

**ORIGINAL RESEARCH****A prospective study on the treatment of chronic non-healing ulcers using autologous platelet rich plasma****<sup>1</sup>Neharika, <sup>2</sup>Brajesh Pathak, <sup>3</sup>Sanjiv Bhatia, <sup>4</sup>Ravi Pratap Singh Rajey****<sup>1,2</sup>Assistant Professor, <sup>3</sup>Professor & HOD, <sup>4</sup>Resident, Department of General Surgery, Hind Institute of Medical sciences, Barabanki, Uttar Pradesh, India****Correspondence:****Ravi Pratap Singh Rajey****Resident, Department of General Surgery, Hind Institute of Medical sciences, Barabanki, Uttar Pradesh, India****Email: [ravirajey141988@gmail.com](mailto:ravirajey141988@gmail.com)****ABSTRACT****Aim: To assess the effect of Platelet Rich Plasma (PRP) in non-healing ulcers.****Material and methods: It was a hospital based prospective cross-sectional & observational study between July 2020 to June 2021, at Hind institute of medical sciences, Safedabad, Barabanki. The history and examination were done bedside. In cases where the condition of patients doesn't warrant giving history, their relatives or attendants were interviewed. A predesigned pretested semi-structured questionnaire was filled by the interviewer. Data was entered in Microsoft Excel sheet. The study area was General Surgery, Orthopaedics & medicine department, of Hind Institute of Medical Sciences, Safedabad, Barabanki. Pus culture and sensitivity were sent then after cleaning the wound with normal saline and betadine solution, already centrifuged platelet rich plasma was injected subcutaneously inside, in the periphery of wound and spread over floor of ulcer then dress with non-absorbent dressing. 1st dressing was changed on the 3rd day of injection then dressing was changed on every 7th day. We evaluated the effect of 1 dose of platelet rich plasma injection efficacy in wound/ulcer healing & reduction in wound/ulcer size and follow-up by visual inspection.****Results: At admission, the mean wound area was  $388.6 \pm 25.35 \text{ mm}^2$  which was reduced to 15.54% at 3<sup>rd</sup> day and this reduction was found to be statistically significant ( $p < 0.001$ ).****Further reduction was observed at 14<sup>th</sup> day (49.66%) and 21<sup>st</sup> day (64.40%) and these reductions were statistically significant ( $p$  value  $< 0.001$ ). At 21st day the % change in volume was maximum for venous ulcer ( $69.25 \pm 4.43$  %) and minimum for post traumatic ( $65.27 \pm 9.11$  %). No significant difference was found in % change in volume at 21st day among various ulcer types ( $p$  value = 0.336).****Conclusion: Based on this study observation and result, we proposed that platelet rich plasma is a safe bio-compatible, cost-effective, less time-consuming procedure and does not require sophisticated equipment.****Keywords: PRP, Diabetes Mellitus, Ulcer****INTRODUCTION**

Chronic ulcers or non-healing ulcers are defined as wounds that have failed to proceed through an orderly & timely reparative process to produce anatomic & functional integrity over a period of 3 months [1], with an underlying aetiology that may be related to systemic disease or local disorders [2]. Chronic ulcers are projected to become more common as the

population ages and as risk factors for atherosclerotic occlusion rise, including smoking, obesity, and diabetes.

Diabetic foot ulcers are prone to infection, and diabetic neuropathy complicates the healing process, resulting in persistent non-healing ulcers. Venous illness is responsible for the majority of chronic lower extremity ulcers, as venous hypertension damages vessel walls, resulting in skin breakdown.[2] In the general population, the prevalence of venous non-healing ulcers is between 1 and 2%, accounting for almost 75–80% of all vascular ulcers. [3-4]

For wounds with a vascular insufficiency, topical growth factor solutions are often used as adjuvant treatments in addition to the standard of care for diabetic foot ulceration, which includes debridement, off-loading, regular dressing changes, and compression. Previous research has demonstrated the efficacy of these medicines, and their significance cannot be overstated. Debridement is the most critical stage in treating a diabetic foot ulcer, with the purpose of eliminating all devitalized tissue, such as callus, necrotic, and infected tissue, and leaving only healthy tissue, effectively transforming a chronic wound into an acute wound. Debridement can be done on both neuropathic and venous ulcers to reduce the bacterial burden of the ulcer even if there is no overt infection. Previous research has found that debrided foot ulcers heal faster than those that aren't, regardless of treatment, implying that frequent debridement is a key adjuvant treatment in these wounds.[5]

Excess exudate is removed, a moist environment is maintained, contamination is prevented, removal does not cause trauma, and no debris is left on the wound bed. Traditional dressings are more likely to be connected with infections than dressings that preserve moisture. There is no evidence to support one type of dressing over another[6,7] and no one dressing is suitable for every type of ulcer and location. Saline or hydrocolloid dressings generate a moist wound bed that promotes healing and prevents the wound from drying out.[8]

Compression is regarded first-line therapy for venous ulcers, because it is more effective than no compression in healing these ulcers. Stockings, multilayer bandages, high-pressure compression boots, intermittent pneumatic compression, and Unna boots are some of the compression methods available. Compression helps the calf muscle pump function and decreases edema and stasis by minimizing distention in superficial veins.[9-10]

Platelet rich plasma has seen a significant surge in use in a variety of diseases and situations, including wound healing, in recent years. In case series and controlled studies of cutaneous ulcers, favorable results have been documented in a range of etiologies. Platelets in platelet rich plasma may play a role in the host defense system at the wound site by generating signaling proteins that attract macrophages[11]. Platelet rich plasma development systems that are predictable and efficient can be employed in both office and hospital settings. While medical professionals are permitted to apply blood products in the office, such as platelet rich plasma, they are not permitted to infuse or re-infuse blood or blood products. There is no need for reinfusion because platelet rich plasma producing systems only require a little volume of blood to manufacture, and studies have shown that these frequent but small blood draws have no effect on hemoglobin, hematocrit, or platelet count.[12] The aim of the study was to assess the effect of Platelet Rich Plasma (PRP) in non-healing ulcers.

## **OBJECTIVES**

1. To assess the efficacy of platelet rich plasma in wound/ulcer healing.
2. To evaluate the percentage reduction in wound/ulcer size.
3. To study the safety and feasibility of autologous platelet rich plasma injections, time to wound/ulcer healing, improvement in pain or discomfort, and quality of life.
4. To study the time period after platelet rich plasma treatment & wound to get prepared for surgical coverage.

## MATERIAL AND METHOD

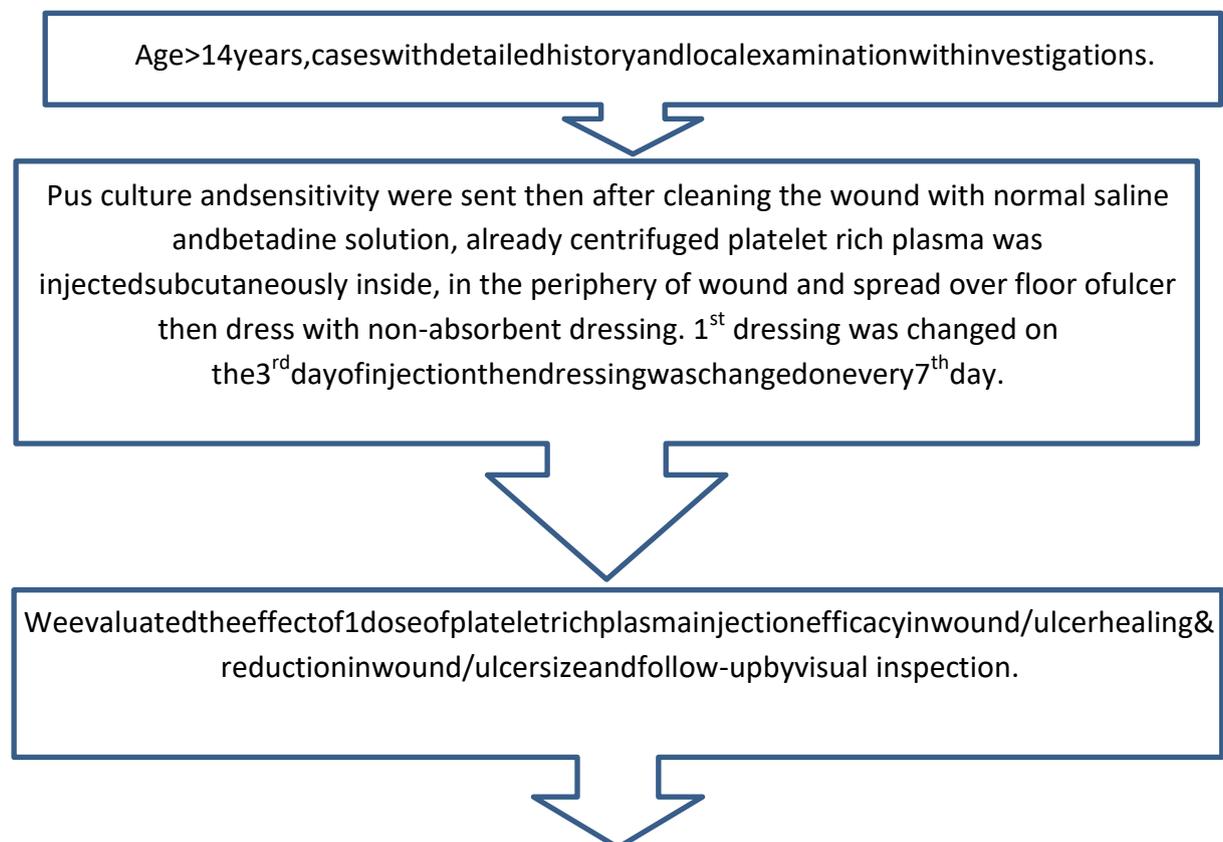
It was a hospital based prospective cross-sectional & observational study between July 2020 to June 2021, at Hind institute of medical sciences, Safedabad, Barabanki. Clearance of the institutional ethics and scientific committee were obtained prior to commencement of the study. Patients who came in General Surgery, Orthopaedics & Medicine department in out patients or in-patients were enrolled in the study. Informed and written consent were taken prior to the study from each patients/attendant (in case of minors), who were enrolled in the study and confidentiality of the information collected was ensured. The history and examination were done bedside. In cases where the condition of patients doesn't warrant giving history, their relatives or attendants were interviewed. A predesigned pretested semi-structured questionnaire was filled by the interviewer. Data was entered in Microsoft Excel sheet. The study area was General Surgery, orthopaedics & medicine department, of Hind Institute of Medical Sciences, Safedabad, Barabanki. Hundred patients were enrolled in study according to inclusion and exclusion criteria.

All types of non-healing ulcers excluding burns & malignant ulcers, age >14 years and diabetic patients were included in the study. Patients having acute traumatic wounds, on immunosuppressant therapy, pregnant women & lactating mothers, burn wounds, ulcers being diagnosed as malignant ulcers, anti-platelet therapy, bleeding disorder and patient not willing for consent and follow-up were excluded from the study.

## MATERIALS

1. 40-60ml of venous blood.
2. Blood vacutainers for collection of blood.
3. Standard calibrated REMI 8c centrifuge
4. Non-absorbent sterile transparent sheet.

## METHODOLOGY



Granulation tissue was observed in follow up with dressings and repeat culture, then the patient was planned for further surgical management.



**Figure 1: Taking patients own blood for centrifugation and preparation for plateletrichplasma.**

### POST PROCEDURE PROTOCOL

On each Visit clinical assessment of ulcer outcome was assessed according to the format. Patient was followed up after 3<sup>rd</sup> day, and every 7<sup>th</sup> day till the time of granulation tissue and photographs was taken on each visit.

Data was collected and subjected to statistical analysis using SPSS software version 24.

### RESULTS

The mean age among the 100 patients was  $49.02 \pm 16.75$  year with maximum cases from age group 55-64 yrs. (30%) followed by the age range  $\geq 65$  yrs. Out of 100 study cases, majority 62 (62%) cases were males and rest 38 (38%) cases were females. Post traumatic was the most common diagnosis (30%) followed by the diabetic foot (24%) and bed sores (23%). 14% incidence was found for venous ulcer while trophic was found in least proportion (9%). The distribution of cases according to site shows that foot was the most frequent site (37%) followed by the upper limb (18%) as shown in table 1.

**Table 1: Distribution of subjects according to diagnosis, site and diabetes mellitus**

Diagnosis	N	%
Venousulcer	14	14.0
DiabeticFoot	24	24.0
BedSores	23	23.0
PostTraumatic	30	30.0

Trophic	9	9.0
<b>Site</b>		
UpperLimb	18	18.0
Foot	37	37.0
Hip	15	15.0
Ankle	13	13.0
Heel	17	17.0
<b>Diabetes Mellitus</b>		
Present	29	29
Absent	71	71

At admission, the mean wound area was  $388.6 \pm 25.35 \text{ mm}^2$  which was reduced to 15.54% at 3<sup>rd</sup> day and this reduction was found to be statistically significant ( $p < 0.001$ ). More 31.57% mean reduction was observed at 7<sup>th</sup> day and this reduction was found to be statistically significant ( $p \text{ value} < 0.001$ ). Further reduction was observed at 14<sup>th</sup> day (49.66%) and 21<sup>st</sup> day (64.40%) and these reductions were statistically significant ( $p \text{ value} < 0.001$ ). Hence significant improvement was found due to applied treatment.

**Table 2: Improvement of area of the ulcer with Time (N=100)**

Time	Wound area ( $\text{mm}^2$ )		% imp	Significance	
	Mean	SD		t-value	p-value
At adm	388.57	25.35	-	-	-
3rd day	328.19	26.34	15.54	81.80	<0.001
7th day	265.88	29.43	31.57	83.71	<0.001
14th day	195.59	28.27	49.66	136.72	<0.001
21st day	138.33	27.01	64.40	209.15	<0.001

At 3rd day, 62% cases showed reduction in area more than 15%, at 7th day, 47% cases showed reduction in area more than 33%, while at 14th day 55% cases showed reduction more than 50% and at 21st day 44% cases showed reduction in ulcer area more than 65% (table 3).

**Table 3: Pattern of Change in area of the ulcer with Time (N=100)**

	Area Change	No.	%
	3rd day	$\geq 15\%$	62
$< 15\%$		38	38.0
7th day	$\geq 33\%$	47	47.0
	$< 33\%$	53	53.0
14th day	$\geq 50\%$	55	55.0
	$< 50\%$	45	45.0
21st day	$\geq 65\%$	44	44.0
	$< 65\%$	56	56.0

At 21st day the % change in area was maximum for diabetic foot ( $66.06 \pm 4.48 \%$ ) and minimum for venous ulcer ( $63.67 \pm 4.30 \%$ ). No significant difference was found in % change in area at 21st day among various ulcer types ( $p \text{ value} = 0.491$ ) as shown in table 4.

**Table 4: Association of Type of Ulcer with % Change in area at 21<sup>st</sup> Day (N=100)**

Type of Ulcer	change in area at 21st day		ANOVA	
	Mean	SD	F-value	p-value
Venous ulcer	63.67	4.30		
Diabetic Foot	66.06	4.48		

BedSores	64.86	3.95	0.86	0.491
PostTraumatic	63.77	6.16		
Trophic	64.87	5.32		

At 21st day the % change in volume was maximum for venous ulcer (69.25±4.43 %) and minimum for post traumatic (65.27±9.11 %). No significant difference was found in % change in volume at 21st day among various ulcer types (p value =0.336) as shown in table 5.

**Table 5: Association of Type of Ulcer with % Change in Volume at 21st Day (N=100)**

Type of Ulcer	% change in Volume at 21st day		ANOVA	
	Mean	SD	F-value	p-value
Venous ulcer	69.25	4.43	1.15	0.336
Diabetic Foot	66.88	5.63		
Bed Sores	65.34	4.76		
Post Traumatic	65.27	9.11		
Trophic	67.93	6.24		

## DISCUSSION

The present study was carried out with an aim to assess the effect of Platelet rich plasma (PRP) in non-healing ulcers with objectives to assess the efficacy of Platelet rich plasma in wound/ulcer healing, to evaluate the percentage reduction in wound/ulcer size, to study the safety and feasibility of autologous Platelet rich plasma injections, time to wound/ulcer healing, improvement in pain or discomfort, and quality of life and to study the time period after platelet rich plasma treatment & wound to get prepared for surgical coverage. For this purpose, a total of 100 patients falling in the sampling frame were enrolled in the study.

Our findings revealed that the mean age of the patients was 49.02±16.75 yr with maximum cases from age group 55-64 yrs (30%) followed by the age range ≥65 yr (21% each). However, a uniform distribution of age was present in the study. Some other implications of the present findings suggest that out of 100 study cases, majority 62 (62%) cases were males and rest 38 (38%) cases were females. So the study consisted males and females in proportion 31:19. The minimum age of the cases was 14 yr. and maximum 75 years. Similar study done by Suthar M et al found that the mean age of the treated patients was 62.5 ± 13.53 years. He also found that among the included patients, 16 (66.6%) were males and 8 (33.33%) were females.[13] Another study by Suryanarayan S et al illustrated the mean age of the patients was 42.5 years±12.48. [14] Mean age revealed by Deshmukh S et al was 42.5 years with 53 males and 17 females.[15] Prabhu R et al in his study observed mean age of 52.34 years.[16] Contrasting results were found in terms of age however, males outnumbered females in line with our study.

The results also confirmed that Post traumatic was the most common diagnosis (30%) followed by the diabetic foot (24%) and bed sores (23%). 14% incidence was found for venous ulcer while trophic was found in least proportion (9%). At 3rd day the % change in area was maximum for diabetic foot (16.91±1.39) and minimum for venous ulcer (14.26±2.28). The significant difference was found in % change in area at 7th day among various ulcer types (p value <0.001). Prabhu R et al found Diabetic foot ulcer 40 (38.4%), Bedsore 20 (19.2%), Venous ulcer 16 (15.3%) and Traumatic non-healing chronic ulcer 24 (23.07%).[16] More than 85% of lower limb amputations are preceded by foot or ankle ulcers and diabetes is one of the major causes of non-traumatic amputations across the world (Brem H et al). [17] Approximately 15–25% of individuals with diabetes develop a foot ulcer, of which an estimated 12% require lower extremity amputation. Prabhu R et al observed 19.2% with bed sores and 38.4% with diabetic foot. [16]

The distribution of cases according to site shows that foot was the most frequent site (37%) followed by the upper limb (18%). Maximum studies included foot as the most common site of ulcer (Suthar M et al; Suryanarayan S et al; Deshmukh S et al ; Prabhu R et al).[14-17] Singh et al found that in platelet rich plasma group most common type of ulcer is venous (29.62%). In NS group most common ulcers were post cellulitis (29.62%) and traumatic (29.62%).[18] (Gopinath VPK) revealed that in all patients ulcers were located in lower limbs with a majority (41.1%) in the medial malleolus followed by shin (17.6%) and big toe (11.8%). Out of 34 ulcers there were 18 (58.8%) venous ulcers, 6 (17.6%) vasculitic ulcers, 4 (11.8%) diabetic foot, 2 (5.9%) each of traumatic, trophic and arterial ulcers. [19]

Out of 100 cases of ulcer wound, 29% were suffered with diabetes mellitus. More than 85% of lower limb amputations are preceded by foot or ankle ulcers and diabetes is one of the major causes of non-traumatic amputations across the world (Brem H et al).[17] Prabhu R et al observed 80.7% with diabetes.[16]

At admission, the mean wound area was  $388.6 \pm 25.35$  mm<sup>2</sup> which was reduced to 15.54% at 3rd day and this reduction was found to be statistically significant (p value <0.001). More 31.57% mean reduction was observed at 7th day and this reduction was found to be statistically significant (p value <0.001). Further reduction was observed at 14th day (49.66%) and 21st day (64.40%) and these reductions were statistically significant (p value <0.001). Hence significant improvement was found due to applied treatment. At 3rd day, 62% cases showed reduction in area more than 15%, at 7th day, 47% cases showed reduction in area more than 33%, while at 14th day 55% cases showed reduction more than 50% and at 21st day 44% cases showed reduction in ulcer area more than 65%. A study by Prabhu R et al revealed such findings such as among those cases, 81.73% (85 patients) healed ulcers were noted at the end of the last visit. The effectiveness of platelet rich plasma dressing was evaluated in terms of complete wound healing or >75% reduction in surface area from the baseline (5.03 cm<sup>2</sup>). For each visit, there was a reduction in the ulcer area. In the last visit, the mean ulcer area became 1.69 cm<sup>2</sup>, which was significant in this study. The reduction in the mean ulcer area directly corresponded with the number of dressings. There was a significant reduction in the mean ulcer surface area in the fifth week (5.03 vs. 1.69) when compared with the baseline value. Regarding mean wound-area reduction, in most of the studies it was superior to 50% at week 4. Mean time to healing ranged between 4 and 10 weeks.[16]

Senet et al also studied the potential benefit of platelet rich plasma in venous ulcers in a randomized double-blind clinical trial of 15 patients (eight in the experimental and seven in the control group). Platelet rich plasma treatment (frozen autologous platelet suspension in saline solution) was applied three times a week, together with hydrocolloids and standardized compression bandages, until either full epithelialization or 12 weeks of treatment.[20] Conde-Montero E observed that Mean percentage reduction in ulcer area was 26.2% in the group versus 15.2% in the placebo group (p value =0.94).[21] Gopinath VPK revealed his findings that the ulcer size was examined before the commencement of treatment and thereafter every week. There was a reduction in mean ulcer area from day zero of  $27.5 \pm 19.3$  to  $7.3 \pm 8.1$  cm<sup>2</sup> at 6th week of follow-up. There was a reduction in mean ulcer volume from day zero of  $28.72 \pm 20.38$  to  $5.47 \pm 7.3$  cm<sup>3</sup> at 6th week of follow-up. Average improvement in the area and volume of ulcers were 85.7% and 90.7% (median) respectively at the end of 6 weeks and this improvement is statistically significant (p < 0.05) (varying from 33% to 100%) within 4 weeks.[19] Singh N et al also revealed that the mean reduction in area and volume of ulcer was  $12.27 \pm 4.10$  and  $6.88 \pm 5.26$  in Platelet rich plasma group whereas in NS group mean reduction in area and volume was  $9.25 \pm 1.89$  and  $4.25 \pm 1.05$ . P value was set less than 0.05 and hence the results were found to be significant.[18]

## CONCLUSION

Based on this study observation and result, we proposed that platelet rich plasma is a safe bio-compatible, cost-effective, less time-consuming procedure and does not require sophisticated equipment. Hence, may be considered as a treatment modality of choice in the management of chronic non healing ulcers. At last, we conclude that as the study was done in a small population group, to validate the result and efficacy of autologous platelet rich plasma in the management of chronic non-healing ulcers, a study with a larger population group must be undertaken.

## REFERENCES

1. Sabiston DC, Townsend CM, Beauchamp RD, Evers BM, Mattox KL. Sabiston textbook of surgery: the biological basis of modern surgical practice. Philadelphia: Wb Saunders; 2001.
2. Greer N, Foman N, Wilt T, Dorrian J, Fitzgerald P, MacDonald R, Rutks I. Advanced Wound Care Therapies for Non-Healing Diabetic, Venous, and Arterial Ulcers: A Systematic Review. 2012.
3. Suryanarayan S, Budamakuntla L, Khadri SIS, et al. Efficacy of autologous platelet-rich plasma in the treatment of chronic non-healing leg ulcers. *PlastAesthet Res*. 2015;1(2):65–9.
4. Sebastian KMS, Lobato I, Hernandez I, et al. Efficacy and safety of autologous Platelet Rich Plasma for the treatment of vascular ulcers in primary care: phase III study. *BMC FamPract*. 2014;15:211.
5. Steed DL, Donohoe D, Webster MW, Lindsley L. Effect of extensive debridement and treatment on the healing of diabetic foot ulcers. Diabetic Ulcer Study Group. *J Am Coll Surg*. 1996;183(1):61–64.
6. Boulton AJ. Pressure and the diabetic foot: clinical science and offloading techniques. *Am J Surg*. 2004;187(5A):17S–24S.
7. Kunimoto BT. Growth factors in wound healing: the next great innovation? *Ostomy Wound Manage*. 1999;45(8):56-64.
8. Mason J, O’Keeffe C, Hutchinson A, McIntosh A, Young R, Booth A. A systematic review of foot ulcer in patients with Type 2 diabetes mellitus. II: treatment. *Diabet Med*. 1999;16(11):889–909.
9. Cullum N, Nelson EA, Flemming K, Sheldon T. Systematic reviews of wound care management: (5) beds; (6) compression; (7) laser therapy, therapeutic ultrasound, electrotherapy and electromagnetic therapy. *Health Technol Assess*. 2001;5(9):1–221.
10. Kantor J, Margolis DJ. Management of leg ulcers. *SeminCutan Med Surg*. 2003;22(3):212–221.
11. AutoloGel System [Internet]. Cytomedix: Regenerative Biotherapies. [place unknown]: AutoloGel System; 2009. [cited 3 May 2009].
12. Fonder MA, Lazarus GS, Cowan DA, Aronson-Cook B, Kohli AR, Mamelak AJ. Treating the chronic wound: A practical approach to the care of nonhealing wounds and wound care dressings. *J Am AcadDermatol*. 2008;58(2):185–206.
13. Suthar M, Gupta S, Bukhari S, Ponemone V. Treatment of chronic non-healingulcers using autologous platelet rich plasma: a case series. *J Biomed Sci*. 2017Feb 27;24(1):16.
14. Suryanarayan S, Budamakuntla L, Khadri SIS, Sarvajnamurthy S.Efficacyofautologousplatelet-richplasmainthetreatmentofchronicnonhealinglegulcers. *PlastAesthetRes*2014;1:65-9.
15. Deshmukh S, Fulare SM, Gupta J, Kate R and Dhamne S. Role of platelet richplasmainchronicnon-healingulcer:Anobservationalstudy.*InternationalJournal of SurgeryScience*2021;5(2): 145-148.

16. Prabhu R, Vijayakumar C, Bosco Chandra AA, et al. Efficacy of Homologous, Platelet-rich Plasma Dressing in Chronic Non-healing Ulcers: An Observational Study. *Cureus*. 2018;10(2):e2145.
17. Brem H, Tomic-Canic M. Cellular and molecular basis of wound healing in diabetes. *J Clin Invest*. 2007;117:1219–22.
18. Singh N, Rathore SS, Choudhary G, Purohit LK. The efficacy of intralesional injection of autologous platelet rich plasma versus normal saline dressing in chronic non-healing ulcers. *Int Surg J* 2021;8:300-6.
19. Gopinath VPK, Simi VM, Basheer Ahammed K, Farisa PM, Ali Rishad CM. Intralesional autologous platelet rich plasma therapy in chronic nonhealing cutaneous ulcers: an interventional study from a tertiary care centre in North Kerala. *Int J Res Dermatol* 2019;5:116-22.
20. Senet P, Bon FX, Benbunan M, Bussel A, Traineau R, Calvo F. Randomized trial and local biological effect of autologous platelets used as adjuvant therapy for chronic venous leg ulcers. *J Vasc Surg*. 2003;38:1342–1348.
21. Conde-Montero E, de la Cueva Dobao P, Martínez González JM. Platelet-rich plasma for the treatment of chronic wounds: evidence to date. *Chronic Wound Care Management and Research*. 2017;4:107-120.