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Research Article / Araştırma Makalesi

# Evaluation of Continuous and Pulsed Ultrasound Treatments on Knee Osteoarthritis: A Randomized and Placebo-Controlled Study

## Diz Osteoartritinde Sürekli ve Kesikli Ultrason Tedavilerinin Değerlendirilmesi: Randomize ve Plasebo Kontrollü Bir Klinik Çalışma

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**Abstract:** This study evaluates effects of therapeutic ultrasound (continue, pulsed, placebo) treatment on the clinical and biochemical parameters in knee osteoarthritis. 30 patients (30-70 aged) diagnosed with knee osteoarthritis were randomly separated into three groups. The 1st group includes 10 patients given continue ultrasound treatment (1 MHz, 2W/cm2), the 2nd group consists of 10 patients given pulsed ultrasound treatment (1MHz, 2 W/cm2, 1:4) and 3th group includes 10 patients given placebo ultrasound treatment (switch off). The patients were assessed before and one month after the treatment with WOMAC, COMP, Hs CRP, MMP-1, MMP-3 levels in serum and CTX-II levels in urine. When the demographic characteristics were compared, no statistically significant difference was found between the three groups in terms of age, body mass index and radiological staging ( p > 0.05). In terms of clinical parameters, there was an improvement in all three groups in the first month after treatment except WOMAC stiffness in pulsed and placebo groups, although no statistically significant difference was observed between the groups ( p > 0.05). In the evaluation of biochemical parameters, there was no significant difference between the groups in terms of pre-treatment and post-treatment 1 month, whereas there was a statistically significant difference between the group 1 and group 3 in favor of group 3 ( p = 0.019). The efficacy of therapeutic US (continuous / pulsed) which was applied short term and alone, on clinical and biochemical markers in knee osteoartrithis, has not been demonstrated.

**Keywords:** knee osteoarthritis, therapeutic ultrasound, biochemical marker

**Özet:** Bu çalışma, diz osteoartritinde, terapotik sürekli, kesikli ve plasebo ultrason uygulamasının, klinik ve biyokimyasal etkinliğini karşılatırmak amacıyla yapılmıştır. Diz osteoartriti tanısı alan 30 hasta üç gruba ayrıldı. 1. gruba sürekli ultrason (1 MHz, 2 W / cm2); 2. gruba kesikli ultrason (1MHz,2W/cm2,1:4); 3. gruba ise plasebo ultrason tedavisi uygulandı. Tüm gruplar 10 gün (5 dakika/seans) tedavi aldı. Hastalar tedavi öncesi ve tedavi sonrası 1. ayda WOMAC, serum hs - CRP, COMP, MMP - 1, MMP - 3 ve idrar CTX - II düzeyleri ile değerlendirildi. Demografik özellikler karşılaştırıldığında, üç grup arasında yaş, vücut kitle indeksi ve radyolojik evreleme açısından istatistiksel olarak anlamlı bir fark bulunmadı ( p > 0.05 ). Klinik parametreler açısından, tedaviden sonraki ilk ayda, plasebo grubunda WOMAC sertlik dışında, her üç grupta iyileşme saptandı, ancak gruplar arasında istatistiksel olarak anlamlı bir fark gözlenmedi ( p > 0.05 ). Biyokimyasal parametrelerin 1. ay değerlendirilmesinde tedavi öncesi ve tedavi sonrası gruplar arasında anlamlı fark bulunmazken, grup 1 ve grup 3 arasında grup 3 lehine istatistiksel olarak anlamlı bir fark vardı ( p = 0.019 ). Diz OA'de tek başına ve kısa süreli uygulanan terapötik ultrasonun (sürekli/kesikli) klinik ve biyokimyasal belirteçler üzerine etkinliği gösterilememiştir.

**Anahtar Kelimeler:** diz osteoartriti, terapotik ultrason, biyokimyasal belirteç

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## 1. Introduction

Osteoarthritis (OA); It is a dynamic process involving all the structures of the joint such as cartilage, synovium, and bone, in response to mechanical and/or inflammatory effects. Although the molecular pathogenesis is unknown; it is thought that various genetic, environmental, metabolic, and biomechanical factors contribute to the pathogenesis (1). In joint cartilage, the imbalance between construction and destruction in favor of destruction causes cartilage damage (2).

Biochemical markers in osteoarthritis are molecules that occur during the physiological cycle of bone and cartilage matrix and detectable in body fluids. The most crucial aim of the biomarker measurement in OA is that early detection of cartilage damage which is not yet radiologically detected. Other reasons for biochemical marker measurement include monitoring the activity of the disease, determining disease severity, predicting prognosis, and evaluating response to treatment (3). Several biochemical markers such as type 2 collagen, proteoglycan, hyaluronan, cartilage oligometric matrix protein (COMP), matrix metalloproteinases (MMP), urine CTX-2 are known to be associated with the diagnosis and radiological progression of OA (4). The most common marker among the destruction markers is COMP (5,6).

The goal of the treatment of osteoarthritis is reducing pain, increasing the range of motion, to dissolve the spasms in the affected muscles or to strengthen the muscles (7-11). Ultrasound is one of the physical therapy agents and is in the group of deep heaters. Deep heaters provide minimal warmth in the skin and subcutaneous tissues, while maximal warming in deep tissues such as muscles, tendons, ligaments, and bones (12). While utilizing the thermal effects in continuous ultrasound treatment, non-thermal effects are utilized in pulsed ultrasound (13).

In vitro and vivo studies on therapeutic ultrasound showed that continuous and pulsed ultrasound had positive effects on cartilage regeneration and clinical outcome (14-19). It has been reported that pulsed and continuous ultrasound performed at the appropriate dose, frequency, and duration increases the type 2 collagen, proteoglycan synthesis and thus accelerate cartilage regeneration (15,20).

In the literature, there is no study investigating the efficacy of pulsed and continuous ultrasound therapy on clinical and biomarkers in knee osteoarthritis. In our study, we aimed to investigate the effect of pulsed and continuous ultrasound treatment on knee osteoarthritis on clinical and biochemical parameters. For this purpose, urine CTX-II which is a cartilage type 2 collagen destruction marker, matrix metalloproteinase 1 (MMP-1) and metalloproteinase 3 (MMP-3), cartilage oligomatrix protein (COMP) levels which are the markers of destruction were evaluated.

## 2. Methods

This study was approved by the decision of the Ethics Committee of the Faculty of Medicine of Eskişehir Osmangazi University, dated 29.8.2012 and numbered 193. Female patients between the ages of 30-70 who were admitted to the outpatient clinic of Eskişehir Osmangazi University Faculty of Medicine Department of Physical Medicine and Rehabilitation Clinic with knee pain were informed about the study. At the end of clinical and laboratory evaluations, 30 patients with primary knee OA according to ACR criteria and who were bilaterally Stage 2-3 diagnosed with Kellgreen Lawrence radiological staging (KGL) were included in the study.

The underlying inflammatory arthropathy, Paget's disease, fracture in the joints, acromegaly, Wilson's disease, fibromyalgia, ochronosis, hemochromatosis, the presence of known collagen gene mutation, pregnancy, malignancy, decompensated heart failure, the presence of hemorrhagic diathesis, a history of severe cognitive disease, the lower extremity in the last year history of previous surgery, history of intra-articular treatment in the last six months, history of physical therapy in the last six months, history of peripheral or

central neurological disease, history of trauma, history of osteoarthritis in the last 6 months, history of use of natural health products, history of ibuprofen use patients were excluded from the study.

Written and verbally informed consent form was obtained from each patient. The study was planned as a prospective, randomized, controlled, single-blind, clinical study. Thirty patients who met the study criteria were randomly divided into three groups by a secure system of numbered 1-2 opaque closed envelopes.

Each group contained ten patients. The first group was given 5 minutes a day for five days, totally two weeks at a frequency of 1 MHz, an intensity of 2W / cm2 continuous ultrasound treatment applied with an applicator with a diameter of 5 cm (Sonopuls 434; Enraf Nonius, Delft, The Netherlands). Supine position was given to patients and sonogel was applied to the area where ultrasound treatment was planned. Then ultrasound was applied to the knee with circular movements. The second group was given five days a week, 5 minutes each day with the same ultrasound equipment and technically 1 MHz frequency, 2W/cm2 dose and 1: 4 pulsed ultrasound treatments was applied for two weeks. The third group was treated with the same equipment and technique for 5 minutes, five days a week for five weeks.

No additional treatment was given to the patients during the study.

The patients were evaluated as clinical (WOMAC Osteoarthritis Index) and laboratory (COMP, MMP-1, MMP-3 and urinary CTX-II) on the day before treatment and in the first month after treatment. Clinical evaluations were performed by a researcher who was blind to the treatment protocol. Blood samples were taken from the patients to evaluate COMP, MMP-1, and MMP-3, and urine samples were taken for CTX-II. Blood samples were centrifuged at 1000 g for 15 minutes. Serum COMP, MMP-1, and MMP-3 were divided into three groups and stored at -80 degrees on the same day. The collected urine samples were centrifuged at 1500 rpm for 3 minutes and then stored in -80 ° C for CTX-II study. COMP, MMP-1, MMP-3, and CTX-II were studied by ELISA (Enzyme-Linked Immuno Sorbent Assay).

The WOMAC score consists of 24 items divided into three subscales: pain, stiffness, and physical function. The global score has a range of 0 (no symptom) to 96 (worst symptoms), with a standardized score to have a range of 0 to 100 (21).

## 3. Results

The patients were randomly divided into three groups. There were ten patients in each group. Seven patients in group 1, seven patients in group 2, and nine patients in group 3 participated in the clinical evaluation at the end of the first month. For biochemical parameters, nine patients in group 1, seven patients in group 2, and five patients in group 3 could be evaluated. When the demographic characteristics were compared, no statistically significant difference was found between the three groups in terms of age, body mass index (BMI) and radiological staging (p> 0.05) (Table1.).

**Table 1.** Demographic characteristics

| | Group I (n=10) (mean±SD) n(%) | Group II (n=10) (mean±SD) n(%) | Group III (n=10) (mean±SD) n(%) | P |
|---|---|---|---|---|
| **(years)** | 59,80±8,18 | 56,10±10,74 | 53,40±10,38 | 0.358 |
| **BMI (kg/m2)** | 29,00±4,72 | 26,96±3,58 | 26,88±4,20 | 0.452 |
| **KGL scala** | | | | |
| **Stage 2** | 4 (%28,60) | 5 (%35,70) | 5 (%35,70) | 0.670 |
| **Stage 3** | 6 (%40,00) | 5 (%33,30) | 4 (%26,70) | |

*BMI: Body Mass Index*

In terms of clinical parameters, there was an improvement in all three groups in the first month after treatment except WOMAC stiffness in the pulsed group, although no statistically significant difference was observed between the groups (p> 0.05) (Table 2, 3).

**Table 2.** Intragroup comparison of the clinical parameters before and first month after the treatment

| | | Group I (n=7) | Group II (n=7) | Group III (n=9) |
|---|---|---|---|---|
| **Womac Total** | BT | [a] 76±13.23 | [a] 79.86±12.12 | 76.13±4.26 |
| | AT | [a] 61.71±22.77 | [a] 65.14±18.85 | 68.63±6.61 |
| **P** | | 0.034 | 0.010 | 0.002 |
| **Womac Pain** | BT | [ê] 14(12.75-15.25) | 15±1.73 | 14.75±1.67 |
| | AT | [ê] 12(7-15) | 11.57±3.16 | 12.50±2.20 |
| **P** | | 0.042 | 0.017 | 0.004 |
| **Womac Stiffness** | BT | [a] 7±2.24 | [ê] 8(6.75-8) | [ê] 6(5.75-7.25) |
| | AT | 5.29±2.36 | [ê] 7(5-7) | [ê] 4(4-6.50) |
| **P** | | 0.037 | 0.102 | 0.002 |
| **Womac Physical Function** | BT | [a] 55.43±9.64 | 58.14±9.23 | 56.63±5.81 |
| | AT | 45.14±16.91 | 47.57±13.89 | 51.88±5.03 |
| **P** | | 0.031 | 0.011 | 0.029 |

*[a]: mean± standart deviation (SD), ê: median (%25-%75)*
*BT: Before the treatment*
*AT: First month after the treatment*

**Table 3.** Intergroup comparison of the clinical parameters before and first month after the treatment

| | | Group I (n=7) | Group II (n=7) | Group III (n=9) | P |
|---|---|---|---|---|---|
| **Womac Total** | BT | 75.5±11.06 | 81.90±11.42 | 76.50±3.95 | 0.282 |
| | AT | 70 (37-81) | 70(60-75) | 70(62-74.25) | 0.988 |
| **Womac Pain** | BT | 14±1.63 | 16.20±2.53 | 14.5±1.72 | 0.051 |
| | AT | 12(7-15) | 13(11-14) | 12(12-13) | 0.942 |
| **Womac Stiffness** | BT | 6.5(5.75-8) | 8(6.75-89 | 6(5.75-7.25) | 0.273 |
| | AT | 5.29±2.36 | 6±2 | 10.67±17.80 | 0.591 |
| **Womac Physical Function** | BT | 55.8±9.11 | 58.70±8.63 | 54.80±6.51 | 0.548 |
| | AT | 52(27-60) | 52(44-55) | 53.5(46.75-56.5) | 0.890 |

*[a]: mean± standart deviation (SD), ê: median (%25-%75)*
*BT: Before the treatment*
*AT: First month after the treatment*

In the evaluation of biochemical parameters, there was no significant difference between the groups in terms of pre-treatment and post-treatment one month, whereas there was a statistically significant difference between the group 1 and group 3 in favor of group 3 (p = 0.019) (Table 4,5).

**Table 4.** Intragroup comparison of the biochemical parameters before and first month after the treatment

| | | Group I (n=9) | Group II (n=7) | Group III (n=5) |
|---|---|---|---|---|
| MMP1 | BT | 6.12(4.65-8.55) | 7.88±4.22 | 6.20±2.82 |
| | AT | 5.92(4.62-6.84) | 8.10±4.29 | 6.94±3.53 |
| P | | 0.953 | 0.256 | 0.112 |
| MMP3 | BT | 13.09±3.52 | 11.75±9.76 | 9.51±2.79 |
| | AT | 13.51±5.08 | 11.72±9.41 | 8.38±2.52 |

| | | | | |
|---|---|---|---|---|
| P | | 0.546 | 0.975 | 0.241 |
| COMP | BT | 301.64(189.88-347.57) | 252.49±139.98 | 154.51±62.86 |
| | AT | 265.72(222.50-334.67) | 233.14±82.35 | 141.31±61.59 |
| P | | 0.953 | 0.534 | 0.704 |
| CTX | BT | 1.89(0.94-2.78) | 2.87(1.7-3.2) | 2.82±2.24 |
| | AT | 1.23(0.95-5.42) | 2.41(1.81-2.74) | 3.96±1.74 |
| P | | 0.767 | 0.176 | 0.248 |

[a]: mean± standart deviation (SD), ê: median (%25-%75)
BT: Before the treatment
AT: First month after the treatment

**Table 5.** Intergroup comparison of the biochemical parameters before and first month after the treatment

| | | Group I (n=7) | Group II (n=7) | Group III (n=9) | P |
|---|---|---|---|---|---|
| MMP1 | BT | 6.41±0.88 | 7.88±1.60 | 6.20±1.26 | 0.602 |
| | AT | 6.05±0.72 | 8.10±1.62 | 6.94±1.58 | 0.483 |
| MMP3 | BT | 11.69(10.17-16.34) | 5.46(5.8-13.4) | 9.84(6.76-12.09) | 0.261 |
| | AT | 12.36(9.34-17.40) | 7.63(5.32-16.2) | 8.03(6.65-10.29) | 0.157 |
| COMP | BT | 279.84±27.50 | 252.49±52.91 | 159.51±28.11 | 0.111 |
| | AT | 302.44±36.33 | 233.14±31.13 | 141.31±27.54 | 0.019 |
| CTX | BT | 1.89(0.94-2.78) | 2.87(1.7-3.2) | 1.35(1.12-5.25) | 0.598 |
| | AT | 1.23(0.95-5.42) | 2.41(1.81-2.74) | 4.9(2.16-5.3) | 0.210 |

*BT: Before the treatment*
*AT: First month after the treatment*

## 4. Discussion

In our study, the effects of therapeutic ultrasound on knee osteoarthritis were evaluated by WOMAC in terms of clinical and functional status. In our study, all groups showed significant improvements after the treatment in all WOMAC scores. However, there was no significant difference between the groups. The effects of therapeutic ultrasound on biochemical markers of knee osteoarthritis were also evaluated. COMP, MMP-1, and MMP 3 were studied from blood samples taken from patients; CTX-II was studied from urine samples. While there were no significant differences in biochemical markers within three groups, there was a significant difference in terms of COMP between placebo treatment and continuous ultrasound treatment in favor of placebo at the 1st month.

Several studies are investigating the effects of therapeutic ultrasound on osteoarthritis biomarkers in the literature. In a study which had similar results to ours showed that pulsed US stimulation increased COMP levels in human cartilage explants in vitro environment (22). In another in vitro study, Tien et al. (17) reported that pulsed US (at the intensity of 48 mW/cm2, a frequency of 1mHz) increased the levels of anabolic markers (agrecan and type 2 collagen) but did not increase proliferation in human condrosit. Lee et al. (18) showed that low-intensity continuous ultrasound (1MHz, 200 mW / cm2, 20 min/day) increased the synthesis of type 2 collagen aggregate synthesis and inhibition of matrix metalloproteinase-2 expression in rabbit mesenchymal stem cells. Also, a systematic review reported that pulsed ultrasound treatment induces chondrocyte proliferation and matrix production in human articular cartilage (23). Contrary to many studies in the literature, the positive effect of therapeutic ultrasound on cartilage degeneration was not observed in our study. In our study, there was a significant difference in biochemical markers between the groups in favor of placebo. However, there was no difference between post and pre-treatment biochemical markers in the placebo group.

Many studies are evaluating the clinical effects of therapeutic ultrasound. Tascioglu et al. (19) found that pulsed US (1:4) was more effective than the continuous US (at the intensity of 2W/cm2, a frequency of 1mHz) in pain relief. In another study which compared pulsed US plus diclofenac sodium (at the

intensity of 120mW/cm2, a frequency of 0.6mHz, 1:5) with placebo US plus diclofenac sodium, was reported that WOMAC scores showed significant improvements in the pulsed US group, at the end of ten days treatment in 106 patients (24). Similar to our study, Cakir S et al. (25) compared the effectiveness of continuous (at the intensity of 1W/cm2, a frequency of 1mHz), pulsed (1:4) and sham US treatments which were combined with home exercise, in 60 knee osteoarthritis patients. They reported a significant improvement in WOMAC in all groups but did not find a significant difference between the groups. A systematic review reported the effects of continuous and pulsed ultrasound on reducing WOMAC scores (pain and physical function scores) (26). In another systematic review, it was found that pulsed US treatment was more effective in terms of both pain relief and functional improvement than the sham US. However, the effectiveness of continuous US only exists in pain relief, when compared with sham US (23). Similar to our study, studies in the literature reported that both continuous and pulsed ultrasound was effective in pain relief.

Application of ultrasound to the patients instead of chondrocytes and having a small number of patients may have caused different results in our study. In the literature, there is no study which evaluated the effects of ultrasound on cartilage with serum and urine biochemical markers except in vitro studies. Therefore, our method makes our work valuable.

When the literature is examined, it is seen that the characteristics of US such as frequency, dose, mode, and duration can change the effectiveness of treatment. Particularly low density and pulsed ultrasound wer suggested to be more effective in clinical and biochemical aspects (meta-analysis summary). Although in our study there was no significant difference in biochemical markers with pulsed ultrasound therapy in contrast to many studies, both literature information and results from our study seem to support the clinically positive effects of therapeutic ultrasound.

The limitations of this study were hading a low number of patients and the lack of using cartilage imaging method which can show cartilage damage. The strengths of the study were use of serum and urine markers and use of ultrasound therapy without any other treatment methods.

According to the results of our study, we believe that there is a need for methodologically well designed, randomized controlled trials with large patient series to assess chondroprotective effects of different treatment protocols in knee OA.

## 5. Conclusion

The efficacy of therapeutic US (continuous/pulsed) which was applied short term and alone, on clinical and biochemical markers in knee osteoarthritis, has not been demonstrated.

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