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EFFICACY OF AUTOLOGOUS PLATELET RICH PLASMA IN THE MANAGEMENT OF PRESSURE ULCER IN PATIENTS WITH SPINAL CORD INJURY

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ABSTRACT

Introduction: Pressure ulcers in spinal cord injury represent a challenging problem for patients, their caregivers, and their physicians. They often lead to recurrent hospitalizations, multiple surgeries, and potentially devastating complications. They present a significant cost to the healthcare system. The incidence of pressure ulcers in the SCI population is 25–66%. Despite treatment, many chronic ulcers fail to heal or persist for months/years and/or recur after healing, requiring additional advanced wound care therapies for adequate healing. Application of autologous Platelet Rich Plasma (PRP) has been a major breakthrough for the treatment of pressure ulcers, as it is an easy and cost-effective method, and provides the necessary growth factors that enhance tissue healing. The purpose of the current study was to determine the safety and effectiveness of treating pressure ulcers with PRP versus a control treatment (normal saline).

Material And Methods: In this Open Level Parallel Randomised Controlled Trial, conducted in the Department of Physical Medicine & Rehabilitation, IPGME&R and SSKM Hospital – Kolkata between 1st January 2018-31st June 2019 (18 months). After getting clearance from the Institutional Ethics Committee, Sample size for this study was calculated on the basis of proportion of subject showing complete ulcer healing in 6 weeks on the basis of an earlier study assuming that complete ulcer healing would occur in 20% case in standard wound care (control) and 60% in PRP group (case), it is established that 22 subjects will be required per group (n=44, 22 in each group). Individual informed written consent was taken from each patient to include in the study group. Every patient was explained properly. Those patients who falls under our inclusion criteria will be included for the study. They will be given a proforma and informed consent was taken after base line laboratory investigations. The patients were evaluated clinically. This included a complete medical history including all reports. If the patient fulfilled the criteria, he or she was put in one of the groups randomly and given PRP as per standard technique. The eschar was adequately removed and pressure ulcer was staged according to the National Pressure Ulcer Advisory Panel & European Pressure Ulcer Advisory Panel. The normal protocol for management of Pressure ulcer at our institute was followed: The pressure ulcer area debrided thoroughly to remove infected tissue and the graded. Group 1(PRP): After PRP injection alternate day dressing was done along with normal saline and Group 2 (Saline): Dressing with normal saline was done daily and repeat debridement are done if needed. PUSH Tool 3.0 & Ulcer area assessment was maintained as per protocol for both the groups. Parameters studied: 1. Area of the ulcer (cm^2), 2. Proportion with complete ulcer healing at 6 weeks, 3. PUSH Tool. Patients were assessed at baseline, 2weeks, 4 weeks, 6 weeks then follow up at 3 months.

Results: Numerical data were compared between groups using student's unpaired t test when normally distributed or by Mann - Whitney's u test if otherwise. All numerical variables in the descriptive statistics tables were normally distributed by Kolmogorov - Smirnov goodness-of-fit test. The Chi – Square test or Fisher's exact test employed for intergroup comparisons of categorical variables. Repeated measures ANOVA followed by Tukey's test was done to compare between two individual time points in both the groups. All analysis was two tailed and p – value <0.05 was considered statistically significant. In PRP group (case) highest number of patients were between 18-29 & 30-39 years, whereas in saline (control) group highest number of patients were found in the age group of 30-39 years. Mean age of the patients assigned to the PRP group was 36.86 years with a SD of 10.9years and median age was 33.0 years. Mean age of the patients assigned to the Saline (control) group was 37.55 years with a SD of 10.80 years and median age was 35.50 years. In both the groups male preponderance were found, in Case (PRP) 72.73% and in Control (saline) 77.27%. Majority of the ulcer were Grade III (59.09%) in case group and Grade II (54.55%) in control group. Most of the SCI patients with pressure ulcer were due to fall from height (54.55%) in each group. SCI resulting from fall of heavy object on the back was lesser in both the groups. Majority of the pressure ulcer site was sacrum for both the study group (PRP- 59.09%) & (Saline- 54.55%). Majority of the NLI was ASIA C (PRP-40.91% & Saline-45.45%) followed by ASIA B. Unpaired t test revealed that there was no statistically significant difference between patients of PRP & Saline group in regards to the age of the patient, mean PUSH Tool and mean Area of the ulcer. A statistically significant difference was found between the two groups when compared for mean PUSH Tool and Area of ulcer at 4wks, 6wks and 3 months. Proportion of the ulcer healed at 6wks was statistically significant in both the groups (P=0.000). Repeated measures ANOVA followed by Tukey's test was done to compare between two individual time points (ANOVA returns $p < 0.05$). Repeated measure ANOVA with multiple comparisons show statistically significant reduction in the mean PUSH tool score of the pressure ulcer in the PRP group when compared to baseline and subsequent visit. Maximum reduction in Mean PUSH Tool 3.0 score (Mean diff = 5.181) was noted in the time period between 6wks to 3 months. Repeated measure ANOVA with multiple comparisons show statistically significant reduction in the mean Area of the pressure ulcer in the PRP group when compared to baseline and subsequent visit. Maximum reduction in ulcer area (mean diff = 14.705) was observed in the time period between Baseline to 2wks. Repeated measures ANOVA followed by Tukey's test was done to compare between two individual time points (ANOVA returns $p < 0.05$). Repeated measure ANOVA with multiple comparisons show statistically significant reduction in the mean PUSH tool score of the pressure ulcer in the Saline group when compared to baseline and subsequent visit. Maximum reduction in Mean PUSH Tool 3.0 score (Mean diff = 6.136) was noted in the time period between 6wks to 3 months. Repeated measure ANOVA with multiple comparisons show statistically significant reduction in the mean Area of the pressure ulcer in the Saline (control) group when compared to baseline and subsequent visit. However, Tukey's Multiple Comparison test did not show significant change in reduction of ulcer area in the time period between 4wks to 6wks. Maximum reduction in ulcer area (mean diff = 6.7500) was observed in the time period between Baseline to 2wks. No major complication was seen after treatment with PRP except burning sensation while injecting the PRP at the site of lesion. PRP application hastens the healing process and lead to rapid wound healing.

Conclusion: In our study, most of the spinal cord injury patients comprised of male belonging to 2nd and 3rd decade. Most of the spinal cord injury patients were due to fall from height (54.55%). Majority of the ulcer were Grade III (59.09%) in case group and Grade II (54.55%) in control group with sacrum being the site of maximum involvement. PRP group shows significant reduction in both the PUSH Tool score 3.0 and

area of pressure ulcer all throughout the follow up period of 2wks, 4wks, 6wks and 3 months interval (p value <0.001). Saline group also showed significant reduction in both the PUSH Tool score 3.0 and area of pressure ulcer was noted at 2wks, 4wks, 6wks and 3 months (p value <0.001). But in regard to reduction in ulcer area not much significant changes was seen between 4 to 6wks. Ulcer healing is more marked, significant and faster in patients who received autologous PRP along with normal saline. PRP may be an ideal therapy for enhancing wound healing process in pressure ulcer.

KEYWORDS : *Autologous Platelet Rich Plasma (PRP), Pressure Ulcer (PrU), Spinal Cord Injury (SCI), PUSH Tool*

INTRODUCTION:

Pressure ulcers in spinal cord injury represent a challenging problem for patients, their caregivers, and their physicians. They often lead to recurrent hospitalizations, multiple surgeries, and potentially devastating complications. They present a significant cost to the healthcare system, they require a multidisciplinary team approach to manage well, and outcomes directly depend on patient's education, prevention, and compliance with conservative and surgical protocols. Pressure ulcers can be life-threatening in end-stage cases as a potential source of overwhelming sepsis. Patients with SCI, its chronic comorbidities and lack of protective sensory perception, are a particularly vulnerable population for developing ulcers and are at high risk for recurrent ulcers.[1] The incidence of pressure ulcers in the SCI population is 25–66%. It has also been reported that patients with higher-level spinal cord injuries are more susceptible than those with lower-level lesions.[2] A pressure injury is localized damage to the skin and underlying soft tissue usually over a bony prominence or related to a medical or other device. The injury can present as intact skin or an open ulcer and may be painful. The injury occurs as a result of intense and/or prolonged pressure or pressure in combination with shear. The tolerance of soft tissue for pressure and shear may also be affected by microclimate, nutrition, perfusion, co-morbidities and condition of the soft tissue.[3]

Despite treatment, many chronic ulcers fail to heal or persist for months/years and/or recur after healing, requiring additional advanced wound care therapies for adequate healing.[4] Application of autologous Platelet Rich Plasma (PRP) has been a major breakthrough for the treatment of pressure ulcers, as it is an easy and cost-effective method, and provides the necessary growth factors that enhance tissue healing. PRP is defined as a portion of the plasma fraction of autologous blood having a platelet concentration above baseline. PRP also has been referred to as platelet-enriched plasma, platelet-rich concentrate, autologous platelet gel, and platelet releasate. Platelet releasates have been used to treat wounds since 1985. PRP serves as a growth factor agonist and has both mitogenic and chemotactic properties. It contains a high level of platelets and a full complement of clotting and growth factors.[5,6]

These modular treatment options are safe and effective and have no side effects. Over the last two decades, emerging cellular therapies such as platelet-rich plasma (PRP) therapy has gathered considerable attention for its potential use in the field of regenerative medicine as a therapeutic agent in a range of chronic conditions and can have an adjunctive role in a standardized, quality treatment plan.[7,8].

The purpose of the current study was to determine the safety and effectiveness of treating pressure ulcers with PRP versus a control treatment (normal saline). The primary objective of the 6-week study was to compare the safety and incidence of complete wound closure between PRP and control-treated wounds at the end of the study. Secondary objectives included comparing the rate of wound healing during the period of 6-weeks and incidence of healed ulcers during a 3-month follow-up period.

REVIEW OF LITERATURE:

As life expectancy is steadily improving through modern spinal unit care, the increased survival in spinal cord injury (SCI) patients is associated with secondary complications, which continue to pose management challenges and impair the quality of life of such patients. Pressure ulcers (PrUs) are one of the major secondary complications of SCI and are a source of suffering for the patients and their caregivers.[9,10] These wounds are typically non-healing, resulting in a downward spiral of chronic inflammation, which can be a source of morbidity and even mortality in immobile populations. Promoting accelerated healing of PrUs would provide an improvement of patient's quality of life and reduce the economic impact that chronic wounds have on the health care system.[11] Wounds on persons with SCI may be even more difficult to heal because of the physiological deficits that an SCI causes.[12] When chronic wounds do not respond, a more aggressive, and sometimes more expensive, treatment is required to stimulate natural healing.[13] The incidence of pressure ulcers in the SCI population is 25–66%.[2]

CLASSIFICATION OF PRESSURE ULCER/INJURY (NPUAP 2016)[3,14]

STAGE 1: Pressure Injury: Non- Blanchable Erythema of Intact Skin
STAGE 2: Pressure Injury: Partial-thickness Skin Loss with Exposed Dermis
STAGE 3: Pressure Injury: Full-thickness Skin Loss
STAGE 4: Pressure Injury: Full-thickness Skin and Tissue Loss

UNSTAGEABLE PRESSURE INJURY: Obscured Full-Thickness Skin and Tissue Loss

DEEP TISSUE PRESSURE INJURY: Persistent Non-Blanchable Deep Red, Maroon or Purple Discoloration

Common sites of pressure ulcer development will vary depending on the most prevalent posture.[15] A prospective study of spinal cord patients not only found that sacral and ischial pressure ulcers were very common (43% and 15%, respectively), as might be expected, but also noted that the second most common location was on the heel (19%).[1]

Wound Assessment:

Assessment of pressure ulcer requires consistent documentation of wound characteristics and is critical for continuing monitoring, identifying infection, progress of wound healing and management. The precise location of the ulcer should be mapped in a transparent sheet and thereafter in a graph paper. Measurement of maximum length and width should be documented and recorded in the PUSH Tool score chart. The PUSH tool is a commonly used tool developed by the NPUAP, which grades pressure ulcers based on size of wound, wound bed tissue type, and exudate amount (Table 1).[16] Another commonly used scale is the Bates-Jensen wound assessment tool which scores wounds based on size, depth, wound edges, tissue undermining, type and amount of necrotic tissue, type and amount of exudate, skin colour, presence of oedema, induration, granulation, and epithelialization.[17] Other similar tools such as the pressure sore status tool and Sessing scale are also of use.[18]

Table 1. Pressure ulcer scale for healing (PUSH tool)[16]

Points	Area, cm2 (Length · Width)	Tissue Type	Exudate Amount
0	0	Closed	None
1	<0.3	Epithelial tissue	Light
2	0.3–0.6	Granulation tissue	Moderate
3	0.7–1.0	Slough	Heavy
4	1.1–2.0	Necrotic tissue	
5	2.1–3.0		
6	3.1–4.0		
7	4.1–8.0		
8	8.1–12.0		
9	12.1–24.0		
10	>24.0		

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The purpose of local wound care is to provide the wound with the most optimal environment for healing. Specific factors that need to be taken into consideration are: **Level of Moisture:** Depending on the pre-existing level of moisture in the wound bed, various dressings can be used to correct the level of moisture in the wound.[19] **Debridement of Necrotic Tissues:** Whenever possible, sharp debridement is the method of choice as it provides the most effective way to remove any necrotic tissue from the wound, though it must be noted that the NPUAP advises against the debridement of eschar in the heel.[20.] **Wound Cleansing:** Facilitates the removal of necrotic materials, exudates any metabolic wastes away from the wound, thus promoting wound healing.[21.] It also may decrease the bacterial load in the wound

tissue; this is important because a bacterial count of greater than 10^5 may be associated with the development of wound infection.[22] Saline is the solution of choice for cleansing. Wound irrigation at a pressure of 4 to 15 psi (pounds per square inch) is recommended.[23] **Protection of Wound:** Achieved by the use of an appropriate dressing to protect the wound from external factors such as further trauma, and bacterial or chemical exposure. **Pressure Relief:** The first step in management is offloading pressure from the wound site. For bedridden patients, strict adherence to repositioning the patient regularly. This may be achieved by the usual on lays and pads, some patients may require specialty beds. Even with these beds, patients still need to be repositioned regularly.[24] **Dressing (Conventional and advanced):** Different types of dressings. Gauze (Dry dressing/Wet-to-dry/Wet-to-moist) may remove granulation tissue and provide moist healing environment. It is commonly used in all stages of pressure ulcer. They help maintain a moist wound environment, and the gauze also serves the role of performing a superficial debridement of biofilm and small amounts of necrotic tissue during dressing changes due to its adherent nature.[25] Advanced dressings include alginates, collagen, composites, films, foams, hydrocolloids and hydrogels. They maintain an ideal moist wound environment by either absorbing exudates (eg: alginates, collagen, composites, films, foams, hydrocolloids) or donating or maintain moisture (hydrogels).[26] **Adjuvant Therapeutic Modalities:** There are many therapeutic modalities used in the treatment of pressure ulcers, including electrical stimulation (ES), hyperbaric oxygen, infrared, ultraviolet, low-energy laser irradiation, and ultrasound. The AHCPR Guidelines[21] supported only the use of hydrotherapy for the cleansing of the ulcers and ES for non-healing Stage III and IV pressure ulcer.

Platelet Rich plasma (PRP) is defined as portion of the plasma fraction of autologous blood having a platelet concentration above baseline. PRP has also been referred to as platelet enriched plasma, platelet rich concentrate, autologous platelet gel and platelet releasate. It contains a high level of platelet and a full complement of clotting and growth.[5.] PRP serves as a growth factor agonist and has both mitogenic and chemotactic properties.[27] The active secretion of these growth factors by platelets begins within 10 min after activation, with more than 95% of the pre-synthesized growth factors secreted within 1 hour. Marx proposed that platelet count of 10 lakh/ ml in 5 mL of PRP, as a working definition of PRP, based on the scientific proof of bone and soft tissue healing enhancement.[28] There is a scarcity of studies stating the concentration at which optimal stimulation occurs. Rughetti et al.[29] studied the relationship between the concentration of platelets in platelet gel and changes in the functional activity of human endothelial cells. The proliferation of endothelial cells and its migration and the invasion of endothelial cells occurred in a bell-shaped manner. The authors found that the stimulation for proliferation of endothelial cells peak at 1.25×10^6 and angiogenesis at 1.5×10^6 platelets/mL, respectively. This signifies the fact that a PRP platelet count 1 million/mL has become the working definition for therapeutic PRP and also reasons out the criticism on not getting the expected best results of PRP, which might be due to lower concentrations of platelets.

Kingsle[30] first used the term PRP to earmark thrombocyte concentrate during experiments related to blood coagulation in the year 1954, and in 1986 Knighton et al.[31] first demonstrated that PC successfully promote healing and they termed it as "platelet-derived wound healing factors (PDWHF)", which was successfully tested for the management of skin ulcers. In 1997 Whitman et al.[32] named their product PRP during preparation but when the end product had a consistency of a fibrin gel and therefore labelled it as "platelet gel". In the year 2009, the first classification about platelet concentrate was proposed by Dohan Ehrenfest et al.[33.]This classification defined 4 main families based on separation of the products using 2 key parameters: The cellular content (primarily leukocytes) and the fibrin architecture: (1) Pure platelet-rich plasma (P-PRP) - or leukocyte-poor platelet rich plasma (LP-PRP);(2) Leukocyte-and platelet-rich plasma (L-PRP); (3) Pure PRF (P-PRF) - or leukocyte-poor PRF; and (4) Leukocyte- and platelet-rich fibrin (L-PRF). Amable *et al.*[34] obtained best performance using parameters of 300×g for 5 min at 12°C and 240×g for 8 min at 16°C for 1st spin. The second spin of 700 × g for 17 min was chosen since it allowed a lower platelet loss into the PPP fraction and produced a pellet that was easily resuspended. The highest platelet recovery efficiency obtained by Slichter and Harker[35] was 80%, using a sample of 250-450 mL of WB centrifuged at 1000×g for a period of 9 min. It was observed that a subsequent centrifugation step of 3000×g for a period of 20 min decreased the platelet viability. Dugrillon *et al.*[36] reported that the number of platelets is not always proportional to the growth factors' quantity. Their study proved that the TGF-β1 and platelet concentration are proportionally related to inversely related to the centrifugal force when forces are above 800×g. Amanda *et al.*[37] demonstrated that the processing of 3.5 mL of blood at 100×g for 10 min (1st spin), 400×g for 10 min (2nd spin) and withdrawing 2/3 of remnant plasma, promoted high platelet recovery (70%-80%) and concentration (5×) maintaining platelet integrity and viability. It has been seen that the two-step procedure renders the highest output.

Mc Aleer et al. (2006) found that the use of autologous PRP was successful in healing a chronic lower extremity wound in a case study of a 57-year-old man with type 2 diabetes and a wound of six months duration. Complete closure of the ulcer was achieved by the fourth week of treatment with PRP.[38] Salemi et al. (2008) evaluated the effectiveness of a combination of autologous adipose tissue and PRP in a lower extremity ulcer of three years duration in a non-diabetic 65-year-old male patient. This study lasted for four weeks with follow-up at one, three, six and 15months. At 15-month follow-up, the wound had healed completely with regained functioning of the limb and enhanced quality of life reported by the patient.[39] Margolis et al. (2001) conducted a retrospective cohort study to estimate the effectiveness of platelet releasate (PR) in the treatment of diabetic neuropathic foot ulcers. Of the 26,599 patients included in the study, 21 percent were treated with PR. Overall, 43.1 percent of patients healed within 32weeks, including 50 percent of patients treated with PR and 41 percent of patients not treated with PR treatment. The investigators concluded that PR was more likely to be used in more severe wounds and was more effective in treating these wounds than the standard of care.[40] O'Connell et al. (2008) presented promising findings from a pilot study involving the treatment of chronic lower-extremity ulcers with autologous platelet-rich fibrin matrix membrane (PRFM). This prospective trial (n=21) of eligible patients aged 18to 85 included 12 patients with 17 venous lower-extremity ulcers and nine patients with13 non venous lower-extremity ulcers, all who had failed to respond to at least four weeks of conventional treatment. Complete healing was achieved in 66.7 % of the patients with venous lower-extremity ulcers in 7.1 weeks (median six weeks) following an average of two applications of PRFM per patient. Of the non-venous lower extremity ulcer group, 44 percent of patients treated with PRFM healed completely during the study period.[41]

Vickie R driver et al.[7] showed that healing process accelerated in diabetic foot ulcer patients when PRP gel was applied. Treating wounds with PRP or saline gel resulted in healing in approximately 6weeks, but in the most common wound sizes, almost twice as many PRP treated wound healed in that time frame. The number of adverse events was minimal. Hemmat M et al.[42] showed epithelization and granulation tissue formation in burn ulcers treatment with PRP. Dressing with PRP was found to be most significant in this respect compared with silver sulfadiazine dressing. R Singh et al.[43] evaluate the local application of platelet-rich plasma (PRP) in relation to pressure ulcers (PrUs) healing. Majority of histopathological pictures of PrUs (case) showed necrosis and suppuration (56%) at the time of enrolment and well-formed granulation tissue and epithelialization (60%) at the 5th week.

AIMS AND OBJECTIVES:

1. To study the efficacy of autologous platelet rich plasma (case) in comparison to standard wound care (control) in the management of pressure ulcer in patients with Spinal Cord Injury

2. To see fifty percent reduction in the ulcer area over the period of 6 weeks in terms of change in ulcer size and PUSH tool 3.0 score.

MATERIALS AND METHODS:

Before the start of the study, clearance from the Institutional Ethics Committee was taken. Individual informed written consent was taken from each patient to include in the study group.

Study Area:

Department of Physical Medicine & Rehabilitation, IPGME&R and SSKM Hospital – Kolkata.

Study Population:

SCI patients admitted at IPGME&R and SSKM hospital, Kolkata - West Bengal were included in the study as per the inclusion and exclusion criteria.

Study Period: 18 months (1st January 2018-31st June 2019)

Sample Size: (n=44, 22 in each group) This study was calculated on the basis of proportion of subject showing complete ulcer healing in 6 weeks on the basis of an earlier study[7] assuming that complete ulcer healing would occur in 20% case in standard wound care (control) and 60% in PRP group (case), it is established that 22 subjects will be required per group in order to assess this outcome with 80% power and 5% probability of type I error, allowing for a 10% dropout rate, the recruitment target was being kept at 25 subjects per group.

Study Design: Open Level Parallel Randomised Controlled Trial.

Inclusion Criteria:
1.Diagnosed cases of Spinal cord injury with pressure ulcer (Grade II/III)
2.Age group >18yrs
3.Clean wound
4.Platelet count > 1.5lakh.

Exclusion Critertia:
1.Severe anaemia
2.Hematologic disease
3.History of hematologic malignant disease
4.Severe cardiovascular disease, infection, immunosuppressive status, DIC
5.Active anticoagulant therapy
6.SCI patients who did not give consent

Study Techniques: Those patients who falls under our inclusion criteria will be included for the study. They will be given a proforma and informed consent was taken after base line laboratory investigations. The patients were evaluated clinically. This included a complete medical history including all reports. If the patient fulfilled the criteria, he was put in one of the groups randomly and given PRP as per standard technique. The eschar was adequately removed and pressure ulcer was staged according to the National Pressure Ulcer Advisory Panel & European Pressure Ulcer Advisory Panel. The normal protocol for management of Pressure ulcer at our institute was followed: The pressure ulcer area debrided thoroughly to remove infected tissue and the graded.

Group 1 (PRP): After PRP injection alternate day dressing was done along with normal saline and Group 2 (Saline): Dressing with normal saline was done daily and repeat debridement are done if needed.

PUSH Tool 3.0 & Ulcer area assessment was maintained as per protocol for both the groups.

PRP was prepared from department of PM&R - details are as follows:[44]
Step 1. Venous blood of 15-30 ml will be drawn put in vacutainer tubes containing sodium citrate. **Step 2**. The samples are gently agitated to thoroughly mix the anticoagulant with the blood. **Step 3.** The blood sample is then centrifuged for 10 mins at 2700 rpm resulting in the following layers: the inferior layer composed of erythrocytes, the intermediate layer composed of leukocytes and the superior layer made up of plasma. **Step 4.** The buffy coat layer together with the plasma layer is collected and centrifuged for another 10 mins at 2000 rpm to separate the leukocytes. **Step 5.** The platelet- poor plasma is first discarded to avoid its mixing up with the PRP. **Step 6:** PRP will be applied locally over the wound and injected around the intra-lesional region. Log book for PRP production, use and disposal are maintained as per decorum.

Parameters Studied:
1.Area of the Ulcer (cm^2)
2.Proportion with complete ulcer healing at 6 weeks
3.PUSH Tool

Assesment Done: At baseline, 2weeks, 4 weeks, 6 weeks then follow up at 3 months.

STUDY TOOLS:
- 4 ml vacutainer tube
- Centrifuge Machine (REMI R-8C)
- Platelet rich plasma
- Anti-coagulant
- Sterile gloves
- Gauze piece
- Normal saline
- Syringe
- Transparent sheet
- Graph paper
- Linear measuring scale (for measurement in cm)

Figure 1: Centrifuge Machine

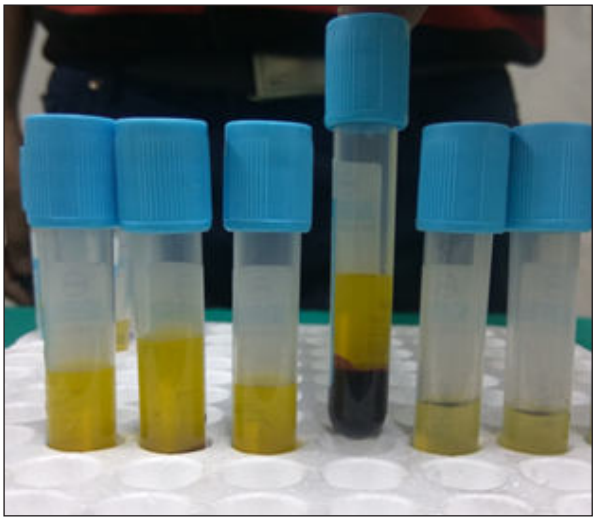


Figure 2: Preparation of PRP

CASE 1 (Figure 3,4,5 and 6)

Figure 3: (At Baseline)

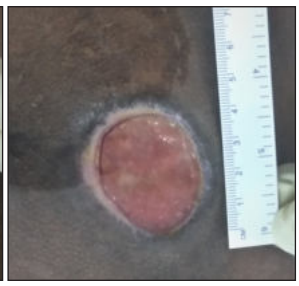


Figure 4: Post PRP (At 2wks)

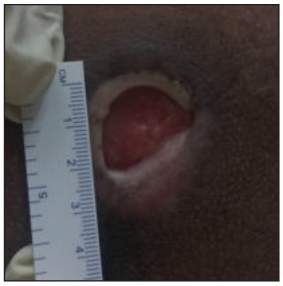


Figure 5: At 4wks

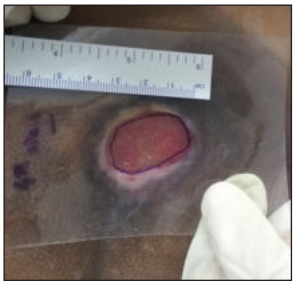


Figure 6: At 6wks

CASE 2 (Figure 7,8,9 and 10)

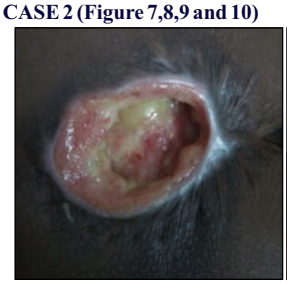


Figure 7: (At Baseline)

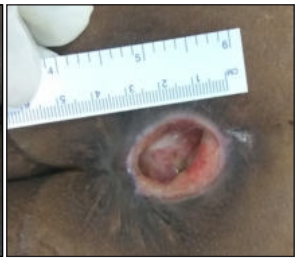


Figure 8: POST PRP (At 2wks)

Figure 9: At 4wks

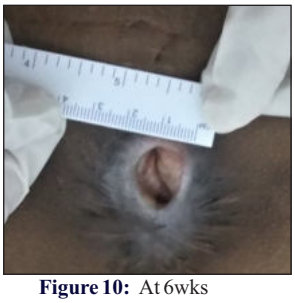


Figure 10: At 6wks

CASE 3 (Figure 11,12,13 and 14)

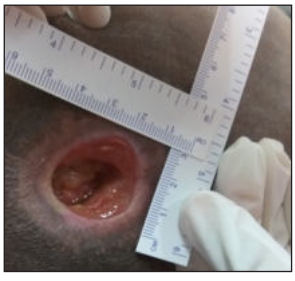


Figure 11: (At Baseline)

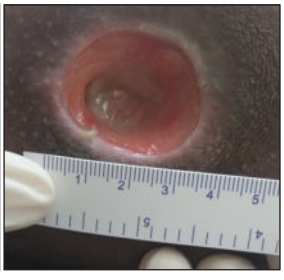


Figure 12: Post PRP (2wks)

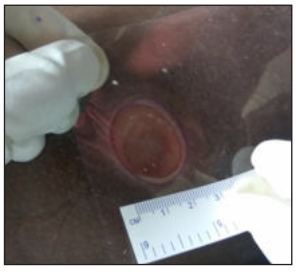


Figure 13: At 4wks

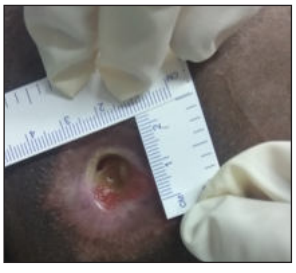


Figure 14: At 6wks

RESULT ANALYSIS:

Numerical data were compared between groups using student's unpaired t test when normally distributed or by Mann - Whitney's u test if otherwise. All numerical variables in the descriptive statistics tables were normally distributed by Kolmogorov - Smirnov goodness-of-fit test. The Chi – Square test or Fisher's exact test employed for intergroup comparisons of categorical variables. Repeated measures ANOVA followed by Tukey's test was done to compare between two individual time points in both the groups. All analysis was two tailed and p – value <0.05 was considered statistically significant.

Table 2: Comparison of Age Distribution between Groups (n=44)

Age in years	Intervention Group		Total Number (%)	Chi square test
	PRP(Case)	Saline (Control)		
18-29	7	6	13 (29.5)	P value 0.265
30-39	7	8	15 (34.1)	
40-49	4	4	8 (18.2)	
50-59	4	3	7 (15.9)	
60 & above	0	1	1 (2.3)	
Total	22	22	44 (100)	

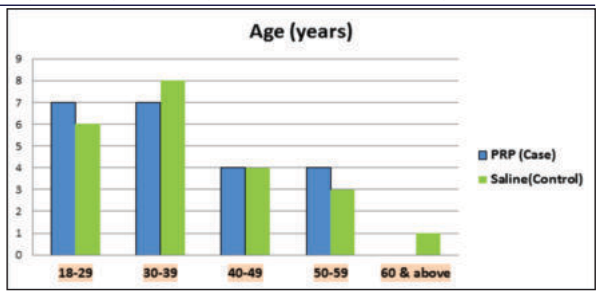


Figure 15: Distribution of Study Population according to Age (n-44)

Comments:

The above table and diagram showing comparison of age distribution between two groups (PRP and Saline). In PRP group highest number of patients were between 18-29 & 30-39 years, whereas in saline (control) group highest number of patients were found in the age group of 30-39 years. However Chi square test shows there is no statistically significant difference (P value 0.265) regarding age between two treatment group under the study .

Table 3:Comparison of Sex Distribution between Groups (n=44)

INTERVENTION GROUP	MALE	FEMALE	Total	Fisher's exact test (2 tailed)
CASE (PRP)	16 72.73%	6 27.27%	22	P-value 1.000
Control (saline)	17 77.27%	5 22.73(%)	22	
TOTAL	33	11	44	

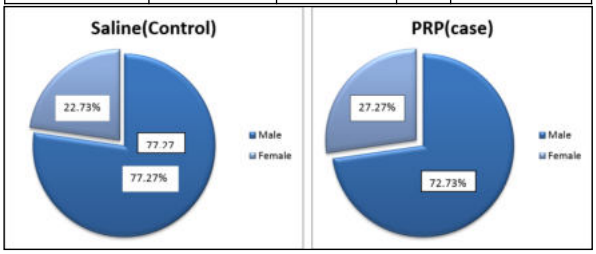


Figure 16: Distribution of Study Population according to Sex (n-44)

COMMENTS:

The above diagram shows distribution of sex between groups. In both the groups male preponderance were found, in Case (PRP) - 72.73% and in Control (saline) - 77.27%.

Fisher Exact test shows there is no statistically significant difference (P-1.000) in proportion of male and female between two groups.

Table 4: Comparison of Ulcer Grade (As per NPUAP Guidelines) between Groups (n=44)

Intervention Group	Ulcer Grade		TOTAL Number(%)	Fisher's exact test
	Grade II	Grade III		
PRP (Case)	9 (40.91%)	13 (59.09%)	22	P-value 0.547
Saline (Control)	12 (54.55%)	10 (45.45%)	22	
Total	23	21	44	

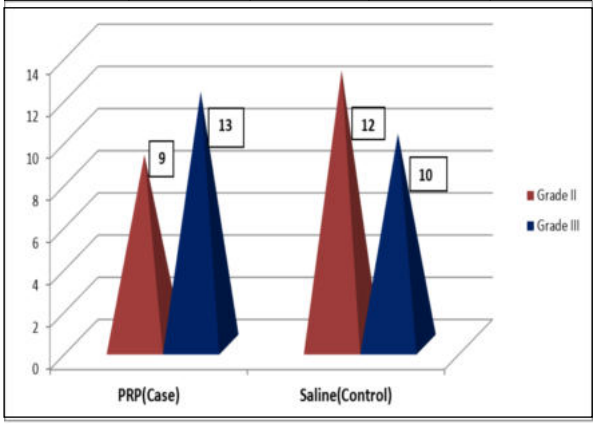


Figure 17: Distribution of Study Population according to NPUAP Ulcer Grading (n-44)

COMMENTS:
Fisher exact test reveals that, two treatment group under the study have no significant difference when compared. Majority of the ulcer were Grade III (59.09%) in case group and Grade II (54.55%) in control group.

Table 5: Comparison of Aetiology of Pressure Ulcer In Case with Spinal Cord Injury between Groups (n-44)

INTERVENTION GROUP	ETIOLOGY OF SCI			Total Number (%)	Chi-Square Test.
	RTA	Fall Of Heavy Object On Back	Fall From Height		
PRP (Case)	8 (36.36 %)	2 (9.09 %)	12 (54.55%)	22	P value-0.822
Saline (Control)	9 (40.91%)	1 (4.55 %)	12 (54.55%)	22	
Total	17	3	24	44	

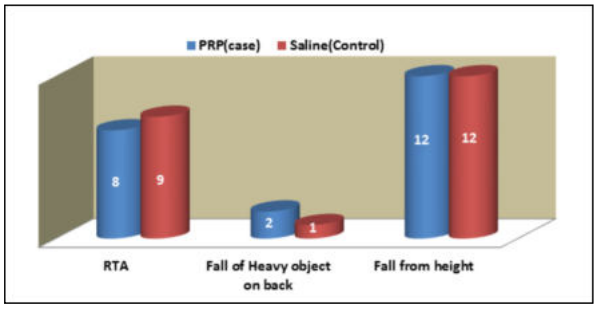


Figure 18: Distribution of Study Population according to Aetiology of SCI in Patients with Pressure Ulcer

COMMENTS: In our study group most of the SCI patients with pressure ulcer were due to fall from height (54.55%) in each group. SCI resulting from fall of heavy object on the back was lesser in both the groups. Chi square test reveals that the two-treatment group under the study were comparable in terms of aetiology.

Table 6: Comparison of Site of Pressure Ulcer between Groups (n=44)

INTERVENTION GROUP	ULCER SITE				TOTAL Number (%)	Chi square Test.
	Sacrum	Ischial tuberosity	Trochanter	Heel		
PRP (Case)	13	1	4	4	22	
	59.09%	4.55%	18.18%	18.18%		P value
Saline (Control)	12	2	5	3	22	0.890
	54.55%	9.09%	22.73%	13.64%		
Total	25	3	9	7	44	

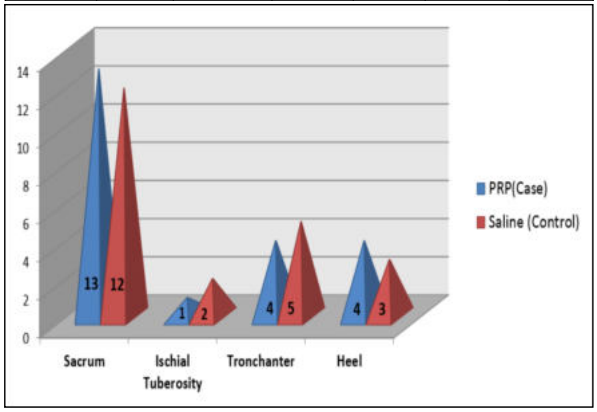


Figure 19: Distribution of Study Population according to Site of Ulcer (n=44)

COMMENTS: Majority of the pressure ulcer site was sacrum for both the study group (PRP- 59.09%) & (Saline- 54.55%). No, statistically significant difference (P-0.890) was found regarding the anatomical site of pressure ulcer between the two treatment groups.

Table 7: Comparison of Neurological Classification of Spinal Cord Injury (As per ASIA Impairment scale) between Groups (n-44)

INTERVENTION GROUP	NLI				TOTAL Number	Chi square Test.
	ASIA A	ASIA B	ASIA C	ASIA D		
PRP (Case)	4 (18.18%)	7 (31.82%)	9 (40.91%)	2 (9.09%)	22	P value 0.897
Saline (Control)	3 (13.64%)	8 (36.36%)	10 (45.45%)	1 (4.55%)	22	
Total	7	15	19	3	44	

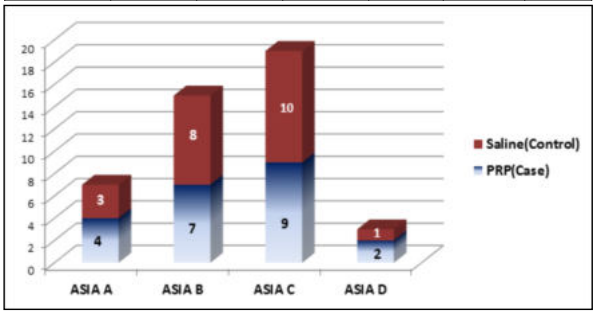


Figure 20: Distribution of study population according to Neurological Classification of Spinal Cord Injury (n=44) [As per ASIA Classification]

COMMENTS:
In comparison between the two groups, majority of the NLI was ASIA C (PRP-40.91% & Saline-45.45%) followed by ASIA B. There was no statistically significant difference (P value 0.897) between the two-study group in regard with Neurological Classification of Spinal Cord Injury.

Table- 8a: Descriptive Statistics of Numerical Variable-Group-1: PRP (n-22)

Variables	Mean	Median	Lower Quartile	Upper Quartile	Std Dev.
Age(years)	36.86	33.00	29	44	10.903
PUSH TOOL 3.0					
PUSH_B	13.23	13.00	12.00	15.00	1.602
PUSH_2w	10.64	11.00	9.00	12.00	1.733
PUSH_4w	8.32	9.00	7.00	9.00	1.810
PUSH_6w	5.82	6.00	4.00	7.00	2.085
PUSH_3m	0.64	0.00	0.00	1.00	0.790
Area of the ulcer(cm^2)					
Area_B	38.14	31.75	25.00	59.00	18.547
Area_2w	23.43	20.25	13.50	35.50	12.551
Area_4w	14.09	11.50	8.50	18.00	8.745
Area_6w	7.16	6.00	3.50	11.00	5.137
Area_3m	0.64	0.00	0.00	1.00	0.902
Proportion of Ulcer healed at 6wks					
UlcerHeal_6w	83.25	81.20	79.70	87.70	1.270

All numerical variables in the descriptive statistics tables are normally distributed by Kolmogorov-Smirnov goodness-of-fit test.

Comments:
1. Mean age of the patients assigned to the PRP group was 36.86 years with a SD of 10.9years and median age was 33.0 years.
2. Mean PUSH Tool score at baseline for PRP group was 13.23 which decreased subsequently with every visit and finally reached to 0.64 at the 3month of final visit.
3. Mean surface area of the pressure ulcer at the beginning of the study for the PRP group was 38.14 cm^2 with S.D of 18.547 cm^2 which was decreased to 0.64 cm^2 at the end of the study.

Table 8b: Descriptive Statistics of Numerical Variable-Group-2: Saline Control (n-22)

Variables	Mean	Median	Lower Quartile	Upper Quartile	Std Dev.
Age(years)	37.55	35.50	28.00	45.00	10.804
PUSH TOOL 3.0					
PUSH_B	12.59	13.00	11.00	14.00	1.469
PUSH_2w	11.23	12.00	10.00	13.00	1.771

PUSH_4w	9.41	10.00	7.00	11.00	1.943
PUSH_6w	8.00	9.00	6.00	9.00	2.000
PUSH_3m	1.86	2.00	3.00	3.00	1.246
Area of the ulcer(mm)					
Area_B	32.68	37.00	15.00	47.00	16.699
Area_2w	25.93	28.75	11.00	36.00	13.861
Area_4w	20.75	22.00	8.00	29.00	11.533
Area_6w	16.27	17.50	6.50	24.50	9.291
Area_3m	1.73	2.00	1.00	2.00	1.232
Proportion of Ulcer healed at 6wks					
UlcerHeal_6w	52.19	53.25	47.70	56.70	7.089

Comments:

1. Mean age of the patients assigned to the Saline (control) group was 37.55 years with a SD of 10.80 years and median age was 35.50 years.
2. Mean PUSH Tool score at baseline for Saline (control) group was 12.59 which decreased subsequently with every visit and finally reached to 1.86 at the 3month of final visit.
3. Mean surface area of the pressure ulcer at the beginning of the study for the Saline (control) group was 32.68 cm^2 with S.D of 16.69 cm^2 which was decreased to 1.73 cm^2 at the end of the study.

Table 9: Comparison of Numerical Variables between Groups 1 and 2 Student's Unpaired T Test

VARIABLES	Mean		Standard Deviation		t value	df	P-value
	PRP group (Case)	Saline group (control)	PRP group (Case)	Saline group (control)			
Age (years)	36.86	37.55	10.903	10.804	-0.2083	42	0.836
PUSH TOOL 3.0							
PUSH_B	13.23	12.59	1.602	1.469	1.3734	42	0.177
PUSH_2w	10.64	11.23	1.733	1.771	-1.1184	42	0.270
PUSH_4w	8.32	9.41	1.810	1.943	-1.9268	42	0.061
PUSH_6w	5.82	8.00	2.085	2.000	-3.5422	42	0.001
PUSH_3m	0.64	1.86	0.790	1.246	-3.9029	42	0.000
Area of the Ulcer (cm^2)							
Area_B	38.14	32.68	18.547	16.699	1.0251	42	0.311
Area_2w	23.43	25.93	12.551	13.861	-0.6270	42	0.534
Area_4w	14.09	20.75	8.745	11.533	-2.1579	42	0.037
Area_6w	7.16	16.27	5.137	9.291	-4.0263	42	0.000
Area_3m	0.64	1.73	0.902	1.232	-3.3512	42	0.002
Proportion of Ulcer healed at 6wks							
UlcerHeal_6w	83.25	52.19	5.956	7.089	15.7344	42	0.000

Comments:

1. Unpaired t test revealed that there was no statistically significant difference between patients of PRP & Saline group in regards to the age of the patient, mean PUSH Tool and mean Area of the ulcer.
2. A statistically significant difference was found between the two groups when compared for mean PUSH Tool and Area of ulcer at 4wks, 6wks and 3 months.
3. Proportion of the ulcer healed at 6wks was statistically significant in both the groups (P=0.000).

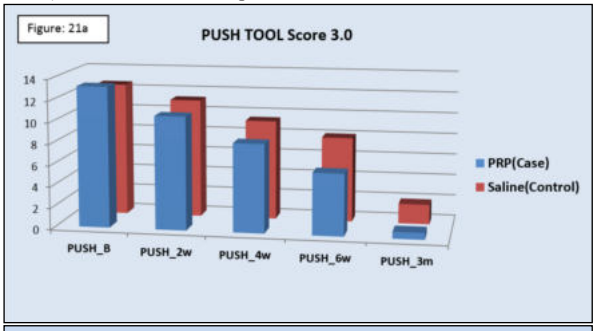
Table 10: Repeated Measure ANOVA

Comparison of change in PUSH Tool 3.0 over time – PRP (Case) (n-22)

REPEATED MEASURE ANOVA	F value 979.20 P value <0.001			
TUKEY'S MULTIPLE COMPARISON TEST	**Mean Diff**	**q**	**P value**	**95% CI of diff**
PUSH_B vs PUSH_2w	2.5909	16.815	< 0.001	1.9818 to 3.2000
PUSH_B vs PUSH_4w	4.9091	31.861	< 0.001	4.3000 to 5.5182
PUSH_B vs PUSH_6w	7.4091	48.086	< 0.001	6.8000 to 8.0182
PUSH_B vs PUSH_3m	12.591	81.717	< 0.001	11.982 to 13.200
PUSH_2w vs PUSH_4w	2.3182	15.045	< 0.001	1.7091 to 2.9273
PUSH_2w vs PUSH_6w	4.8182	31.271	< 0.001	4.2091 to 5.4273
PUSH_2w vs PUSH_3m	10.000	64.901	< 0.001	9.3909 to 10.609
PUSH_4w vs PUSH_6w	2.5000	16.225	< 0.001	1.8909 to 3.1091
PUSH_4w vs PUSH_3m	7.6818	49.856	< 0.001	7.0727 to 8.2909
PUSH_6w vs PUSH_3m	5.1818	33.631	< 0.001	4.5727 to 5.7909

Comments:

1. Repeated measures ANOVA followed by Tukey's test was done to compare between two individual time points (ANOVA returns $p < 0.05$).
2. Repeated measure ANOVA with multiple comparisons show statistically significant reduction in the mean PUSH tool score of the pressure ulcer in the PRP group when compared to baseline and subsequent visit.
3. Maximum reduction in Mean PUSH Tool 3.0 score (Mean diff = 5.181) was noted in the time period between 6wks to 3 months.



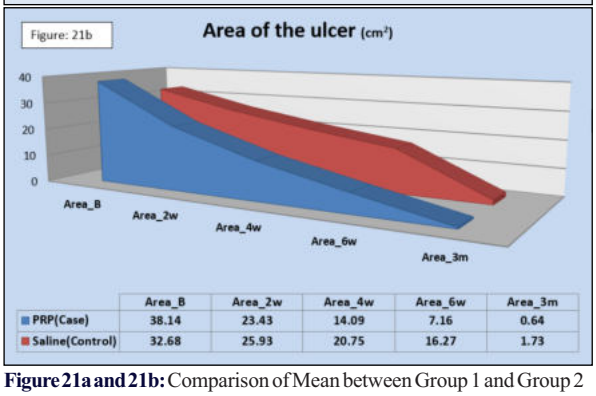


Figure 21a and 21b: Comparison of Mean between Group 1 and Group 2

Table 11: Repeated Measure ANOVA

Comparison of change in Area of the Ulcer over time – PRP (Case) Group

REPEATED MEASURE ANOVA	F value 95.425 P value <0.001			
TUKEY'S MULTIPLE COMPARISON TEST	**Mean Diff**	**q**	**P value**	**95% CI of diff**
Area_B vs Area_2w	14.705	9.7923	< 0.001	8.7685 to 20.641
Area_B vs Area_4w	24.045	16.013	< 0.001	18.109 to 29.981
Area_B vs Area_6w	30.977	20.629	< 0.001	25.041 to 36.913

Area_B vs Area_3m	37.500	24.973	< 0.001	31.564 to 43.436
Area_2w vs Area_4w	9.3409	6.2204	< 0.001	3.4049 to 15.277
Area_2w vs Area_6w	16.273	10.837	< 0.001	10.337 to 22.209
Area_2w vs Area_3m	22.795	15.180	< 0.001	16.859 to 28.731
Area_4w vs Area_6w	6.9318	4.6161	< 0.05	0.99580 to 12.868
Area_4w vs Area_3m	13.455	8.9598	< 0.001	7.5185 to 19.391
Area_6w vs Area_3m	6.5227	4.3437	< 0.05	0.58671 to 12.459

Comments:

1. Repeated measure ANOVA with multiple comparisons show statistically significant reduction in the mean Area of the pressure ulcer in the PRP group when compared to baseline and subsequent visit.
2. Maximum reduction in ulcer area (mean diff = 14.705) was observed in the time period between Baseline to 2wks.

Table 12: Repeated Measure ANOVA

Comparison of change in PUSH Tool 3.0 over time – Saline (Control) Group (n-22)

REPEATED MEASURE ANOVA	**F value 1458.5 P value <0.001**			
TUKEY'S MULTIPLE COMPARISON TEST	**Mean Diff**	**q**	**P value**	**95% CI of diff**
PUSH_B vs PUSH_2w	1.3636	12.519	< 0.001	0.93304 to 1.7942
PUSH_B vs PUSH_4w	3.1818	29.210	< 0.001	2.7512 to 3.6124
PUSH_B vs PUSH_6w	4.5909	42.146	< 0.001	4.1603 to 5.0215
PUSH_B vs PUSH_3m	10.727	98.480	< 0.001	10.297 to 11.158
PUSH_2w vs PUSH_4w	1.8182	16.692	< 0.001	1.3876 to 2.2488
PUSH_2w vs PUSH_6w	3.2273	29.627	< 0.001	2.7967 to 3.6579
PUSH_2w vs PUSH_3m	9.3636	85.961	< 0.001	8.9330 to 9.7942
PUSH_4w vs PUSH_6w	1.4091	12.936	< 0.001	0.97850 to 1.8397
PUSH_4w vs PUSH_3m	7.5455	69.270	< 0.001	7.1149 to 7.9760
PUSH_6w vs PUSH_3m	6.1364	56.334	< 0.001	5.7058 to 6.5670

Comments:

1. Repeated measures ANOVA followed by Tukey's test was done to compare between two individual time points (ANOVA returns $p < 0.05$).
2. Repeated measure ANOVA with multiple comparisons show statistically significant reduction in the mean PUSH tool score of the pressure ulcer in the Saline group when compared to baseline and subsequent visit.
3. Maximum reduction in Mean PUSH Tool 3.0 score (Mean diff = 6.136) was noted in the time period between 6wks to 3 months.

Table 13: Repeated Measure ANOVA

Comparison of change in Area of the Ulcer over time – Saline (Control) Group (n-22)

REPEATED MEASURE ANOVA	**F value 82.425 P value <0.001**			
TUKEY'S MULTIPLE COMPARISON TEST	**Mean Diff**	**q**	**P value**	**95% CI of diff**
Area_B vs Area_2w	6.7500	5.2599	< 0.01	1.6771 to 11.823
Area_B vs Area_4w	11.932	9.2978	< 0.001	6.8589 to 17.005
Area_B vs Area_6w	16.409	12.787	< 0.001	11.336 to 21.482
Area_B vs Area_3m	30.955	24.121	< 0.001	25.882 to 36.027
Area_2w vs Area_4w	5.1818	4.0379	< 0.05	0.10893 to 10.255
Area_2w vs Area_6w	9.6591	7.5267	< 0.001	4.5862 to 14.732
Area_2w vs Area_3m	24.205	18.861	< 0.001	19.132 to 29.277
Area_4w vs Area_6w	4.4773	3.4889	ns	-0.59562 to 9.5502
Area_4w vs Area_3m	19.023	14.823	< 0.001	13.950 to 24.096
Area_6w vs Area_3m	14.545	11.334	< 0.001	9.4726 to 19.618

Comments:

1. Repeated measure ANOVA with multiple comparisons show statistically significant reduction in the mean Area of the pressure ulcer in the Saline (control) group when compared to baseline and subsequent visit.
2. However, Tukey's Multiple Comparison test did not show significant change in reduction of ulcer area in the time period between 4wks to 6wks.
3. Maximum reduction in ulcer area (mean diff = 6.7500) was observed in the time period between Baseline to 2wks.

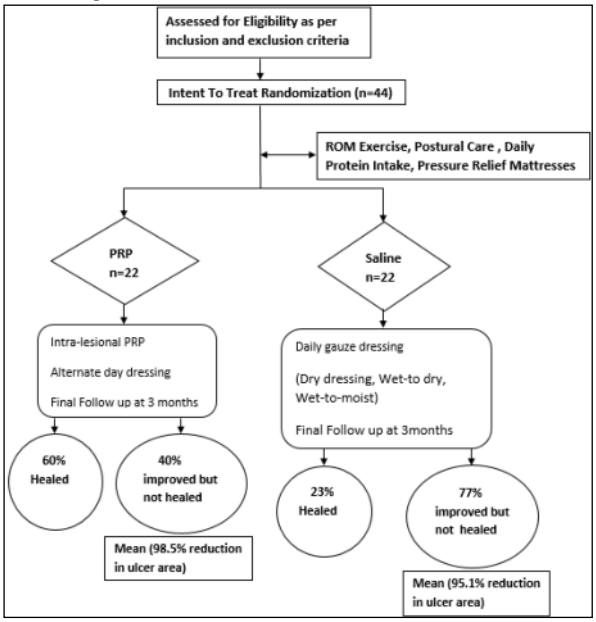


Figure: 22 Trial profile

DISCUSSION:

Patients admitted with pressure ulcer following spinal cord injury is a common scenario noticed in any health care setup. Our study was an Open Level Parallel Randomised Controlled Trial conducted for a period of 18 months in the Department of Physical Medicine and Rehabilitation, IPGME&R. The purpose of the present prospective study was to evaluate the efficacy of autologous platelet rich plasma (case) in comparison to standard wound care (control) in the management of pressure ulcer in patients with Spinal cord injury. Only few studies have evaluated its role in pressure ulcer in the SCI Population.[43,45]

After getting the ethical committee clearance, we included total 44 patients and divided them into two groups (22 in each group). In our study there was no dropout rate and all patients of both the group completed the study and attended all the follow up visit. Mostly male of 2nd and 3rd decade were affected in our study.

There was significant reduction in mean PUSH Tool score of Pressure Ulcer (PrU) (case) (p value<0.001) and PrU (control) (p value<0.001)

at 2wks, 4wks, 6wks and 3 months. The decrease in wound surface area of PrU (case) was statistically significant (p value<0.001), whereas in PrU (control) it was statistically insignificant during the follow up period between 4wks to 6weeks. The mean percentage of ulcer area healed at 6wks as compared to baseline was statistically significant in both the groups (case- 83.25% reduction, control-52.19% reduction) (t=15.7344).

Mazzuco et al.[46] showed 100% healing with use of autologous platelet gel in chronic wounds compared with control group of similar categories in 6wks period. The study also reported angiogenesis in chronic wound with PRP Therapy. Anitua et al.[47] showed that after 8weeks of PRP therapy in chronic wound, the mean percentage of ulcer area healed in PRP group was 72.94 ± 22.25, whereas it was 21.48 ± 33.5 in control group($p<0.05$) which was similar to our study. This study also concluded that platelet derived growth factor and vascular endothelial growth factor induced fibroblast proliferation and angiogenesis in chronic wound. Rappl LM[12] showed average reduction of surface area of PrU in 20 SCI patients (53.81%) after 3-4 weeks of PRP therapy. Sell et al.[11] showed complete healing PrU in 3 SCI patients after PRP therapy. They also reported granulation tissue development, vascularisation and epithelisation in 3 patients after PRP therapy. Gürgen M.[48] reported complete wound healing in 1 patient, decreased in ulcer size to an average of 55.2% in 12 patients 4weeks of PRP treatment in chronic wounds of various aetiologies. Gardner et al.[49] reported that total PUSH score was highly correlated with surface area measurements and this correlation increased over time as wound progressed towards closure. They measured wound surface area using acetate surface tracing, whereas we measured linear dimension (length –times-width).

The present study corroborates the findings with Rappl LM[12] and Scevola et al.[45] who reported that most healing occurred in first 4weeks of treatment of pressure ulcer in subjects with Spinal Cord Injury. There was statistically significant increase in onset of granulation phase of wound healing, the healing process was faster and there was more healing in the first 2 weeks of treatment with PRP gel compares with current wound dressing protocol. Platelet rich plasma have the supreme advantage, they synergistically induce various growth factors, promote angiogenesis and mitogenesis at the wound site,[50,51].

No major complication was seen after treatment with PRP except burning sensation while injecting the PRP at the site of lesion. PRP application hastens the healing process and lead to rapid wound healing.

LIMITATION:

1.Sample size was small in each group.
2.Time period of study was short.
3.Standard protocol regarding yield of maximum quantity of PRP via centrifuge is lacking due to a smaller number of pre-existing studies.
4.There is diversity in site of pressure ulcer for both PrU (case) and PrU (control) group.
5.Comparison of improvement due to PRP with standard literature was difficult due to paucity of previous evidence.

CONCLUSION:

In our study, most of the spinal cord injury patients comprised of male belonging to 2nd and 3rd decade. Most of the spinal cord injury patients in our study were due to fall from height (54.55%) followed by Road Traffic Accident (40.91%). Both the treatment group under the study were comparable in terms of aetiology of ulcer. All patients were complaint during the study and there were no dropouts. Majority of the ulcer were Grade III (59.09%) in case group and Grade II (54.55%) in control group with sacrum being the site of maximum involvement with regard to pressure ulcer. Both PRP and Saline group aids to reduction in pressure ulcer area and PUSH Tool Score 3.0. Our study concluded that PRP group shows significant reduction in both the PUSH Tool score 3.0 and area of pressure ulcer all throughout the follow up period of 2wks, 4wks, 6wks and 3 months interval (p value <0.001). Our study similarly shows that in Saline group significant reduction in both the PUSH Tool score 3.0 and area of pressure ulcer was noted at 2wks, 4wks, 6wks and 3 months (p value <0.001). But in regard to reduction in ulcer area not much significant changes was seen between 4 to 6wks. Overall rate of ulcer healing was good in both form of treatment. Although the reduction is more marked, significant and faster in patients who received autologous PRP along with normal saline. Our study failed to take into consideration certain variables like nutritional status of the patient, depth of the ulcer, histopathological finding from the ulcer area in regards to seeing the pattern of wound healing. Larger studies in different patient population are needed to validate this observation further as the result of this study were promising. With the advantage of simple preparation, biocompatible safety, low cost and significant clinical effectiveness, it may be beneficial to study the effect of PRP on larger scale to validate it as an ideal therapy for enhancing wound healing process in Pressure ulcer.

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