Role of Platelet Rich Plasma in Management of Chronic Venous Leg Ulcers

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ABSTRCT

Objectives*: The aim of this study is to compare between the short term results and healing rates between platelet rich plasma and standard four layer compression technique for treatment of chronic non ischemic venous leg ulcers (VLUs).* ***Patients and method:*** *From October 2015 till October 2017, 73 ulcers in 68 patients from the attendees to Zagazig university hospitals were assigned in two groups, group (A) including 42 ulcers managed by standard four layer compression therapy only and group (B) including 31 ulcers managed by four layer compression therapy and PRP. Time for complete epithelization and pain scale were followed every week.* ***RESULTS****: complete epithelization occurred in 19/36 ulcers (52.7%) and 18/27 ulcers (66.6%) in group A and B respectively after 12 week (p value=0.014). Visual analogue scale (VAS) mean values showed no statistical difference between both groups before (p value = 0.64) or after (p value = 0.114) enrollment in the study.* ***Conclusion****: PRP is feasible and safe adjunctive therapy in the management of chronic venous leg ulcers and when the technique is used in combination with multiple layer compression therapy it improves significantly both the rate of ulcer healing and pain reduction.*
Keywords*: venous, ulcer, Platelet, plasma, compression*

INTRODUCTION

Venous leg ulcers (VLUs) are an important medical problem. The chronic and recurrent nature of VLUs causes morbidity, severely reduces quality of life, and increases the cost of health care. Standard evidence-based care includes compression therapy and the use of adjunctive agents, which have been shown to accelerate healing, improve quality of life, and likely reduce cost[1]

One of the newly adjuvant therapies is platelet rich plasma (PRP). Platelets carrying a wide range of inflammatory mediators, chemo-attractants, chemokines, and growth factors are involved in not only the establishment of the initial clot but in the recruitment of cells involved in wound healing (neutrophils, macrophages, stem cells, etc.) [2,3].

Platelets contains many growth factors like; Platelet-derived growth factor, transforming growth factor beta1 and beta2, epithelial growth factor, insulin-like growth factor type I, vascular endothelial growth factor (A and C), basic fibroblastic growth factor (FGF-2), hepatocyte growth factor, Bone morphogenetic protein (BMP) and connective tissue growth factor (CTGF).[4]

Many reviews have addressed the question of whether PRP is effective in inducing wound healing or not and have come to the relatively consistent conclusions that the evidence is mixed and that further studies are required to make a definitive decision one way or the other. [5]

This study aimed to assess the adding benefit of usage autologous PRP with standard compression therapy in management of chronic leg venous ulcers.

PATIENTS AND METHODS

Our study was designed as a prospective controlled trial treating patients with chronic venous ulcers (i.e. Chronic wounds are defined as those that do not heal completely after 30 days of standard medical treatment)[6] and were characterized by impaired remodeling of the extracellular matrix (ECM), prolonged inflammation and periods of relatively steady healing interposed with plateaus in which the wounds did not improved.

The patients were assigned to control group (treating with compression therapy only) and study group (combining autologous PRP with compression therapy). Patient's enrollment in each group was under patients request and financial resources.

From October 2015 till October 2017, 73 ulcers in 68 patients from the attendees to Zagazig

university hospitals were selected to our study according to the following inclusion and exclusion criteria.

The ***inclusion*** criteria were patients with single typical venous ulcer not healed for more than 30days, intact distal pulsation with ankle/brachial index 0.8-1.2, and normal hemogram with platelets count > 150,000/ml.

The exclusion criteria were patients with ulcer > 5cm in diameter, chronic ulcer more than 6 months, patients with debilitating disease as liver or renal impairment, heart failure, malignancies, uncontrolled diabetes (HbA1C> 7.5%), bleeding or platelet disorders, low immunity or corticosteroid therapy and history of operation for venous disorder in the same limb.

Full medical and surgical history taking, general assessment, vascular examination and neurological assessment were done for all patients. Laboratory investigations (pre-operative), arterial duplex and culture from the ulcers were done routinely. All patients signed informed consent.

Two weeks before assignment, all our patients underwent standard wound care in the form of mechanical debridement of dry slough when present, cover the wound with saline soaked gauze, single layer of elastic compression bandage were applied and antibiotics administration according to culture and sensitivity.

All the ulcers were divided in to two groups, group (A) including 42 ulcers managed by standard four layer compression therapy only and group (B) including 31 ulcers managed by four layer compression therapy and PRP.

The size of ulcers was recorded before treatment and every week till complete healing or the end of the study period (weekly visits during 12 weeks). For calculating the size of the ulcer we used Kundin's method " ulcer surface area = length x width x 0.785 (correction factor)" this correction factor was needed to compensate ulcers surface irregularities. [7]

The primary end point of our study was complete epithelization of the ulcer or after the patient underwent 12 weeks sessions. Our secondary end point was the recording of major complications (e.g appearance of new ulcers) or increasing the size of the ulcer.

Pain reduction was evaluated at the beginning and at the end of our study using visual analogue scale (VAS)

The standard four layer compression therapy was performed in all cases on weekly visits using several layers of four materials: orthopedic wool cotton, crepe bandage, and self-cohesive bandage. All bandages were wrapped using figure of 8 techniques thereby increasing the number of overlapping layers reaching a sub-bandage pressure equal 40mmHg.

For PRP preparation 25 mL of the patient blood was collected then one single centrifuge was done at 5000 rpm for 10 min then extraction of the platelet-rich fraction of the plasma, situated immediately above the red cell layer. Activation by 10% Calcium chloride (0.05 ml for each ml of plasma) followed by warming of the plasma obtained to 37◦C for 5 min. A PRP gel is obtained and topically applied to the surface of the ulcer and standard dressing technique with four layer compression was applied. The procedure was repeated every week till the end of the study period (12 weeks).

Statistical analysis

Data collected throughout history, basic clinical examination, laboratory investigations and outcome measures coded, entered and analyzed using Statistical Package for the Social Sciences (SPSS version 20.0) software for analysis. According to the type of data qualitative represented as number and percentage, quantitative continues group represented by mean±SD. Differences between frequencies (qualitative variables) and percentages in groups were compared by Chi-square test or Fisher exact test, differences between parametric quantitative independent groups were compared by independent t test and paired t test in paired data. For comparing both groups out comes we used Kaplan Meier survival test with Log Rank (Mantel-Cox) test. In all our statistical analysis we considered P value <0.05 to be significant and <0.001 to be high significant results.

RESULTS

73 venous ulcers in 68 patients fulfilled our inclusion criteria and were assigned in our study, 42 venous ulcer for group (A) and 31 for experimental group or group (B). 10 patients did not complete our 12 week study period; 6 patients

were from group A (three because of appearance of new ulcers, two cases lost from the follow up and one patient died), and four patients from group B (two patients were non-compliant to the cost and two cases lost from our follow up).

Both groups were homogenous regarding demographic criteria and the pre-existing medical condition with no statistical significant difference as shown in table (1)

Table (1): Patients' criteria in both groups

	Group (A) N=42	**Group (B) N=31**	**P value**
Age (mean) in years± SD deviation	44.6±10.32	40.58±10.92	0.114
Sex(male: female) Male%	24:18 (57.1%)	17:14 (54.8%)	0.516
Diabetes (N, %)	7(1.66)	4(12,9)	0.46
Hypertensive patients (N, %)	16 (38.1%)	15 (48.3%)	0.261
History of smoking (N, %)	12 (28.5%)	9 (29.01%)	0.589
History of ischemic heart disease (N, %)	4 (9.5%)	3(9.6%)	0.641

As shown in table (2), regarding the characters of the venous ulcers treated in our study, there was no statistical difference between both groups of patients as regard ulcer size, age of the ulcers in months, history of recurrence at the same site, type of venous deficiency and vas scale for pain before enrolment in the study.

Table (2): Clinical presentation

	Group (A)=42	**Group (B)=31**	**P value**
Size of the ulcers in cm2	9.07±4.97cm2	9.81±4.66cm2	0.52
Age of the ulcers in months	3.8±0.2m	3.1±0.5m	0.17
Vas scale pre (mean+SD)	7.07±1.29	7.23±1.45	0.64
History of deep venous thrombosis (N, %)	36(85.7%)	27(87.1%)	0.574
History of recurrence at the same site	7 cases (7.1%)	3 cases (16.1%)	0.31

Treatment outcome:

From our 73 ulcers 10 patients did not complete our 12 weeks study period (6 patients of group A and 4 patients of group B). The remaining 63 ulcers (36 ulcers of group A and 27 ulcers of group B) completed our study either till they completed 12 weekly sessions or complete epithelization of the ulcer occurred before the end of this time period. In group A complete epithelization occurred in 19 of 36 ulcers (52.7%) while in group B complete epithelization occurred in 18 of 27 ulcers (66.6%). Data of ulcer healing time in our cases were processed using Kaplan Meier survival test and our results are shown in figure (1). Using Log Rank (Mantel-Cox) test p value=0.014 which was considered statistically significant different between both groups of patients.

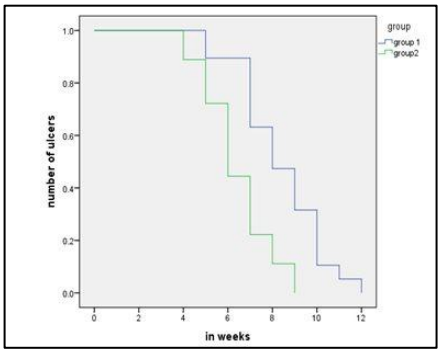

Figure (1): Kaplan Meier survival analysis for healing of ulcers in both groups

At the start of the study the VAS mean values for pain scale in all our patients showed no statistical difference between both groups with p value = 0.64 as shown in table (2).

After exclusion of the 10 censored cases the remaining VAS values before and after 12 weeks study period were shown in table (3).

Table (3): Shows the means of VAS values pre and post intervention in our cases that completed our study period

	At the start	**After study period**	**P value**
Group A (36 patients)	6.05±2.67	3.26±1.6	0.001
Group B (27patients)	6.32±2.83	2.68±1.44	0.001
P value	0.68	0.114	

Figure (2): Chronic venous ulcer over medial malleolus before treatment.

Figure (3): 10 weeks after PRP gel applications and complete healing.

Adverse effects:

No adverse events were recorded in our cases with absence of any signs of infection.

Figure (4): Application of PRP gel before spreading.

Figure (5): Four layers compression technique overlying the ulcer.

DISCUSSION

Chronic venous leg ulcers are considered by many physicians as serious health problem that add much burden on patient's quality of life. Healing is influenced by patients and wounds characteristics. For a long period of time compression therapy was considered as the only non surgical line of treatment for chronic VLUs. Compression therapy was offered by different techniques using stocking and bandages with little if any difference between both regarding time for ulcer healing. [8]

Numerous studies have demonstrated the safety of the application of PRP and supported its use in a wide variety of clinical applications, including chronic ulceration of the lower extremities of various etiologies.[9-11]

There are few studies that were concerned with the benefits of using PRP in chronic VLUs. Due to the lack of standardization as regard collecting and processing of PRP and methods of application on wounds, that hinder the possibility of clear evidence based guidelines for using PRP in treating chronic venous ulcers.

Although Stacey et al.[12] and Senet et al.[13]denied any significant values of PRP application on the healing of venous ulcers, many other research groups as O'Connell et al.[11], Ficarelli et al.[14], Park et al.[15], Frykberg et al.[16], Alvarez et al.[17], and De Leon et al.[18]had results that confirm the efficacy of using PRP as adjuvant line of treatment of VLUs, we think that the difference in methods of preparation and application of the PRP may explain these contradictory results in addition to the reduced number of patients and different follow up period, keeping in our mind that neither of the previously mentioned studies was randomized control trial (RCT).

Anitua in 2008, with his research group[19], characterized a simple and safe technology for preparation of autologous PRP and they studied its therapeutic potential in tissue repair and wound healing. They founded a statistical significant difference between using PRP in experimental group compared to a group of chronic leg ulcers treated by standard dressing care alone as regard to the percentage area healed in favor PRP group (72.94 ± 22.25 compared to 21.48 ± 33.56; $p< 0.05$). Nonetheless, the number of the patients in the study was few beside that only 64% of the ulcers were of venous aetiology.

Cardenosa and his colleagues[20] conducted a randomized control study on 102 venous ulcers assigned in experimental group (PRP topical application) and control group (gauze soaked with saline with single layer pressure bandage) after 24 weeks the study results showed that the average percentage healed area in the PRP group in comparison to control group was 67.7±41.51, 11.17± 24.4 respectively with highly significantly p value =0.001.

Our study was prospective controlled study with two groups of patients suffering of chronic VLUs using topical PRP, collected with the same technique of Anitua et al. [19], we thought that as complete epithelization of the VLUs is the main target of the patient so we decided to use time of healing of the VLUs as main comparing parameter instead of ulcer size reduction. Also we used four layer compression therapy instead of single layer pressure bandage in all our patients as some RCT shown that multiple layered compression is more effective than single layer compression in healing of VLUs.[21]

Regarding the age and the initial size of the VLUs, many studies postulates that the bigger size of the ulcer and its longer age were unfavorable prognostic factors.[22-25] Moffat and co-workers[24] found that the number of healed VLUs significantly decreased if the sizes of the ulcer exceed 10 cm^2 and the ulcer age was greater than 6 months. Marston et al.[26] reported that 57% of VLUs seen in clinical practice treated with compression healed in 10 weeks and 75% healed in 16 weeks. Larger ulcers (>20 cm2) associated with delayed healing. Other risk factors for poor healing include long-standing VLU. Along the same line Cardenosa et al.[20] found a direct correlation between initial sizes of the VLUs, its clinical course and the reduction rate of the VLUs treated by either compression therapy alone or with PRP and compression.

63 ulcers completed our 12 session with a week in between (36 ulcers of group A and 27 ulcers of group B) In group A complete epithelization occurred in 19 of 36 ulcers (52.7%) while in group B complete epithelization occurred in 18 of 27 ulcers (66.6%). there was statistical significant difference between our both groups of patients (p value=0.014). Our results coincide with that of Anitua et al. [19] and Cardenosa et al. [20]

which stated that PRP had statistical significant favorable impact in regard to the mean healed area.

Salazar-Alvarez et al.[17], prospectively selected 11 patients with non ischemic chronic leg ulcers, 7 of these were of venous aetiology 7/11, they prepared PRP as Anitua [19] and applied it both topically and peri-lesional injection in the subcutaneous tissue through 4 weekly sessions and by assessed the quality of life (SF-12 questionnaire) and pain (visual analogue scale) before and after PRP sessions they observed a significant reduction in pain ($P < .05$) and a significant improvement in the physical and mental components of the SF-12 questionnaire ($P < .05$). The mean reduction in ulcer size was 60%, and complete healing was achieved in 5 cases. No adverse effects were observed.

In our study as regard pain associated with VLUs we used the VAS also and it was not an amazing when our data showed a significant decrease in VAS values before and after applying the 12 session of compression in our both groups. As in literature all trial on compression therapy with VLUs showed significant reduction in pain scale before and after enrollment but we were surprised when we calculated the p value between our both groups post intervention (using independent t test) and it was 0.114 which meant that although mean pain scale for PRP group was less than for control group, the deference between both groups still insignificant and this results were in contrast with Cardenosa et al.[20] study that recorded a significant reduction in VAS between groups after 24 week (study period) with a greater reduction in the PRP group. We believe that this contrast may be due to longer research period (24 weeks) and more cases in Cardenosa et al. [20] study. In addition we used independent t test instead Wilcoxon test used by Cardenosa et al. [20] to compare between mean pain variables.

It is well known that infection is considered as major risk factor that affect ulcer healing on the other hand the effectiveness of using oral antibiotics and topical antiseptics for healing of venous leg ulcers cannot be proved [27] therefore we did not routinely use systemic antibiotics for our patients during the study but we only used antibiotics according to culture and sensitivity for all our cases for two weeks before assignment in our study. None of our cases showed signs of local infection in both groups

Study limitations: we hope to have the opportunity to apply this study on more wide scale of patients with standard randomization and extended follow up period to detect the recurrence rate in both groups also the possibility to sort the ulcers by the initial size of the ulcer into multiple strata may end in more precise results.

CONCLUSION

From our study we can conclude that PRP is feasible and safe adjunctive therapy in the management of chronic venous leg ulcers and when the technique is used in combination with multiple layer compression therapy it improves significantly both the rate of ulcer healing and pain reduction.

Conflict of interest: No conflict of interest.

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