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Cell Proliferation Marker Response to Estrogen Iontophoresis in Treatment of Chronic Lower Limb Ulceration

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Abstract : Purpose: evaluate the efficacy of estrogen iontophoresis as physical therapy modality in the treatment of diabetic foot ulcers. **Methods:** Forty patients who had diabetic foot ulcers for longer than three months. Their ages were ranged from 40-50 years with mean value 53.95±2.846 years. The patients were selected from Deraya University Physical Therapy Center in the period between Feb 2015 and Jul 2016. Patients who met the selection criteria were divided randomly into two equal groups, Group (A) received estradiol iontophoresis (-ve) electrode by intensity 1-5 mA for 10 min, 3 sessions per week for 6 weeks and medical treatment. Group (B) received medical care only 6 weeks. **Measurements:** Wound surface area (WSA) was assessed by Digital Camera and **ImageJ 1.49. v** computer software, wound volume and Ki-67% were assessed before treatment and after 6 weeks of treatment. **Results:** The findings of this study indicated significant decrease in WSA and wound volume with significant increase in the Ki-67% after treatment in both groups A and B ($P<0.0001$). There was significant difference between both groups after treatment in WSA ($P<0.0001$), wound volume ($P=0.004$) and Ki-67% ($P<0.0001$) with favored results in group A. **Conclusion:** results showed that estrogen iontophoresis for 6 weeks is an effective adjuvant therapy in treatment of diabetic foot ulcers through accelerating wound healing, reducing WSA, wound volume, and improving the cells proliferation rate.
Key words: (Estrogen iontophoresis, diabetic foot ulcers).

Introduction

Foot ulcers are defined as any break in the cutaneous barrier, but usually extend through the full thickness of the dermis. Certain infections of the foot as cellulitis or osteomyelitis can occur without break in the skin. A wound may be acute or chronic, the latter could be defined as a wound that is not continuously progress toward healing, any wound that remains unhealed after 4 weeks is a cause for concern, as it is associated with worse outcome , including amputation[1].

Chronic wounds may be due to arrest of wound healing in the state of chronic inflammation, with an imbalance between protease activity and growth factor expression. The chronic wound environment has an overload of matrix metalloproteases (MMPs), reduced amounts of tissue inhibitors of MMPs (TIMPS), senescent and dysfunctional cells with decreased proliferative and synthetic activities, and shortages in growth

factors and growth factor receptors. This situation suppresses fibroblasts proliferation, motility and protein production so that the wound remain in a chronic state[2].

The cost of wound care is significant. The most important components are the costs of wound-related hospitalisation and the opportunity cost of nurse time. The 32% of patients treated in hospital accounted for 63% of total costs[3].

Series of clinical studies has identified estrogen as being endogenous enhancers of healing processes. The administration of 17b-estradiol, either systemically or topically, has been shown to reverse the fundamental repair defects observed in postmenopausal women. By contrast, androgenic species retard repair and interfere with the accumulation of the structural proteins that reconstitute the damaged dermis. Since estrogen-based hormone replacement therapy produces wide-ranging effects, not all of which are considered to be desirable, more recent studies have sought to identify downstream mediators of estrogenic effects in order to formulate better targeted[4].

Iontophoresis primarily involves delivery of "ions" (as well as some molecules through the process of electroendoosmosis) whereas Phonophoresis involves the delivery of "molecules". Although the exact mechanism is not known, drug absorption may involve a disruption of the stratum cornea, lipids allowing the drug to pass through the skin[5].

Iontophoresis is a topical application of an ionized substance through the intact skin by the application of a continuous direct electric current. It is used to deliver medications directly to soft tissues limiting systemic absorption. The theory is simple. If a drug is applied to the skin in an electrode of the same charge as the drug (for example, lignocaine/anode) and electric current is applied the drug will pass with the current and deposited not only superficial but in the deeper subcutaneous tissue[6].

Therefore, this study was conducted to investigate the efficacy of evaluate the efficacy of estrogen iontophoresis as physical therapy modality in the treatment of diabetic foot ulcers in the lower limb through reducing wound surface area (WSA) and wound volume.

Experimental

Subjects

The study was conducted on in the period between Feb 2015 and Jul 2016. Fourty patients who had chronic unhealed diabetic foot ulcer for longer than three months were participate in this study. Their ages were ranged from 40-50 years. The patients had been selected from Deraya University Physical Therapy Center. The exclusion criteria were as follows: Patients who had severe anemia, internal fixation in the area of application, implanted cardiac rhythm devices, uncontrolled hypertension or uncontrolled cardiac patients, pregnant women, skin allergy or cortisone therapy and presence of a tumor or cutaneous lesion that could interfere with the procedure. All patients were given a full explanation of the treatment protocol and a written informed consent form giving agreement to participation and publication of results was signed by the patients.

Study Design

This was randomized, controlled, pre-test and post-test design study. Patients who met the selection criteria were divided randomly into two equal groups, Group (A) iontophoresis group: this group included 20 patients with chronic unhealed diabetic foot ulcers for longer than three months. They received estradiol that transmitted through iontophoresis (-ve) electrode by intensity adjusted between 1 and 5 mA increased very slowly until the patient reports feeling a tingling or prickly sensation for 10 min, 3 sessions per week for 6 weeks and medical treatment[7]. Group (B) Medical Treatment Group: This group included 20 patients with diabetic foot ulcers for longer than three months. They received medical treatment (according to the case) only.

Assessment

All medical and demographic data of patients was collected and the role of physical therapy importance in improving their condition was explained.

The pre- and post-intervention assessments were the WSA assessment and Wound volume assessment. The assessment was carried out before start of treatment and after 6 weeks at the end of treatment program.

Computerised photographic WSA assessment:

By using Kodak Easy share P712 Zoom Digital Camera and **ImageJ 1.47.** Computer software. A square adhesive 4 × 4 cm^2 in size with 16 square grids of 1 cm^2 each, fixed as near as possible for the wound. An ordinary digital camera (Kodak Easy share P712 Zoom) used for capturing a photograph for the wound (showing the adhesive 4x4 cm^2 square). Image J ^{TM} free open source software will be used to analyze the photograph as following: The edges of the wound were marked and the number of pixels falling under the marked wound was calculated. The edges of the square adhesive marker were marked and the number of pixels falling under the marked square adhesive marker were calculated. Since the dimensions of the square are known (16 cm2), it was possible to derive the exact size of the marked area of the wound by dividing the size of wound by the size of the square in the photo then multiplied by the actual size of the square (16 cm^2) [8].

Wound volume assessment

Using sterilized siring and Terramycin ointment. Filing the sterilized siring of 5 cm^3 with Terramycin ointment. Then filing the wound by the kwon volume of the Terramycin ointment. It is an easy accurate method for wound volume estimation.

Treatment

Preparatory procedures

Each patient was informed about experimental process as well as the significance of study and write a consent. All equipments were checked up, calibrated and prepared before application

Procedure estradiol iontophoresis therapy:

Group A patients received 0.5% solution of estradiol was used over the target zone into an absorbent rapier covered electrode (electrode for iontophoresis) the electrode is applied. The time of treatment was adjusted (for 10 min). The intensity was adjusted to (1-5 mA) be increased very slowly until the patient reports feeling a tingling or prickly sensation. Then the treatment was start. Remove electrode and end treatment session[9].

Outlines of medical care

The outlines of medical care that patients of both groups received were as following: Prevention of infection, off-loading of wound area, debridement if necessary, applying medication or dressings to the ulcer day after day and managing blood glucose and other health problems.

Statistical Analysis

Statistical analysis was conducted using SPSS for windows, version 18 (SPSS, Inc., Chicago, IL). In this study, the descriptive statistics (the mean and standard deviation) were be calculated for all patients in all groups of the study for age, weight, height, BMI, WSA and wound volume variables. Comparisons between mean values of WSA and wound volume in both groups pre-treatment and post-treatment was made by independent t-test. Paired t- test was used to compare mean values of WSA and wound volume before and after treatment in the same group. P-value ≤ 0.05 was considered statistically significant.

Results

Baseline and demographic data

There were no statistically significant differences ($P>0.05$) between patients in both groups concerning age (Table 1). There were also no statistically significant differences between groups for any outcome variables at baseline (pre-intervention).

Table (1): Ages of all patients

	Mean± SD		t-value	p-value
	Group (A)	Group (B)		
Age (years)	53.65±2.720	54.25±3.7	1.639	0.112

* : significant difference

Wound surface area (WSA):

As indicated at table (2) and illustrated at figure (1) "Paired t test" revealed that there was a significant reduction of WSA (t-value= 9.041, P-value =0.000*) in group A. In addition, "Paired t test" revealed that there was significant reduction in WSA (t-value= 5.388, P-value =0.000*) in group B. Unpaired t test revealed that the mean values of the "pre" treatment between both groups showed there was no significant differences (t-value= -0.0928, P=0.36). Comparison between the mean values of the "post" treatment mean values in both groups showed there was significant difference of WSA (t-value= -5.299, p=0.000*) with better improvement in Group A. More over figure 2 shows complete healing of diabetic foot wound after 6 weeks of treatment application.

Table (2): Wound surface area pre and post treatment comparison in both groups and between groups pre and post treatment.:

WSA	Before treatment	After treatment	t_p value	P-value
	Mean± SD	Mean± SD		
Group A	10.9265±6.43	3.648±3.53	9.041	0.000*
Group B	12.676±5.46	10.3575±4.43	5.388	0.000*
t_p value	-0.93	-5.30		
P-value	0.36	0.000*		
SD: Standard Deviation	tp: paired t-test			
* = Significant	N.S. = non-significant			

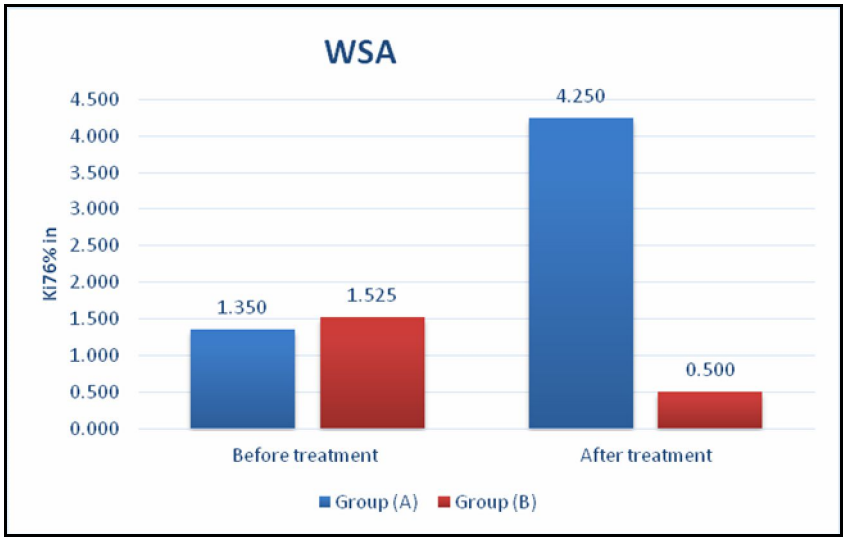

Figure. (1): The mean values of WSA of pre and post treatment in both groups of the study (A&B).

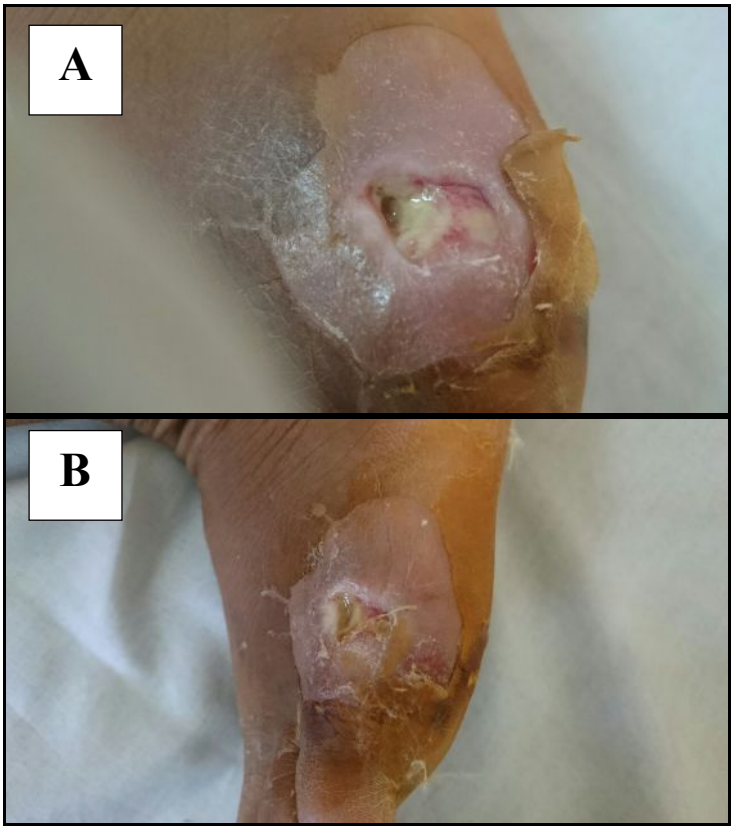

Figure (2): Diabetic ulcer in the left foot (A) before treatment and (B) after treatment estradiol iontophoresis and medical care.

Wound volume:

As indicated at table (3) and illustrated at figure (3) "Paired t test" revealed that there was a significant reduction of Wound Volume (t-value= 4.8, P-value =0.000*) in group A (Figure 3). In addition, "Paired t test" revealed that there was significant reduction in Wound Volume (t-value= 6.383, P-value =0.000*) in group B. Unpaired t test revealed that the mean values of the "pre" treatment between both groups showed there was no significant differences (t-value= -0.588, P=0.56). Comparison between the mean values of the "post" treatment mean values in both groups showed there was significant difference of Wound Volume (t-value= -3.057, p=0.004*) with better improvement in Group A.

Table (3): Wound Volume pre and post treatment comparison in both and between groups pre and post treatment.

Wound Volume	**Before treatment**	**After treatment**	**t_p value**	**P-value**
	Mean± SD	**Mean± SD**		
Group A	3.652±3.38	1.0115±1.16	4.80	0.000*
Group B	4.1995±2.44	2.6445±1.09	6.383	0.000*
t_p value	-0.59	-3.06		
P-value	0.56	0.000*		
SD: Standard Deviation	tp: paired t-test			
* = Significant	N.S. = non-significant			

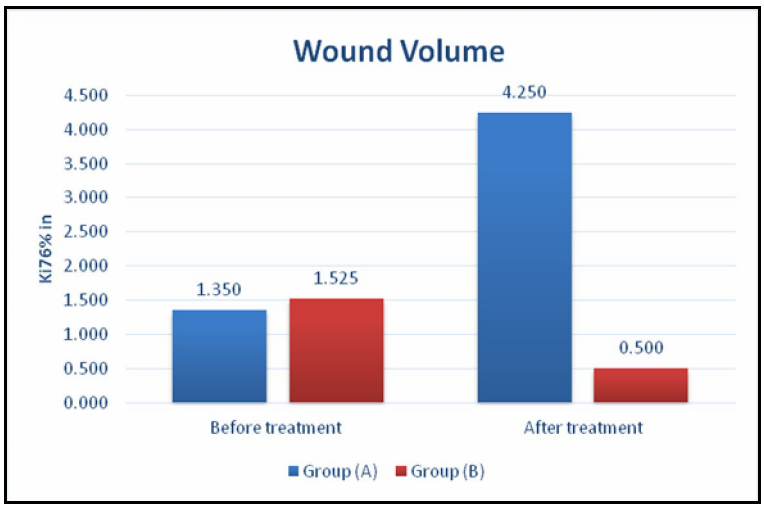

Figure. (3): The mean values of Wound Volume of pre and post treatment in both groups of the study (A&B).

Discussion

This study was conducted to determine the therapeutic efficiency of estrogen iontophoresis in managing diabetic foot ulcers by reducing ESA, Wound volume.

The pre- treatment results of the present study revealed no significant difference of the mean values of WSA and wound volume between both groups. The post - treatment results of this study showed reduction in the WSA and wound volume after the treatment for Group (A) and (B) with a percentage of 66.6%, 18.3% respectively for WSA and 72.3% and 37% respectively for wound volume.

Also compression between both groups post - treatment showed significant difference of the mean values of WSA (p-value= 0.000), and wound volume (p-value= 0.004).

The results of our study consistent or supported by the works reported by Sunkari et al. [14]; Tomoda et al. [10]; Han et al. [16]; Khaksar et al. [17]; Campbell et al. [15]; Prentice, [11]; Essa et al. [9]; Essa et al. [12] and Arora et al. [13].

Tomoda et al. [10] observed higher amount of estradiol was delivered through skin when estradiol was loaded in nanoparticles than estradiol was free molecules. Also, iontophoresis was applied to enhance the permeability of nanoparticles. When iontophoresis was applied, permeability of estradiol-loaded PLGA nanoparticles was much higher than that obtained by simple diffusion of them through skin, since they have negative surface charges. They were found to penetrate through follicles mainly. Also, enhanced permeability effect of estradiol by using nanoparticle system and iontophoresis were observed in vivo. The combination of charged nanoparticle system with iontophoresis is useful for effective transdermal delivery of therapeutic agents.

Prentice [11] reported that iontophoresis decreases the absorption lag time, while it increases the delivery rate when compared with passive skin application.

Essa et al. [9] stated that Iontophoresis (0.8 mA/cm2, for 8 h) improved drug penetration over passive delivery for all systems, with ultradeformable vesicles performing best although the penetration parameters of estradiol from ultradeformable vesicles were higher than those from traditional liposomes.

Essa et al. [12] concluded that estradiol iontophoresis (up to 0.8 mA/cm2) further promoted delivery of estradiol by the liposomal structure in a proportional fashion i.e. increased current densities raised fluxes. Such improvements may be due to high deformability of the vesicular membrane that enables the liposomes to penetrate intact through the skin, and/or the negative charge on the vesicular surface.

Arora et al. [13] examened the effect of estrogen on endothelium-dependent relaxation in the cutaneous microcirculation of women. Results indicate that estrogens might enhance endothelium-dependent and endothelium-independent vasodilatation in the microcirculation of women.

Estrogen topical application also enhances the wound healing according to the following studies:

Sunkari et al.[14] concluded that esterogen receptor beta (ERb) -mediated signaling increased angiogenesis when is impaired in diabetic mice wound. This observation is important for potential therapeutically use of selective modulators of ER (SERM) that could be potentially used for stimulating wound healing in diabetes.

Independent of the mechanism, a repressed angiogenesis seems to have a central pathogenic role for impaired wound healing in diabetes since loss-of-function of ERb in nondiabetic wounds is followed by a worse wound healing rate secondary of a defect in cellular migration[15].

In another similar observation by Han et al.[16] on esterogen receptor alpha (Era) -mediated signaling which reported to have positive vascular effects through both EPCs induction and improvement of the vascular reaction.

Khaksar et al. [17] investigated the possible effects of systemic and topical estrogen were investigated on wound healing in normal and diabetic male rats. The results expressed that systemic and topical estrogen can improve the impaired healing of diabetic wounds.

Iontophoresis enhance estrogen transport through the skin can be explained that by Electromigration mechanism in which electrical field enhance the transport of negatively charged drugs (estrogen) from the cathode (-ve electrode) by repulsion forces between the similar charges which may called active ionic transport.

Also possible intracellular mechanisms that electrical stimulation in general stimulate fibroblastic responses include activation of transcription and translation of mRNA to make available important protein precursors, [18] increased ATP production to supply necessary energy demands, [19] membrane permeability that would allow increased intracellular stores of calcium, [20] and production of membrane receptors for important cytokines such as epidermal growth factor [21].

Epithelial cell activity during repair also seems to be affected by electrical current. In particular, in vitro studies have shown that epithelial cell proliferation[22] and differentiation[23] can be activated in epidermal cells by electrical stimulation. In addition, keratinocyte migration can be influenced by the application of an electrical field, and the synthesis and secretion of growth factors by epithelial cells can be stimulated to a greater extent by the application of an electrical current[20]. Correspondingly, several authors have reported that exogenous application of electrical currents to various animal models can accelerate wound re-epithelialization [24].

In addition to accelerated activities of fibroblasts and epithelial cells during the proliferative stage of healing, electrical current has also been shown to augment angiogenesis. Clinical studies have detected a greater density of capillaries within newly formed granulation tissue analyzed in tissue biopsies taken from individuals with chronic venous leg wounds when they were pretreated with electrical current [25].

Increased local vasodilation and improved tissue oxygenation have been reported to occur in individuals with peripheral vascular disease following treatment with electrical current [26]. Gilcreast et al [26] and Faghri et al have demonstrated that electrical stimulation can enhance perfusion of ischemic limbs. In addition, Im et al [27] showed enhanced survival rates of skin flaps pretreated with electrical stimulation that was attributed to improved blood perfusion observed in skin flaps under the negatively charged cathode.

Sex hormones play an important role in decrease wound infection as estrogen improves neutrophil phagocytic ability, suggesting that higher levels of estrogen can aid clearance of infection through increased neutrophil function [28]. Estrogens and androgens have complex interactions with immune cell function, and it is important to note that they can either positively and/or negatively regulate the immune response by aiding resolution or by compounding morbidity and mortality depending on which immune responses are being observed [29]. Estrogens are generally thought to enhance the humoral immune response[30], while androgens act to suppress both cell-mediated and humoral responses[31].

So it can be claimed that, there was greater improvement after application of estrogen iontophoresis and so enhancing the treatment of diabetic foot ulcers by decreasing the WSA and wound volume. Also cost effectiveness, finally regaining to work quickly.

Conclusion

The results of current study showed that estrogen iontophoresis therapy for 6 weeks is an effective adjuvant therapy in treatment of unhealed diabetic foot ulcers through accelerating wound healing, reducing wound surface area (WSA) and wound volume.

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