Osteoarthritis in patients with familial Mediterranean fever: genetic basis and anti-inflammatory effect of laser acupuncture

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Received 20 February 2019
Accepted 20 June 2019

Middle East Journal of Medical Genetics
2019,8:33–41

Background
Familial Mediterranean fever (FMF) is an autosomal recessive autoimmune disease characterized by recurrent attacks of fever, peritonitis, pleuritis, arthritis, and renal amyloidosis. Arthritis could be the presenting symptom in many cases. Laser acupuncture has analgesic and anti-inflammatory effects on joint arthritis.

Aim
To highlight the genetic basis of osteoarthritis in patients with FMF and to highlight the anti-inflammatory effect of laser acupuncture on osteoarthritis of knee joint in Egyptian patients with FMF.

Patients and methods
A randomized controlled study was performed on 40 Egyptian patients with FMF. Molecular analysis of *MEFV* gene mutations was performed for all selected patients. Twenty patients with FMF were exposed to low-level laser acupuncture sessions (laser acupuncture group) and 20 other patients with FMF did not receive low-level laser acupuncture sessions (control group). Twelve low-level laser acupuncture sessions were implemented for the group treated with laser acupuncture three times per week for a duration of 4 weeks. All patients were checked before and after laser acupuncture sessions for pain intensity, radiography changes, and some inflammatory markers (white blood cell, eosinophils, lymphocytes, erythrocyte sedimentation rate, and C-reactive protein).

Results
M694V mutation was detected in 40% of patients. Patients with FMF with knee arthritis exposed to low-level laser acupuncture sessions showed significant improvement in pain intensity compared with the control group ($P < 0.001$). Statistical significance improvement of the treated knees started from the third follow-up laser session revealed by significant improvement in the radiography findings ($P < 0.001$). The inflammatory markers white blood cell, eosinophils, lymphocytes, erythrocyte sedimentation rate, and C-reactive protein became significantly lower ($P = 0.037$, $P < 0.001$, $P < 0.001$, $P < 0.001$, and $P < 0.001$, respectively).

Conclusion
M694V variant of *MEFV* gene is more frequent in the Egyptian patients with FMF with osteoarthritis. Low-level laser acupuncture therapy is effective in ameliorating pain of osteoarthritis in patients with FMF.

Keywords:
patients with familial Mediterranean fever, low-level laser acupuncture, *M694V*, osteoarthritis

Middle East J Med Genet 8:33–41

2090-8571

Introduction

Familial Mediterranean fever (FMF) OMIM (2017) is an autosomal recessive disorder and is associated with a missense mutation in the *MEFV* gene located on chromosome 16p13.3 (Akkoc and Gul, 2011). FMF mainly affects people of Mediterranean descent (Sönmez *et al.*, 2016) with an ethnic distribution to the Turkish, Armenian, Jews, and Arab population (Sarı *et al.*, 2014). It also affects other Mediterranean populations, such as Italians and Greeks (Ben-Chetrit and Touitou, 2009). On the contrary, it was found to affect non-Mediterranean populations, like the Japanese population (Tsuchiya-Suzuki *et al.*, 2009).

The *MEFV* gene encodes for mutated protein pyrin (marenostrin), which is mainly expressed in the neutrophils, eosinophils, dendritic cells, and fibroblasts (Cantarini *et al.*, 2012). Therefore, it has an important effect on the innate immune system cells, including neutrophils, eosinophils, and cytokine-activated monocytes. These cells play a role in the exaggerated inflammatory response through the excess production of interleukin-1 (IL-1) (Davtyan *et al.*, 2008). Pyrin

is an immune-regulatory protein made up of 781 amino acids. It interacts with caspase-1 and other inflammasome components to regulate IL-1β production, nuclear factor-κB, and leukocyte apoptosis. Inflammasomes are multiprotein complexes that play a major role in both innate and adaptive immune systems (Masters *et al.*, 2009; De Torre-Minguela *et al.*, 2017). The most important inflammasome is the nucleotide-binding domain, leucine-rich repeat/pyrin domain-containing-3 (NALP3) which is required for the synthesis of IL-1β, which is implicated in the pathogenesis of FMF and other auto-inflammatory diseases (Campbell *et al.*, 2016).

The clinical manifestations of FMF are characterized by recurrent periods of fever, peritonitis, pleuritis, arthritis, erysipelas-like erythema, and long-term complications, mainly renal amyloidosis (Ben-Chetrit and Levy, 1998; Khalil *et al.*, 2018).

Arthritis is the second most common symptom and the attacks are usually mono-articular typically involving large joints of the lower limbs (hips, knees, and ankles) and develop in childhood. The attack lasts for 5–7 days (Usluer and Bircan, 2007; Kucuk *et al.*, 2014). The frequency of arthritis in FMF was reported to range between 21 and 77% in different ethnic groups (Jarjour and Dodaki, 2011). Arthritis is the presenting finding of FMF in some patients, and sometimes it remains as the only major manifestation of the disorder. Although the most common type of arthritis is recurrent, self-limited, and short lived acute inflammation of the joints, chronic forms with protracted joint effusion were occasionally reported (Majeed and Rawashdeh, 1997; Sneh *et al.*, 1997). Risk of secondary amyloidosis was also increased in patients with FMF with arthritis (Cefle *et al.*, 2005). *M694V* variation of the *MEFV* gene that is usually associated with the most severe clinical form was found to be more frequent in patients with arthritis (Brik *et al.*, 1999; Tunca *et al.*, 2005; Jarjour and Dodaki, 2011).

Clinical manifestations of arthritis in patients with FMF include joint pain, stiffness, decreased range of motion, muscle weakness, proprioceptive changes (Kaufman *et al.*, 2001), and difficulties in daily living activities such as walking, climbing/descending stairs, and housekeeping (Bennell *et al.*, 2007). Deformities and instabilities were also observed but joint pain is the dominant symptom which becomes accentuated when the joint is moved and relieved with rest. Persistent pain even during rest or at nocturnal rest may be a sign of advanced osteoarthritis (Michael *et al.*, 2010). Pain and stiffness are the two primary reasons for daily life activity and mobility disability, adversely affecting the quality of life of these patients (Hunt *et al.*, 2013). Management aims to relieve pain and improve quality of life. It also aims to increase joint mobility and function with knee stabilization, reduction of the load on the joint and most importantly, prevention of deformities and slowing the progression of the disease (Huleatt *et al.*, 2014).

Low-level laser therapy (LLLT) has been used in arthritis and for other painful conditions without any observed adverse effects (Baltzer *et al.*, 2017). LLLT has many clinical applications in the past few years. The effect of LLLT plays a beneficial effect by modulating the inflammatory process. LLLT has analgesic, anti-inflammatory, and regenerative effects providing pain relief in joint arthritis by improving the microcirculation (Hegedus *et al.*, 2009). It also exerts a positive influence on ATP synthesis on the cellular level with an increase in the cellular metabolism (Hashmi *et al.*, 2010) and cellular-molecular level on fibroblasts (Van Breugel and Bär, 1992) as well as collagen synthesis (Lam *et al.*, 1986). LLLT inhibits the pro-inflammatory mediators such as PGE2, TNF-α, IL-1β, and COX-2 and reduces MMP activity. Furthermore, it has an analgesic effect through direct irradiation without thermal adverse effect (Carlos *et al.*, 2014).

Acupuncture is one of the most popular treatments applied in traditional Chinese medicine and was used for relieving pain in musculoskeletal diseases (Liu *et al.*, 2015). Manual or electrical stimulation of the acupuncture point is the most popular form of acupuncture therapy (Carbioglu *et al.*, 2006; Mao and Kapur, 2010). Laser acupuncture using a low-intensity laser is a therapeutic method equivalent to needle acupuncture with respect to the selection of acupuncture points (Chow *et al.*, 2012). Laser beams are characterized by being monochromatic, coherent, and collimated. Energy power, wavelength, and energy density are the three important parameters of laser acupuncture. Energy power of the LLLT was considered as the treatment dose (Glazov *et al.*, 2016). Stimulating certain acupuncture points on the human body can release certain chemical mediators like dynorphins and endorphins which have anti-inflammatory effects and play a major role in pain relief (Mohammed *et al.*, 2018).

The aim of the present study was to highlight the genetic basis of osteoarthritis in patients with FMF and to assess the effectiveness of laser acupuncture on the reduction of pain and improvement in the function of the knee joint. Additionally, we aimed to investigate the anti-inflammatory effect of laser acupuncture on patients with FMF by investigating the effect of laser acupuncture on the inflammatory markers [white blood cell (WBC), eosinophils, lymphocytes,

erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP)] in patients with FMF.

Patients and methods

A randomized controlled study was performed on 40 Egyptian patients with FMF. Patients were recruited from the Clinical Genetics Department in the National Research Centre, Egypt, and were referred to the Complementary Medicine Department in the center of excellence, National Research Centre, Egypt, from October 2017 to November 2017. The study was approved by the Ethical Research Committee of the National Research Centre (ethical approval number 17153) and was conducted in accordance with National Research Centre by the laws for human research. It conforms to the provisions of the Declaration of Helsinki in 2000. Parents or caregivers gave written informed consent to the study.

Patients were subjected to detailed history (including demographic data, age at the onset, detailed pedigree construction, and analysis, with special emphasis on parental consanguinity, a similar disease in the family, and treatment modalities). The diagnosis was confirmed by molecular analysis of *MEFV* gene mutations by PCR amplification followed by DNA sequencing analysis of exon 10 and RFLP for E148Q mutation in exon 2 (recorded hot-spots) (Zarouk *et al.*, 2018). All selected patients experienced knee osteoarthritis at least for 3 months that did not resolve on ordinary painkillers and anti-inflammatory drugs in spite of regular doses of colchicine.

Patients were divided into two groups:

Group A (study group): it included 20 patients with FMF. Their ages ranged from 7 to 15 years, and they were exposed to laser acupuncture sessions (laser acupuncture group)

Group B (control group): it included 20 age-matched and sex-matched patients with FMF who served as controls. Their ages ranged from 6 to 14 years; they did not receive laser acupuncture sessions (control group).

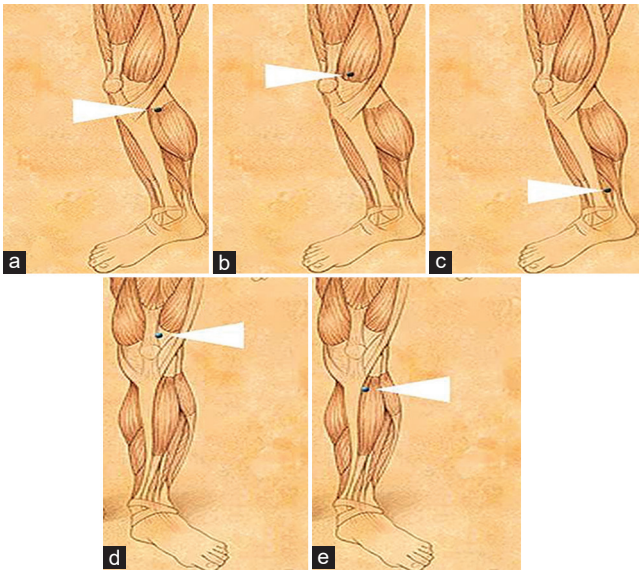
Twelve laser acupuncture sessions were performed to group A patients (20 patients with FMF) three times per week for a duration of 4 weeks using a single probed cold semiconductor laser device, with power = 100 mw and wavelength = 808 nm. The maximum dose (in J) will be 5 J/cm^2 which is suitable for children according to the World Association of Laser Therapy rules 2017 (World Association of Laser Therapy, 2017). We used the device on traditional Chinese acupoints for knee osteoarthritis as follows: Spleen 9 (Yanglinquan, SP9), Spleen 10 (Xuehai, SP10), Stomach 34 (Liangqui, ST34), Stomach 36 (Zusanli, ST36), and Spleen 6 (Sanyinjiao). Acupoints are points for maximum pain in the joint, which differs from one case to the other. Only extra-articular points around the knee joint were selected, to reduce the chance of infection. Different sites are illustrated in Fig. 1.

All patients were examined before and after low-level laser acupuncture sessions depending on pain intensity [no pain, mild, moderate, and severe pain according to visual analog scale (VAS)]. The linear scale is the visual representation of the range of pain that a patient believes he or she might experience. The range is represented by a line usually 100 mm in length with or without marks at each centimeter. One end represents 'no pain' whereas the other represents the worst pain the patient could imagine. Pain VAS score ranges from 0 to 100 mm: no pain (0–4 mm), mild pain (5–44 mm), moderate pain (45–74 mm), and severe pain (75–100 mm) (Hawker *et al.*, 2011).

Investigations

It included radiography findings and inflammatory markers (WBC, eosinophils, lymphocytes, ESR, and CRP). Plain radiography was performed to verify the degree of knee osteoarthritis. Radiography findings were divided into five grades based on Kellgren and

Figure 1



(a) Spleen 10: with the knee flexed, 2 cun (cun = 2.5 cm) above the superior medial border of the patella on the bulge of the medial portion of quadriceps femoris (vastus medialis). (b) Stomach 34: when the knee is flexed, on the anterior aspect of the thigh, on the line connecting the anterior superior iliac spine and the lower lateral border of the patella, 2 cun above the patella. (c) Spleen 6: 3 cun directly superior to the tip of the medial malleolus on the posterior border of the tibia. (d) Spleen 9: under the medial condyle of the tibia, in the depression posterior and inferior to the medial border of the tibia. (e) Stomach 36: 2 cun lateral to the tibial tuberosity.

Lawrence system for classification of osteoarthritis of knee [grade 0: no radiographic features of osteoarthritis were present, grade 1: doubtful joint space narrowing (JSN) and possible osteophytic lipping, grade 2: definite osteophytes and possible JSN on anteroposterior weight-bearing radiograph, grade 3: multiple osteophytes, definite JSN, sclerosis, and possible bony deformity, and grade 4: large osteophytes, marked JSN, severe sclerosis, and definite bony deformity] (Kellgren and Lawrence, 1957). A 5-ml blood sample was taken for a complete blood count to test for the total leukocytic (WBCs) count with differential for the eosinophils and lymphocytes in addition to ESR level and CRP, before and after the laser sessions.

Statistical analysis

The collected data were coded, tabulated, and statistically analyzed using IBM SPSS statistics (Statistical Package for Social Sciences) software (version 18.0, 2009; IBM Corp., Chicago, Illinois, USA).

Descriptive statistics were done for quantitative data as minimum and maximum of the range as well as mean ± SD for quantitative normally distributed data, median and first and third interquartile range for quantitative nonnormally distributed data, whereas for qualitative data as number and percentage.

Inferential analyses were done for quantitative variables using independent *t* test in cases of two independent groups with normally distributed data and paired *t* test in cases of two dependent groups with normally distributed data. In qualitative data, inferential analyses for independent variables were done using χ^2 test for differences between proportions and Fisher's exact test for variables with small expected numbers. The level of significance taken at *P* value less than 0.050 is significant.

Results

Demographic data for patients with FMF and controls are represented in Table 1. There were no statistically significant differences between the study and control groups. Positive consanguinity was found in 60% of cases in the study group and 55% of cases in the control group. Positive family history was present in 85% in both groups. Overall, 40% of patients with FMF had *M694V* mutation (Table 2 and Fig. 2).

All 20 patients with FMF in the study population completed the treatment regimen with good compliance, and the regimen was well tolerated. The knee joints were treated by 12 laser acupuncture sessions three times per week for a duration of 4 weeks. No adverse effects or any patient discomfort was reported during the treatment course. There was no significant difference between the study group and the control group regarding the basal pain (*P* value using Fisher's exact test 0.827), which became significantly lower in the study group than in the control group

Table 1 Demographic characteristics among the studied groups

Variables	Study (*n*=20)	Control (*n*=20)	*P*
Age (years)			
Mean±SD	10.4±2.5	10.5±2.5	0.898[a]
Range	7.0-15.0	6.0-14.0	
Sex [*n* (%)]			
Male	11 (55.0)	12 (60.0)	0.749[b]
Female	9 (45.0)	8 (40.0)	
Duration (years)			
Mean±SD	3.9±2.5	4.1±2.3	0.794[a]
Range	1.0-10.0	1.0-9.0	
Consanguinity [*n* (%)]	12 (60.0)	11 (55.0)	0.749[b]
Family history [*n* (%)]	17 (85.0)	17 (85.0)	1.000[b]

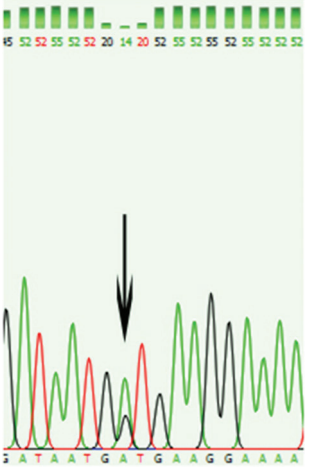
[a]Independent *t*-test. [b]χ^2 test.

Table 2 Mutation results of 40 patients with familial Mediterranean fever with knee osteoarthritis

Mutation	Number of mutations in patients with FMF with arthritis [*n* (%)]
M694V	16 (40)
M694I	10 (25)
M680I	6 (15)
E148Q	4 (10)
V726A	2 (5)
R761H	-
A744S	2 (5)
K695R	-
P369S	-
F479L	-
I692del	-

FMF, familial Mediterranean fever.

Figure 2



Partial exon 10 sequence chromatogram of *MEFV* gene showing heterozygous M694V [AG] variant.

after treatment ($P < 0.001$). Improvement outcomes for the treated knees reached statistical significance starting from the third follow-up session (Table 3). Most of the improvements in pain sensation occurred between the third and fifth follow-ups. A comparison between the study and the control groups regarding the basal radiography findings showed no significant difference ($P = 1$). A significant improvement was noticed in the study group after treatment than in the control group ($P < 0.001$). Radiography findings in the study group before and after treatment showed a significant difference ($P < 0.001$) (Table 4). There was no significant difference between the study and the control groups regarding the basal levels of WBC, eosinophils, lymphocytes, ESR, and CRP ($P = 0.97$, 0.845, 0.657, 0.985, and 1, respectively), which became significantly lower in study group than in the control group after treatment ($P = 0.037$, $P < 0.001$, $P < 0.001$, $P < 0.001$, and $P < 0.001$, respectively). The levels of WBC, eosinophils, lymphocytes, ESR, and CRP in the study group before and after treatment decreased significantly ($P = 0.027$, $P < 0.001$, $P < 0.001$, $P < 0.001$, and $P < 0.001$, respectively) (Table 5). The overall significant improvements in all the studied factors in the study group compared with the control group are illustrated in Figs. 2 and 3.

Table 3 Pain among the studied groups

Time	No	Mild	Moderate	Severe	From basal[a]
Study group (*n*=20) [*n* (%)]					
Before	0 (0.0)	6 (15.0)	28 (70.0)	6 (15.0)	-
FU1	0 (0.0)	6 (15.0)	29 (72.5)	5 (12.5)	1.000
FU2	0 (0.0)	6 (15.0)	29 (72.5)	5 (12.5)	1.000
FU3	1 (2.5)	15 (37.5)	19 (47.5)	5 (12.5)	<0.001*
FU4	9 (22.5)	22 (55.0)	9 (22.5)	0 (0.0)	<0.001*
FU5	14 (35.0)	25 (62.5)	1 (2.5)	0 (0.0)	<0.001*
FU6	28 (70.0)	12 (30.0)	0 (0.0)	0 (0.0)	<0.001*
FU7	30 (75.0)	10 (25.0)	0 (0.0)	0 (0.0)	<0.001*
FU8	39 (97.5)	1 (2.5)	0 (0.0)	0 (0.0)	<0.001*
FU9	40 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	<0.001*
FU10	40 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	<0.001*
FU11	40 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	<0.001*
After	40 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	<0.001*
Control group (*n*=20) [*n* (%)]					
Before	0 (0.0)	6 (15.0)	30 (75.0)	4 (10.0)	-
After	0 (0.0)	4 (10.0)	32 (80.0)	4 (10.0)	1.000

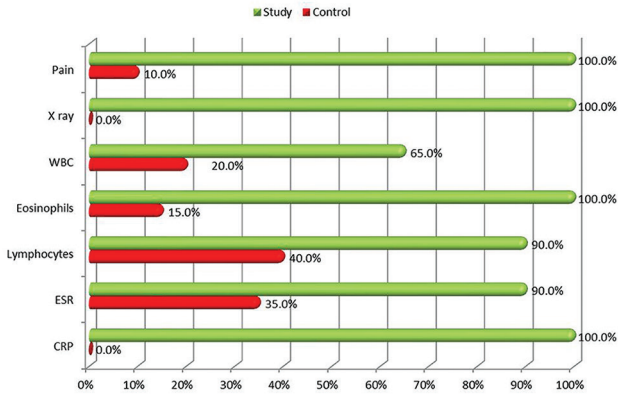
FU, follow up. [a]McNemar test. *Significant.

Discussion

FMF is characterized by recurrent febrile attacks of peritonitis, pleuritis, and arthritis. The high-grade fever and severe pain leave the patient bedridden during the attack. Arthritis is a major and common feature of FMF. The most common arthritic attack of FMF is acute large joint mono-arthritis, most often affecting the knee and hip joints, lasting for several days (Onen, 2006). Patients with FMF with arthritis have a younger age of onset and are more prone to erysipelas-like erythema, myalgia, and vasculitis (Tunca *et al.*, 2005).

There are no specific criteria to differentiate arthritis accompanying FMF from arthritis of other inflammatory conditions. This may lead to delayed diagnosis and the emergence of complications. Therefore, molecular analysis is an essential tool to confirm the diagnosis and give the appropriate treatment. In the present study, *MEFV* gene mutation was present in all patients with FMF (100%) who presented with knee osteoarthritis which supports the role of *MEFV* gene mutation in the inflammatory process and development of arthritis in these patients. Tunca *et al.* (1999) showed in their study that patients with FMF with *MEFV* mutations had increased levels of inflammatory markers. The relationship of *MEFV* gene mutation and many inflammatory diseases was also supported by studies that showed that *MEFV* mutation plays a role in the course and severity of

Figure 3



Improvements among the studied groups.

Table 4 Radiography findings among the studied groups

Time	Grade	Study (*n*=40) [*n* (%)]	Control (*n*=40) [*n* (%)]	Study/control[b]
Before	Grade 1 and 2	8 (20.0)	8 (20.0)	1.000
	Grade 3 and 4	32 (80.0)	32 (80.0)	
After	Grade 0	33 (82.5)	0 (0.0)	
	Grade 1 and 2	7 (17.5)	8 (20.0)	<0.001*
	Grade 3 and 4	0 (0.0)	32 (80.0)	
Improvement		40 (100.0)	0 (0.0)	<0.001*
Before/fter[a]		<0.001*	1.000	

[a]McNemar test. [b]χ^2 test. *Significant.

Table 5 Laboratory findings among the studied groups

Variables	Study (*n*=20)	Control (*n*=20)	*P* (study/control)
WBC (×10³/mm³)			
Before			
Mean±SD	7.8±2.4	7.9±1.8	0.970[a]
Range	5.0-12.0	5.0-11.0	
After			
Mean±SD	6.4±2.4	7.8±1.6	0.037*,[a]
Range	3.0-12.0	5.0-11.0	
P (before/after)[b]	0.027*	0.716	
Eosinophils%			
Before			
Mean±SD	4.5±2.5	4.7±2.4	0.845[a]
Range	1.0-9.0	1.0-9.0	
After			
Mean±SD	0.9±1.0	4.7±2.4	<0.001*,[a]
Range	0.0-3.0	0.0-9.0	
P (before/after)[b]	<0.001*	0.666	
Lymphocytes%			
Before			
Mean±SD	56.3±7.4	57.4±8.8	0.657[a]
Range	42.0-70.0	40.0-74.0	
After			
Mean±SD	39.7±7.8	56.1±8.2	<0.001*,[a]
Range	20.0-50.0	40.0-72.0	
P (before/after)[b]	<0.001*	0.433	
ESR (mm/h)			
Before			
Mean±SD	16.2±8.5	16.1±8.5	0.985
Range	3.0-33.0	3.0-34.0	
After			
Mean±SD	6.3±2.7	16.1±8.5	<0.001*
Range	3.0-11.0	4.0-34.0	
P (before/after)[b]	<0.001*	0.379	
CRP [*n* (%)]			
Before			
Mean±SD	16 (80.0)	17 (75.0)	1.000[d]
Range	4 (20.0)	3 (15.0)	
After			
Mean±SD	20 (100.0)	17 (75.0)	<0.001*,[d]
Range	0 (0.0)	3 (15.0)	
P (before/after)[c]	<0.001*	1.000	

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; WBC, white blood cell. [a]Independent *t* test. [b]Paired *t* test. [c]McNemar test. [d]Fisher's exact test. *Significant.

such diseases like ankylosing spondylitis, rheumatoid arthritis, Behcet disease, ulcerative colitis, and juvenile rheumatoid arthritis (Booth *et al.*, 2001; Ozen *et al.*, 2003; Rabinovich *et al.*, 2005; Cosan *et al.*, 2006; Giaglis *et al.*, 2006; Rabinovich *et al.*, 2007; Kholoussi *et al.*, 2018).

In our study, molecular analysis of the *MEFV* gene revealed the M694V variant in 40% of the patients. The other most common mutations detected in our patients were M694I (25%), M680I (15%), E148Q (10%), V726A (5%), and A744S (5%). This was consistent with other studies which reported a higher incidence of M694V variant in cases of patients with FMF with arthritis (Brik *et al.*, 2001; Olgun *et al.*, 2005; Tunca *et al.*, 2005; Jarjour and Dodaki, 2011; Sarikaya *et al.*, 2012). However, some studies did not find any genotype–phenotype correlation (Yalcinkaya *et al.*, 2000; Ertekin *et al.*, 2005).

Higher consanguinity in our patients (57.5%) was consistent with the autosomal recessive inheritance of FMF. Although FMF affects both males and females in a similar ratio, male affection was slightly higher (55–60%) than female affection in our patients, whereas positive family history was present in 85% of our cases. This was consistent with Sohar *et al.* (1967) who reported a male predominance.

In Asian traditional medicine, acupuncture is considered as one of the most effective treatment modalities, especially for diseases accompanied by severe pain (Singh, 1998). Acupuncture aims to stimulate anatomical points. This could be achieved by needles, manual pressure, electrical stimulation, magnets, low-level laser, heat, and ultrasound. The purpose of using this noninvasive technique is to achieve the best healing effect with the minimal anatomical and physiological interventions to reach the highest standards of health care and minimize rehabilitation needs (Dong, 2018). Manual or electrical stimulation of the acupuncture point is the most popular form of acupuncture therapy by stimulation of thin, solid, metallic needles inserted into the skin (Carbioglu *et al.*, 2006). A meta-analysis done by Vickers *et al.* (2018) revealed that acupuncture was effective for the treatment of chronic pain, including osteoarthritis. In addition, a study done by Tu *et al.* (2019) showed a significant effect on using manual and electro-acupuncture for knee osteoarthritis.

Many studies showed the beneficial effect of using LLLT throughout certain wavelength application on the body surface to treat joint inflammation by relieving the pain and improving the cell function (Jamtvedt *et al.*, 2007; Bjordal *et al.*, 2008; de Paula Gomes *et al.*, 2018). The advantage of using LLLT is that it is an effective, noninvasive, safe, easily applied and cost-effective technique to treat the joint inflammation (Cetiner *et al.*, 2006).

The present study revealed significant pain relief on using low-level laser acupuncture therapy in knee osteoarthritis. All patients showed full relief to mild pain, based on the pain VAS score. This is consistent with a study done by Bjordal *et al.* (2007) who reported pain relief in knee osteoarthritis after 2–4 weeks of LLLT. In addition, Al Rashoud *et al.* (2014) studied the effect of LLLT applied to acupuncture points on the knee joint for treatment of grade 2 knee osteoarthritis

and showed a significant improvement in pain on VAS and increase in serum beta-endorphin. Moreover, David (2015) reported in his study the beneficial effect of using LLLT in pain relief of knee osteoarthritis and mentioned that LLLT reduced the need for joint replacement surgery. However, Brosseau *et al.* (2004) reported conflicting results, and they owed this conflict to the variability in the method of application of LLLT by the wavelength, frequency, duration, dosage, and site of application points of LLLT. They recommended the use of the dosage indicated by the World Association for Laser Therapy. Another study conducted by Huang *et al.* (2015) showed no benefit of LLLT on knee osteoarthritis.

The protein pyrin which is encoded by the *MEFV* gene is an immune-regulatory protein and has a significant effect on the innate immune system cells including neutrophils and eosinophils leading to exaggerated inflammatory response (Davtyan *et al.*, 2008). Similarly, perivascular autoimmune infiltrate of lymphocytes occurs nonspecifically as part of the inflammatory process especially joint arthritis (Barzilai *et al.*, 2000; Pettit *et al.*, 2001). Our study showed an elevation in the inflammatory markers WBC, eosinophils, lymphocytes, ESR, and CRP. This indicated the significant role of these elevated markers in osteoarthritis in patients with FMF. This was in agreement with previous studies that revealed elevated WBC, ESR, CRP, and other acute-phase reactants during the acute attacks in patients with FMF (Gang *et al.*, 1999; Ben-Zvi and Livneh, 2011). Additionally, Mercan *et al.* (2015) revealed in their study elevated ESR and CRP levels and attributed the persistently high ESR and CRP in patients with FMF to active FMF disease, spondyloarthritis, and inflammatory bowel disease. Many studies demonstrated shifts in platelet, lymphocyte, neutrophil, and monocyte counts in autoimmune inflammatory disorders like rheumatoid arthritis, systemic lupus erythematosus, and inflammatory rheumatic disease (Uslu *et al.*, 2015; Wu *et al.*, 2016; Gasparyan *et al.*, 2019).

As observed in our study, there was a significant improvement in the inflammatory markers after the laser treatment. This indicates the effect of LLLT in reducing the inflammatory process in patients with FMF. Furthermore, a study by Erer *et al.* (2016) used the inflammatory markers ESR, CRP, and serum amyloid A to monitor the disease activity.

Conclusion

In conclusion, M694V variant of the *MEFV* gene is more common in the Egyptian patients with FMF with osteoarthritis. In addition, low-level laser acupuncture offers therapeutic benefits for the treatment of knee osteoarthritis in patients with FMF, through the reduction of painful swollen joints. These therapeutic effects may also be through reducing inflammation in patients with FMF after laser acupuncture. We suggest mandatory molecular analysis for all patients with FMF with monitoring disease activity, applying laser acupuncture to the affected joints, and follow-up by the inflammatory markers.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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