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ORIGINAL ARTICLE

Effect of Hyaluronic Acid Iontophoresis on Healing of Chronic Venous Ulcer

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ABSTRACT

Purpose: The purpose of the study is to evaluate the effect of hyaluronic acid iontophoresis on healing of chronic venous ulcer. Methods: Forty patients of both genders suffering from chronic venous ulcer in area below the knee and above the ankle with area (5-20 cm2) with less than 50% necrotic tissue participated in this study. Their ages ranged from (45 to 60) years. They were selected from Vascular Surgery Department, Al Ahrar Teaching Hospital between June 2022 and January 2023. Patients were divided randomly into 2 equal groups; each one had 20 patients. Group (A) received Hyaluronic acid iontophoresis 1 session per day, 3 sessions per week, for 4 weeks, and follow up 4 weeks after the end of the treatment. In addition to conventional, medical treatment and traditional care. Group (B) received conventional medical treatment and traditional care (ulcer cleansing, debridement, dressing, compression, medication, exercise). Measurements were done by photographic digital camera for wound Surface area (WSA) and wound volume using saline injection into a wound cavity before treatment and after 4 weeks of treatment. Results: The findings of this study indicated significant decrease in WSA and wound volume after treatment in all groups A, B ($p<0.001$) at day 60 post treatment. There was significant decrease in ulcer surface area and ulcer volume at day 60 post treatment of study group (group A) compared with that of control group (group B) ($p = 0.001$).Conclusion: Hyaluronic acid iontophoresis for 6 weeks is an effective and safe adjuvant therapy in treatment of unhealed venous ulcers through accelerating wound healing, reducing Wound Surface Area (WSA) and wound volume.

Keywords: Hyaluronic Acid, iontophoresis, Chronic Venous leg ulcers, Wound Healing.

INTRODUCTION

Venous ulcer is open skin lesion that occurs in an area affected by venous hypertension**[1].** The prevalence of venous ulcers in the United States ranges from 1% to 3%. In the United States, 10% to 35% of adults have chronic venous insufficiency, and 4% of adults 65 years or older have venous ulcers. Poor prognostic signs for healing include ulcer duration longer than three months, ulcer length of 10 cm (3.9 inch) or more, presence of lower limb arterial disease, advanced age, and elevated body mass index**[2]**. Adequate wound and skin management is central to promoting the healing process, and regular wound cleansing and debridement to remove necrotic tissue and fibrin are key elements of therapy. If conservative measures fail to provide a satisfactory outcome, further treatment should be considered; options include topical and systemic medications, sclerotherapy, and surgery**[3]**.

MATERIALS AND METHODS

A statistical power analysis suggested that sample size was (40) participants (20) per group were required to achieve more than 80% power. Forty patients of both genders suffering from chronic venous ulcer in area below the knee and above the ankle with area (5-20 cm2) with less than 50% necrotic tissue participated in this study. Their ages ranged from (45 to 60) years complaining from stage 3 of chronic venous ulcer located between knee and ankle joint 2 months and 1 year duration. They were randomly divided into 2 equal groups; each one has 20 patients (Group A and Group B). They were selected from Vascular Surgery Department, Al Ahrar Teaching Hospital, Zagazig, sharkia government, Egypt. Patients were excluded from this study if they had Skin diseases in the area to be treated, Pregnancy or epilepsy. Patients with associated diseases (Diabetes, infectious diseases and autoimmune disease) that will interfere with the healing process. Group A received Hyaluronic acid iontophoresis transmitted through (–ve) electrode by intensity adjusted between 1 and 5 mA increased very slowly until the patient reports feeling a tingling or prickly sensation for 20 min., 1 session per day, 3 sessions per week, for 4 weeks, and follow up 4 weeks after the end of the treatment. In addition to their conventional, medical treatment and traditional care. Group B received their conventional medical treatment and traditional care (ulcer cleansing, debridement, dressing, compression, exercise and medication).

Study design and randomization: The study was designed as a prospective randomized clinical trial in which patients were assigned randomly into two groups. Randomization was used to eliminate the researches' bias and was carried out by a blinded and an independent research assistant who opened sealed envelopes that contained a computer generated randomization card. Measurements were done before the starting of treatment and at the end of treatment 4 weeks and follow up 4 weeks for patient of two groups

Computerized photographic WSA assessment was performed by using (digital camera Nikon D3500 and J Image 1.53e software) for measurement of wound size (length and width). The subject's wound was placed under a lighted, and a digital camera was mounted 50 cm perpendicular to the subject's wound it took a clear photo of the ulcer area. Image J free open source software was used to analyze the photograph by marking the edges of the wound and calculating the number of pixels under the marked wound area to measure distances and angles.**[4]** It can create density histograms and line profile plots. Photographs were visualized with Image J software and wound surface measurement was achieved by using a straight line tool a line was pulled along the ruler. The examiner pulled 1 cm over the ruler. The distance in pixels of the line was calculated by the software then set scale then known distance the unit of length (mm). The software automatically recalculated the number of pixel/mm. By using "freehand selection "tool the wound boundaries were be drawn and traced according to the wound shape with the track pad of the laptop. Analyze menu then measure. The area in mm2 was calculated.[5] Wound volume assessment was performed by using sterilized syringe and saline was injected to wound area for measuring wound depth filing the sterilized syringe of 5cm3 with Saline. Then filing the wound by the known volume of the saline. It is an easy accurate method for wound volume estimation**.[6]**

A verbal explanation about the treatment of the research and main points of experimental procedure design was explained to every patient. Patient history was taken in relation of the study. Patient was informed of the treatment and its purpose. For group A; gel containing HA (0.2%) was applied to the negative electrodes as a carrier of the electric current. Placed the electrode a rectangular adhesive 4 X 6 cm2 in size with 24 square grids of 1 cm2 each, fixed as near as possible for the wound proximal and distal to the ulcer site20 min was applied.[7] In addition they received

their conventional, medical treatment and traditional care. For group B; they received conventional medical treatment and traditional care only (ulcer cleansing, debridement, exercise,dressing, compression, medication)

Statistical analysis: All statistical measures were performed through the statistical package for social studies (SPSS) version 25 for windows. Descriptive statistics and unpaired t-test were conducted for comparison of age between groups. Chi squared test was conducted for comparison of sex distribution between groups. Normal distribution of data was checked using the Shapiro-Wilk test. Levene's test for homogeneity of variances was conducted to test the homogeneity between groups ANOVA with repeated measures was conducted for comparison of ulcer surface area and ulcer volume between day 1, 14, 28 and 60 in each group. Unpaired t test for comparison of ulcer surface area and ulcer volume between groups. The level of significance for all statistical tests was set at $p < 0.05$.

RESULTS

Table (1) showed the subject characteristics of the study and control groups. There was no significant difference between groups in age, of sex, affected side and type of surgery distribution ($p>0.05$).

Table 1: Basic characteristics of participants.

	Study group	Control group	p-value
	Mean ± SD	Mean ± SD	
Age (years)	51.1 ± 5.53	51.7 ± 5.59	0.73
Sex, n (%)			
Females	6 (30%)	8 (40%)	0.51
Males	14 (70%)	12 (60%)	

SD, standard deviation; p-value, level of significance

Table 2: Mean ulcer surface area and volume at day 1, 14, 28 and 60 of study and control groups:

	Day 1	Day 14	Day 28	Day 60
	mean ± SD	mean ± SD	mean ± SD	mean ± SD
Ulcer surface area (cm^2)				
Study group	9.33 ± 2.52	6.48 ± 1.83	4.15 ± 1.86	2.45 ± 1.9
Control group	8.75 ± 2.37	7.91 ± 2.12	5.75 ± 2.11	4.95 ± 2.36
MD	0.58	-1.43	-1.6	-2.5
t- value	0.76	-2.27	-2.54	-3.68
	p = 0.45	p = 0.02	p = 0.01	p = 0.001
Ulcer volume (ml)				
Study group	2.02 ± 0.27	1.42 ± 0.42	0.76 ± 0.45	0.31 ± 0.17
Control group	2.15 ± 0.38	1.85 ± 0.47	1.07 ± 0.39	0.5 ± 0.23
MD	-0.13	-0.43	-0.31	-0.19
t- value	-1.31	-3.04	-2.3	-2.97
	p = 0.19	p = 0.004	p = 0.02	p = 0.005

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

Table 3: Comparison of ulcer surface area and ulcer volume between day 1, 14, 28 and 60 post treatment in study group.

	Ulcer surface area (cm^2)			Ulcer volume (ml)		
	MD	% of change	p value	MD	% of change	p value
Day 1- Day 14	2.85	30.55	0.001	0.6	29.70	0.001
Day 1- Day 28	5.18	55.52	0.001	1.26	62.38	0.001
Day 1- Day 60	6.88	73.74	0.001	1.71	84.65	0.001
Day 14- Day 28	2.33	35.96	0.001	0.66	46.48	0.001
Day 14- Day 60	4.03	62.19	0.001	1.11	78.17	0.001
Day 28- Day 60	1.7	40.96	0.001	0.45	59.21	0.001

Mean difference; p-value, level of significance

Table 4: Comparison of ulcer surface area and ulcer volume between day 1, 14, 28 and 60 post treatment in control group.

	Ulcer surface area (cm^2)			Ulcer volume (ml)		
	MD	% of change	p value	MD	% of change	p value
Day 1- Day 14	0.84	9.60	0.001	0.3	13.95	0.001
Day 1- Day 28	3	34.29	0.001	1.08	50.23	0.001
Day 1- Day 60	3.8	43.43	0.001	1.65	76.74	0.001
Day 14- Day 28	2.16	27.31	0.001	0.78	42.16	0.001
Day 14- Day 60	2.96	37.42	0.001	1.35	72.97	0.001
Day 28- Day 60	0.8	13.91	0.001	0.57	53.27	0.001

Mean difference; p-value, level of significance

Effect of treatment on ulcer surface area and volume: Between group comparison; there was no significant difference in ulcer surface area and volume between study and control groups at day 1 ($p > 0.05$).

There was a significant decrease in ulcer surface area and volume of study group at day 14, 28 and 60 post treatment compared with that of control group ($p < 0.05$). (Table 2)

Within-group comparison revealed a significant decrease in ulcer surface area and ulcer volume in both groups at day 14, 28 and 60 compared with day 1 ($p < 0.001$), a significant decrease in ulcer surface area and ulcer volume at day 28 and 60 compared with day 14 ($p < 0.001$) and a significant decrease in ulcer surface area and ulcer volume at day 60 compared with day 28 ($p < 0.001$). The percent of change of ulcer surface area and ulcer volume between day 1 and day 60 of study group was 73.74 and 84.65% respectively and that in control group was 43.43 and 76.74% respectively. (Table3-4)

DISCUSSION

The pre-treatment results of the present study revealed no significant difference between the mean values of WSA and wound volume.

Post-treatment results of group A of showed reduction in the WSA and wound volume with mean difference between day 1 and day 60 was 6.88 cm^2 and the percent of change was 73.74%. Respectively for WSA and with mean difference between day 1 and day 60 was 1.71 ml and the percent of change was 84.65% respectively for wound volume. So there was a significant decrease in ulcer surface area and in ulcer volume in this group. Ibraheem et al., (2022) evaluated the efficacy of 0.2% hyaluronic acid gel and 0.01% hyaluronic acid (HA) spray in the healing of extraction wounds using the ruler and digital planimetry method. The wound closure with the ruler method was 43.01% for the control group, 67.01%, and 65.82% for the gel group and spray group respectively. The wound closure with the digital planimetry method was 47.97% for the control group, 69.08% for the gel group, and 66.94% for the spray group. The gel showed better results of wound closure as compared to the spray.[8]

Harikrishna and Athirah, (2022) investigated the recovery of chronic wounds treated with hyaluronic acid-collagenase, which facilitates enzymatic debridement, helping prepare the wound bed for healing and closure on top of first-line therapy. The study observed a minimal to 100% reduction in wound size, notably diminished exudate excretion, healthy wound edge, and lower pain score.[9] Nosseir et al., (2018) compared between the effectiveness of estrogen iontophoresis as physical therapy modality and stem cell therapy in the treatment of diabetic foot ulcers. Results showed that estrogen iontophoresis for 4 weeks in combination with stem cell is an effective adjuvant therapy, better that each method alone, in treatment of diabetic foot ulcers through accelerating wound healing and reducing WSA, wound volume.[10] Mendes et al., (2020) demonstrated significant improvements in outcome measures when used iontophoresis and hyaluronic acid (HA) combined with a gold nanoparticle (GNP) solution in an excisional wound model. The animals were induced to a circular excision, and treatment started 24 h after injury with microcurrents (300 MA.) containing gel with HA (0.9%) and/or GNPs (30 mg/L) in the electrodes (1 mL) for 7 days.[11] In addition, Dohnert et al., (2012) had demonstrated that the GNPs associated to the HA + photobiomodulation contributed for a higher wound repair process.[12] Victor et al., (2012) showed that HA, a molecule widely used in clinical practice, with GNPs, which have shown beneficial effects on tissue repair.[13] Mendes et al., (2020) developing therapeutic interventions that promote the formation of epithelial tissue In addition, this study used an electrophysical agent (MIC) capable of stimulating the infiltration of these molecules and promoting faster healing.[11]

Silveira et al., (2014) and Kim et al., (2013) stated that the associated use of MIC with GNPs-HA favors the tissue repair process by modulating important pathways related to inflammation and tissue formation.[14,16] Lee et al., (2010) MIC allows for the transport of bioactive molecules when associated with some drugs, in a process called iontophoresis.[15] Prentice, (2011) reported that

iontophoresis decreases the absorption lag time, while it increases the delivery rate when compared with passive skin application.[17] Wang et al., (2021) compared MIC with other similar technologies in regard to the transdermal permeation of drugs have stated that iontophoresis guarantees the delivery of drugs in an optimized and controlled manner through the mechanisms of electro-migration and electro-osmosis, considered the main means of increased drug transport through the skin to the systemic circulation.[18] Tomoda et al., (2012) observed that iontophoresis was applied to enhance the permeability of nanoparticles. [19] Humbert et al., 2013 this was RTC (n=89); venous leg ulcers Percentage of wound size reduction at day 45: 73 ± 4.6% in HA vs. 46 ± 9.6% in control (p=0.011); Number of healed ulcers: 31.1% in HA vs. 9.3% in control at day 45 and 37.8% vs. 16.3% at day 60; Pain intensity based on visual analogue scale lower in HA vs. control.[20] Essa et al., (2002) stated that Iontophoresis (0.8mA/ cm2, for 8h) improved drug penetration over passive delivery for all systems, with ultra-deformable vesicles performing best.[21]

Group B showed that the post-treatment results of this study showed reduction in the WSA and wound volume after the treatment, the mean difference between day 1 and day 60 was 3.8 cm^2 and the percent of change was 43.43% for WSA and, the mean difference between day 1 and day 60 was 1.71 ml and the percent of change was 84.65% forwound volume, so there was a significant decrease in group (B) regarding the ulcer surface area ($p = 0.001$)and wound volume. Hettrick, (2009) stated that Compression therapy, delivered using compression hosiery or multi-layer bandaging, is the mainstay of treatments for venous leg ulcers results that demonstrate that one of the goals of compression therapy is to enhance healing of a venous leg ulcer. [22] Almond A., (2007); the exercise group participants performed an aerobic exercise and a resistance exercise with 8–12 repetition maximum (RM), recommended as an optimal intensity to enhance muscle strength. No significantly better effect of the conventional treatment plus tailored exercise training program on calf muscle strength when compared to the conventional treatment alone.[23] Lanting et al. (2017) 12-week supervised exercise programmer improved measures of cutaneous micro vascular reactivity in people being treated with compression therapy for venous ulceration.[26]

Kulprachakarn et al., (2022) Adding the tailored exercise training incorporated with the conventional treatment may provide a greater tendency on wound healing and have the potential to improve ankle mobility than the conventional treatment alone.[24]

Cardinal et al., (2009) two controlled, prospective, randomized pivotal trials of topical wound treatments on 366 VLUs and 310 DFUs over 12 weeks. VLUs had a significantly higher median wound surface area reduction following clinical visits with surgical debridement as compared with clinical visits with no surgical debridement (34%, p=0.019).[25]

CONCLUSIONS

From the obtained data of the present study, the most notable conclusions were: HA iontophoresis for 6 weeks is an effective adjuvant therapy in treatment of unhealed venous ulcersthrough accelerating wound healing, reducing Wound Surface Area (WSA) and wound volume. The results of current study showed that combination between HA iontophoresis and conservative treatment for ulcer care is the most effective in comparison to use of either method alone in treatment of unhealed venous ulcers.

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