Efficacy of autologous platelet-rich fibrin in chronic cutaneous ulcer: a randomized controlled trial

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Abstract

Background: Platelet derived growth factors have been used as topical adjuvant therapy in chronic cutaneous ulcer. However the clinical evidence for effectiveness is limited. Therefore, this study was performed to evaluate the efficacy of topical platelet rich fibrin (PRF) dressing in the treatment of chronic cutaneous ulcer.

Aims: To evaluate the efficacy of topical platelet rich fibrin (PRF) dressing in the treatment of chronic cutaneous ulcer in comparison to conventional dressing.

Methods: An assessor-blinded, parallel group, randomized controlled trial was undertaken in 50 patients with chronic cutaneous ulcers of more than 6 weeks duration attending the outpatients, department of dermatology, BPKIHS, Nepal and followed up for 8 weeks. All patients were 18 years or older with ulcers size >0.5 cm2 to ≤50cm2, baseline hemoglobin >9 gram/dl, platelets >1,00,000 cells/cm3, Serum protein concentrations above 6 gram/dl, fasting blood glucose <140 mg/dl and postprandial <200 mg/dl.

Results: The PRF and the conventional groups were equivalent for ulcer size, ulcer duration and other characteristics. Median 100% healing was seen earlier in PRF group (4th Week) in comparison to conventional group (7th week). The Kaplan Meir median time to complete healing in the PRF group was 3.5 weeks compared to 4.19weeks in the conventional group.

Limitations: single center study.

Conclusion: In comparison to conventional dressing, platelet rich fibrin dressings had shown faster healing of chronic cutaneous ulcer. PRF dressing is a safe nonsurgical adjuvant therapy for chronic cutaneous ulcers.

Keyword: Ulcer, Platelet rich fibrin, Dressing, Healing.

Introduction

Chronic ulcers are ‘‘ulcers that fail to heal through the normal, orderly, and timely sequence of repair.[11] It has significant psychological and economical impact on the patients.[15]

Blood platelets have a major role in the initiation of cutaneous wound healing. They adhere, aggregate and release adhesive molecules, lipids, and numerous growth factors. The clinical evidence for the effectiveness of platelet-rich products is limited. A meta-analysis favored the healing of diabetic foot ulcers, the Cochrane review did not favor autologous platelet-rich plasma (PRP) for treating chronic ulcers.[21,14]

Currently, platelet rich fibrin (PRF) is being used in reconstructive, orthopedics and oral maxillofacial surgery and found significant improvement in tissue healing with no adverse effects.[20] However to our knowledge, no randomized controlled clinical trial has evaluated the efficacy of platelet-rich fibrin on chronic cutaneous ulcers.

Methods

Study Design: All consecutive patients with acutaneousulcer of more than 6 weeks duration were randomized in a prospective, assessor-blinded, parallel group, randomized control trial comparing the efficacy of dressing versus conventional dressing between May 2014 and august 2015. The study was approved by the institutional ethics review board.

Study population: Patients with chronic cutaneous ulcers more than 6 weeks duration, aged more than 18 years, with an ulcer area between 0.5 and 50 cm^2, Hemoglobin concentration >9 mg/dl, Platelet count >1,00,000/mm^3, Fasting blood glucose <140 mg/dl, postprandial <200 mg/dl and Serum protein concentrations >6 gram/dl. We excluded women who were pregnant or nursing. We also excluded ulcers exposed with bone, muscle, ligament or tendon. Participants with systemic infection and/or clinical manifestations compatible with active infection of ulcer, Irradiation, wounds containing malignant cells, terminal disease, Uncontrolled systemic disease, current use of anticoagulants or immunosuppressant drugs were also excluded. If the patients met the following criteria, they were described about the study (risks / benefits, voluntary participation, procedures). Patients were given adequate time to reflect on the information, their questions were answered and gave free and voluntary consent. Patients then provided written informed consent.

Study interventions: Patients with chronic cutaneous ulcer were randomly assigned to receive either the PRF dressing or conventional dressing. The study protocol included baseline assessment and a treatment period of 8

weeks or earlier in cases of complete healing of ulcer. The ulcer was thoroughly cleaned using normal saline and to make ulcer free of necrotic debris and foreign bodies, gauze as mechanical debridement or sharp debridement was done the size of the ulcer was measured using image J software.

PRF group

The PRF was prepared following the protocol developed by[3] For PRF preparation we used a table centrifuge (Remi R-8C DX) and collection kit (20 ml syringe, 5 ml blood collection tubes). A few minutes before wound care, 20 ml blood was drawn by venipuncture under aseptic precautions in four sterile borosil glass tubes of 5-ml capacity without anti-coagulant for every 4cm^3 of ulcer. The glass tubes were then placed at opposite sites in a centrifugal machine at 3000 rpm (704.34g / radius 7 cm) for 10 minutes and immediately centrifuged. Blood centrifugation immediately after collection created a well-structured and resistant fibrin clot in the middle of the tube, just between the red corpuscles at the bottom and straw colouredacellular plasma at the top[3] The upper straw colored layer was then removed and middle fraction was collected and cut at the erythrocyte zone as close as possible to the fibrin clot (2 mm below lower dividing line) as PRF where, platelets trap massively in the fibrin meshes.

Then the PRF were used to cover the floor of the ulcer and the ulcer was further dressed with non-adherent dressing.

After one week, the dressing was removed completely and a new PRF sheet was applied. This procedure was repeated weekly for the maximum of 6 weeks depending on the healing response. The patients were instructed to get alternate day dressing by the health worker without removing the PRF sheet at home.

Conventional group

Patients randomized to conventional dressing received the standard care, which consisted of application of paraffin mesh (Jelonet^{tm} Smith & Nephew) followed by non-adherent dressing.

The patient was instructed to change the dressing strictly at every 48 hours. The first dressing was changed in the hospital.

This standard care procedure was repeated weekly for the maximum of 6 weeks depending on the healing response and the patients were instructed to get alternate day dressing. In addition, the patients received the standard treatment for the underlying cause of the ulcer.

At the end of the 8 weeks follow up period, the healed ulcer patients were released from the study and unhealed ulcers were treated at a facility of his/her choice or as per the clinician's treatment protocol .

Outcomes Variables: The primary efficacy variable was considered as the proportion of ulcer size healed & Proportion of patients with a completely healed ulcer (100% epithelized) at 8 weeks. Ulcer evaluation were performed at baseline, 3rd day, 7th day and then every week till 8 weeks after enrolment for ulcer area, characteristics of ulcer exudates (presence, color, amount, and odor), necrotic tissue & granulation tissue, pain, infection and complete healing. The ulcer area at baseline was set at 100% and all measurement at other assessment times was calculated as percentage changed in relation to the initial area to this time point. The percentage of surface area healing was estimated as initial ulcer surface - final ulcer surface/initial ulcer surface X 100. Complete healing was defined as complete closure of ulcer as 100% epithelized.

Ulcer area was evaluated by measuring wound depth and length by metric measure and four successive numerical photographs at each visit and assessment was done using digital planimetry using Image J software. Photographs were obtained with a Nikon digital camera and was standardized for light and distance from the ulcer.

Secondary endpoints included pain, exudates, adverse effects and infection.

Pain assessment were done using Visual analogue scale (VAS scale: 0-10). Amount of wound exudates were assessed by inspecting the gauze, closest to the ulcer after removal of dressing using quantitative variable (<0.5ml, 0.5-1.5ml, >1.5ml).

Ulcer swab for bacterial culture were done in all patients before debridement and at every week for the assessment of bacterial contamination and load. Patients were assessed for any adverse events (AEs) like irritant/allergic contact dermatitis, venipuncture site infection and were documented at each visit.

Sample size: The sample size was estimated based on the findings reported by[7]as the difference of 39% in percentage reduction of ulcer size in patients with chronic cutaneous ulcer using topical platelet rich plasma gel (PRP) dressing (81%) with conventional dressing (42%) at 8 weeks follow up, power 80%, two sided and alpha level of 5 percent. A total of 23 in each group were needed however to allow for potential participant loss to follow up, 25 patients in each group were recruited.

Randomization and allocation concealment: The randomization schedule was generated through random permuted blocks (size 4 & 6) with the help of Ralloc software program in a 1:1 ratio. The patients were assigned randomly to either the platelet-rich fibrin group or the conventional group by means of a sequentially numbered envelop in which the allocation is indicated. The sealed envelopes were numbered from 1 to 50 and were opened in ascending order.

Blinding: Due to nature of the procedure only the assessor was blinded.

Statistical analysis

The statistical package for the Social Sciences (SPSS 11.5 for windows, SPSS Inc., Chicago) was used for the analysis of data. Patient's data were analyzed by intention to treat. The baseline characteristics of the study patients were expressed as numbers and percentages for categorical variables and as means ± standard deviations (SD) for continuous variables. Between and within group comparisons of efficacy variables were assessed by using the Mann-Whitney U test and the Wilcoxon signed rank for paired samples, respectively. Categorical variables were compared with the chi-square (X^2) test. Time to healing analysis was done with Kaplan-Meier method and survival curves were compared with the log-rank test. A p value of less than 0.05 was considered statistically significant.

Results

Initially 73 cutaneous ulcer patients were screened (Fig. 1). Of these patients, 23(31.5%) were excluded as they could not meet the inclusion criteria. Finally, a total of 50 patients were enrolled in the study and randomized into two groups (PRF group and conventional group).One patient was lost to follow up in the PRF dressing group at 4th week and 3 patients were lost to follow up in the conventional dressing group, one at 2nd week, one at 4th week and one patient from conventional group underwent split skin grafting at 2nd week. The patients were recruited from (1st may 2014 – 30th august 2015)

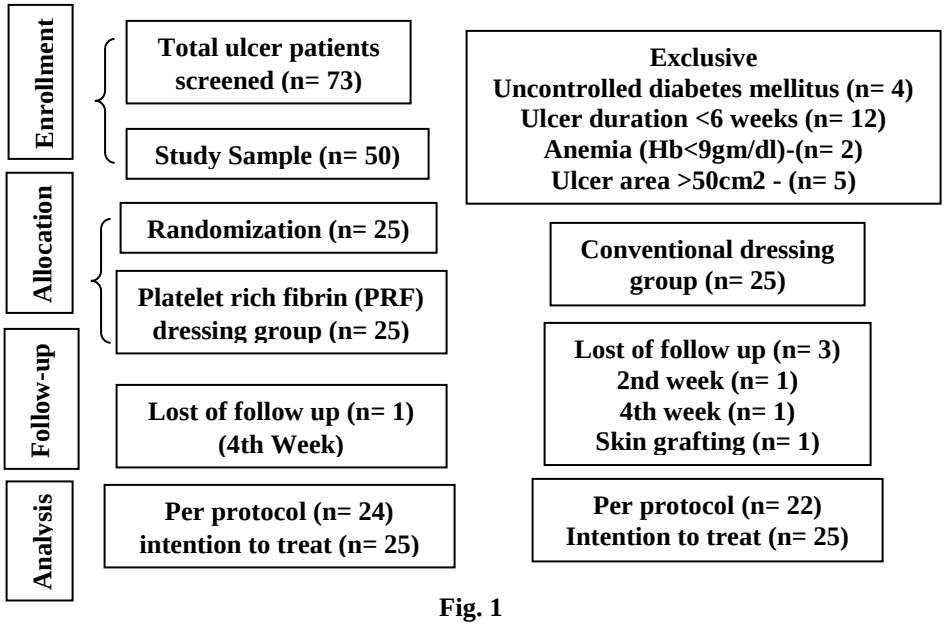


Fig. 1

The baseline socio-demographic, ulcer, biochemical and exudate characteristics of the two groups were similar assummarized in Table 1,2,3,4.

Table 1: Baseline Socio-demographic characteristics in both treatment groups

		Platelet rich fibrin n=25(%)	Conventional n=25(%)	Chi square test*/ t-test**/mannwhitney U test ***	P-value
Age (years) Mean ± Standard deviation Range Median		35.16 ± 15.824 18-70 30	41.08 ± 16.731 18-69 42	1.124**	0.267
Gender	Male / female	18(72) / 7(28)	15(60) / 10(40)	0.802*	0.370
Marital status	Married / unmarried	19(76) / 6(24)	18(72) / 7(28)	0.104*	0.747
Religion	Hindu / Muslim	24(96) / 1(4)	25(100) / 0	****	1.00
Education	No	6(4)	13(52)	5.246*	0.155
	Up to 10th	13(52)	7(28)		
	Up to 12th	4(16)	2(8)		

	Graduate and above	2(8)	3(12)		
Socio economic status	Lower	2(8)	4(16)	1.667*	0.435
	Middle	20(80)	20(80)		
	Upper	3(12)	1(4)		
Alcohol	Yes / no	2(8) / 23(92)	6(24) / 19(76)	****	0.247
Smoking	Smoker	1(4)	0	1.022	0.600
	Non-smoker	22(88)	23(92)		
	Ex-smoker	2(8)	2(8)		

Table 2: Ulcer characteristic's at baseline

		Platelet rich fibrin n=25(%)	**Conventional n=25 (%)**	**Chi square test*/ t-test**/mannwhitney U test ***/fischer's exact test****	**p-value**
Number	1 / 2	24(96) / 1(4)	24(96) / 1(4)	****	1.00
Localization	Upper	5(20)	4(16)	1.138*	0.566
	Lower	19(76)	18(72)		
	Trunk	1(4)	4(8)		
Pain	No	12(48)	10(40)	1.525*	0.677
	Mild	9(36)	11(44)		
	Moderate	3(12)	4(16)		
	Severe	1(4)	0		
Ulcer area(cm2) Mean ± SD Median		12.07±9.53 9.125	12.00±9.99 9.86	308***	0.930
Duration in weeks Mean ± SD Median		12.36±13.156 7	11.44±11.125 8	312***	0.992
etiology	Traumatic	17(68)	12(48)		0.243
	Infection	7(28)	12(48)		
	Venous	1 (4)	0		
	Others (bed sore)	0	1(4)		

Table 3: Biochemical characteristics at baseline

	Platelet rich fibrin (n=25)	**Conventional (n=25)**	**t-test**	**p-value**
Hemoglobin (mg/dl) Mean± Standard deviation	12.65±1.72	12.20±1.28	1.021	0.313
Total leucocyte count (cells/mm^3) Mean± Standard deviation	9624±3343.18	9108±3429	0.522	0.593
Platelet count (cells/mm^3) Mean± Standard deviation	286360±91313.5	337520±105115	1.837	0.072
Fasting blood sugar (mg/dl) Mean± Standard deviation	90.72±9.91	89.48±6.72	0.518	0.607
Post prandial sugar (mg/dl) Mean± Standard deviation	120.60±22.17	129.52±18.77	1.535	0.131
Total protein (mg/dl) Mean± Standard deviation	8.12±.76	8.15±.68	0.117	0.907
Albumin (mg/dl) Mean± Standard deviation	4.77±.53	4.96±.36	1.428	0.160

SGOT (units/L) Mean ± Standard deviation	23.96±7.63	24.52±7.40	0.263	0.793
SGPT (units/L) Mean ± Standard deviation	26.16±6.34	25.92±5.46	0.143	0.887
Urea (mg/dl) Mean ± Standard deviation	25.72±10.44	27.56±5.98	0.764	0.448
Creatinine (mg/dl) Mean ±Standard deviation	0.70±.21	0.69±.18	0.141	0.888

Table 4: Comparison of presence of exudate between the platelet rich fibrin group and conventional group

Exudate presence	Platelet rich fibrin(n=25)	Conventional (n=25)	Chi square*/fishers exact test**	p-value
2nd day	23 (92%)	24 (96%)	**	1
1st week	22 (88%)	21 (84%)	**	1
2nd week	16 (64%)	18 (72%)	**	1
3rd week	12 (48%)	14 (56%)	**	1
4th week	3 (12%)	11 (44%)	**	1
5th week	0	4 (16%)	**	1
6th week	0	1	**	1
7th week	0	0		
8th week	0	0		

Table 5: Objective Surface Area improvement in each follow up as per intention to treat (ITT)

Follow up	Percentage of surface area healed		Mann whitney U test value	P value
	Platelet Rich Fibrin{n=25}	Conventional {n=25}		
2nd day Mean ± Standard deviation (Median)	15.59 %± 20.47 (10.65)	7.14 %± 12.31 (2.20)	179	**0.010**
1st week Mean± Standard deviation (Median)	44.55 %± 24.39 (35.38)	23.94 %± 22.08 (17)	122.5	**0.000**
2nd week Mean± Standard deviation (Median)	67.52 %± 22.77 (69)	41.63%± 31.25 (31.44)	129.5	**0.000**
3rd week Mean ± Standard deviation (Median)	81.55 %± 19.31 (81.56)	52.43 %± 29.33 (47)	132.50	**0.000**
4th week Mean± Standard deviation (Median)	90.35 %± 14.49 (100)	64.38 %± 29.80 (66.67)	147.50	**0.000**
5th week Mean± Standard deviation (Median)	94.73 %± 11.92 (100)	73.60 %± 29.64 (78.35)	156.00	**0.001**
6th week Mean± Standard deviation (Median)	97.10 %± 10.99 (100)	81.99 %± 29.82 (88.90)	185.00	**0.001**
7th week Mean± Standard deviation (Median)	98.06 %± 87.06 (100)	87.06 %± 30.44 (100)	250.00	**0.044**
8th week Mean ± Standard deviation (Median)	98.06 %± 9.68 (100)	88.93 %± 30.66 (100)	286	0.274

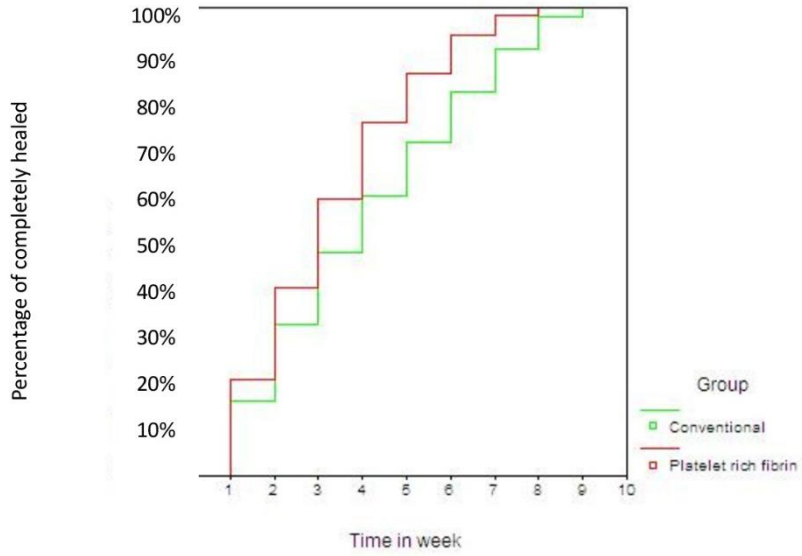
Infection: There was a single case of infection (staphylococcus aureus) in the PRF group at 1st week, which was subsequently resolved after one week of treatment with tablet cefixime 200mg twice daily orally for one week.

Efficacy Outcomes

Objective surface area improvement survival analysis (Kaplan-Meir survival analysis)

The survival analysis performed on the objective improvement showed significant difference between the two treatment groups as seen in the graph with a p value of 0.005 as shown in Fig.2. The Kaplan-Meir median time to complete healing in the PRF group was 3.52 weeks compared to 4.19 weeks in the conventional group. Life table in Fig. 3 shows healing of ulcer over time for both PRF and conventional dressing.

Fig. 2: Kaplan-Meir (1-survival analysis) objective surface area between the two group



Chi-square: 7.945 degree of freedom: 1 p-value: 0.005
-2 log likelihood: 2599.533.

Fig. 3: Life table showing healing of ulcer over time for both the PRF and conventional dressing

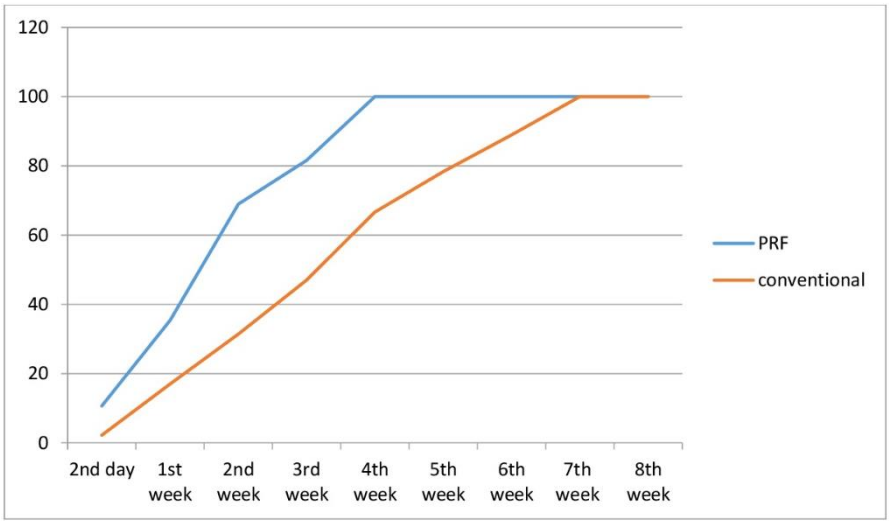
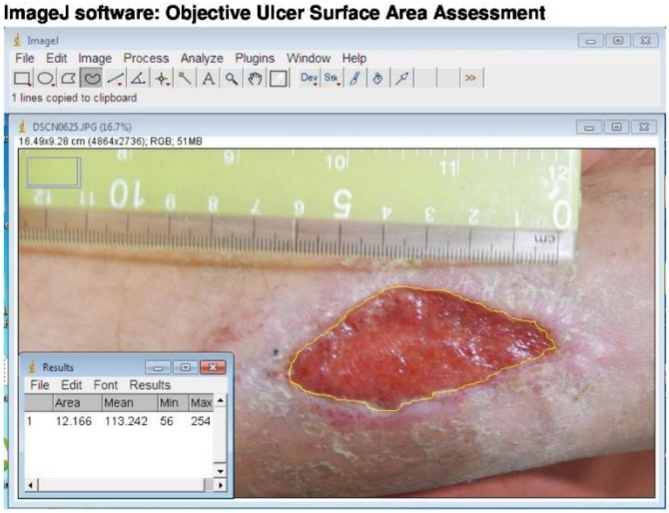


Fig. 4

ILLUSTRATIONS

ImageJ software: Objective Ulcer Surface Area Assessment



Patient no. 26: Objective ulcer surface area assessment at Baseline - 12.166 cm²

PLATELET RICH FIBRIN GENERATION KIT (PRF)

Centrifuge Machine (Remi-8C-DX)

Fig. 5

PLATELET RICH FIBRIN

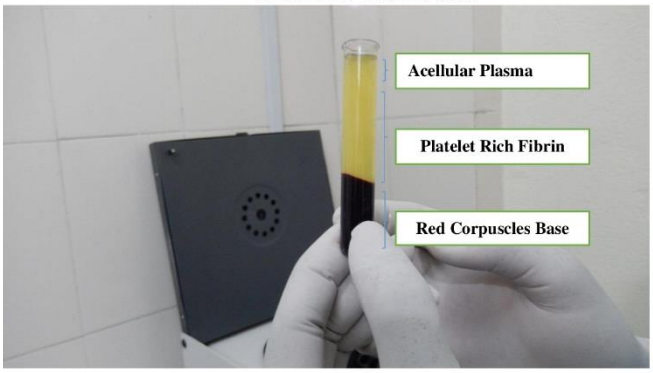


Fig. 6

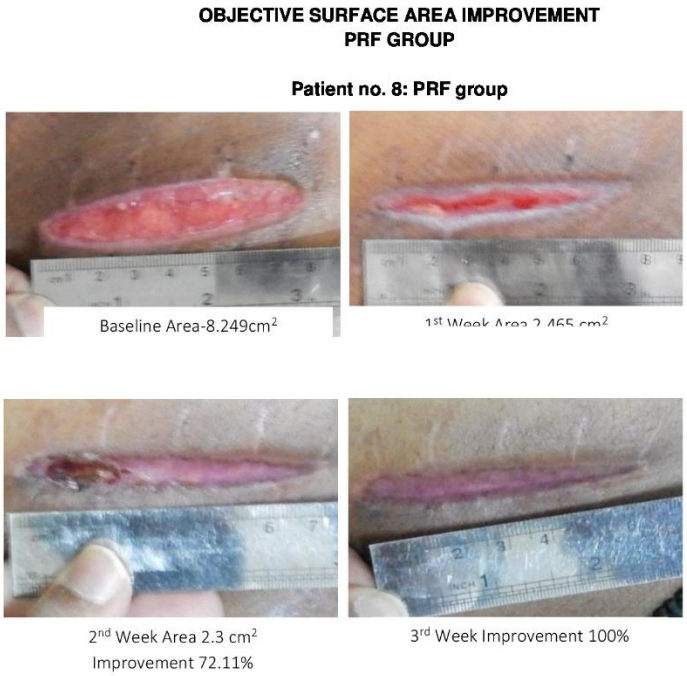


Fig. 7

Discussion

In our study we have evaluated the effectiveness of PRF in treatment of chronic cutaneous ulcer. The hypothesis for our study is the presence of myriad amount of growth factors in PRF. To the best of our knowledge this is the first randomized controlled trial to know the efficacy of platelet rich fibrin in chronic cutaneous ulcers.

In our study, the baseline platelet count in PRF group was 2,86,360 cells/cm^3 while it was 3,37,520 cells/cm^3 in the conventional group, although the platelets were significantly more in the conventional group the difference was not statistically significant. The findings were in corroboration with the study done by,[7] in which the mean platelet count were 2,64,000 cells/cm^3 in the PRP group and 2, 63,000 cells/cm^3 in the conventional group. Platelet rich plasma containing platelets with high concentrations of growth factors (GFs) are thought to facilitate healing.[13] When these GFs are released from the platelets they trigger a tissue regeneration process.[9]

Platelets derived products have been tried since many decades for wound healing and got mixed responses. A Cochrane review and meta-analysis in 2012 which evaluated 9 RCT's[1,7,8,9,10,16,19,18,22] showed no difference in the rate of wounds completely healed when comparing platelet rich plasma with conventional dressing. None of the studies had used platelet rich fibrin.

Blood platelets adhere, aggregate and release numerous growth factors like PDGF, transformed growth factor (TGF-b), VEGF, basic fibroblast growth factor, EGF, type-I insulin-like growth factor (IGF-I), and hepatocyte growth factor (HGF). Senet et al[18] used frozen autologous platelets that had no significant adjuvant effect on healing of chronic venous leg ulcers.[17] found neoangiogenetic abilities to platelet derived wound healing factors, but not any significant clinical advantage.

When using topical application of active agents with autologous platelet-derived products, it is important to determine the optimal method of application available to be taken up by the wound. While in the case of platelet rich fibrin the fibrin meshwork potentially protects the growth factors and a slow sustained release of the growth factors is established.

In our study, there was significant difference in the subjective surface area improvement at 2nd day, 1st week, 2nd week, 3rd week, 4th week, 5th week with p values ranging from 0.000 to 0.014. The Kaplan-Meir (1-survival analysis) of the subjective surface area improvement between the two groups showed there was a significant difference between the two groups with a p value of 0.004. When objective surface area improvement data was analyzed there was significant difference in healing times between the PRF dressing and conventional dressing group with improvement at 2nd day, 1st week, 2nd week, 3rd week, 4th week, 5th week with p values of 0.015, 0.001, 0.002, 0.000, 0.002, 0.014. The Kaplan-Meir (1- survival analysis) performed on the objective surface area improvement showed significant difference between the two treatment protocols with p value of 0.005.

In our study, we found significant differences in both the subjective surface area and the objective surface area improvement with better healing in the PRF group.

The fibrin network in PRF has a homogeneous 3-dimensional organization, even more highly coherent than natural fibrin clots. These preliminary data therefore imply that PRF would not only be a new generation of platelet gel, but a completely usable healing concentrate.[6]

Choukroun's PRF protocol is a simple and free technique developed in France by Choukroun et al.[3] Following centrifugation of venous uncoagulated blood, three layers are formed: the RBC base layer, acellular plasma top layer and a PRF clot in the middle. The PRF clot forms a strong fibrin matrix with a complex three-dimensional architecture, in which most of the platelets and leucocytes from the harvested blood are concentrated.[6] When pressed between two gauzes, the PRF clot becomes a strong membrane. Some applications of this autologous biomaterial have been described in oral,[4] maxillofacial,[5] ENT (ear, nose, throat)[5] and plastic surgery.[2]

Unlike the PRPs, Choukroun's PRF does not dissolve quickly after application; instead, the strong fibrin matrix is slowly remodelled in a similar way to a natural blood clot.[6]

The literature on the use of PRF on hard-to-heal wounds is limited. A 2006 review of its use on venous leg ulcers called it 'promising'.[1] A study showed that these leukocytes and platelet rich patch can provide a way of transferring concentrated cells and signals directly to a surface and would be beneficial for the healing of recalcitrant wounds.[12]

In our study, it was also seen that PRF was especially helpful in volume deficient wounds where the PRF applied transformed into corresponding adjacent tissues muscle, subcutis and skin, although we could not quantify its benefit since volume of ulcer was not measured in each visit.

Weekly PRF (platelet rich fibrin) dressings has shown better healing when compared to conventional dressing.

Limitations

1) Single centre study.
2) Our study does not compare the efficacy of PRP versus PRF.

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