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Ten week pulsed electromagnetic therapy and exercise training on strength of femur bone & estrogen hormone in Sprague Dawley rat

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ABSTRACT

BACKGROUND: Osteoporosis is mentioned for reduction of skeletal mass and destruction of microscopic structure of bone and these changes result the increase of frangibility and at last the increase risk of bone fraction. **Purpose**: the purpose of current study is to examine the effect ten week pulsed electromagnetic therapy and exercise training on strength of femur bone & estrogen hormone in Sprague Dawley rat **METHODS:** Thirty female 2 months old Sprgue Dawley rats with the weight of 200±5 gram in every phase of menopause provided from Shiraz University Laboratory. Animals were randomly divided into 3 equal groups including Group 1 as control (C), Group 2 undergoing training exercise (E) & Group 3 undergoing electromagnetic therapy. All animals were housed identically as five rats per cage in a condition of 12 hours light/dark cycle with environmental temperature of 21±2°C and relative humidity of 60±5%. The exercise training consisted of running on a flatted treadmill (IRAN) 3 days/weak for 10 weeks. The speed of treadmill gradually increased from 15 m/min to 19 m/min and the duration of each exercise started from 6 min in the first week and reached1 8 min in the last week of exercise training (10th week).and electromagnetic therapy with 51 MT/10 intensity and 30 min duration 3times a week. After end of 10 weeks exercise training all rats from each group was bleeding for determination of estrogen hormone and then killed for assigning of strength of femur. **RESULTS:** in this research it was observed that there is significant difference in strength of femur (P<0.05).but no significant difference in estrogen hormone (P>0.05). **CONCLUSION:** According to the results of this study can be said that electromagnetic therapy and training exercise can significantly contribute to the strength of the femur. It is recommended this study was conducted in a clinical setting to allow the results to determine design guidelines for the treatment of this condition and perhaps more appropriate to measure estrogen levels during exercise

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INTRODUCTION

One of the problems that occur after the age of 40-50 years is Osteoporosis. Osteoporosis is a disease characterized by reduced density and quality of bone, leading to weakness of the skeleton, fractures, mostly hospitalization [1,2,3,4], there is no treatment for osteoporosis therefore, the best way of preventing osteoporosis is to maximize peak bone mass during the growth period and reduce risk factors for bone loss [5,6,7,8,9,10]. Most fractures caused by osteoporosis have significant side effects. The devastating consequence of the effects of osteoporosis is femur fractures that are associated with many deaths. It is estimated that proximately 740000 deaths per year are associated with hip fracture. In the United States, about 7 percent of survivors suffer permanent disability of all types of fractures caused by osteoporosis and 8% eventually require long-term nursing home care [11]. Hip fractures are always in need of hospitalization and patients with get acute complications of immobility, such as pressure ulcers, urinary tract infections and also has a significant effect on

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a person's ability walking.40% of patients were disable to walk independently after the first year after the fracture and 60% of those who need help in at least one of the basic activities of daily living (For example, dressing, bathing) 80% unable to perform at least one activity of daily living such as driving or shopping [12].

Numerous studies have demonstrated the effectiveness of exercise in order to promote and maintain bone density, strength and balance which can reduce the risk of bone fractures. Should be noted that although the rate of bone loss during menopause significantly increases but fractures related to, osteoporosis after menopause it reaches highest level [13, 14]. The disease process begins after age 35 in men. In adults, bone mineral gradually reduced if bone mineral doesn’t not enough in life a person is greatly at risk for bone fractures. Hip fractures caused by osteoporosis, it takes more than a third of medical costs**.** That reflecting the broad impact of fractures -related of osteoporosis in elderly people used not only for economic but also for the whole world is engaged in the healthcare and osteoporosis prevalence is higher in Asian countries [15,16].

We have a lot of research to find effective programs to prevent this disease. Several studies have shown that low levels of estrogen in patients after menopause is associated with low levels, bone mass [17,18,19,20]. Magnetic therapy, or magnotherapy, including the use of a magnetic field. Doctors claim that exposure to certain magnetic fields are beneficial health effects to the body and the protein hemoglobin, which carries oxygen, is weakly magnetic effects and Magnetic can be effective in increasing blood circulation. Pulse Electro-magnetic therapy increase formation of osteoblast cells with Continuous decrease in the production of local factors [21,22]. It also increases the speed of recovery of the connective tissue and can be production and regulation of extracellular matrix [23,24. So the purpose of current study is to examine the effect ten week pulsed electromagnetic therapy and exercise training on strength of femur bone & estrogen hormone in Sprague Dawley rat.

MATERIALS AND METHODS

Thirty female 2 months old Sprgue Dawley rats with the weight of 200±5 gram provided from Laboratory Animal Center of Shiraz University of Medical Sciences were randomly divided into 3 equal groups including Group 1 as control (C), Group 2 undergoing training exercise (E) & Group 3 undergoing electromagnetic therapy.

All animals were housed identically as five rats per cage in a condition of 12 hours light/dark cycle with environmental temperature of 21±2°C and relative humidity of 50%. They were fed with standard pellets and had access to food and water ad libitum. The enrolled rats were in all menstrual cycles (proestrous, estrous, diestrous, metestrous). Animal selection, all experiments, subsequent care and sacrifice procedure were all adhered to the same guide lines under supervision of Animal Care Committee of Iran Veterinary Organization. All experiments were carried out under aseptic conditions in Comparative Medicine Research Center of Shiraz University of Medical Sciences. The protocol of anesthesia, surgical procedure, postoperative care and sacrifice were identical for all animals.

Exercise training:

The exercise training in groups of 2 consisted of running on a flatted treadmill (Shiraz, Iran) 3 days/weak for 10 weeks. The speed of treadmill gradually increased from 15 m/min to 19 m/min and the duration of each exercise started from 6 min in the first week and reached1 8 min in the last week of exercise training (10th week)(table 1-4) .All exercises were performed in the morning and for adaptation to further experiments, they underwent exercise for one week (5 times/week) with a speed of 12 m/min and the duration of 3 min. No electric shock or artificial stimulation was used at during the study.

دهم	نهم	هشتم	هفتم	ششم	پنجم	چهارم	سوم	دوم	اول	
19	19	19	19	19	15	15	15	15	1	سرعت(متر بر دقیقه)
18	18	15	15	12	12	9	9	6	6	زمان(دقیقه)

Exercise training protocol table1 (25):

Electromagnetic therapy protocle:

Electromagnetic therapy with BTL 5000 Seri ES magnet in besat physiotherapy clinic in shiraz city which 51 MT/10 intensity and 30 min duration 3 times a week for 10 weeks. After 10 weeks, all rats were anesthetized with a mixture of 2% xylazine and 10% ketamine (8 and 90 mg/kg respectively).

Blood sample:

They were bled under anesthesia from their heart. The blood sample was centrifuged (Model: Behdad Iran) with 3000 rpm speed for 10 min. The collected serum samples were transferred into tubes and were kept in -20°C for further tests. Then using ELISA test, estrogen (Monobind,USA, 92630) levels were determined.

Biomechanical Testing:

Mechanical strength of the diaphysis long bones was evaluated by compression test using a material testing machine (H505ks, honsfield, England) in the Fars Standard Institute.

Each bone was placed horizontally between two quadrate plate shapes and was cut using an electric saw at medial of diaphesis. A speed of 50mm/min for femur fracture occurred ultimate load was determined from the load number by connected computer.

Statistical analysis:

Statistical analysis was performed using SPSS software (version 16.0, Chicago, IL, USA) If data was normal we used Shapiro-wilk test [26], Scaling Groups (Levene statistic) with One-Way ANOVA and Scheffe following test and if data was abnormal we Kruskal- Wallis H and Mann-Whitney U tests and a $p \leq 0.05$ was considered significant.

Results:

After 10 weeks, strength of femur bone 7/05, 8/29 and8/60 **mm/m** in groups C, E and M respectively while the changes was statistically significant between groups C and E (p=0.036) and groups C and M (p=0.03) (Figure 1,a)

For estrogen level, the changes were respectively 10/45, 11/33 and 13/68 pg/ml in groups C, E and M respectively while between groups, the differences were not significant (Figure 1b).

Discussion:

Recent studies have shown that the risk of osteoporosis is lower for rat who are active, and used electromagneto therapy especially those who do load-bearing, or weight-bearing activities at least three times a week[27,28].

Bone mass has been estimated to account for up to 65% of the variation in bone strength and hence fractures risk; at any age this is a reflection of the amount of bone laid down during growth (peak bone mass) and the amount of bone lost subsequently [29,30].

Osteoporotic fractures are a crippling occurrence and are an obvious impediment for most of the activities of daily living. A multitude of factors determine bone strength: genetic, nutritional (calcium), physical activity, and hormonal factors. As the population ages, medical and social costs associated with skeletal fragility resulting in huge economic burden on society. Osteoporosis is one of the biggest health problems in the world that causes fragility bones. It possible complication of osteoporosis begins in childhood that poor nutrition and physical inactivity is combined together and prevents sufficient calcium deposits. This disease is very common among the human populations and is one of the most serious health problems in the elderly men because the effect of reducing the amount of calcification of bone tissue [31].

This finding is similar with research of Hong-fei Shi et al that observed electromagnetic pulse is effective in a three-month delay between the 16 weeks to 6 months in patients with long-bone fusion were fracture treatment. Patients treated with an early application of Pulsed electromagnetic field got a significantly increased rate of union and an overall reduced suffering time compared with patients that archive Pulsed electromagnetic field after the 6 months or more of delayed union, as described by others [32].

Assiotis et al(2012) observed that pulsed electromagnetic therapy can be efective as a non-invasive method for repairing not infection the delayed unions and nonunions tibia[31].

Fredericks et al (2000) observed that pulsed electromagnetic therapy can be effective on bone healing in an animal model of rabbit tibial osteotomy and rabbits gain rapid callus formation in tibia and increased torsional strength of bone [33]. Also in this research observed that exercise caused a significant increase in hip strength compared with the control group. Both human and animal studies have shown that moderate exercise can add a significant amount of new bone to the growing skeleton [34,35,36,37].

The mechanical load imposed by exercise results in gains in bone mass and strength [38]. It is also not surprising to find that there is a dynamic regulatory system in bone that tunes its strength by alterations in the amount and orientation of bone present at each location in the skeleton [39]. In all studied, researchers investigated effect of electromagnetic pulses in patients with non-union or delayed union of the fracture. In this regard, the effect of magnetic therapy on healthy bone strength is not investigated. That had an impact on healthy bone rats hip. According to the results of this study can be said that electromagnetic therapy and training exercise can significantly contribute to the strength of the femur. It is recommended this study was conducted in

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a clinical setting to allow the results to determine design guidelines for the treatment of this condition and perhaps more appropriate to measure estrogen levels during exercise

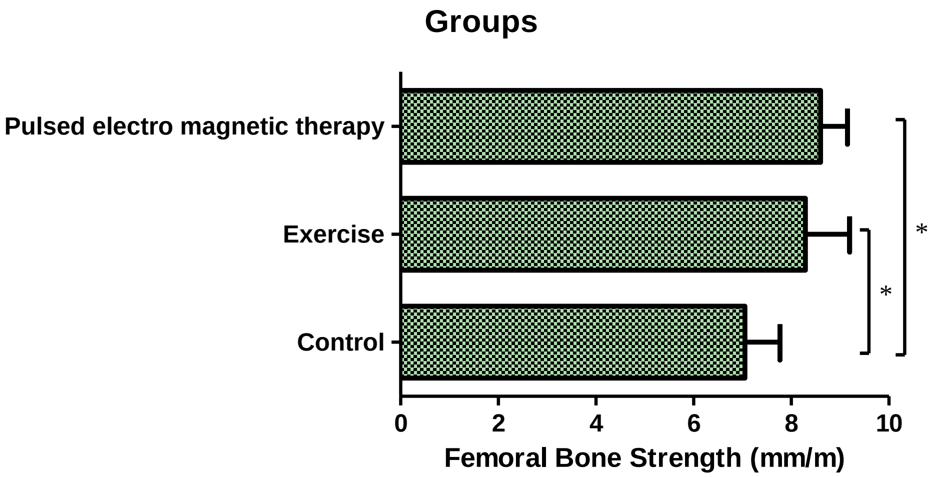

Fig.1,a: The femoral bone strength

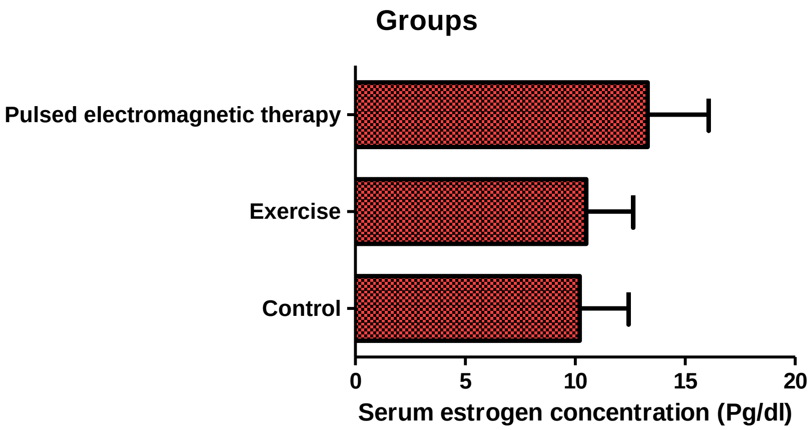

Fig. 1b: The level of estrogen hormone after 10 weeks

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