

EFFICACY OF ULTRASONIC TRANSDERMAL PLATELET RICH PLASMA ON CHRONIC LOWER LIMB VENOUS ULCER HEALING

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Abstract Aim: The present study was carried-out to examine the impact of Ultrasonic Transdermal Delivery of platelet rich plasma (PRP) on chronic lower limb venous ulcer healing.

Design: Randomized controlled trial.

Patients and methods: The present work was conducted on sixty patients with chronic lower limb venous ulcers who took-part in this study, they aged from 40 to 60 years. The participants were recruited from Mataria teaching hospital and were randomized into two groups; each group consisted of 30 patients. Group A (Ultrasonic PRP group) received Ultrasonic Transdermal Delivery of autologous PRP twice weekly for a month in addition to medical care and group B (Control group) received medical care in form of antimicrobial dressing, wound care and debridement of ulcers. Saline method was used to measure the ulcer volume. Digital photography/imaging and surface area estimation software (Image J) was used to measure ulcer surface area.

Results: After treatment, there was significant decline in wound surface area as well as volume of PRP group in comparison with the control group ($p < 0.001$). Conclusion: Adding Ultrasonic transdermal delivery of platelets rich plasma to medical treatment of chronic venous ulcer can enhance healing process by reducing surface area and ulcer volume and it is considered a safe, simple, inexpensive and cost effective modality.

Keywords: Venous ulcer, platelet rich plasma (PRP), Ultrasonic transdermal deliver

1. INTRODUCTION

An ulcer is a lesion in which tissue is being lost gradually due to some internal cause or disease (as in trauma). When dealing with ulcer, the variations in ulcer patients' appearance, demography, anatomical locations, physiology, pathology, response to treatment, and potential consequences become much more crucial. Increasing rates of atherosclerotic obstruction risk factors such as smoking, obesity, as well as diabetes are contributing to the rising incidence of ulcers (1). In medical terms, an ulcer is an open sore that has "full thickness depth" as well as a "slowly healing propensity." Complete loss of epidermis, dermis, and occasionally subcutaneous fat can occur with skin ulcers (2).

Several medical procedures involve the use of platelet-rich plasma (PRP) extracted from the patient's natural blood. Previously, chronic ulcers were treated with bandages, but this has now been superseded. Platelets serve as a physiologic warehouse for growth factors, that are essential in tissue regeneration and responsible for PRP's therapeutic effects. Platelets are known to include a number of growth factors that have potential medical applications. There are claims that ultrasound may speed up tissue healing, reduce pain by relaxing tight muscles, increase blood flow to the skin, and increase tissue temperature (3). Ultrasound may be used to penetrate the skin and reach the deeper tissues underneath. This specific kind of ultrasonic therapy is known as phonophoresis is characterization of the final products (4).

Phonophoresis is the practice of using ultrasonic to improve the absorption of topical medicines. In order to increase the effectiveness of topical analgesics and anti-inflammatory drugs (5).

Up to the knowledge of the primary author, this study is the 1st to test whether or not phonophoresis of PRP accelerates the healing of venous ulcers in preparation for its clinical use. To further understand how Ultrasonic Transdermal Delivery of PRP influences the healing of chronic venous lower leg ulcers, this research was designed. The purpose of this research was to draw attention to the positive impact of PRP phonophoresis on healing process by reducing wound surface area as well as wound volume.

2. SUBJECTS AND METHODS

• Subjects

Sixty people (32 men and 28 women) with chronic lower limb venous ulcer took part in the research. They ranged in age from forty to sixty. The patients were recruited from El Mataria Teaching Hospital patients and randomized into two groups of the same size. Each individual was given detailed information about the diagnostic and therapy processes. Patients were asked to report any adverse reactions they experienced while undergoing therapy or testing. Individuals who fulfilled the following criteria were eligible to participate in the study: (1) Both gender with age 40- 60 participated in the study (2) Vein ulcers of the lower extremities were seen in all cases. (3) All research participants provided written informed permission. Patients who had met one of the subsequent criteria were excluded from the study : (1) Lower extremity arterial occlusion. (2) Cardiovascular disease. (3) Lower extremity cellulitis. (4) Deep venous thrombosis. (5) Any disease affects the results of the study

• Sample size determination

Utilizing G*POWER statistical software (*version 3.1.9.2; Franz Faul, Universitat Kiel, Germany*), the minimum number of individuals needed for the present study was determined to be 30 for each group. Allocation ratio N2/N1 = 1 was used in the calculations with a significance level of 0.05, power of 80%, and effect size of 0.74.

• Design

The Ethical Committee of the Department of Physical Therapy at Cairo University approved this study (*P.T.REC/012/003433*). Informed consent was obtained after a thorough explanation of the study was provided. The patients were divided into two groups at random using the envelope method. Patients who agreed to take part in the study were randomly assigned into either the Ultrasonic Transdermal Delivery of PRP group) " or the 'control group' by having a physiotherapist who was blinded to the study's purpose choose one of the sealed envelopes containing a card with their group designation written on it. Thirty patients in Group A were given Ultrasonic Transdermal Delivery of PRP, while the same number in Group B were given standard medical care. The assigned therapy was started at a predetermined date after the initial week of randomization. The examining physiotherapist was not a part of the randomization process and did not know who would be receiving therapy. During the evaluation with a physiotherapist, patients were instructed to keep their therapy assignment a secret. Throughout the course of treatment, participants were encouraged to report any adverse events they experienced.

• Measurements

(A) Measurement of surface area:

A digital camera with 12 megapixels (*Sony - Alpha 7R*) was used to take a picture of the wounds at a distance of 20 centimeters and at an angle of 90 degrees, with a graduated ruler positioned next to the wound as a reference value; the picture was then processed using the Image J software, this is a valid as well as reliable technique for measurement the wound surface area. The area was determined using Java-based image processing program created by the National Institutes of Health's Laboratory for Optical as well as Computational Imaging (6).

(B) Measurement of wound volume:

The amount of normal saline drained into the wound was determined by first filling the wound with the solution and then measuring the volume to which it penetrated (7). All assessment procedures were done pre-treatment and at the end of treatment.

• Therapeutic procedures:

Group A: (Ultrasonic Transdermal Delivery of PRP)

The patient was positioned in a relaxing chair. Normal saline was then used to cleanse the ulcer. Platelet-rich plasma was administered to the skin (1 ml PRP/cm²). The therapist applied ultrasound to the wound while wearing a sterile glove. The ultrasound treatment was administered for 6 minutes at a pulsed duty cycle of 40% (4 ms on, 6 ms off) with a power density of 0.5 W/cm² (8). Once the treatment was complete, a sterile dressing was applied to the wound. Patients attended therapy twice weekly for a full month. The process of obtaining PRP including blood samples, centrifugation and degranulation of platelets by thrombin were performed by a professional lab specialist in El Mataria Teaching Hospital.

Group B:(control group).

The patient was given the necessary medical treatment in form of antimicrobial dressing, wound care and debridement of ulcers.

• **Statistical analysis**

- The age of the groups was compared using an unpaired t-test. The Shapiro-Wilk test was used to ensure that the data followed a normal distribution. To examine whether or not there was homogeneity in the variances among groups, Levene's test was carried out. Wound surface area as well as volume were compared using a mixed design repeated measures MANOVA. Multiple comparisons were addressed by post hoc tests utilizing the Bonferroni correction. All statistical tests were conducted with a p-value of less than 0.05 considered significant. IBM SPSS Statistics Version 25 for Windows (*Chicago, Illinois, USA*) was used for all statistical analysis.

3. RESULTS

- **Subject characteristics:**

Comparison of Group A and Group B individuals is shown in **Table 1**. The distributions of ages and genders weren't significantly different between the groups ($p > 0.05$).

Table 1. Subject characteristics

	Group A	Group B	MD	t- value	p-value
	Mean ± SD	Mean ± SD			
Age (years)	51.13 ± 6.73	51.8 ± 4.64	-0.67	-0.44	0.65
Sex, n (%)					
Females	13 (43%)	15 (50%)		$\chi^2 = 0.26$	0.61
Males	17 (57%)	15 (50%)			

SD, standard deviation; MD, mean difference; χ^2 , Chi squared value; p-value, level of significance.

• **Impact of treatment on wound surface area and volume:**

A significant interaction between treatment and time was found ($F = 23.72$, $p < 0.001$, $\eta^2 = 0.45$). The time factor had a significant main impact ($F = 504.71$, $p < 0.001$, $\eta^2 = 0.94$). Treatment had a significant main impact ($F = 16.16$, $p < 0.001$, $\eta^2 = 0.36$).

- **Within group comparison**

There was a significant decrease in wound surface area and wound volume of group A as well as B post treatment in comparison with that pre-treatment ($p < 0.001$). The percentage of change of wound surface area as well as wound volume of group A was 76.56 and 82.11% respectively and that of group B was 45.9 and 63.6% respectively. (**Table 2**).

- **Between groups comparison:**

before treatment, there was no statistically significant difference among the groups. After treatment, a comparison of the two groups showed that wound surface area as well as wound volume were significantly reduced in group A in comparison with group B ($p < 0.001$). (**Table 2**).

Table 2. Mean wound surface area and volume pre and post treatment of group A and B:

	Group A	Group B	MD (95% CI)	P value
	Mean \pm SD	Mean \pm SD		
Surface area (cm ²)				
Pre treatment	14.76 \pm 3.36	15.75 \pm 2.70	-0.99 (-2.56: 0.58)	0.21
Post treatment	3.46 \pm 1.21	8.52 \pm 1.54	-5.06 (-5.78: -4.35)	0.001
MD (95% CI)	11.3 (10.42: 12.17)	7.23 (6.35: 8.1)		
% of change	76.56	45.9		
	p = 0.001	p = 0.001		
Wound volume (cm ³)				
Pre treatment	4.08 \pm 1.33	4.45 \pm 1.08	-0.37 (-0.99: 0.26)	0.24
Post treatment	0.73 \pm 0.38	1.62 \pm 0.42	-0.89 (-1.09: -0.67)	0.001
MD (95% CI)	3.35 (3.03: 3.67)	2.83 (2.51: 3.15)		
% of change	82.11	63.6		
	p = 0.001	p = 0.001		

SD, Standard deviation; MD, Mean difference; CI, Confidence interval; p-value, Level of significance

4. DISCUSSION:

This is the first study to our knowledge to evaluate the effectiveness of transdermal PRP by low intensity ultrasound for the healing of persistent venous ulcers. Both the wound surface area as well as volume in both groups decreased significantly post treatment when compared to pre- treatment (p 0.001). Ultrasonic PRP group achieved higher significant improvement in wound surface area and volume by 76.56% and 82.11 % respectively, whereas controlled group showed 45.9 % and 63.6% respectively. Results from other studies comparing PRP with conventional medical therapy and concluding PRP is helpful in treating chronic venous ulcers are consistent with the findings of the current research, which demonstrate the superiority of ultrasonic transdermal PRP (9-14).

Scientific explanation about the positive effects of ultrasound transdermal PRP in the acceleration of ulcer healing, decreasing ulcer surface area and ulcer volume in our study can be illustrated as follow, in addition to concentrated platelets and fibrin, autologous PRP also contains growth factors (GFs) including platelet-derived GF, vascular endothelial GF, epidermal GF, insulin-like GF, as well as transforming GF. Thus, it promotes epithelialization, granulation tissue formation, as well as collagen production in addition to angiogenesis, cellular proliferation, differentiation, and migration. Each of these may be found in both the replicative and restorative stages. Therefore, PRP has been suggested as a means of facilitating the healing of chronic VLU (15). By collaborating with macrophages, PRP may lower inflammation and cytokine production in chronic wounds, allowing for more rapid tissue regeneration, vascularization, and epithelialization. Additionally, the platelets in PRP aid the body's immune system as well, the production of cytokines that recruit macrophages to the injury site. (16)

PRP may as well include a limited number of leukocytes, which may create interleukins in the context of a general immunological reaction (17). Moreover, PRP has been demonstrated to have antimicrobial properties against a wide variety of microorganisms, including *Escherichia coli*, *Staphylococcus aureus* (such as methicillin-resistant *S. aureus*), *Candida albicans*, as well as *Cryptococcus neoformans*. (18). Also taking into consideration the benefits of using ultrasound in wounds healing, as early ultrasound intervention should hasten the shift into the proliferative phase, speed up the acute inflammatory phase, and make the patient more comfortable at this time ultrasound stimulates tissue regeneration by increasing the production of growth factors, migration, and cell proliferation, thermal ultrasound is used to alter collagen extensibility and enzyme activity, hence increasing the tensile strength of the repairing tissue also ultrasound improves the absorption of topical medicines which could in turn increase the PRP efficiency (19).

A total of 294 individuals with lower extremity venous ulcers were included across 6 studies; of these, 148 received PRP and 146 received conventional treatment. Six trials with 294 participants provided moderate evidence for a treatment's effect on the Formula of an elliptical at the conclusion of treatment, with a mean change of 1.19 (95% CI 1.80 to 0.58, P<0.001). a statistically significant difference has been detected in recovery rates among the experimental and control groups (risk ratio [RR] 5.73; 95% confidence interval [CI] 3.29-9.99; 5 trials; 248 individuals; intermediate level of evidence). This comprehensive review of the literature demonstrates that PRP improves wound healing rates and shortens the healing time for lower extremity venous ulcers(9).Thirty individuals with chronic leg venous ulcers took part in a study and randomly assigning to receive either an injection or topical PRP, where treatment outcomes were evaluated by complete healing ratio which was the same (47%) in both groups, with healing scores of 62.22 and 68.45 percent, respectively(10).

In a study including 24 patients and 33 ulcers, six sessions of topical PRP therapy resulted in a mean healing duration of 5.6 weeks (SD: 3.23). The average volume and surface area of the ulcers decreased by 91.7%. (SD 1657

18.4 percent). (11). In an alternative study, twenty-three patients were split into two groups: those who received a silicone dressing (n = 11) and those who received PRP (n = 12). There was an 82% decrease in ulcer area in the PRP group in comparison with a 24% reduction in the control group after 8 weeks. With a P value of .001, the PRP group healed their ulcers much faster than the control group (9.6 vs. 23.7 weeks) (12). Moreover, PRP was evaluated on patients with persistent unhealed venous ulcers for 6 weeks, the improvement of ulcer surface area within PRP group was 67.6% while in the controlled group was 13.67% (13). Also, another study where 11 patients having venous ulcers were injected with PRP around ulcer and applied topically in 4 sessions for one month, the mean decline in ulcer size was 60% (14).

Several studies support the usage of phonophoresis of different materials to speed up in wound healing (20-22), study was carried-out to investigate the impact of topical hydrocortisone, pulsed ultrasound (US), and pulsed phonophoresis in aiding the healing of the Achilles tendon (tendo-calcaneus) in rats after tenotomy. The results confirmed that ultrasonic stimulation elicits a response from the collagen molecule and that the hydrocortisone delivered topically to the treated group did not enter the body through the skin. Based on these findings, it was inferred that ultrasound stimulates tissue regeneration and triggers therapeutic levels of hydrocortisone to be transported trans dermally to the tendon(20). In a study where 100 patients with chronic venous ulcers or diabetic ulcers comparison was done between Bee Venom gel phonophoresis and ultrasound, evaluation of wound surface area, volume and Ki-67 biomarker demonstrated that bee venom delivered by phonophoresis is an efficient adjunct treatment for venous as well as diabetic foot ulcer healing (21).

Additionally, 35 local rabbits were divided into seven groups using a random number generator: pulsed as well as ultrasonic, topical N. sativa oil, pulsed as well as continuous phonophoresis, topical MEBO ointment, as well as control. Surface area of wounds were measured using images taken on days 0, 7, and 21 utilizing metric graph paper. There were statistically significant improvements in wound healing from pre- to post-treatment in all treatment groups excepting the control group, suggesting that pulsed phonophoresis was the cause of these improvements. Low results, in contrast, were discovered with continuous ultrasound group (22).

Another explanation for the outcomes is the enhanced efficacy of drug delivery system through skin using ultrasound as phonophoresis enhances a drugs transdermal penetration through mechanical effects including cavitation property as well as acoustic power (23). The drugs are incorporated into the cytoplasm, intercellular space, and cell projections. Medications have a rapid effect when delivered via ultrasound because blood flow is increased to the target tissues and the substance is able to pass the stratum corneum of the skin and enter the bloodstream (24). We believe that all these factors play significant roles in wound healing and management.

Ultrasonic transdermal delivery of PRP was shown to assist in the healing of chronic lower-extremity venous ulcers with no reported adverse effects, and this study provides the preliminary evidence needed for deploying this technique as a standard part of chronic venous ulcer treatment, however, There are limitations that must be taken into account while interpreting these findings; The inability to examine the long-term effects of the medication was the primary issue with this study, thus future trials should include patients' follow up, moreover, unclear gold standard techniques for PRP production is one of the study's limitations, also to minimize human suffering and financial expenses, it is vital to raise awareness regarding the protection, early diagnosis, and timely therapy of chronic venous ulcers.

CONCLUSION:

Adding Ultrasonic transdermal delivery of platelets rich plasma to medical treatment of chronic venous ulcer can enhance healing process by reducing surface area and ulcer volume and it is considered a safe, simple, inexpensive and cost effective modality.

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