Biochemistry and Biophysics Reports 9 (2017) 316–321

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

Comparative study of the efficacy of pulsed electromagnetic field and low level laser therapy on mitogen-activated protein kinases

Ayman M. El-Makakey[a], Radwa M. El-Sharaby[b], Mohammed H. Hassan[c,*], Alaa Balbaa[d]

[a] Department of Surgery, Qena Faculty of Physical Therapy, South Valley University, P.O. Box No. 83523, Qena, Egypt
[b] Department of Clinical Pathology, Faculty of Medicine, Tanta University, P.O. Box No. 31527, Tanta, Egypt
[c] Department of Medical Biochemistry and Molecular Biology, Qena Faculty of Medicine, South Valley University, P.O. Box No. 83523, Qena, Egypt
[d] Department of Physical Therapy for Orthopedic, Faculty of Physical Therapy, Cairo University, P.O. Box No. 12613, Cairo, Egypt

ARTICLE INFO

Keywords:
Pulsed electromagnetic field
Low level laser therapy
Mitogen-activated protein kinases
Peripheral lymphocytes
Flow cytometry

ABSTRACT

Mitogen-Activated Protein Kinases (MAPKs) consist of three major signaling members: extracellular signal-regulated kinase (ERK), p38 and C-JUN N-terminal kinase (JNK). We investigated physiological effects of Pulsed Electromagnetic Field Therapy (PEMFT) and Low Level Laser Therapy (LLLT) on human body, adopting the expression level of mitogen-activated protein kinases as an indicator via assessment of the activation levels of three major families of MAPKS, ERK, p38 and JNK in the peripheral lymphocytes of patients before and after the therapies. Assessment for the expression levels of MAPKs families' were done, in the peripheral lymphocytes of patients recently have appendectomy, using flow cytometric analysis of multiple signaling pathways, pre and post LLLT and PEMFT application (twice daily for 6 successive days) on the appendectomy wound. There were non-significant differences in the expression levels of MAPKs families' pre- therapies application. But there were significant increase in the ERK expression levels post application of LLLT compared to its pre application ($p < 0.01$). Also, there was significant increase in the ERK, p38 and C-Jun N terminal expression level values post application of PEMFT compared to its pre application expression levels ($p < 0.01$ for each). The present study demonstrates that PEMFT has a powerful healing effect more than LLLT as it increase the activation of ERK, P38 and C-Jun-N Terminal while LLLT only increase the activation of ERK. LLLT has more potent pain decreasing effect than PEMFT as it does not activate P38 pathway like PEMFT.

1. Introduction

Lasers are often described by the kind of lasing medium they use-solid state, gas, exciter, dye or semiconductor. Lasers are also characterized by the duration of laser emission-continuous wave or pulsed laser (according to Oregon state university- faculty of Physics classification). The most common use of LLLT in the field of physical therapy is to promote healing process and decrease pain sensation after injury as LLLT has been suggested to enhance the activity of macrophages and fibroblast migration and proliferation and its subsequent increase in type I and III procollagen mRNA synthesis [1], it promote revascularization of wound [2], it also enhances bone healing, it also has visible stimulation of blood microcirculation in case of tissue heating on 0.8–1 °C or more [3], so it has potent effect in enhancing the healing process and in management of pain [4].

LLLT promote ATP synthesis [5] as cell absorb the radiated photons from laser and convert its energy to ATP which is a necessary source of energy for living cells in most of its functions. ATP is formed in cell mitochondria using glucose and oxygen in Krebs cycle to convert ADP to ATP. LLLT increases mitochondrial membrane potential and ATP synthesis in C2C21 myotubes with a peak response at 3–6 h [6]. This effect depends on chromophores which are light absorbing components in the cells and other cell components as cytochrome c, porphyrins and flavin also have a light absorbing capability [7]. Given explanation of this effect by the forming of singlet oxygen, reactive oxygen species (ROS), or nitric oxide. The enhanced ATP formation promote essential functions in the cell like cell homeostatic function, mitosis and proliferation by enhancing mRNA activity. The effect of laser depend on the time of application, intensity of the energy and exposure area, while, the depth of penetration depends on the wave length of the applied laser. High energy laser lead to cell damage [8,9]. While LLLT has a high effect on injured cells more than sound one that

Abbreviations: MAPKs, Mitogen-Activated Protein Kinases; ERK, Extracellular signal-Regulated Kinase; JNK, C-JUN N-terminal Kinase; PEMFT, Pulsed Electromagnetic Field Therapy; LLLT, Low Level Laser Therapy; DMEM, Dulbecco's Modified Eagle Medium

* Corresponding author.
E-mail addresses: Mohammedhosnyhassaan@yahoo.com, mohammedhosnyhassaan@med.svu.edu.eg (M.H. Hassan).

http://dx.doi.org/10.1016/j.bbrep.2017.01.008
Received 21 November 2016; Received in revised form 22 January 2017; Accepted 24 January 2017
Available online 25 January 2017

Table 1
The ERK expression levels values (%), pre and post application of LLLT, PEMF on both groups.

ERK	Pre PEMF	Pre LLLT	Post PEMF	Post LLLT
Range	0.15–0.59	0.31–0.56	0.65–0.84	0.66–0.85
Mean ± SD	0.389 ± 0.120	0.455 ± 0.065	0.764 ± 0.062	0.768 ± 0.055
	Pre PEMF & Post PEMF	**Pre LLLT & Post LLLT**	**Post PEMF & Post LLLT**	
T. test	10.748	14.627	0.193	
P. value	0.001	0.001	0.853	

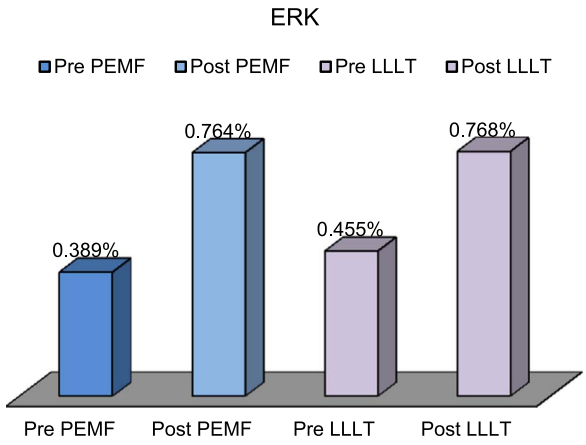

Fig. 1. The mean ERK expression levels (%) pre and post application of LLLT, PEMF on both groups.

give improve the hypothesis that laser promotes the normal cell function rather than changes it. Pulsed electromagnetic field therapy (PEMFT) creates micro currents in the body's tissues. These micro currents lead to certain biological and physiological responses depending on field parameters such as amplitude, frequency and wave form.

PEMFT is used worldwide and the most uses were to improve wound healing and fractures and pain modulation. Many theoretical hypotheses underlying this healing ability, one of them is due to increased blood circulation and microcirculation by significant arteriolar vasodilatation [10], anti-inflammatory effect [11], decrease edema and swelling [12], improve cell proliferation and differentiation potentials [13] and finally modulation of pain [14].

MAPKs are serine- threonine protein kinases that are activated by diverse stimuli ranging from cytokine, growth factors, neurotransmitters, hormones, cellular stress and cell adherence. MAPKs are expressed in all eukaryotic cells [15], they regulate diverse processes in the cell via transcriptional and non transcriptional control as they control the activity rhythm of large number of genes functionally related to each other's [16]. They consist of three major signaling members, the extracellular signal-regulated kinase (ERK), p38 and C-JUN N-terminal kinase (JNK). ERK regulates mitosis, proliferation, differentiation and survival of mammalian cells during development and neuronal plasticity in adult [17], while, p38 and JNK play essential roles in regulating inflammatory responses, neurodegeneration, cell death and pain hypersensation [18,19]. As conformational changes of any protein molecule affects its interaction with ligand and its biological partners at different level, so to understand the signal molecules interaction analysis, this could be explored by performing molecular docking with long term molecular dynamics simulations [20–22].

MAPKs are relates to various kinds of cellular stimulation and stress induced by heat, UV and chemicals, and they are important research targets in the field of biology and medicine. Application of electromagnetic field to therapy is also an interesting subject in which many things including its physical and biological mechanisms are still unclear. The current study provides the mechanisms of LLLT and PEMFT application in management of various human pathologies such as pain and wound healing via studying their effects on different signaling components of MAPKs pathway which is involved in various cellular regulatory functions including pain and wound healing process. Also the present study solves the conflicted studies regarding the effect of PEMFT on pain.

2. Material and methods

2.1. The study population

Fifty patients (30 male and 20 female) with age range (20–32) years old not suffering from any relevant diseases or take any medication that interfere with the application of treatment or measuring procedures and with no relevant history of smoking or addiction or bad habits or alcoholism who recently undergo appendectomy (3 days post-operative, as an example of injury) admitted at the department of surgery-Faculty of Medicine-Tanta University after obtaining approval of university hospital ethics committee and informed consent from the included patients. They were divided into 2 main groups: Group A (PEMFT) formed of 25 patients to whom the PEMFT has been applied. Group B (LLLT) formed of 25 patients to whom the LLLT has been applied.

2.2. PEMFT and LLLT applications

Portable magnetic therapy device (EASY Qs) was used for PEMFT application. This device has frequency from 5 to 100 Hz and intensity from 1 to 60 Gauss. Unidirectional quasi-rectangular waveform with strength 15 gauss and frequency less than 20 Hz was applied to the wound for 20–30 min each time. Pulsed wave diode laser (CEI 76-2/1999-1, Italy), was used for LLLT application, emitting wavelength of 905 nm with peak power 25 W and impulse duration 100nS. Linear application of LLLT on para-incisional line on both sides of the wound for 15–30 s for each point (total application period 20–30 min). Each of the two modalities was used twice daily for 6 successive days at the

Table 2
The P38 expression levels values (%), pre and post application of LLLT, PEMF on both groups.

P 38	Pre PEMF	Pre LLLT	Post PEMF	Post LLLT
Range	0.47–0.77	0.59–0.78	0.97–1.80	0.63–0.77
Mean+SD	0.669 ± 0.075	0.690 ± 0.061	1.269 ± 0.289	0.690 ± 0.039
	Pre PEMF & Post PEMF	**Pre LLLT & Post LLLT**	**Post PEMF & Post LLLT**	
T. test	7.783	0.001	7.686	
P. value	0.001	0.998	0.001	

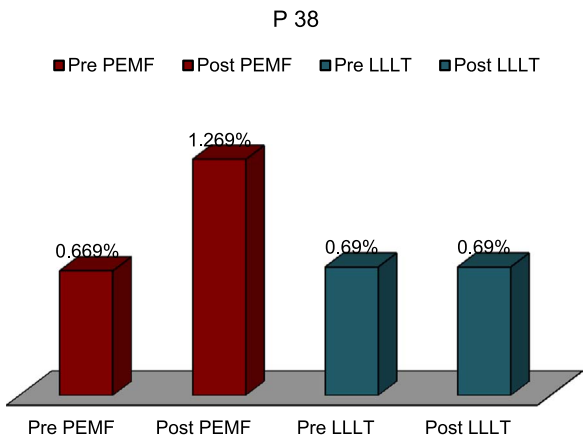

Fig. 2. The mean P38 expression levels (%) pre and post application of LLLT, PEMF on both groups.

Table 3
The C-JUN N-terminal kinase (JNK) expression levels values (%), pre and post application of LLLT, PEMF on both groups.

c – JUN	Pre PEMF	Pre LLLT	Post PEMF	Post LLLT
Range	0.47–0.78	0.59–0.79	0.96–1.20	0.60–6.80
Mean + SD	0.659 ± 0.079	0.689 ± 0.063	1.088 ± 0.095	1.885 ± 2.476
	Pre PEMF & Post PEMF	**Pre LLLT & Post LLLT**	**Post PEMF & Post LLLT**	
T. test	13.448	1.869	1.253	
P. value	0.001	0.072	0.223	

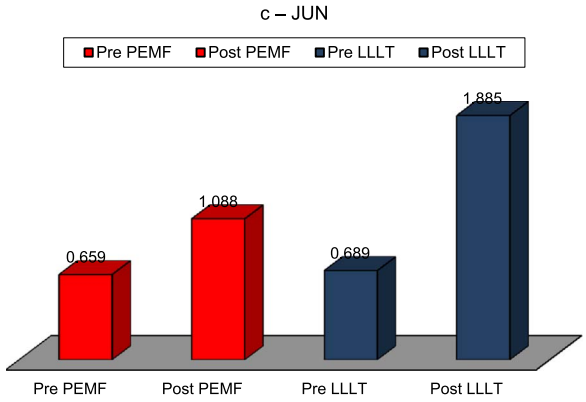

Fig. 3. The mean c-JUN N terminal expression levels (%) pre and post application of LLLT, PEMF on both groups.

site of the appendectomy wound (3–4 cm) around the McBurney's point.

2.3. Flow cytometric Analysis of MAPKs mediated signaling molecules

Patients in both groups were assessed for the expression levels of MAPKs signaling components pre and post PEMFT and LLLT in the peripheral Lymphocytes. Two blood samples (5 cc of venous blood using EDTA as the anticoagulant) were taken, in each time of PEMFT and LLLT applications, from every included patient, one before and the other after the applications, the pre-application sampling were taken to assess if there were cumulative effect of the PEMFT or LLLT from the previous application or not to be taken into consideration during calculation of the results. Peripheral mononuclear cell suspension pellets were obtained from each anti-coagulated blood sample using density gradient centrifugation on Ficoll-Hypaque media, were the blood samples layered onto a Ficoll-sodium metrizoate gradient of specific density following centrifugation, mononuclear cell pellets were collected from the plasma-Ficoll interface.

2.3.1. Lymphocytes isolation

Culture media was prepared by transferring 9 ml of DMEM media (lot n s13365L0103 biowest) to the labeled flask, 100 µl Fungizone (10 µl/1 ml media) and 100 µl Pen/Strep solution (10 µl/1 ml media) were pipette to the same flask. 1 ml of suspended cell pellet was added to the previous mixture with gentle mixing. The mixture was incubated for 2 h at 37 0 C in 5% CO_2. The flask was removed from the incubator and placed in the hood. The media containing non-adherent cells was collected from the flask by a sterile pipette.

2.3.2. Activation of ERK, P38 and c-jun in the isolated lymphocytes

After isolation of lymphocytes, cells were suspended at 1×106/ml in tissue culture medium (αMEM) plus 10% fetal bovine serum (FBS lot n 41G5834K)) at 37° water bath. Activation was done for 10 min using 40 nM phorbol myristate acetate [(PMA; #P8139), which obtained from Sigma, and used at a 40 mM working dilution dissolved in 100% ethanol and the concentration adjusted to 1×10^6/ml in α MEM (lot number M0200) (modified Egle media) containing 10% fetal calf serum]. While maintaining sample at 37°, fixation was done (at 37° for 10 min) by adding 10% formaldehyde (Ultrapure M Grade) to give a final concentration of 2% formaldehyde. Tubes were placed on ice for 2–3 min, then 100% ice cold methanol was added followed by gentle vortex mixing, to give a final concentration of 90% methanol, held on ice for 30 min, then processed for antibody labeling. The tubes were centrifuged, aspirated, and washed once using 2 ml PBS containing 4% FBS. Centrifugation and re-suspended at 10^6 cells in 100 ml PBS plus 4% FBS was done. Then Labeling with phospho-specific polyclonal antibody (from Abcam Company) to ERK1/2(lot n s13365L0103), P38 (lot n ab170099) and JNK (lot n ab31419) separately was done for 15 min at room temperature, then washed once using 2 ml PBS plus 4% FBS, all these antibodies used at a 1/100 dilution. The samples were run on flow cytometer (BD FACS Calibur flow cytometer, USA); fluorescence signals were collected using band pass filters excitation at 525 nm and emission at 575 nm. The expression levels were automatically calculated by the flow cytometry by measuring the percent (%) of expression in the activated lymphocytes appeared at the upper right quadrant of the graph.

2.4. Statistical analysis

Statistical analyses of the data were carried out using Graph Pad prism version 5.0 (Graph pad Software, Inc., CA, and USA). Data comparisons were performed using paired *t*-test. The levels of significance were accepted with $p < 0.05$ and all relevant results were graphically displayed as mean ± SD.

3. Results

In this study according to the expression ERK levels (%), it was found that, there were statistical significant difference between Pre PEMFT & Post PEMFT (p=0.001), Pre LLLT & Post LLLT (p=0.001) and there were no statistical significant difference between Post PEMFT & Post LLLT (p=0.853) (Table 1 and Fig. 1).

According to the expression P38 levels (%), there was statistical significant difference between Pre PEMFT & Post PEMFT (p=0.001), Post PEMFT & Post LLLT (p=0.001) and there were no statistical significant difference between Pre LLLT & Post LLLT (p=0.998) (Table 2 and Fig. 2).

According to the expression C-JUN N-terminal kinase (JNK) levels

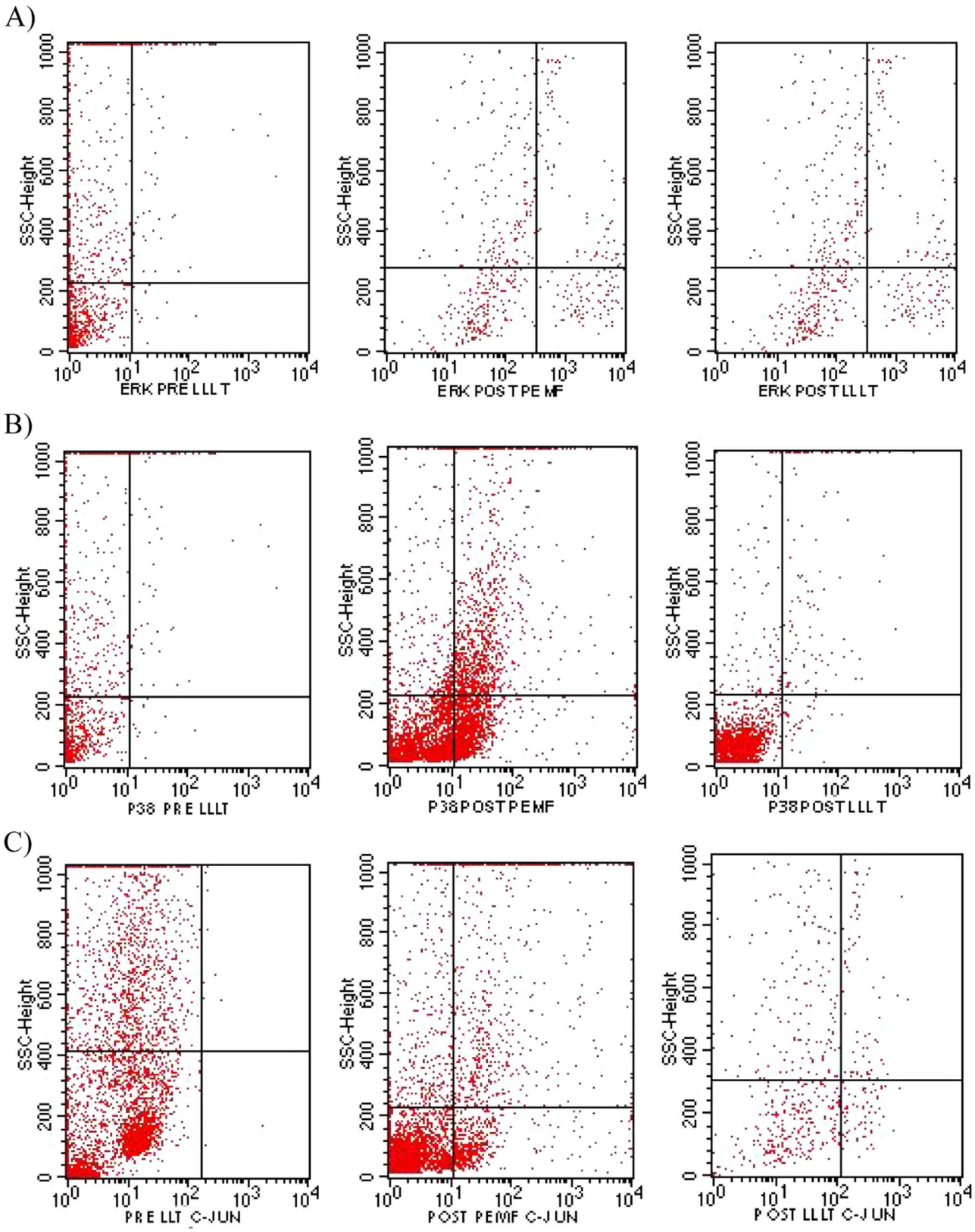

Fig. 4. Flowcytometric expression levels of ERK (A), P38 (B) and C-JUN N-terminal kinase (JNK) (C), pre & post application of PEMFT and LLLT. The expression levels were automatically calculated by the flow cytometry by measuring the expression percent in the activated lymphocytes at the upper right quadrant of the graph. The vertical axis represents the side scatter (SSC)-height and the horizontal axis represents the cell concentration.

(%), we found that there were statistical significant difference between Pre PEMFT & Post PEMFT (p=0.001) and there were no statistical significant difference Post PEMFT & Post LLLT (p=0.223) and between Pre LLLT & Post LLLT (p=0.072) (Table 3 and Fig. 3).

Some samples' results for flow cytometric expression levels of ERK, P38 and C-JUN N-terminal kinase (JNK), pre & post application of

PEMFT and LLLT were presented (Fig. 4).

4. Discussion

The findings of the present study revealed that LLLT has a direct increasing effect on ERK which was in concurrent with the results of Kiyosaki et al. [23], Meng et al. [24] and Aleksic et al. [25], as they demonstrate an increase in the osteoblast and neural cells proliferation and differentiation by the direct effect of LLLT on activation of ERK pathway. The results of our study also showed the direct activation effect of PEMFT on ERK pathway which was in agreement with the results of Li et al. [26], Pan et al. [27] and Teven et al. [28], they examined the PEMFT effect on angiogenesis, osteoprogenitor cells and the microcirculation by measuring the activation levels of ERK and they noted the direct increasing effect of PEMFT on ERK pathway activation level but it was noted that LLLT has no effect on both P38 and C-Jun N terminal activation levels.

Gavriela et al. [29] test the skeletal cell activation by the influence of low energy laser exposure by the role of MAPK ERK, P38 and C-JUN N terminals and he noted no effect on P38. Aleksic et al. [25] used Low-level Er: YAG laser irradiation to enhances osteoblast proliferation through activation of MAPK/ERK and reported that there were no effect on P38 nor C-Jun N terminal activation level by radiation of laser. PEMFT, on contrast has a potent effect on activation P38 level as noted in the present study, also when Hou et al. [30] tested the effects of magnetic field on MAPK signaling pathways of human retinal pigment epithelial cells, he noted the elevated level of P38.. Kim et al. [31] also report this result when he tested repetitive exposure to a 60-Hz time-varying magnetic field induces DNA double-strand breaks and apoptosis in human cells. Soda et al. [32] found this effect when examined the effect of exposure to an extremely low frequency-electromagnetic field on the cellular collagen with respect to signaling pathways in osteoblast-like cells. PEMFT has a powerful healing effect more than LLLT as it increase the activation of ERK, P38 and C-Jun-N Terminal while LLLT only increase the activation of ERK. LLLT has more potent pain decreasing effect than PEMFT as it does not activate P38 pathway like PEMFT.PEMFT has a high cellular biological stimulatory effect as it activates the three signaling pathways of MAPKs so it should be used with great caution concerning time and repetition of its application. PEMFT should not be used as pain killer modality in acute pain but it's very useful to be used in chronic pain management with hypothalamic –ve Ingram due to its stimulatory effect on P38 which lead to increase pain activation and perception that leads to subsequent increase the endogenous endorphins and encephaline leading to late relief of pain that explains the contradictions between researches who reported the active effect MFT on increase pain as Del et al. [33], Kavaliers et al. [34,35] and Sartucci et al. [36] and on other hand the researches that noted the active role of MFT in decreasing pain as Kavaliers et al. [37], Thomas [38] and Fleming et al. [39].

In conclusion: these data collectively demonstrate that PEMFT stimulates all pathways of MAPKs including ERK, P38 and C-Jun-terminal while LLLT stimulates only the ERK pathway in MAPKS activation pathways. So, PEMFT has a powerful healing effect more than LLLT as it increase the activation of ERK, P38 and C-Jun-N Terminal while LLLT only increase the activation of ERK. LLLT has more potent pain decreasing effect than PEMFT as it does not activate p38 pathway like PEMFT. Such achievements are valuable for the community in plasma medicine.

4.1. Recommendations

PEMFT has a high cellular biological stimulatory effect as it activates the three pathways of MAPKs so it should be used with great caution concerning time and repetition of its application. PEMFT should not be used as pain killer modality in acute pain but it's very useful to be used in chronic pain management with hypothalamic –ve Ingram due to its stimulatory effect on P38. Further larger scale studies regarding the potential advantages of using PEMFT versus LLLT in wound healing and pain management are recommended. Also further studies using molecular dynamic simulations to confirm the findings of this study are recommended.

Declaration of conflicting interests

None.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Ethical approval

The Research Committee at Faculty of Medicine-Tanta University approved this study (20/01/015).

Contributorship

AME shared in research design and gaining ethical approval, obtained informed consent from patients, enrolled patients, obtained data from the enrolled patients, applying PEMFT and LLLT, shared in interpretation of results and wrote the first draft of manuscript. RME and MHH shared in research design, obtained samples from the enrolled patients, carried out the flow-cytometric analysis, shared in statistical analysis and interpretation of results and reviewing the litrature. AB shared in interpretation of results and manuscript final revision. All authors revised and edited the manuscript and approved its final version.

References

[1] R.P. Abergel, C.A. Meeker, T.S. Lam, R.M. Dwyer, M.A. Lesavoy, J. Uitto, Control of connective tissue metabolism by lasers: recent developments and future prospects, J. Am. Acad. Dermatol. 11 (1984) 1142–1150.
[2] I.B. Kovacs, E. Mester, P. Gorog, Stimulation of wound healing with laser beam in the rat, Experientia 30 (1974) 1275–1276.
[3] D.A. Rogatkin, A.V. Dunaev, Stimulation of blood microcirculation at low level laser therapy: monitoring tools and preliminary data, JMRD 3 (2014) 100–106.
[4] T. Ozen, K. Orhan, I. Gorur, A. Ozturk, Efficacy of low level laser therapy on neurosensory recovery after injury to the inferior alveolar nerve, Head. Face Med. 2 (2006) 3.
[5] U. Oron, S. ILIC, T.L. DE, J. Streeter, J, Laser (Ga-As (808 nm) irradiation enhances ATP Productio in human neuronal cells in culture, Photomed. Laser Surg. 25 (2007) 180–182.
[6] F. Cleber, K. Beatriz, A. Pinar, H. Ying-Ying, M.V.P. de Sousa, V.S. Bagnato, N.A. Parizotto, A. nivaldo, M.R. hamblin, Low-level laser (light) therapy increases mitochondrial membrane potential and ATP Synthesis in C2C12 myotubes with a peak response at 3–6 h, Photochem. Photobiol. 91 (2015) 411–416.
[7] T. Karu, Photobiology of low power laser effect, Health Phys. 56 (1989) 691–704.
[8] S. Fine, E. Klein, W. Nowak, R. Scott, Y. Laor, L. Simpson, J. Crissey, J. Donoghue, V.E. Derr, Interaction of laser radiation with biologic systems. I. studies on interaction with tissues, Fed. Proc. (1965) 24–35.
[9] R. Lubart, H. Friedmann, M. Sinyakov, Change in calcium transport in mammalian sperm mitochodria and plasma membranes caused by 780 nm radiation, Laser Surg. 21 (1997) 493–499.
[10] T.L. Smith, D. Wong-Gibbons, J. Maultsby, Microcirculatory effects of pulsed electromagnetic fields, J. Orthop. Res. 22 (2004) 80–84.
[11] R. Selvamb, K. Ganesana, K.V.S.N. Rajub, A.C. Gangadharanc, B.M. Manohard, R. Puvanakrishnan, Low frequency and low intensity pulsed electromagnetic field exerts its anti-inflammatory effect through restoration of plasma membrane calcium ATPase activity, Life Sci. 80 (2007) 2403–2410.
[12] G.L.Y. Cheing, J.W.H. Wan, S.K. Lo, Ice And pulsed electromagnetic field To reduce pain And swelling After distal radius fractures, J. Rehabil. Med. 37 (2005) 372–377.
[13] L.Y. Sun, D.K. Hsieh, T.C. Yu, H.T. Chiu, S.F. Lu, G.H. Luo, T.K. Kuo, O.K. Lee, T.W. Chiou, Effect of pulsed electromagnetic field on the proliferation and differentiation potential of human bone marrow mesenchymal stem cells, Bioelectromagnetics 30 (2009) 251–260.
[14] V. Graak, S. Chaudhary, B.S. Bal, J.S. Sandhu, Evaluation of the efficacy of pulsed electromagnetic field in the management of patients with diabetic polyneuropathy,

Int J. Diabetes Dev. Ctries. 29 (2009) 56–61.
[15] C. Widmann, S. Gibson, M.B. Jarpe, G.L. Johnson, Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human, Physiol. Rev. 79 (1999) 143–180.
[16] G.L. Johnson, R. Lapadat, Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases, Science 298 (2002) 1911–1912.
[17] S. Torii, K. Nakayama, T. Yamamoto, E. Nishida, Regulatory mechanisms and function of ERK MAP kinases, J. Biochem. 136 (2004) 557–561.
[18] Y.J. Gao, R.R. Ji, Activation of JNK pathway in persistent pain, Neurosci. Lett. 6 (2008) 180–183.
[19] C. Huang, K. Jacobson, M.D. Schaller, MAP kinases and cell migration, J. Cell Sci. 117 (2004) 4619–4628.
[20] V. Rajendran, Structural analysis of oncogenic mutation of isocitrate dehydrogenase 1, Mol. Biosyst. 12 (2016) 2276–2287.
[21] V. Rajendran, R. Purohit, R. Sethumadhavan, In silico investigation of molecular mechanism of laminopathy caused by a point mutation (R482W) in lamin A/C protein, Amino Acids 43 (2012) 603–615.
[22] V. Rajendran, R. Sethumadhavan, Drug resistance mechanism of PncA in Mycobacterium tuberculosis, J. Biomol. Struct. Dyn. 32 (2014) 209–221.
[23] T. Kiyosaki, N. Mitsui, N. Suzuki, N. Shimizu, Low-level laser therapy stimulates mineralization via increased Runx2 expression and ERK phosphorylation in osteoblasts, Photo. Laser Surg. 28 (2010) 167–172.
[24] C. Meng, Z. He, D. Xing, Low-level laser therapy rescues dendrite atrophy via upregulating BDNF expression: implications for Alzheimer's disease, J. Neurosci. 14 (2013) 13505–13517.
[25] V. Aleksic, A. Aoki, K. Iwasaki, A.A. Takasaki, C.Y. Wang, Y. Abiko, I. Ishikawa, Y. Izumi, Low-level Er: YAG laser irradiation enhances osteoblast proliferation through activation of MAPK/ERK, Lasers Med Sci. 25 (2010) 559–569.
[26] Y. Li, X. Yan, J. Liu, L. Li, X. Hu, H. Sun, J. Tian, Pulsed electromagnetic field enhances brain-derived neurotrophic factor expression through L-type voltage-gated calcium channel- and Erk-dependent signaling pathways in neonatal rat dorsal root ganglion neurons, Neurochem Int. 75 (2014) 96–104.
[27] Y. Pan, Y. Dong, W. Hou, Z. Ji, K. Zhi, Z. Yin, H. Wen, Y. Chen, Effects of PEMF on microcirculation and angiogenesis in a model of acute hind limb ischemia in diabetic rats, Bioelectromagnetics 34 (2013) 180–188.
[28] C.M. Teven, M. Greives, R.B. Natale, Y. Su, Q. Luo, B.C. He, D. Shenaq, T.C. He, R.R. Reid, Differentiation of osteoprogenitor cells is induced by high-frequency pulsed electromagnetic fields, J. Craniofac Surg. 23 (2012) 586–593.
[29] S. Gavriela, O. Uri, I. Andrey, W. Anton, H.O. Halevy, Skeletal muscle cell activation by low-energy laser irradiation: a role for the MAPK/ERK pathway, J. Cell. Physiol. 187 (2001) 73–80.
[30] X. Hou, Y.N. Hui, Q.H. Han, X.G. Zhang, D. Hu, C.M. Guo, Effects of magnetic field on MAPK signaling pathways of human retinal pigment epithelial cells bound with beads in vitro, Zhonghua Yan Ke Za Zhi. 42 (2006) 1103–1108.
[31] J. Kim, C.S. Ha, H.J. Lee, K. Song, Repetitive exposure to a 60-Hz time-varying magnetic field induces DNA double-strand breaks and apoptosis in human cells, Biochem Biophys. Res Commun. 400 (2010) 739–744.
[32] A. Soda, T. Ikehara, Y. Kinouchi, K. Yoshizaki, Effect of exposure to an extremely low frequency- electromagnetic field on the cellular collagen with respect to signaling pathways in osteoblast-like cells, J. Med. Investig. 55 (2008) 267–278.
[33] C. Del Seppia, P. Luschi, S. Ghione, E. Crosio, E. Choleris, F. Papi, Exposure to a hypogeomagnetic field or to oscillating magnetic fields similarly reduce stress-induced analgesia in C57 male mice, Life Sci. 66 (2000) 1299–1306.
[34] M. Kavaliers, K.P. Ossenkopp, M. Hirst, Magnetic fields abolish the Enhanced nocturnal analgesic response to morphine in mice, Physiol. Behav. 32 (1984) 261–264.
[35] M. Kavaliers, K.P. Ossenkopp, Magnetic fields inhibit opioid-mediated 'analgesic' behaviours of the terrestrial snail, Cepaea nemoratis, J. Comp. Physiol. 162 (1988) 551–558.
[36] F. Sartucci, L. Bonfiglio, C. Del Seppia, P. Luschi, S. Ghione, L. Muiri, F. Papi, Changes in pain perception and pain-related somatosensory evoked potentials in humans produced by exposure to oscillating magnetic fields, Brain Res. 769 (1997) 362–366.
[37] M. Kavaliers, K.P. Ossenkopp, Repeated naloxone treatments and exposures to weak 60-Hz magnetic fields have 'analgesic' effects in snails, Brain Res. 620 (1993) 159–162.
[38] W. Thomas, M. Kavaliers, F.S. Prato, K.P. Ossenkopp, Antinociceptive effects of a pulsed magnetic field in the Eand snail, Cepaea nemoralis, Neurosci. Lett. 222 (1997) 107–110.
[39] J.L. Fleming, M.A. Persinger, S.A. Koren, Magnetic pulses elevate nociceptive thresholds: comparisons with opiate receptor compounds in normal and seizure-induced brain-damaged rats, Electro- Magn. 13 (1994) 67–75.