5 times) within fifteen days.

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Conclusion	Conclusion
In the study its found that the difference in all the objective parameters between controls and PRP groups were statistically significant ($P<0.05$).	The outcome of all the assessment parameters was found to be statistically significant with a$P \leq 0.05$. The used PRP in all types of wounds irrespective of the aetiology yielded favorable results. In comparison to conventional methods of anchorage,Autologous PRP ensured instant skin graft adherence to woundbed.

1

DISCUSSION

Platelet Rich plasma is defined as a platelet of at least 10,00,000 platelets / µL in 5 ml of plasma. There is a 3-5 times increase in the concentration of growth factors.(66)

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Protein studies have shown that platelets contain more than 800 proteins with posttranslational changes, resulting in more than 1,500, resulting in over 1,500 protein based bioactive factors.(52)

The primary aim of application of PRP prior to resurfacing of skin graft is to show its instant stable adhesion to the wound bed without mechanical fixation. Other benefits of hemostasis, reduction in operating time and frequency of post-operative dressings is achieved. Citrate phosphate dextrose-adenine (CPD-A)was the preferred anticoagulant during preparation of PRP. The citrate binds calcium to create the anticoagulation. The dextrose, buffers and other ingredients are known to support platelet metabolism and viability.(67)"

As surgery itself lead to platelet activation and the coagulation system, blood is drawn before the surgery commencement.(68) both buffy coat and supernatant plasma in which platelet concentration was more than white blood cells is used . particularly intra-operatively, where the risk of infection is greater,it is possible that leukocytes could play an important antimicrobial role in PRP,.(69,70,71)Due to the presence of hepatocyte GF,platelet activation also results in an increase in anti-inflammatory cytokines .(72)

Platelets are activated both by native and exogenous molecules, like collagen,plateletactivating factor, magnesium, serotonin, calcium,, thromboxane A2 (TXA2), adenosine diphosphate (ADP), and thrombin.(73)platelets are activated by calcium chloride and/or thrombin for activation, degranulation and release of growth factors in PRP preparations,.

Due to the formation of antibodies against factor V, factor XI and thrombin,Topical bovine thrombin when used as an activator has been reported to produce fatal coagulopathies.(6,7

In some patients Calcium chloride is known to induce arrhythmias.(68

PRP is not activate with either calcium or thrombin but used the fresh preparation on the wound bed as studies have shown that platelet activation also takes place when it comes in contact with collagen in the injured vessel wall, In view of these side effects reported.(73) PRP has been found to be stable for 8 hrs, after preparation with anticoagulant.(2)

Platelet activation results in exocytosis, cytoplasmic α degranulation and an initial burst of growth factors such as platelet-derived growth factor (PDGF, vascular endothelial growth factor (VEGF)and epidermal growth factor (EGF), transforming growth factor(TGF), insulin-like growth factor 1 (IGF-1),fibroblast growth factor (FGF), , throughout the platelets life span.(52,69,70)

In various Studies, PRP used in particular etiological groups,(74-78) In this study, irrespectiveof the etiology, PRP is been used in all types of wounds and has been yielded favourable results. The phases of split skin graft are; in first 24-48hours is

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stage of plasmatic imbibition, second stage of Inosculation or capillary in growth and lastly the stage of revascularisation. To the overall success of graft take, graft survival in the first two phases is critical.(74,79,80)

Applying PRP on wound bed prior to application of skin graft causes hemostasis and provides a sticky surface for instant adherence of graft. Within seconds there was instant adherence of skin graft to wound bed in all 50 patients in the PRP test group as compared to control group in whom instant adherence did not happen as was seen in other studies but has been compared in a large group of patients .(74,79,80)

"In view of the adhesive nature of PRP,(81) the necessity of securing skin graft to wound margins or bed with sutures, staples or glue was not required in the PRP group as was seen in a study of on forty patients conducted by Gibran et al.(79)This not only saves the operative time but also the surgeon's time and effort of removing sutures/staplers in the post-operative period. It is known to provide plasmatic nutrition to graft in the early post graft period.(79)"

Up to first two to three days with an increase in weight by30-50%skin graft edema is noticed till the circulation and venous drainage is established in the graft. By 6-7 days post grafting good circulation to the graft is found to be restored.

we observed only 10% with graft edema in the PRP treated group, compared to 68% of patients in control group had graft oedema for more than a week. Skin graft and the inner dressings was found to be dry in the PRP group

application of PRP accelerates the stage of early circulation and capillary inosculation, thereby reducing graft oedema much earlier, platelets are known to stimulate angiogenesis.(7,77)

In PRP group, 4% of the patients had haematoma under the graft and required Secondary grafting compared to 16% in the control group.

Intake of anti-platelet drugs (oral aspirin), Diabetes and hypertension and were relevant factors detrimental to graft take.

In view of its haemostatic properties, PRP was beneficial who were on aspirin analogues, in hypertensive patients .

In 94% of patients who underwent first graft inspection on the tenth day, grafts were found to be dry, non-oedematous and firmly adherent including diabetic patients.

About 86% in the control group underwent frequent dressings within 15 days compared to 95% in the PRP group who underwent only two dressings within fifteen days. Reduction in the frequency of dressings. The mean hospital stay was ten days compared to the control group which was15 days. In this study PRP group, scar hypertrophy was not seen probably due to early adhesion, lesser incidence of graft edema and collection under the graft similar to other studies.(76,77)

A prospective, randomized crossover study conducted by Knighton et al of repair in chronic, nonhealing ulcers using platelet derived wound healing formula (PDWHF) in

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which 32 patients were randomized and treated with PDWHF or placebo. By 8 weeks, 81% of PDWHF-treated patients had 100% epithelialization, whereas only 15% of controls did (p 0.0001). After crossover, unhealed placebo patients also healed.(3)"
A multicentre, randomized controlled clinical study conducted by Gibran N et al in 40 patients to investigate efficacy and safety of a fibrin sealant (FS 4IU) for attachment of autologous sheet grafts in comparison with staples. They found that its safety profile was excellent with lack of any serious adverse experiences and percent area of hematoma/seroma were less for FS 4IU treated sites and. They concluded that FS 4IUiseffectiveandsafe for skin grafts fixation and in the post-operative period saves both operative time and the time and effort of removing sutures/staplers. It provides plasmatic nutrition to graft in the early post graft period. (79)"
Schade VL and Roukis TS conducted study to observe the time to >/=90% primary healing of ssg augmented with application of PRP and it showed a healing time of 16+/- 4.2 days. So concluded that the addition of PRP to ssg recipient sites enhances primary healing and reduces healing time.(80
In their study of clinical applications of PRP and methodological aspects Marques LF et al reviewed and discussed the bioactive properties of PRP, and its therapeutic use in different fields of regenerative medicine.(82)
Waiker VP and Shivalingappa S conducted a single centre based randomized controlled prospective study where 200 patients with wounds were divided into two equal groups. Autologous PRP was applied on wound beds in PRP group and conventional methods like staples/sutures used to anchor the skin grafts in a control group. They found that 94% of PRP group who underwent first graft inspection on the tenth day showed dry, non-oedematous firmly adherent graft. Also 86% in control group underwent frequent dressing within 15 days compared to 95% in the PRP group who underwent only two dressings within fifteen days. They concluded that autologous PRP ensures instant skin graft adherence to wound bed compared to conventional methods.(1)In this study its found that the difference in all the objective parameters between controls and PRP groups were statistically significant ($P<0.05$). and is found it to be highly beneficial in many aspects both to the patient and surgeon based on results.

6.1. LIMITATIONS OF OUR STUDY

1. More studies required for using PRP under flaps, full thickness skin grafts and split skin graft donor areas and their results.
2. In each etiological group larger samples with extrapolation of results and
3. No follow up done beyond 3months,the patients were followed up only for 3monthsand to assess the nature of scar, long term follow-ups required

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SUMMARY

This study was conducted in ESIC MC & PGIMSR BangaloreI and the findings are tabulated.

The present study is a prospective comparative study, A COMPARATIVE STUDY OF CONVENTIONAL GRAFT FIXATION VERSUS AUTOLOGOUS PLATELET RICH PLASMA (PRP) FIXATION IN WOUND BEDS PRIOR TO SPLIT SKIN GRAFTING, conducted from November 2022 to November 2023.

Here patients will be divided into two groups, A (PRP group) and B (Control group) in a group of 50 each.

Age, gender, etiology of wounds, anatomical distribution, co-morbidities and patients on aspirin analogues were compared in both groups . Skin grafts were found to adhere instantly within seconds to the wound bed in all patients of the PRP group which was not seen in the control group.

In the PRP group, 94% of patients underwent first post-graft dressing after one week, among them, 94% underwent first graft inspection between 10 and 12 post-graft days and the graft was found to be well adhered and dry. In view of wetness of dressing, pain, hematoma and smell , 86% underwent first graft inspection and dressing within one week in the control group.

Graft edema was insignificant in PRP group and observed in 64% of patients in the control group .

Seropurulent discharge was seen at the graft site in 2% of PRP group and 20% in control group.

There was hematoma under the graft with significant graft loss necessitating secondary grafting in 16% of patients in the control group which was 4% in PRP group.

Hence, 94% of patients in the PRP group underwent only two post-operative dressings, one on the tenth and the second on the fifteenth day, whereas 86% in the control group required frequently (at least 3-5 times) within fifteen days.

About 92%of patients were discharged between 10 and 12 post-graft days in the PRP group and only 33% of patients were discharged on fifteenth day in the control group.

In the PRP group, 15% and in control group, 11% were lost in follow-up after discharge.

Compared to PRP group the overall expenditure was 8 times more in the control group which was spent on staples/sutures, operating time, change of dressings, secondary grafting and hospital stay.

The outcome of the assessment parameters,

1) Instant adhesion,

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2) Graft edema,
3) Day of first graft inspection. >1 week,
4) Frequency of dressings. (1-2 times),
5) Stay in general surgery unit. (10 days)
was found to be statistically significant with a P value of 0.05. The used PRP in all types of wounds irrespective of the aetiology yielded favourable results

CONCLUSION

In conclusion, the results from our study showed that ,PRP is a safe and effective treatment modality for autologous platelet rich plasma (PRP) fixation in wound beds prior to split skin grafting.
1) In comparison to conventional methods of anchorage, Autologous PRP ensured instant skin graft adherence to wound bed.
2) Graft edema was found insignificant in PRP group.
3) Underwent first post-graft inspection and dressing after one week in the PRP group.
4) Patients were discharged between 10 and 12 post-graft days in the PRP group .
This not only saves the operative time but also the surgeon's time and effort of removing sutures/staplers in the post-operative period. It is known to provide plasmatic nutrition to graft in the early post graft period.
In view of its safety, low cost, ease of preparation, hemostatic, adhesive and healing properties, it can be used as adjuvant in the management of wounds mainly in developing countries as it reduced the financial burden.
As suggested in this study results, it recommend the use of autologous PRP routinely in all age groups and all types of wounds especially in patients on anti-platelet drugs diabetics, prior to resurfacing to ensure better and faster healing.
To ensure the benefits of early healing, this study recommends the use of autologous PRP routinely on wounds prior to resurfacing.
In each etiological group larger samples with extrapolation of results and no follow up done beyond 3months, the patients were followed up only for 3months and to assess the nature of scar, long term follow-ups required.
Further research and randomized controlled clinical trials on larger patient population are necessary to validate the results.
This conclusion is in par with findings of conventional methods like staples/sutures used to anchor the skin grafts.

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