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Platelet Rich Plasma (PRP) as an Enhancer of Wound Healing in Problematic Wounds: Our University of Port Harcourt Teaching Hospital (UPTH) Experience

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ABSTRACT

Introduction: Problematic wounds are difficult to heal wound, as the timely and orderly manner of acute wound healing is disrupted and have defied the normal wound healing protocols, Platelet Rich Plasma {PRP} in the enhancement of tissue repair and cellular regeneration. Objective: To evaluate the effect of topical PRP in wound healing about time to heal, level of pain, and number of dressing changes. Methods: This is a prospective, randomized clinical study conducted at the outpatient clinic with 21 well-matched patients fulfilling the inclusion criteria. A 10 ml sample of fresh blood was drawn and centrifuged at 1800rpm for 10 minutes then 3500 rpm for 15 minutes to isolate the PRP. All patients with chronic ulcers were treated to receive dressings impregnated with PRP. Patients were followed up for 8 weeks to evaluate the wound characteristics, healing process, quality of granulation tissue, presence of exudate or slough, need for frequent changes of dressings, and associated pain levels. Data Analysis: collected were entered in Microsoft Excel 2010. Analysis was done using Epi-info version 7.0. Result: All patients with chronic ulcers received dressings infused with PRP. Assessments were conducted over an 8-week monitoring wound characteristics, healing progression, granulation tissue quality, presence of exudate or slough, necessity for frequent dressing changes, and associated pain levels. Conclusion: The results of this study depicted that Platelet Rich Plasma (PRP) is highly effective in the treatment of Problematic Wounds of various etiologies. PRP reactivates the healing and regeneration in problematic wounds.

Keywords: Problematic wounds, PRP.

INTRODUCTION

Wound healing is a complex, dynamic process, requiring the interactive functions of multiple cell types and growth factors coupled with molecular pathways. Historically, wound healing is classified into four main overlapping phases: hemostasis, inflammation, proliferation, and remodeling[1]. A variety of factors such as diabetes, infections, and a failure in blood supply can turn a wound into a non-healing wound termed chronic ulcers when the duration has exceeded 6 weeks[2]. Chronic wounds are a significant burden to health care systems all over the world;

the estimated annual cost of chronic wounds in the United States ranges from $28 billion to $96 billion[3]. The prevalence of chronic wounds in Nigeria is estimated at 12.6%[4-5]. Management of chronic wounds requires a multidisciplinary approach involving debridement, appropriate dressings, and advanced therapies, biomedical engineering and researches. Platelet Rich Plasma (PRP) is one such product that is invaluable in developing countries with limited resources for medicare[6].

In our institution, the University of Port Harcourt Teaching Hospital, we commonly use various wound dressing agents, which include povidone iodine, gentamicin ointment, honey, hydrogels, hydrocolloids, alginate-based dressings, and recombinant epidermal growth factor preparations[16]. These agents either reduce the bioburden of the wound or provide growth factors that directly stimulate wound healing. In particular, because it is widely available, cheap, and accessible to patients, povidone iodine is one of the most commonly used agents.

Of special interest in the tissue regeneration field is the use of Platelet Rich Plasma (PRP) for recalcitrant wounds[17]. PRP is a second-generation platelet concentrate that uses only autologous materials derived from the patient and subsequently used locally to stimulate wound healing and facilitate cell regeneration. Growth factors, which play a crucial role in the process of tissue regeneration, are found in abundance in the platelet concentrate. The first description and use of PRP were by Choukroun et al. in France in 2001[17].

PRP preparation is a simple procedure that can be done in an office or clinical setting. The procedure involves the collection of 5 milliliters of venous blood, which is placed into two sterile vacutainer tubes that do not contain anticoagulants (plain tubes).[18] The blood samples are promptly transferred to a centrifuge within approximately two minutes and are spun at a speed of 3,500 revolutions per minute (rpm) or 800g for a duration of 10 minutes; then respinning at 1500rpm for 15 minutes[19]. This process separates the blood into three distinct layers: the straw-colored, acellular plasma forms the upper layer; the lower layer is red and consists of red blood cells (RBCs); and a middle layer that is gel-like in nature, which contains the fibrin clot. The fibrin clot is a collection of platelets encapsulated within a fibrin meshwork. This technique of PPRP preparation is known as the Choukroun[20] Protocol. The fibrin gel is then put on the wound, and dressings are covered over it, allowing a slow release of growth factors for a minimum of seven days, maximum of 28 days[21].

In Plastic Surgery, it has been used as a biological dressing for acute and chronic skin wounds. In 2013, a study by Chirag et al.[22] proved its efficacy in healing a defect in the lower lip and hence introduced the term "Modified Secondary Intention Healing" by the authors.[23]

The use of Platelet Rich Plasma (PRP) in chronic ulcers is of great interest to plastic surgeons. PRP is a second-generation platelet concentrate applied in several surgical sub-specialties to accelerate tissue healing and promote cellular regeneration. PRP is, therefore, an autologous and natural source of growth factors beneficial in wound healing and tissue regeneration.

Platelet-rich plasma (PRP) is a solution in which, by centrifugation of the patient's own blood, platelets and their associated growth factors are concentrated[7]. PRP has been used in numerous applications across the fields of Plastic and Reconstructive Surgery, Orthopedics, Dentistry, Dermatology and Clinical Haematology due to its healing properties in tissue repair and

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regeneration[8]. The application of PRP in wound healing depends on its capacity to deliver a high concentration of growth factors, including PDGF, VEGF, and TGF-β, directly into the wound site[9]. These Growth Factors play a very significant role in promoting cellular proliferation, differentiation, and migration. These are imperative in effective wound healing processes[10].

Numerous studies have confirmed the efficacy of PRP in wound healing for various types of wounds, including diabetic foot ulcers, pressure ulcers, and surgical wounds[11-13].

Further research is required on this application of PRP for low- and middle-income countries like Nigeria, as most studies are conducted in high income countries[14]. The study aimed to assess the efficacy and safety of PRP in wound healing amongst our patient population.

This will lead to the introduction of Platelet Rich Plasma (PRP) as an adjuvant treatment for wound healing in our armamentarium of Advanced Wound Healing Products at the University of Port Harcourt Teaching Hospital[15].

Objectives

1. To assess the effect of topical platelet rich plasma (PRP) on the amount of wound exudate and the frequency of dressing changes.
2. To assess the effect of topical platelet rich plasma (PRP) on the wound culture of the patients.
3. To establish whether topical platelet rich plasma will diminish the healing duration of the wound, therefore, decreasing the need for skin coverage

METHODOLOGY

This prospective study was performed in an outpatient clinic with 21 well-matched patients who met the inclusion criteria. A 10 ml sample of fresh blood was collected and centrifuged at 3500 rpm for 10 minutes then 1500rpm at 15 minutes to prepare the PRP. All patients suffering from chronic ulcers received dressings containing PRP. Follow-up assessments were carried out over a period of 8 weeks to assess wound characteristics, wound progression, rate of healing, quality of granulation tissue, presence of exudate or slough and dressing change frequency.

Inclusion criteria were Patients with chronic ulcers at any site of the body with dimensions ≤ 6cm x 6cm, who were willing to undergo the study and had given consent, female and male patients in the age group of 18-60 years, Ulcers more than 12 weeks duration, diabetic patients (having 1-3 above) with poorly controlled blood sugar and Sickle cell disease patients (having 1-3 above) with haemoglobin levels of ≤ 8g/dl.

The exclusion criteria comprise patients who were below 18 years and above 65 years, Patients with cardiovascular instability, patients with haemoglobin levels below 10g/dl and sicklers with < 8g/dl, patients with extensive chronic wounds exceeding the preferred dimensions of ≤6cm x 6cm area, patients who had not given consent for the study, patients with a history of bleeding disorder, clinical features suggestive of malignant ulcers, chronic osteomyelitis, cellulitis, and very ill patients. The following variables were observed: wound characteristics, pain, analgesic requirements, wound exudation, frequency of dressing change, and dressing cost.

DATA ANALYSIS

Data collected were entered into Microsoft Excel 2010. The data were analyzed using Epi-info version 7.02 (CDC, WHO). Descriptive statistics were carried out with frequency and percentages for the categorical variables and means with standard deviations for continuous variables. Inferential statistical data analysis was carried out with Chi-square (χ^2) test and Students T-test. Chi square test was performed between categorical variables. The Student T-test was used when any comparison was being sought between continuous variables. A P-value of ≤ 0.05 is considered statistically significant.

RESULTS

The study had 42 patients recruited and were monitored over an 8-week period. The ages of the patients varied from 18 to 65 years, with an average age of 42.69 ± 13.70 years. The age group of 19 years and younger exhibited the lowest frequency, accounting for 2 patients (4.76%), while the highest frequency was observed in the 50-59 years age group, comprising 10 patients (23.81%).

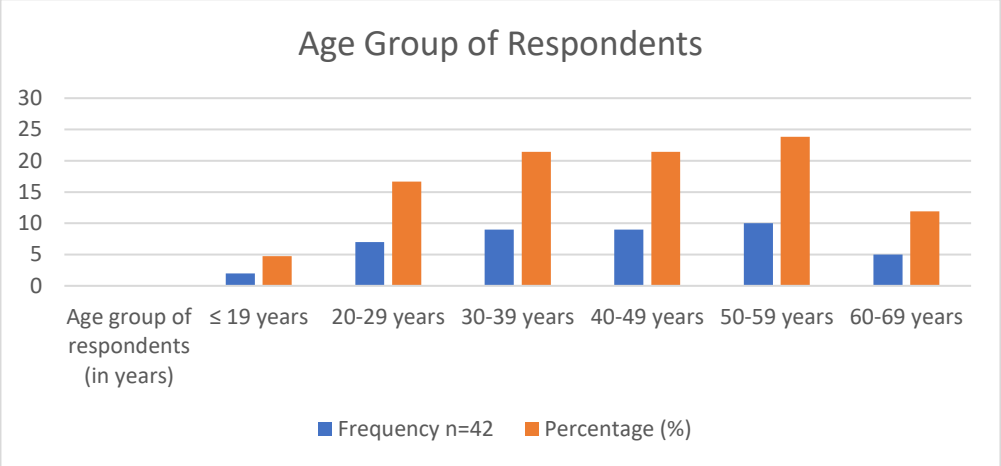

Figure 1: Age distribution of the respondents

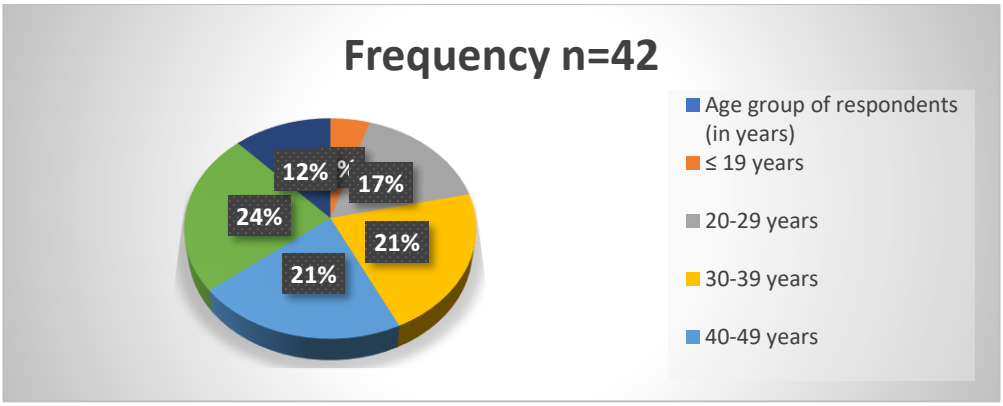

Figure 2: Age Distribution of the Respondents

Majority of the patients were male, totaling 31 (73.81%), with the largest occupational groups being civil servants, 10 (23.81%), and labourers, 6 (14.29%). The least represented occupations, each with a frequency of 1 (2.38%), included an athlete, a hair stylist, and a seamstress.

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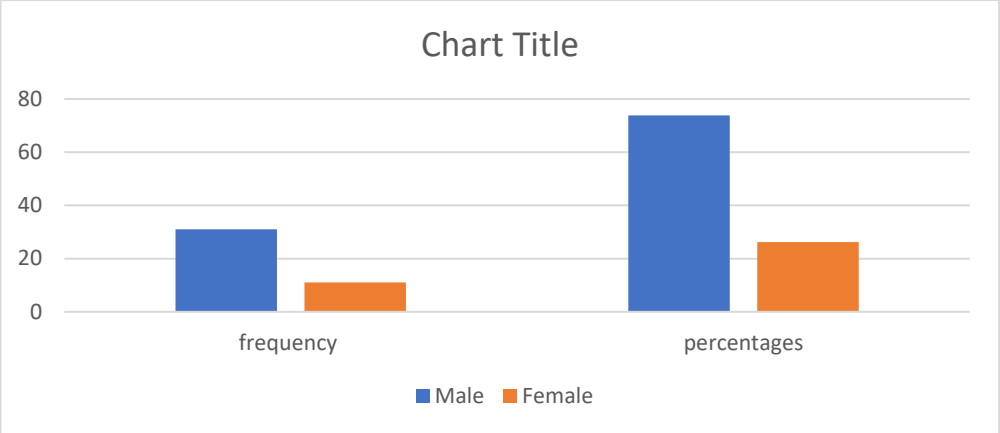

Figure 3: Gender Distribution of the Respondents

Aeitiology of the Wound

The most common cause of the ulcers was diabetes mellitus, which caused 12 (28.57%) of diabetic foot ulcers. Post-traumatic ulcers were the second most common cause, occurring in 7 cases (16.67%), followed by post-infective ulcers at 6 cases (14.29%). The least common cause was post-surgical wound breakdown, occurring in 3 cases (7.14%).

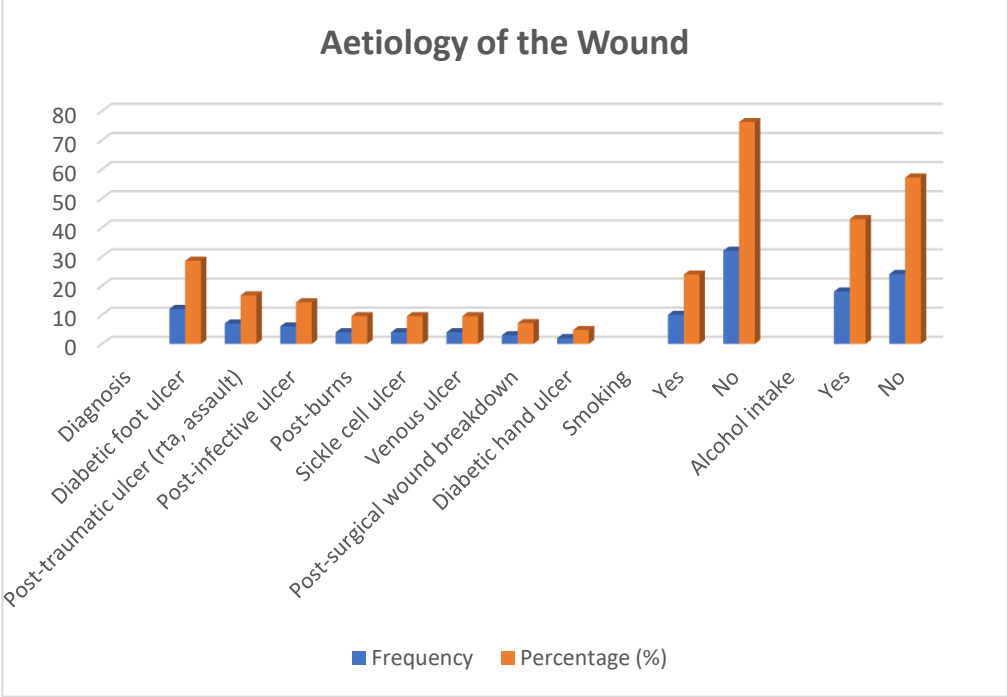

Figure 4: Distribution in Percentages of the Aetiology of the Wound

In the first week, infection rates were high in the patient treated with PRP with a value of 11(52.38%). However, the pattern rapidly changed with infected wound cases falling to 2 (9.52%) and this continued to fall over the succeeding weeks.

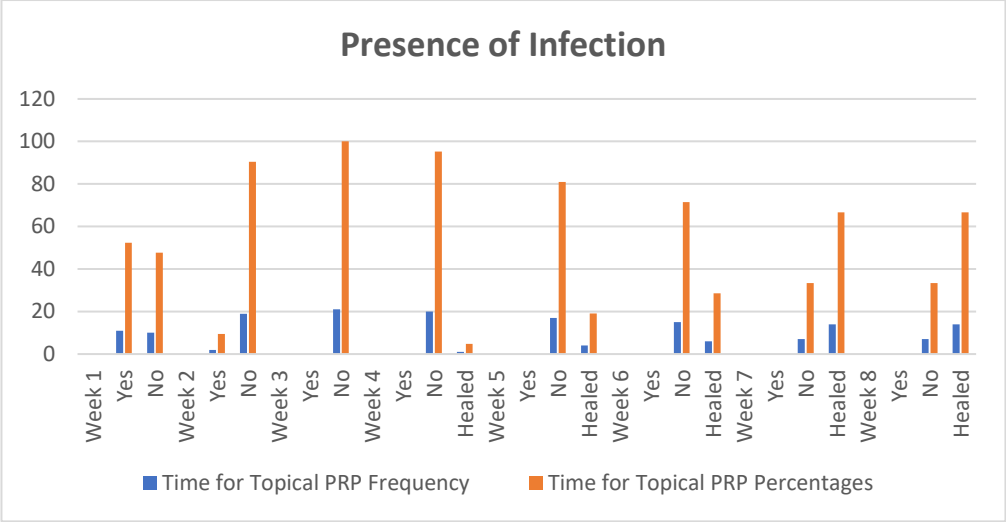

Figure 5: Bar Charts showing the distribution in Frequency and Percentages of the Presence of Infections

The presence of slough rates for the patients treated with PRP declined rapidly from 3 (4.29%) in the second week to 0 (0.0%) in the fourth week. This level was maintained till the end point of the study. The wound of the patient treated with PRP exhibited initial signs of re-epithelialization as early as the second week, with a measurement of 11 (52.38%). In both study groups, there was a notable increase in re-epithelialization rates, with early healing observed in both populations by the fourth week. However, the patients receiving PRP demonstrated statistically significant rates of re-epithelialization during the second, third, fourth, seventh, and eighth weeks.

Granulations Tissue

Healthy granulation tissue was noted at statistically significant levels for patients receiving PRP during the second, third, fourth, seventh, and eighth weeks. The peak values were observed in the third week, where the PRP patient exhibited a percentage of 20 (95.24%).

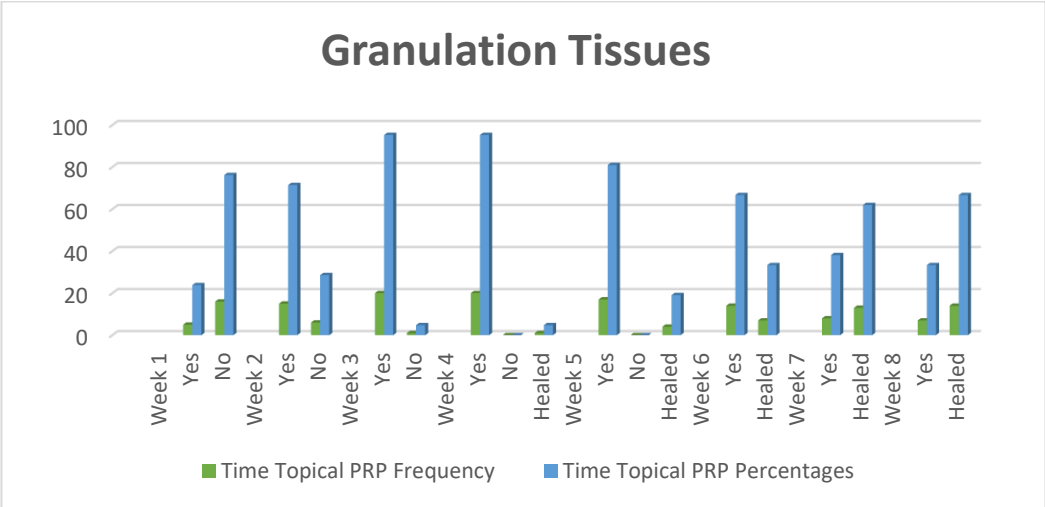

Figure 6: Bar Charts showing the distribution in Frequency and Percentages of the Presence of Granulation tissues

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There was a marked difference in the sizes of the wounds as observed for the patient treated with PRP from the second week till the endpoint of the study. The values were also uniformly statistically significant for the patient treated with PRP.

Presence of Exudate

The values for wound exudation were increasing and reducing in the patient treated with PRP patients from the second week till the study ended.

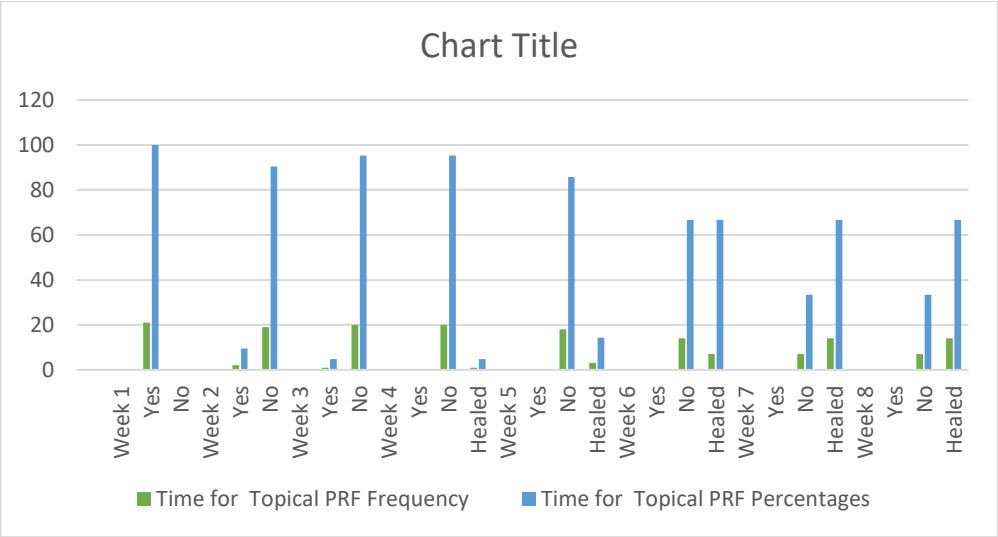

Figure 7: Bar Charts showing the distribution in Frequency and Percentages of the Presence of Exudate

The patient treated PRP patients had no frequent dressing changes with a percentage of 21(100.00).

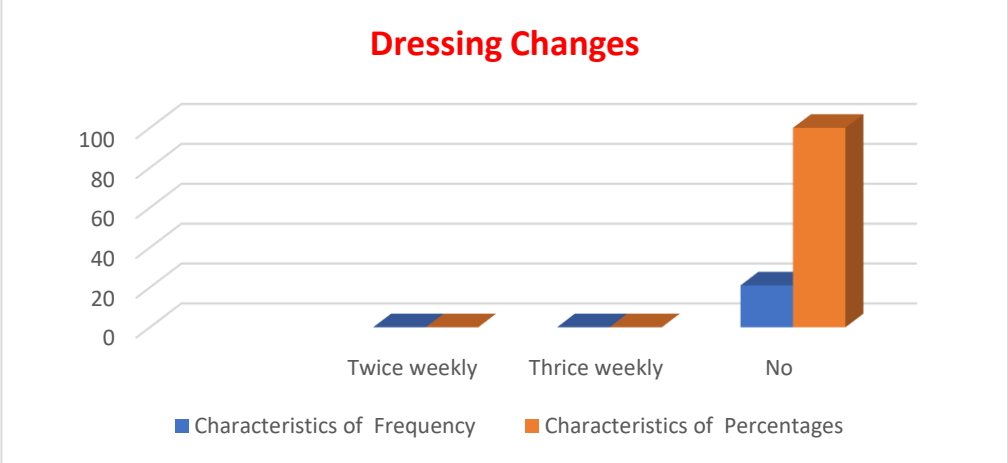

Figure 8: Bar Charts showing the distribution in Frequency and Percentages of Dressing Changes

At the beginning of the study, a vast majority, i.e., 13 (61.90%) of the PRP patients reported moderate levels of pain during the first week. During the second week, the pain in 11 (52.38%) of the PRP patients had subsided to a mild form. The pain continued to be constantly mild in all

subjects of the P group throughout the study until its end. On the other hand, a higher number of patients in the I group reported moderate pain levels throughout the study from its commencement to the end.

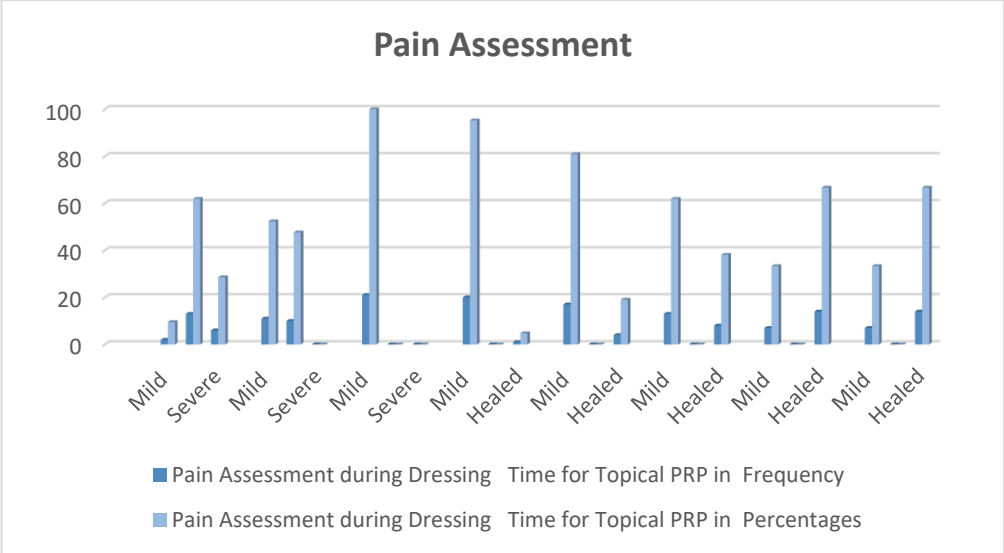

Figure 9: Bar Charts showing the distribution in Frequency and Percentages of Pain Assessment

Most of the ulcers (47.62%) continued for 5 to 9 months, and a few even up to 15 to 19 months (4.76%). The mean duration of all ulcers was 13.311 ± 18.76 months. Amongst the patients, 27 (64.29%) had no history of previous ulcer healing, but 15 (35.71%) had ulcers that healed in the past. During dressing changes, 9 patients required analgesics, while 28 patients required some form of analgesic following the dressing change.

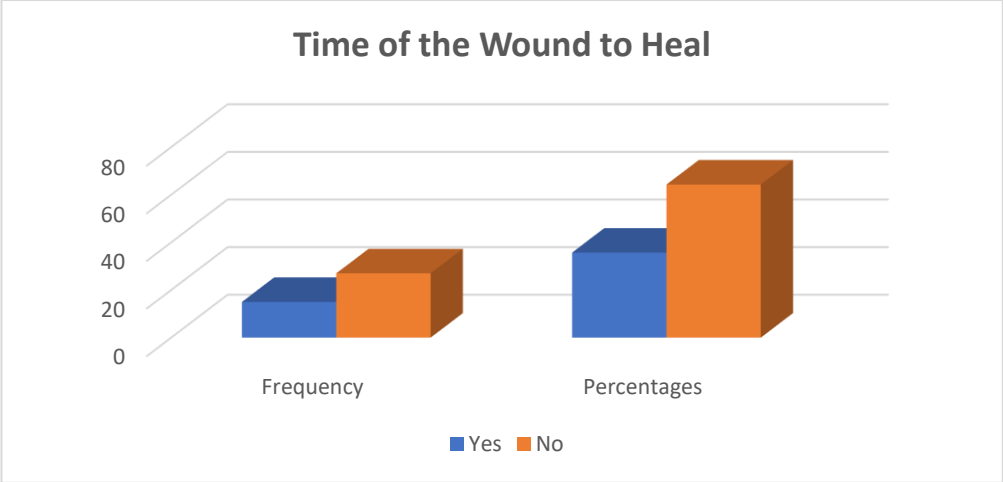

Figure 10: Bar Charts showing the distribution in Frequency and Percentages of Time taken for the Wound to heal

DISCUSSION

10 (23.81%) of the study population fell within the age bracket of 50-59 years. This finding is consistent with an earlier study by Guo and Di Petro and Gould et al.,[24] who found a higher incidence of chronic wounds among the geriatric population[25-26]. However, in our study we had

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65 years as oldest participant. Those below 40 years was 21.43% and those above the age of 60 years were 11.90%. Age has a great impact based on this study which reveals that wound heal more and faster in the younger than the elderly since the ageing process are not old.

The study also had a greater number of males than females as in the pie chart. This could be the reason for the observed disparity in the healing of acute wounds in males, which might result in chronic conditions, as observed in several studies27. Several studies have shown that estrogen might accelerate wound healing, particularly in post-menopausal women as observed in ovariectomized rats. In contrast, androgens were found to impair wound healing, as reported by Ashcroft and Mills28. There was also a fair share of civil servants, either serving 10 (23.81%) or retired 3 (7.14%), within the study.

As it is the case with illiterate vs. educated population in general, those who have education are much better informed and would most likely resort to regular medical services when needed more frequently. As it turns out, that wound incidence among these people can explain the cause.

Total 14(33.33%) cases were identified as diabetes mellitus-related ulcers. Post-surgical wound breakdown was the least common etiology. The incidence rate of diabetes mellitus is quite important in this study population[29]. The prevalence of diabetes mellitus and its related complications, such as diabetic foot ulcers, have been increasing. 9.52% (4 patients) of the studied subjects had sickle cell disease[30]. A total of 19 patients, which is 45.24%, had never received any treatment for their wounds before. Most of the wounds had been present for 5 to 9 months. Previous history of healing was found in 15 patients, that is, 35.71%. Though 9 patients, 21.43%, required analgesia during the dressing of the wound, 28 patients, 66.67%, required it only after the dressing change. Also, 10 patients (23.81%) were smokers, while 32 patients (76.19%) were non-smokers. In addition, 18 patients (42.86%) consumed alcoholic beverages, and 24 patients (57.14%) did not.

The number of patients developing infection after PRP treatment for varying numbers of weeks 3. The highest infection rates were registered during the first week and reduced progressively in the patients with PRP. The low incidence of infected wounds in this group might be explained by the stimulation of expression of various chemokines and growth factors, such as Human beta-defensin-3 (hBD-3). As stated by Bayer et al.,[32] "Human beta-defensin-3 (hBD-3) is an antimicrobial peptide inducibly expressed in human keratinocytes, particularly upon wounding." Other immunity-enhancing growth factors are also produced during the centrifugation process.

The study enumerates the presence of slough in the different wounds, with the lowest rates found in the PRP patients; this was, again, observed much earlier than in other groups. This further underscores the immune-boosting properties that have been documented by other authors. Evidence of re-epithelialization is given in this study, which has pointed out the better capacity of PRP in tissue regeneration and in stimulating keratinocytes' proliferation. The wounds of patients under PRP treatment showed signs of re-epithelialization earlier, evidenced by a greyish-blue color of advancing epithelial cells at the wound margins. By the fifth week, an additional three wounds in patients treated with PRP had attained healing[33].

Contrary to the results of this study, Bayer et al.,[33] in their study on the effects of platelet-released growth factors on primary human keratinocytes, came to the conclusion that the favorable clinical results seen with autologous platelet concentrate in the treatment of chronic, non-healing wounds are not due to increased keratinocyte proliferation. There was a higher incidence of healthy granulation tissue formation in patients treated with PRP[34]. This finding is in agreement with previous studies, including that by Desai, which showed that PRP not only accelerated wound healing and the formation of granulation tissue but also had anti-inflammatory properties. The term "modified secondary intention wound healing" was coined to describe the use of PRP in wound healing[35]

Furthermore, this study showed progressive and significant reduction of wound size, another determinant in accelerated healing. The dimensions of the ulcer wounds progressively reduced during the whole period of investigation, while their rate of healing was higher in the group of patients treated with PRP. It has to be underlined that starting from the second week, all obtained results were statistically significant, thus complementing similar findings also noted by Pinto et al.[36] For purposes of this study, an equal amount of dressing materials was issued to all patients at the commencement.

The effect of PRP on pain modulation is illustrated in, which is shown that the patients who had been treated with PRP reported moderate degrees of pain initially, which reduced to mild pain as the study proceeded from week two to week eight, and the outcomes were statistically significant. This observation gives credence to the analgesic effect of PRP on wounds, which corroborates the findings by Lokman et al.,[37] who have shown that PRP significantly reduced post-operative pain, trismus, and the number of analgesics taken by the patients undergoing impacted third molar surgery.

CONCLUSION

Platelet Rich Plasma (PRP) has shown excellent effectiveness in the treatment of chronic ulcers of multifactorial etiologies, and this study serves to confirm that. What is amazing is the way PRP reawakens healing in ulcers stuck in the healing phase and rapidly leads to the development of healthy granulation tissue. Moreover, compared to many other conventional dressings, the application of PRP on wounds dressing every week was relatively painless, less expensive, and it also presented lesser wound exudation, diminished amount of slough and lessened the offensive odor of the wounds. Therefore, the alternative hypothesis of difference in the duration of wound healing, wound characteristics-such as level of exudation, interventions required, patient symptomatology-that is pain during dressing changes, the frequency of hospital visits and the total cost of the patient care when using topical PRP compared with povidone iodine was accepted.

Recommendations

1. The application of PRP should be adopted in our hospitals as part of the standard management of chronic wounds.
2. A follow-up study using newer wound care products is warranted to see if similar results can be replicated.
3. Studies to compare the tensile strength of newly formed skin on both groups will provide valuable information.

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