ORIGINAL ARTICLE

UDC: 616.61-008.6-08:[616.71-08:615.82/.84
https://doi.org/10.2298/VSP160617212R

The effects of extreme low frequency pulsed electromagnetic field on bone mineral density and incidence of fractures in patients with end – stage renal disease on dialysis – three year follow up study

Efekti pulsnog elektromagnetnog polja ekstremno niske frekvencije na gustinu kosti i incidenciju preloma kod bolesnika sa terminalnom bubrežnom slabošću na dijalizi: trogodišnja studija praćenja

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Abstract

Background/Aim. A variety of physical therapy options has been developed for the treatment of musculoskeletal disorders including those characterized with low bone mineral density (BMD). Extreme low frequency pulsed electromagnetic field (ELF-PEMF) can accelerate bone formation. Patients with end stage of renal disease (ESRD) are predisposed to high incidence of fractures due to bone disorder with multifactorial pathogenesis. Vitamin D, calcium supplements, antiresorptive and anabolic drugs in those patients have changed pharmacodynamics and pharmacokinetics and have minimal or limited effects. The aim of this study was to assess the effectiveness of long-term ELF-PEMF therapy applied in concordance with physical exercise on bone mass, incidence of new bone fractures and parathyroid hormone concentrations in ESRD patients on dialysis. **Methods.** In this 3-year prospective clinical trial, 151 patients with ESRD on dialysis program were subjected to treatment with ELF-PEMF (18 Hz, 2 mT) applied during 40 min after 10 consecutive dialysis procedures, 4 times through one year (120 treatments in total during three years) together with kinesitherapy (study group) or only with kinesitherapy (control group) on the voluntary basis. **Results.** Total of 124 patients have completed the study. In the study group (n = 54), regardless of sex, significant improvements of BMD, T-score and Z-score on both lumbar spine and femoral neck were achieved after 3-year treatment with ELF-PEMF. In the control group (n = 70), significant decreases of BMD, T-score and Z-score as well as the higher incidence of new bone fractures were recorded. **Conclusion.** ELF-PEMF could be a convenient and safe non-pharmacological therapeutic strategy for fracture prevention in nephrology practices.

Key words:
kidney failure, chronic; renal dialysis; electromagnetic fields; bone density; fractures, bones; incidence; treatment outcome.

Apstrakt

Uvod/Cilj. Različite metode fizikalne terapije koriste se u lečenju mišićno-skeletnih oboljenja uključujući i ona koja se karakterišu sniženom mineralnom koštanom gustinom (BMD). Pulsno elektromagnetno polje ekstremno niske frekvencije (ELF-PEMF) stimuliše formiranje koštanog tkiva. Zbog poremećaja koštanog tkiva multifaktorijalne patogeneze bolesnici sa terminalnom bubrežnom slabošću (TBS) imaju visoku učestalost preloma. Vitamin D, suplementi kalcijuma, antiresorptivni i anabolički lekovi kod ovih bolesnika, zbog izmenjene farmakodinamike i farmakokinetike imaju minimalne ili ograničene efekte. Cilj ovog rada bio je da se ispitaju efekti dugotrajne primene ELF-PEMF u kombinaciji sa kineziterapijom na BMD, učestalost novih preloma kostiju i koncentraciju parathormona kod bolesnika sa TBS na programu hemodijalize. **Metode.** U 3-godišnjoj prospektivnoj kliničkoj studiji bolesnici, njih 151, sa TBS na programu hemodijalize na dobrovoljnoj bazi, svrstani su u dve grupe: studijska grupa (ELF-PEMF, 18 Hz, 2 mT, pri-

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menjivana tokom 40 min posle 10 uzastopnih procedura hemodijalize, četiri puta tokom jedne godine, ukupno 120 tretmana tokom tri godine uz kineziterapiju) i kontrolna grupa (samo kineziterapija). **Rezultati.** Ukupno 124 bolesnika je završilo ispitivanje. U studijskoj grupi (n = 54), nezavisno od pola, posle tri godine primene ELF-PEMF postignuto je značajno poboljšanje BMD, T-skora i Z-skora na lumbalnoj kičmi i vratu butne kosti. U kontrolnoj grupi (n = 70), primećeno je značajno smanjenje BMD, T-skora i Z-skora uz veću incidencu novih preloma kostiju. **Zaključak.** Tehnika ELF-PEMF mogla bi predstavljati korisnu i bezbednu nefarmakološku metodu u programu prevencije preloma kod bolesnika sa TBS.

Ključne reči:
bubreg, hronična insuficijencija; hemodijaliza; elektromagnetna polja; kost, gustina; prelomi; incidenca; lečenje, ishod.

Introduction

In the treatment of musculoskeletal disorders, a variety of physical therapy options has been developed. Among them, pulsed electromagnetic fields (PEMF) have reached significance and attracted attention in both clinical and basic research [1].

Following convincing evidence that electromagnetic currents can accelerate bone formation, PEMF have been used as therapeutic agent for over the 40 years. It seems that most of different effects strongly depend on parameters of applied electromagnetic fields [2, 3]. Extreme low frequency PEMF (ELF-PEMF), available and applicable in biomedicine, are electromagnetic fields with frequency below 60 Hz, induction value 1 pT- 15 mT, volume 130 V/m and triangle or 4 angle oscillations magnetic field. They are sufficient to maintain bone mass even in the absence of physical activity and reducing the frequency to 15 Hz made the field extremely osteogenic [4]. Since 1979, on the basis of strong empirical evidence, PEMF have been approved by the Food and Drug Administration (FDA) for treating non-healing fractures and related problems in bone healing [5]. ELF-PEMF has also analgesic (antinociceptive) effects and this method of physical therapy is suggested as adjunctive therapy in other chronic pain medical conditions such as painful diabetic peripheral neuropathy and a variety of different disorders including spasticity in multiple sclerosis and benign prostate hyperplasia [6, 7].

There is no discomfort or known risk associated with ELF-PEMF and therefore it is a non-invasive, long-term safe and easy to apply, low-cost method [4]. The occurrence of adverse events is indicated by a relative risk of 1.4.

Chronic kidney disease (CKD) affects 5–10% of the world population and is associated with many adverse outcomes including bone disorders and fractures [8]. Decreased bone mineral density (BMD) and disruption of micro architecture occur early in the course of CKD and worsen with the progressive decline in renal function, so that at the time of initiation of dialysis at least 50% of patients had a fracture [9]. The etiology of fractures in patients with CKD on dialysis is multifactorial [10]. Dialysis modality, sex, age, presence of cardiovascular disease, diabetes, diuretics, steroids, vitamin D and low BMD had statistically significant associations with hip fracture [11]. Furthermore patients with end stage renal disease (ESRD) are predisposed to many risk factors of low bone strength, including low dietary calcium intake, reduced exercise, heparin therapy, low body weight, amenorrhea, and premature menopause. The term renal osteodystrophy failed to describe the entire spectrum of bone and mineral abnormalities that include mineral disturbance and abnormal metabolism of bone, its regulating hormones, as well as, various calcifications of soft tissues and cardiovascular system. According to Kidney Disease: Improving Global Outcomes (KDIGO) recommendations, this term has been replaced with the term chronic kidney disease-mineral and bone disorder (CKD-MBD) [12].

The first step towards decreasing the morbidity and mortality associated with fractures in patients with ESRD on dialysis is to direct appropriate preventative and treatment strategies. Changed pharmacodynamics and pharmacokinetics of vitamin D, calcium supplements, antiresorptive drugs including bisphosphonates, and anabolic drugs, as well as, a multifactorial pathogenesis of bone and vascular disease in ESRD patients on dialysis, are responsible for undesirable, adverse, minimal or limited effects [13]. Therefore, the physical therapy, especially ELF-PEMF, could be a convenient non-pharmacological step in the strategies for fracture prevention in nephrology practices.

The aim of this study was to assess the effectiveness of long-term osteogenic ELF-PEMF therapy applied in concordance with physical exercise on BMD, frequency of new bone fractures and parathyroid hormone (PTH) concentrations in ESRD patients on dialysis.

Methods

Patients

This study was preformed as a 3-year prospective clinical trial. All study protocols were in accordance with the Declaration of Helsinki and International Committee on Harmonization-Good Clinical Practice (ICH-GCP) and were approved by the Independent Ethics Institutional Review Committee of the University hospital “Zvezdara” as part of the School of Medicine, University of Belgrade, Serbia on April 19, 2011. All patients have signed written informed consent for the entry into the clinical trial on the voluntary basis.

Total of 151 patients of both sexes were initially included in the study. All patients had a chronic renal failure of a different origin (primary chronic glomerulonephritis, tubulo-interstitial nephritis, nephroangiosclerosis, diabetic nephropathy) and were on dialysis program with hemodialysis product 36, for at least one year. Further inclusion criteria required patients to be at least 25 years old. All patients have continued with their basic therapeutic regimen (vitamin D, calcium and phosphate binder supplementation) during the observation period. Exclusion criteria were: any relative or absolute contraindication for either ELF-PEMF or kinesitherapy treatment, any disorder affecting the bone metabolism (except renal failure and hyperparathyreoidism) and any medication affecting the bone metabolism (except vitamin D, calcium and heparin during hemodialysis). Early menopause was defined as having occurred before the age of 40.

Collection of demographic and case history data was performed by reviewing case notes and treatment records.

According to the applied physical therapy procedure patients were divided into two groups. Patients included in the study group (n = 64) were subjected to treatment with ELF-PEMF together with kinesitherapy, while patients assigned to the control group (n = 87) were subjected only to kinesitherapy.

Physical therapy procedures

ELF-PEMF (18 Hz, 2 mT) was applied during 40 min after 10 consecutive dialysis procedures, 4 times through one year (120 treatments in total during 3 years). The source of magnetic field was a Magomil 2 pad (35 × 27 × 13 cm) with computed device for ELF-PEMF (Electronic Design Medical, Belgrade, Serbia).

Kinesitherapy treatment (active and passive-assisted exercises per segments in two series with 10 repeats) was dosed individually according to general shape during 30 min after every hemodialysis procedure by the same physiotherapist who had been trained in the treatment scheme according to the usual program.

BMD measurements

All subjects underwent dual-energy x-ray absorptiometry (DXA) densitometry (Hologic explorer, USA). Lumbar spine and femoral neck BMD (g/cm^2) were measured twice: at the beginning of the study (baseline) and after three years. Results are reported as actual values and T and Z scores, that reflect the number of standard deviations (SDs) by which a patient's value differs from the mean of a group of young normal (T score) or age- and sex-matched controls (Z score).

Biochemical measurements and body mass index (BMI) calculation

Blood sampling was performed routinely using standard certified procedures for measuring of investigated parameters. Serum urea, creatinine, albumin, calcium, and phosphate were measured using standard autoanalyser techniques. Calcium levels were corrected for albumin concentration. Intact PTH levels were measured by a chemiluminescent enzyme immunometric assay performed with an automated analyzer (Immulite®, Diagnostic Products Corporation). The weight used for the calculation of body mass index (BMI) was the average of 3 postdialysis weights recorded in the week prior to entry.

Statistical analysis

For statistical analysis the patient data were entered on a computer Excel® (Microsoft Office) sheet and subsequently analyzed with the Origin Pro 8.5 statistical software (Stata Corporation, College Station, TX, USA). Group data are expressed as mean ± standard deviation (SD). One-sample Kolmogorov-Smirnov test was used for testing of normal distribution of data. Summary statistics, including mean, SD, range and percentiles were calculated for demographic data, fracture incidence, BMDs, T-scores, Z-scores, urea, creatinine, PTH, thyroid-stimulating hormone (TSH), calcium, phosphate serum concentrations and alkaline phosphatase activity. One way ANOVA and *t*-test for depended samples were used to investigate differences between groups for parametric variables and χ^2 test for nonparametric ones. Observations were considered significant if two-tailed *p* values were below 0.05.

Results

Of 151 patients initially enrolled in the study (64 in the study group and 87 in the control group), total 124 patients (54 in the study group and 70 in the control group) have completed all treatments and testing after 3 years. Ten patients in the study group and 17 in the control group dropped out of the study: 2 (one from each group) due to change in concomitant therapy and 25 (9 from the study and 16 from the control group) due to death related to cardiovascular events. During the follow-up period, not a single patient underwent renal transplantation, was transferred to another dialysis center or changed the dialysis mode. Finally, there were 29 females and 25 males in the study group and 36 females and 34 males in the control group.

Demographic and clinical data of the patients that have completed the study are presented in Table 1 for female and male patients. It is important to note that the patients in finally analyzed groups were comparable in relation to age, duration of dialysis, BMI, smoking history, presence of bone fractures, parameters measured by DXA and PTH levels at the beginning of investigation.

Effects of 3-year follow-up on DXA results, frequency of new bone fractures and concentration of PTH in female patients on dialysis in the study and control group are presented in Table 2.

In the study group, females achieved significant improvements of BMD, T-score and Z-score (on both lumbar spine and femoral neck) after 3-years treatment with ELF-PEMF. However, after the same period, significant decrease of BMD and T-score on both lumbar spine and femoral neck and Z-score only on femoral neck was recorded in females from the control group. Also, the higher frequency of new bone fractures was noticed but this change did not reach statistical significance. Concentrations of PTH were not changed in both groups.

Table 1

Demographic and clinical data of female/male dialysis patients in the study and the control group at the beginning of investigation

Parameter	Study group (n = 29/25)	Control group (n = 36/34)	*p*
Age (years), mean ± SD	56.9 ± 6.4/63.2 ± 7.4	61.2 ± 7.6/61.2 ± 13.6	F = 1.89; *p* = 0.13/ F = 0.55; *p* = 0.85
Duration of dialisis (years), mean ± SD	9.3 ± 5.6/8.8 ± 3.7	9.2 ± 6.6/8.7 ± 3.4	F = 1.64; *p* = 0.17/F = 1.46; *p* = 0.20
BMI (kg/m^2), mean ± SD	23.7 ± 3.2/25.9 ± 2.8	24.9 ± 5.4/23.7 ± 3.5	F = 2.15; *p* = 0.09/ F = 10.9; *p* = 0.08
[1]Duration of menopause (years), mean ± SD	9.0 ± 4.5	10.8 ± 6.2	F = 1.72, *p* = 0.15
[1]Early menopause (% of patients)	20.7	16.7	χ = 0.07, *p* = 0.98
Smoking history (% of patients)			
ever smoked	44.8/72.0	47.2/61.7	χ = 0.011, *p* = 0.99/χ=0.131; *p*=0.87
present smoking	20.7/40.0	19.4/41.1	χ=0.006, *p*=0.99/χ=0.002; *p*=0.99
Bone fractures (% of patients)	31.0/24.0	22.2/20.5	χ = 0.264, *p* = 0.88/χ = 0.043; *p* = 0.99
BMD L1-L4, (g/cm^2), mean ± SD	0.812 ± 0.114/0.774 ± 0.065	0.993 ± 0.182/1.060 ± 0.143	F = 0.52, *p* = 0.88/F = 4.74; *p* = 0.18
T-score L1-L4, mean ± SD	-2.8 ± 1.2/-2.9 ± 0.8	-1.7 ± 1.4/-1.3 ± 1.1	F = 1.83, *p* = 0.14/F = 1.45; *p* = 0.39
Z-score L1-L4, mean ± SD	-1.3 ± 1.1/-1.3 ± 1.0	-1.4 ± 1.4/-0.9 ± 1.1	F = 1.39, *p* = 0.31/F = 3.04; *p* = 0.057
BMD femur (g/cm^2) , mean ± SD D	0.866 ± 0.132/0.831 ± 0.173	0.745 ± 0.174/0.831 ± 0.146	F = 1.17, *p* = 0.51/F = -64.48; *p* = 1
T-score femur, mean ± SD	-1.9 ± 0.9/-2.9 ± 0.8	-2.4 ± 1.2/-2.1 ± 1.0	F = 1.93, *p* = 0.12/F = 0.46; *p* = 0.89
Z-score femur, mean ± SD	-0.7 ± 0.9/-1.0 ± 0.5	-1.1 ± 1.2/-1.2 ± 0.8	F = 1.34, *p* = 0.36/F = 0.46; *p* = 0.89
PTH (pg/mL) , mean ± SD	760.7 ± 125.0/795.5 ± 119.4	788.4 ± 147.2/774.0 ± 114.7	F = 1.08, *p* = 0.61/F = 1.18; *p* = 0.55

[1]Getting data on women only.

BMI – body mass index; BMD – bone mineral density; PTH – parathyroid hormone; SD – standard deviation.

Baseline and closing results of DXA measurements, frequency of new bone fractures and concentrations of PTH in male patients on hemodialysis after 3 years are presented in Table 3. The results are similar to those found in the females, except for the absence of significant decrease of T-score and Z-score on femoral neck in the control group.

During the investigation period, no side-effects of ELF-PEMF were noticed.

Discussion

In this study, the results of a 3-year follow-up investigation of the effects of ELF-PEMF on osteodensitometric parameters and incidence of new bone fractures in patients with ESRD treated with dialysis are presented. At the beginning, the study and control groups were similar according to demographic and all investigated parameters. In the study group, compliance to ELF-PEMF was very high, no one dropped out because of poor adherence.

Our results clearly demonstrated that ELF-PEMF significantly increased BMD, T-scores as well as Z-scores at all measured sites. Although there is some controversy about the significance of measuring BMD in ESRD patients [9], our findings strongly indicate beneficial effects of this physical procedure in ESRD patients. Evaluation of the effects of ELF-PEMF in our patients did not have the aim to investigate the effects on osteoporosis because, as mentioned above, the role and usefulness of DXA in assessing bone status is not well defined. But it was demonstrated that patients with ESRD and low BMD have a significantly shorter survival and that reduced BMD is also predictive of increased all-cause mortality and cardiovascular mortality [14–17]. According to the eldest cross-sectional study of von der Recke et al. [17], low hip BMD seems to predict all-cause mortality in ESRD patients after adjustment for age, years of menopause, presence of hypertension, smoking, and abnormalities in the lipid profile. Indices of osteoporosis predict also cardiovascular mortality. In the study of Kohno et al. [18] the relationship of BMD reduction with increased mortality in hemodialysis patients was examined as a single-center prospective observational study conducted on 269 male hemodialysis patients followed for 61 months. The results suggested that BMD reduction might be a clinically relevant marker that predicts an increased risk of mortality in male hemodialysis patients. According to Matsubara et al. [19], even after adjustment for several confounders and risk factors, all-cause and cardiovascular mortality remained significantly associated with low BMD as an independent predictor in ESRD patients. The association between arterial calcification and bone loss is believed to be one of the links that explains the relationship between decreased BMD and poor cardiovascular outcomes [10, 18–20]. BMD in these patients has been shown to be inversely associated with vascular calcifications. The lack of an association between lumbar spine bone mass measurements and mortality was not unexpected and explained by the fact that spinal osteophytes and abdominal aortic calcification may elevate lumbar BMD and therefore obscure any associations with other factors [17]. The number of patients in our study is too small to bring daring conclusions, but the results demonstrated that low BMD may be a predictor of mortality in maintenance hemodialysis patients. The overall mortality rate was 1.7 times greater in the control group. On the other hand, overall mortality in our control group is similar as expected in clinical trials (about 7.9 deaths/100 person-years) [17].

Table 2

Effects of 3 year treatment with ELF-PEMF on bone mineral density, frequency of new fractures and concentration of PTH in female patients on dialysis in the study and the control groups

Parameter	Study group (n = 29)			Control group (n = 36)		
	before treatment	after treatment	*t*; DF; *p*	before treatment	after treatment	*t*; DF; *p*
BMD L1-L4 (g/cm^2), $\bar{x}$ ± SD	0.812 ± 0.114	0.906 ± 0.188	4.28; 28; < 0.05	0.993 ± 0.182	0.917 ± 0.179	4.02; 35; < 0.05
T-score L1-L4, $\bar{x}$ ± SD	-2.8 ± 1.	-2.3 ± 1.0	3.12; 28; < 0.05	-1.7 ± 1.4	-2.1 ± 1.4	14.06; 35; < 0.05
Z-score L1-L4, $\bar{x}$ ± SD	-1.3 ± 1.1	-0.9 ± 0.8	6.79; 28; < 0.05	-0.4 ± 1.4	-0.5 ± 1.4	0.89; 35; 0.38
BMD femur (g/cm^2), $\bar{x}$ ± SD	0.866 ± 0.132	1.094 ± 0.291	3.26; 28; < 0.05	0.745 ± 0.174	0.625 ± 0.097	5.55; 35; < 0.05
T-score femur, $\bar{x}$ ± SD	-1.9 ± 0.9	-1.4 ± 0.6	-4.10; 28; < 0.05	-2.4 ± 1.2	-2.877 ± 0.804	3.27; 35; < 0.05
Z-score femur, $\bar{x}$ ± SD	-0.7 ± 0.9	-0.3 ± 0.5	10.19; 28; < 0.05	-1.1 ± 1.2	-1.5 ± 0.9	2.73; 35; < 0.05
Bone fractures (% of patients)	31.0	34.4	χ = 0.026; 1; 0.88	22.2	41.6	χ = 1.065; 0.37
PTH (pg/mL), $\bar{x}$ ± SD	760.7 ± 125.0	724.5 ± 85.0	1.03; 28; 0.31	788.4 ± 147.2	791.7 ± 115.4	*t* = -0.88; 35; 0.38

ELF-PEM – extreme low frequency pulsed electromagnetic field; FBMD – bone mineral density; PTH – parathyroid hormone; $\bar{x}$ – mean value; SD – standard deviation.

Table 3

Effects of 3 year treatment with ELF-PEMF on bone mineral density, frequency of new fractures and concentration of PTH in male patients on diaiysis in the study and the control group

Parameter	Study group (n = 25)			Control group (n = 34)		
	before treatment	after treatment	*t*; DF; *p*	before treatment	after treatment	*t*; DF; *p*
BMD L1-L4 (g/cm^2), $\bar{x}$ ± SD	0.774 ± 0.065	0.906 ± 0.188	4.02; 24; < 0.05	1.060 ± 0.143	0.917 ± 0.179	4.28; 33; < 0.05
T-score L1-L4, $\bar{x}$ ± SD	-2.9 ± 0.8	-2.3 ± 1.0	14.06; 24; < 0.05	-1.3 ± 1.1	-2.1 ± 1.4	3.12; 33; < 0.05
Z-score L1-L4, $\bar{x}$ ± SD	-1.3 ± 1.0	-1.2 ± 0.5	11.25; 24; < 0.05	-0.9 ± 1.1	-1.4 ± 0.9	2.66; 22; < 0.05
BMD femur (g/cm^2), $\bar{x}$ ± SD	0.831 ± 0.173	0.850 ± 0.058	6.92; 24; < 0.05	0.831 ± 0.146	0.997 ± 0.115	3.59; 33; < 0.05
T-score femur, $\bar{x}$ ± SD	-2.3 ± 0.4	-1.4 ± 0.6	6.95; 24; < 0.05	-2.1 ± 1.0	-2.6 ± 1.0	1.95; 33; 0.06
Z-score femur, $\bar{x}$ ± SD	-1.0 ± 0.5	-0.6 ± 0.4	5.67; 24; < 0.05	-1.2 ± 0.8	-1.4 ± 0.8	1.01; 33; 0.32
Bone fractures (% of patients)	6 (24.0%)	8 (32.0%)	χ = 0.142; 1; 0.94	7 (20.5%)	12 (35.2%)	χ = 0.658; 1; 0.91
PTH (pg/mL), $\bar{x}$ ± SD	795.5 ± 119.4	712.2 ± 52.6	1.21; 24; 0.25	774.0 ± 114.7	792.0 ± 123.3	1.76; 33; .38

ELF-PEM – extreme low frequency pulsed electromagnetic field; BMD – bone mineral density; PTH – parathyroid hormone; $\bar{x}$ – mean value; SD – standard deviation.

The presence of fractures in the ESRD patients on dialysis can significantly influence their outcome [21]. The important finding of the present study is lower incidence of new fractures in the ESRD patients subjected to the treatment with ELF-PEMF, especially in females. The CKD–MBD clinical practice guideline by KDIGO suggests that BMD does not predict fracture risk as it does in general population, although this evidence level is 2B, meaning a weak recommendation with moderate grade of evidence [12]. However, Iimori et al. [22], followed-up 485 hemodialyzed patients during 6 years and demonstrated a significant predictive power of BMD. These authors found that BMD, especially at the total hip and other hip regions, was useful to predict any type of incident of fracture in females with low PTH or to discriminate prevalent spine fracture for every patient. Furthermore, among 13 cross-sectional studies which were the basis for KDIGO CKD–MBD guideline for the association between BMD and fractures in CKD [12], 7 studies did not find a relationship between BMD and fracture rate, whereas 6 studies found a relationship at least in one skeletal site. If only the studies that used DXA for BMD in ERSD receiving hemodialysis are selected, then 9 studies (4 negative and 5 positive results) remained [22].

It is well known and proved in previous studies, that age, gastric acid suppression therapy, female gender, age at menarche, history of previous fractures and especially serum PTH levels, were identified as important negative determinants of BMD in chronic hemodialysis patients [14]. Secondary hyperparathyroidism, common among patients with ESRD directly affects bone turnover and mineralization and is associated with pain and fractures [23]. Our results did not show any effects of ELF-PEMF on PTH levels.

There is a large body of evidence that ELF-PEMF has high potential in osteogenesis, but the mechanisms has not been clarified yet. It seems that in effect on bone repair a number of different mechanisms are included [4]. PEMF has been shown to stimulate calcification in the extracellular space between the bone cells, to increase blood supply that arises due to PEMF effects on ionic calcium channels, to have an inhibitory effect on the resorptive phase in bone remodeling, leading to the early formation of osteoids and calluses and to increase the rate of bone formation by osteoblasts. On subcellular level, there are at least two aspects - biomechanical and biochemical.

ELF-PEMF can mimic and potentiate effects of physical activity on osteogenesis [4]. The frequencies and field intensities when ELF-PEMF technique is used are most effective in the exogenous stimulation of bone formation when they are similar to those produced by normal physical activity. The application of physical stress on bones promoted the formation of very small electric currents, piezoelectric potentials that are related to bone formation [24]. Piezoelectric potentials are due primarily to movement of fluid-containing electrolytes. When these electrolytes move in the bone channel, which has organic constituents with fixed charges, they generate streaming potentials transforming mechanical stress into an electrical phenomenon capable of stimulating synthesis of matrix components. Using an *in vivo* model, it was also demonstrated that the bone resorption can be prevented or even reversed by the exogenous induction of electric fields [4]. Importantly, the manner of the formation, turnover or resorption are exceedingly sensitive to subtle changes in electric field parameters induced at frequencies between 50 Hz and 150 Hz for 1 h/day. They were sufficient to maintain bone mass even in the absence of function and reducing the frequency to 15 Hz made the field extremely osteogenic [4]. We used similar very low frequency, 18 Hz, which is safe for use in applied therapeutic regiments.

Time varying EMF also generates changes in metabolic activity in the living bone. Interaction between cell membrane and PEMF modulates critical events in signal transduction mechanisms such as Ca^{2+} influx and mobilization, surface receptors redistribution and protein kinase C activity [25]. Cellular production of cAMP in response to PTH is significantly reduced. PEMF can produce a modification of membrane cytoskeleton organization, together with an alteration of protein kinase activity, modify membrane structure and interfere with initiation of signal cascade pathway.

PEMF stimulation is reported to enhance the osteoblast differentiation and to increase bone formation through protein kinase A, protein kinase C and protein kinase G pathways, transcriptional upregulation of bone morphogenic proteins (BMP) 4, 5 and 7, increased levels of BMP-2 and BMP-4 messenger rilonucleic acid (mRNA). The similar effects are observed in mesenchymal stem cells. Several cellular mechanisms, including increases in growth factors, have been implicated as the possible causes of osteogenesis from PEMF stimulation. On the other side, PEMF can also target osteoclasts through increasing the number of adenosine A2a receptors which lead to a decrease in lysosomal enzyme activities [26].

Significant reduction of proinflammatory cytokines like tumor-necrosis factor alpha (TNFα) and interleukin (IL)-6 and inflammatory mediators like prostaglandin PGE2 are noticed.

PEMF increase serum bone formation markers, including osteocalcin and N-terminal propeptide of type 1 procollagen with minor inhibitory effects on bone resorption markers, including C-terminal crosslinked telopeptides of type I collagen and tartrate-resistant acid phosphatase 5b [27]. Bone histomorphometric analysis demonstrated that PEMF increased mineral apposition rate, bone formation rate, and osteoblast numbers in cancellous bone, but PEMF caused no obvious changes on osteoclast numbers. Real-time PCR showed that PEMF promoted gene expressions of Wnt1, LRP5, β-catenin, OPG, and OC, but did not alter receptor activator of nuclear factor kappa-B ligand (RANKL), receptor activator of nuclear factor K (RANK), or Sost mRNA levels [28]. PEMF attenuated deterioration of bone microarchitecture and strength in rats by promoting the activation of Wnt/LRP5/β-catenin signaling rather than by inhibiting RANKL-RANK signaling [29]. The results of some studies show that PEMF frequency is an important factor with regard to the induction of human mesenchymal stem cell differentiation. Furthermore, a PEMF frequency of 50 Hz was the most effective at inducing human mesenchymal stem cell osteoblast differentiation *in vitro* [30]. In mice models the expression levels of angiopoietin-2 and fibroblast growth factor-2 in the bone marrow were significantly higher by the PEMF [31]. Such angiogenesis acceleration represents one possible mechanism for the acceleration of bone fracture healing by PEMF. The results found in rat models demonstrate that PEMF stimulation can efficiently suppress bone mass loss through promoting transforming growth factor (TGF)-beta1 secretion and inhibiting IL-6 expression [32]. Some studies hypothesized and confirmed that PEMF increase nitric oxide (NO), which induces vasodilation, enhances microvascular perfusion and tissue oxygenation [33]. PEMF can facilitate the osteogenic differentiation of bone marrow mesenchymal stem cells *in vitro* [29]. The PEMF stimulation, could induce expression of osteoblast specific genes and proteins including alkaline phosphatase and osteocalcin, as well as gene expression of BMP-2, Runx2, β-catenin, Nrf2, Keap1 and integrin β1.

Conclusion

Our study provides evidence for a beneficial effect of ELF-PEMF on BMD and risk of fracture in ESRD patients on dialysis. Physical therapy in general and magnetobiology in particular provide non-invasive, safe and easy to apply methods to directly treat the site of injury or the source of pain, inflammation and dysfunction. As observed earlier, ELF-PEMF has a marked osteogenic potential proved by clinical, animal and tissue culture studies over a period of 20 years. Our findings suggest that ELF-PEMF has clinical relevance as a successful adjuvant option in the management of low BMD in ESRD for the first time without reports of side-effects. In future study, design ELF-PEMF effects need to prove this assumption in order to consider accurate results. A clearer definition of the mechanisms might also help in choosing patients and modalities that are more likely to benefit from such a treatment. The limitation of the study is a lack of possibility to study subgroups by energy levels or other parameters of treatment in order to produce recommendations.

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Received on June 17, 2016.
Accepted on July 13, 2016.
Online First July, 2016