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Ki-67% Response to Pulsed Radio Frequency Energy on Chronic Wound Healing in Patients with Diabetic Foot Ulcer

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Abstract: Objectives: Diabetic foot is a serious complication of diabetes which aggravates the patient's condition whilst also having significant socioeconomic impact. The aim of the present study is to examine the effectiveness of pulsed radio frequency in the treatment of chronic lower limb ulcers. **Materials and Methods:** Forty patients with persistent diabetic foot ulcers for more than three months participated in the study. Their ages ranged from 40 to 50 years. Patients were chosen from Deraya University Physical Therapy Center between July 2019 and February 2020. Patients were randomly assigned to one of two groups: Group (A): The Pulsed Radio Frequency Group received PRFE with a maximal power 400 Watts, a pulse width of 400 microseconds, a frequency of 144 pulses per second, and an average power of 23 watts for 30 minutes, three times a week for six weeks, as well as medical treatment. Only medical treatment was provided to Group (B) (Medical Treatment Group). Ki-67 percent was used to measure cell proliferation before and after treatment. **Results:** the Wilcoxon sum ranking test revealed that there was a significant increase in Ki-67% after 6 weeks of treatment application in group A when compared with corresponding pre-treatment value where P-value = 0.00008. While there was no significant difference between pretreatment and posttreatment data in group B regarding the Ki-67% where P-value = 0.496. Mann-Whitney test revealed that there was no statistically significant difference between groups regarding the pretreatment data where P-value = 0.562. While there was a statistically significant difference between groups regarding posttreatment data where P-value less than 0.00001. **Conclusion:** Pulsed radio frequency energy accelerates wound healing and increases cell proliferation, making it an important adjuvant therapy for chronic lower limb ulcers**.**

Keywords: Ki-67%, Lower limb ulcers, Pulsed radio frequency energy.

1 Introduction

Diabetic foot is one of the most significant and devastating complications of diabetes and is defined as a foot affected by ulceration that is associated with neuropathy and/or peripheral arterial disease of the lower limb in a patient with diabetes. The prevalence of diabetic foot ulceration in the diabetic population is 4–10%; the condition is more frequent in older patients. It is estimated that about 5% of all patients with diabetes present with a history of foot ulceration, while the lifetime risk of diabetic patients developing this complication is 15% **[**1–2**].**

Chronic wounds are a public healthcare challenge and have massive consequences for the quality of life of patients and caregivers, and for the use of both material and professional services. Damage to the skin may have a long-lasting negative effect on the overall health of the patient, with some patients spending years in the health care system. **[**3**].**

The most common etiology of chronic wounds is multifactorial and involves local causes (e.g. venous or arterial insufficiency, infection and local pressure) and systemic factors (e.g. diabetes and nutritional status); severe injuries or surgery are another common cause **[**4**].**

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In developing countries, chronic, soft tissue wounds in the lower limbs, identified as ulcers that remain for more than 3 months and require specialized treatment, have a prevalence of 1% in the adult population, including 3.6% in people older than 65 years **[**5**].**

Chronic wounds with an excess of protease activity and growth factor expression may be the result of wound healing being stopped in a state of chronic inflammation. In the chronic wound environment, there are too many matrix metalloproteases (MMPs), too few MMP tissue inhibitors (TIMPS), senescent and defective cells with low proliferative and synthetic activity, and too few growth factors and growth factor receptors. This situation inhibits fibroblast proliferation, motility, and protein synthesis, resulting in a chronic wound. **[**6**].**

Prolonging the recovery time for chronic wounds affects the quality of patient's life and raises the cost of their care. The cost of moist wound dressing products to the largest provider of primary health care estimated at nearly EUR 6 million in 2010 in some countries **[**7**].**

Chronic ulceration of the lower legs is a relatively common condition amongst adults, and ulcer symptoms usually include increasing pain, friable granulation tissue, foul odor, and wound breakdown instead of healing. This results in social distress and considerable healthcare and personal costs [8].

There are many physiotherapy modalities used in wound healing as laser and exercises **[**9, 10**].** Pulsed radio frequency electromagnetic energy (PRFE) electrotherapy has recently been given a new focus, with a range of case reports showing positive results in improving hyperplasia and chronic wound healing **[**11, 12**].**

The use of pulsed radio frequency electromagnetic field (PEMF) therapy, also referred to as pulsed radio frequency energy (PRFE) therapy, has shown considerable success in chronic wound healing **[**13**]**. PEMF is the non-ionizing energy of the electromagnetic spectrum's shortwave radiofrequency band, commonly at a frequency of 27.12MHz. PEMF therapy is not invasive and is administered through the dressing of the wound, and no unwanted side effects have been shown to date. With promising studies documenting PEMF as an efficient therapy in the literature, its wider use as an adjunct therapy seems justified **[**14**].**

Pulsed radiofrequency energy therapy can up-regulate mechanisms involved in tissue repair including growth factors and cytokines important for the wound healing process. A number of cellular studies show PRFE has effects on production of nitric oxide, increased cell proliferation and in vivo vasodilation in rat muscle **[**15**].**

This study was therefore carried out to examine the effectiveness of pulsed radio frequency energy as a modality of physical therapy in the treatment of chronic lower limb ulcers.

2 Materials and Methods

Subjects

The study was carried out in the period from July 2019 to February 2020. This study involved forty patients who had persistent unhealed lower limb ulcers (diabetic foot ulcers) for more than three months. Their ages varied from 40-50 years, with an average value of 43.99±2.843 years as presented in Fig. (1). Patients were chosen from Deraya University Physical Therapy Center. The exclusion requirements were as follows: patients suffering from extreme anemia, internal fixation in the region of application, cardiac rhythm devices inserted, patients with uncontrolled hypertension, pregnant women, and the presence of a tumor or cutaneous lesion which could interfere with the procedure. A complete description of the treatment procedure was given to all patients and a written informed consent form was signed by the patients to agree to participate and to publish the findings.

Study Design

This study was a randomized controlled trial with pre-post assessment. The patients who met the selection criteria were divided into two equal groups at random. Category (A): The Pulsed Radio Frequency Group. It includes 20 patients who received PRFE three sessions per week for six weeks. Group (B) Medical Care Group: This group consisted of 20 patients who received only medical care.

The numbered envelops system was used to apply randomization. The patients were blind to the group’s assignment

Assessment

Both patients' medical and demographic data were gathered and the importance of physical therapy in improving their health was clarified.

Cell proliferation was the pre- and post-intervention evaluation. The evaluation was conducted prior to the start of therapy and After 6 weeks at the end of the treatment program

Histology:

Wound tissue biopsy was taken from each subject in both groups (A and B) by taking 1 mm skin key punch biopsy from the edge of the wound during laying in comfortable position before treatment and 6 weeks after treatment **[**16**]**. The biopsy was coded and fixed in 10% buffered formalin, processed in ascending grades of alcohol (70%, 90% and 100% absolute) and xylene then Embedded in paraffin

blocks which were produced serially sectioned and were stained by Haematoxylin-eosin (H and E) stains **[**17**].**

Immunohistochemistry Using Ki-67 Proliferation Marker

De paraffinized samples were put into a plastic rack placed in a trough containing 250 ml of 10 mm citric acid (Ph adjusted to 6.0 using 2 M sodium chloride) microwaved at 750 W (4 min pulses) then lifted 10 min in room temperature. After that the samples were rinsed in TBS, drained and the section circled with risen pen then washed in running tap water. Slides were stained by Monoclonal Mouse Anti-Human Ki-67 Antigen (dilution 1:75-1:150), next the slides were washed three times in TBS, Biotinylated anti-mouse antibody (diluted 1:4000) was applied for one hour, washed for three times in TBS and had AB Complex/ Strept Ab Complex applied for further one hour. DAB solution was applied for 5 min and sections rinsed in distilled water, washed under running tap water and counterstained with Haematoxylin for 60 sec. They were then washed again, dehydrated and mounted in (Distyrene Plasticizer Xylene) DPX. The stained slides were analyzed at high power light microscope (Olympus CX41) for total number of cells interfollicular basal and first suprabasal layer. Cells with clear cut and strong nuclear staining by ki-67 were counted. For positive control, breast carcinoma tissue sections (Figure 11), already known to have high Ki-67 proliferation index were used; applying the same immunohistochemical procedure. For negative control, tissue sections from the same patients' specimen was used, applying the same procedure, but omitting the primary antibody. Positive immunoreactivity for Ki-67 was visualized as nuclear brownish staining. **[**18**].**

Treatment

Preparatory Procedures

The treatment protocol was explained to each patient, as well as the importance of testing and consent writing. Prior to use, the PRFE device was thoroughly tested, calibrated, and prepared.

Procedure of PRFE Therapy Technical Steps: -

Thermatur 200 by Uniphy in Belgium. Deliver PRF up to 400 W peak power, 2 ms/0.4 ms pulse width, up to 350 Hz pulse frequency and 27.12 generator frequency.

Patients in Group A received 30 minutes of PRFE as follows:

The following are the system parameters: maximal power of 400 watts, pulse width: 400 microseconds, frequency: 27.12 MHz, pulse frequency: 144 Hz. Each patient received a 30-minute treatment with a 23 watts energy exposure. The system applicator was put as near as possible to the treatment area. The therapy was then started without any thermal or sensory effects.

Outlines of Medical Care

Preventing infection, healing the wound region, debridement, if necessary, day-to-day application of medication or dressings to the ulcer, and management of blood glucose and other health issues were among the medical care outlines received by patients from both groups.

Statistical Analysis

SPSS for windows, version 22 (SPSS, Inc., Chicago, IL) was used to perform statistical analysis. In this study, descriptive statistics (mean, standard deviation) for all patients were determined for age, weight, height, BMI, and median for histopathology and cell proliferation index variables (ki-67%). Mann-Whitney test was conducted to compare the mean ranking of the study and the control group. Wilcoxon sum ranking test was used to compare the pretreatment and posttreatment data of the same group. The alpha level of significance was set less than 0.05.

3 Results and Discussion

Demographic data

There were no statistically significant differences (P>0.05) between patients in both groups concerning age, weight, height, and BMI (Table 1).

Table 1: Demographic data

	Mean± SD		**t-value**	**p-value**
	Group (A)	**Group (B)**		
Age (years)	43.65± 2.72	44.25±3.0065	0.661	0.512
Height (cm)	172±2.92	173.05±5.7	0.7332	0.468
Weight (Kg)	83.65±7.17	83.8±7.25	0.0658	0.948
BMI (Kg/m^2)	28.28±1.8	27.99±1.54	0.5475	0.587

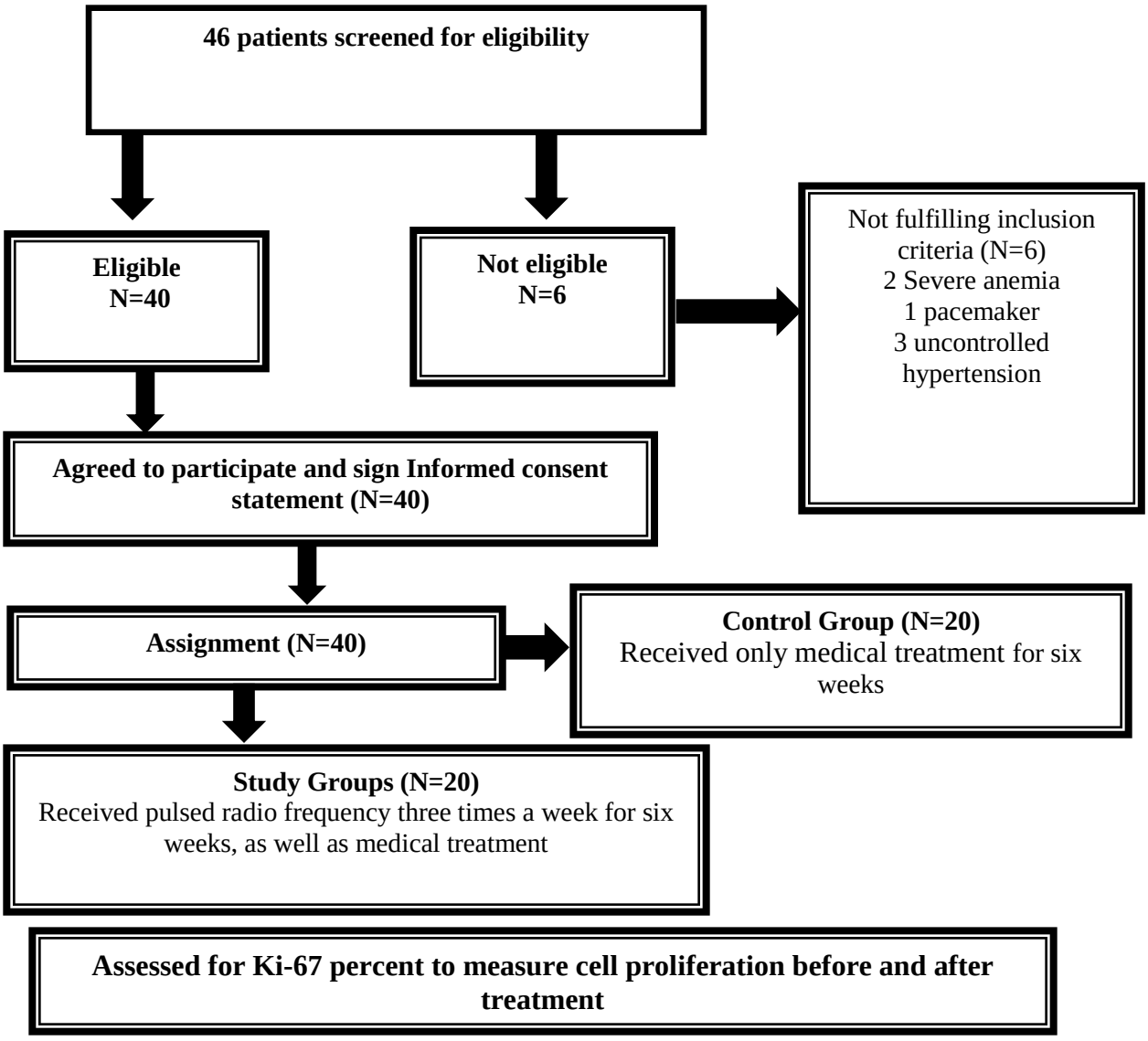

Fig.1: Flow Chart.

Cell Proliferation Index (ki-67%):

As indicated in table (2), the Wilcoxon sum ranking test revealed that there was a significant increase in Ki-67% after 6 weeks of treatment application in group A when compared with corresponding pre-treatment value where P-value = 0.00008. While there was no significant difference in the Ki-67% after 6 weeks of treatment application in group B when compared with the corresponding pre-treatment value where P-value = 0.496. Mann-Whitney test revealed that there was no statistically significant difference between groups regarding the pretreatment data where P-value = 0.562. While there was a statistically significant difference between groups regarding post-treatment data where P-value less than 0.00001.

From the histopathological analysis point of view before treatment; there was covering of stratified squamous epithelium at wound edge showed very low Ki-67 proliferation index; less than 1% within nuclei (Figure 2.A). In some patients; nuclei of stratified squamous epithelium at wound edge were totally negative for Ki-67 (Figure 2.B).

After treatment (figure 2.C& D), covering stratified squamous epithelium at wound edge, showed increase in Ki-67 proliferation index; more than 5% positivity within nuclei.

Table 2: Intra and intergroups comparisons of Ki-67% results.

Ki-67%	Before treatment	After treatment	Z value	P-value
	Median	Median		
Group A	1	5	-3.919	0.00008*
Group B	2	1	-0.682	0.496
Z value	-0.582	5.383		
P-value	0.562	< 0.00001*		
* = Significant				

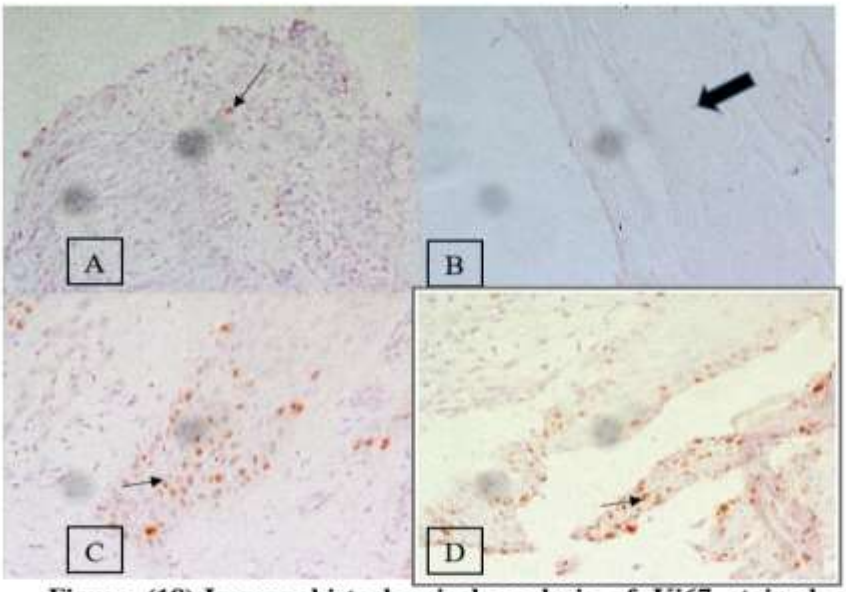

Figure (18):Immunohistochemical analysis of Ki67 stained sections (A) Low proliferation index Ki67 (thin black arrow) less than 1% in nuclei of covering stratified squamous epithelium at the wound edge in patients before treatment. (B) Stratified squamous epithelium at the wound edge totally negative for Ki67 (thick black arrow) in patient before treatment. (C) and (D): High proliferation index Ki67 more than 5% positivity within nuclei (thin black arrow) of covering stratified squamous epithelium at the wound edge (Immunoperoxidase X 300)

Results of Histopathological Assessment:

Wound edge biopsy of most patients before treatment (Figure 3) showed total ulceration of covering stratified squamous epithelium. Dense fibrinous exudate was seen entangling scattered inflammatory cells. Nuclear debris was seen with several karyorrektic fragments. In few patients, starting minimal granulation tissue was seen devoid of inflammatory cells; within the ulcer gap.

After treatment (Figure 3); the process of healing granulation tissue formation improved. Dense inflammatory cellular infiltrate was noticed; formed of numerous polymorphs, pus cells and small mature lymphocytes. Fibrous tissue with basophilic spindle shaped nuclei showing tapering ends was increased. Neovascularization became evident with numerous newly formed, thin-walled blood vessels. Collagenization of the fibrous tissue was focally noticed. Adjacent covering stratified squamous epithelium was seen proliferating with moderate to marked acanthosis.

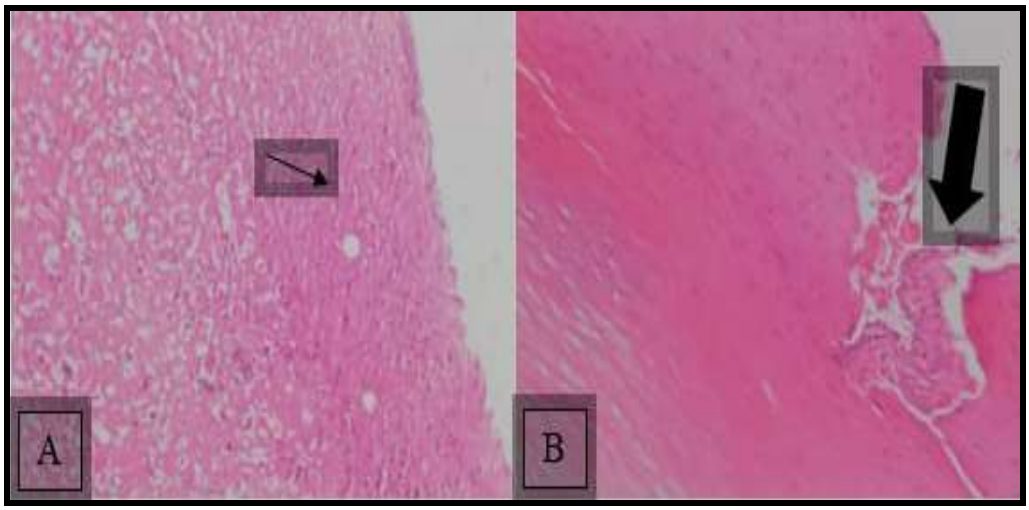

Fig.3: Wound edge biopsy before treatment; (A) Totally ulcerated surface epithelium, with dense fibrinous exudate (thin black arrow) and scattered inflammatory cells. (B) Minimal granulation tissue formation (thick black arrow)

The effectiveness of pulsed radio frequency energy as a physical therapy modality in the treatment of chronic lower limb ulcers by accelerating wound healing and increasing cell proliferation was explored in this research.

The results of this study showed a significant increase in Ki-67 percent (a cell proliferation marker) in group A (P=0.00008). While there was no significant difference in the Ki-67% after 6 weeks of treatment application in group B when compared with the corresponding pre-treatment value where P-value=0.496. In addition, there was no statistically significant difference between groups regarding the pretreatment data where P-value = 0.562. While there was a statistically significant difference between groups regarding posttreatment data where P-value less than 0.00001.

The findings of this study were in line with **Rawe and Vlahovic,** who evaluated the effect of using PFRE system on 4 patients who had ulcers for more than 3 months and did not heal after traditional therapy. As a procedure, a portable PRFE type system was introduced and used 6-8 hours per day for a duration of 6 weeks. All patients showed improvement after 1 week of therapy and wound size was seen to decrease. Patient 1 who had a venous stasis ulcer after 2 weeks of treatment, reported pain relief. After 3 weeks of therapy, patients 2 and 3 reached full healing, and patients 1 and 4 had a 95 percent and 88 percent reduction in wound size after the 6 week of therapy. After the 6-week study period, both these patients continued to complete the healing through using the PRFE unit **[**19**].**

According to **Conner-Kerr and Isenberg,** who assessed the benefit of PRFE therapy in the treatment of chronic pressure ulcers via the Provant Therapy System, the outcome of this study was that 89 patients showed a 51 percent median decrease in wound surface area and accelerate the rate of wound healing after 4 weeks, so that the median of wound healing was 0.13 cm / d. The study concluded that the treatment of PRFE is a beneficial adjuvant therapy for the healing of chronic pressure ulcers **[**20**].**

The outcome of the current research, is supported by **Frykberg et. al.,** who conducted a study to explore the role of PRFE on the rate of wound healing. outcomes, found that the mean percent decrease in wound area after 4 weeks was 49 percent ± 6 percent for pressure ulcer (PU) (P<0.0001), 38 percent ± 6 percent for diabetic foot ulcer (DFU) (P<0.0001), 44 percent ± 5 percent for venous leg ulcer (VLU) (P<0.0001), and 39 percent ± 9 percent for ulcers of different forms. The median wound reduction rate (range -4.14-2.21) was 0.08 cm (2)/day. Reports showed that a significant proportion of these wound ulcers treated with PRFE have healed with continuing therapy **[**21**].**

There is additional support for the role of pulsed radiofrequency energy in the diabetic mouse model. They suggest that pulsed radiofrequency energy induced increased wound closure mainly resulting from contraction, and the biological effects of such therapeutic strategies may relate to specific targeting of dermal cells in a manner that induces a hyperproliferative state in dermal fibroblasts and myofibroblasts that encourages a robust granulation tissue

response with collagen deposition and wound contraction. **[**22**].**

Pulsed RF energy therapy has been reported to promote healing of chronic wounds otherwise nonresponsive to standard of care treatment, such as chronic lower extremity wounds in patients with diabetes. Effective wound therapeutics that obviate the need for surgical intervention are especially important in this particular population, which has a high incidence of nontraumatic lower limb amputation **[**23**].**

4 Conclusions

The current study found that 6 weeks of pulsed radio frequency energy is an efficient adjuvant therapy for treating chronic lower limb ulcers by accelerating wound healing and increasing cell proliferation.

Conflict of interest:

No conflict of interest

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References

[1] C. A. Abbott, A. L. Carrington, H. Ashe, S Bath, L. C. Every, J. Griffiths, A. W. Hann, A. Hussein, N. Jackson, K. E. Johnson, C. H. Ryder, R. Torkington, E. R. E. Van Ross, A. M. Whalley, P. Widdows, S. Williamson, A. J. M. Boulton. North-West Diabetes Foot Care Study. Diabet Med., **19(5)**, 377-384(2002).

[2] S. Lauterbach, K. Kostev, T. Kohlmann. Prevalence of diabetic foot syndrome and its risk factors in the UK, J Wound Care,19: 333-337 (2010). doi: 10.12968/jowc.2010.19.8.77711.

[3] S. Bateman. Principles of preventative foot care. Br J Community Nurs.,**19**, 30-38 (2014). doi: 10.12968/bjcn.2014.19.sup3.s30.

[4] M.C. Ferriera, P. Tuma, V.F. Carvalho, F. Kamamoto. Complex wounds, Clinics (Sao Paulo)., **61**, 571-578 (2006). DOI: 10.1590/s1807-59322006000600014

[5] F. Werdin, M. Tenenhaus, H.O. Rennekampff. Chronic wound care, Lancet 29;372(9653):1860-1862 (2008). DOI: 10.1016/S0140-6736(08)61793-6

[6] E. Martin, E. Tierney, A. Tallis, G.R. Frykberg. Use of human fibroblast Derived Dermal Substitute (HFDDS) to Close a Complex Chronic Wound in the presence of peripheral arterial disease, The Journal of Diabetic Foot Complications,5, 39-43 (2013).

[7] N.G. Puig, C.V. Teixidó, J.L. Estébanez Perpina, S.F. Bosch. Prevalence of injuries in the field of primary care, regarding the registry variants, Nursing Goals, 16, 6-10 (2013).

[8] S.S. Chatterjee. Venous ulcers of the lower limb: Where do we stand? Indian J Plast Surg., **45(2)**, 266-274 (2012). doi:10.4103/0970-0358.101294

[9] S.A. Tantawy, W.L. Abdelbasset, D.M. Kamel, S. M. Alrawaili. A randomized controlled trial comparing helium-neon laser therapy and infrared laser therapy in patients with diabetic foot ulcer. Lasers Med Sci., **33(9)**,1901-1906. doi: 10.1007/s10103-018-2553-2 (2018).

[10] S.A. Tantawy, H.M. Zakaria. The role of physical therapy intervention in the management of diabetic neuropathic foot ulcers, Med. J. Cairo Univ., **78**, 103-109 (2010).

[11] H.M. Elgohary, S.A. Tantawy. Pulsed electromagnetic field with or without exercise therapy in the treatment of benign prostatic hyperplasia. J Phys Ther Sci., **29(8)**, 1305–1310 (2017). https://doi.org/10.1589/jpts.29.1305.

[12] M. Maier, Pulsed radio frequency energy in the treatment of painful chronic cutaneous wounds: a report of two cases. Pain Med., **12(5)**, 829-832 (2011). doi: 10.1111/j.1526-4637.2011.01081.x.

[13] J.A. Larsen and J. Overstreet, Pulsed radio frequency energy in the treatment of complex diabetic foot wounds: two cases. J Wound Ostomy Continence Nurs.,**35 (5)**, 523-527 (2008). doi: 10.1097/01.WON.0000335966.98607.1c.

[14] I. Rawe, Pulsed radio-frequency electromagnetic field (PEMF) therapy as an adjunct wound healing therapy. Wounds International,**3**, 32-34 (2012).

[15] H.M. Lee, U.H. Kwon, H. Kim, H.J.Kim, B. Kim, J.O. Park, E.S. Moon, S.H. Moon. Pulsed electromagnetic field stimulates cellular proliferation in human intervertebral disc cells. Yonsei Med J.,**51**, 954-959 (2010). DOI: 10.3349/ymj.2010.51.6.954

[16] S. Chakraborty, D. Sampath, M.O. Yu Lin, et al. Agrin-Matrix Metalloproteinase-12 axis confers a mechanically competent microenvironment in skin wound healing. Nat Commun., **12**, 6349 (2021). https://doi.org/10.1038/s41467-021-26717-7

[17] M.M. Tsamouri, M. Rapti, P. Kouka, et al. Histopathological evaluation and redox assessment in blood and kidney tissues in a rabbit contrast-induced nephrotoxicity model. Food and Chemical Toxicology: an International Journal Published for the British Industrial Biological Research Association.;108(Pt A):186-193 (2017). DOI: 10.1016/j.fct.2017.07.058.

[18] J. Bullwinkel, B. Baron-Lühr, A. Lüdemann, C. Wohlenberg, J. Gerdes, T. Scholzen. Ki-67 protein is associated with ribosomal RNA transcription in quiescent and proliferating cells. J Cell Physiol., **206(3)**, 624-35 (2006). doi: 10.1002/jcp.20494.

[19] I.M. Rawe, T.C. Vlahovic. The use of a portable, wearable form of pulsed radio frequency electromagnetic energy device for the healing of recalcitrant ulcers: a case report, Int. Wound J., **9(3)**, 253-258 (٢٠١٢). doi: 10.1111/j.1742-481X.2011.00853.x.

[20] T. Conner-Kerr, R.A. Isenberg. Retrospective analysis of pulsed radiofrequency energy therapy use in the treatment of chronic pressure ulcers. Adv Skin Wound Care., **25(6)**, 253-260 (2012). doi: 10.1097/01.ASW.0000415342.37554.ed.

[21] R.G. Frykberg, V.R. Driver, L.A. Lavery, D.G. Armstrong, R.A. Isenberg. The use of pulsed radio frequency energy therapy in treating lower extremity wounds: Results of a retrospective study of a wound registry, Ostomy Wound Manage.,**57(3)**, 22-29 (2011).

[22] Q. Li, H. Kao, E. Matros, C. Peng, G.F. Murphy, L. Guo. Pulsed radiofrequency energy accelerates wound healing in diabetic mice, Plast Reconstr Surg.,**127(6)**, 2255-2262 (2011). DOI: 10.1097/PRS.0b013e3182131bb5

[23] L. Guo, N.J. Kubat, T.R. Nelson, R.A. Isenberg. Meta-analysis of clinical efficacy of pulsed radio frequency energy treatment. Ann Surg., **255(3)**,457-67 (2012). doi: 10.1097/SLA.0b013e3182447b5d.