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Serum RANKL levels and bioelectric impedance assessments in knee osteoarthritis patients

Diz osteoartritli hastalarda serum RANKL düzeyleri ve biyoelektrik empedans değerlendirmeleri

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Abstract

Aim: Osteoarthritis (OA) is a common joint disease that is caused by mechanical, genetic, and biochemical factors, and knee OA is one of the reasons of mobility limitation and disability. The receptor activator of NF-κB ligand (RANKL) is directly involved in the differentiation of osteoclasts through its receptor RANK. In this study, we aimed to study circulating serum levels of RANKL, and assess knee bioelectric impedance in control and patients with knee OA.

Methods: In this case-control study, OA severity was evaluated by the Kellgren–Lawrence grading scale, based on which we categorized patient groups. There were 22 control individuals (Grades 0 and 1), 11 early (Grade 2) and 30 late OA patients (Grades 3 and 4). We evaluated the performance of the bioimpedance phase angle values at 50 kHz. The RANKL protein levels in the serum were quantified using Enzyme-Linked Immunosorbent Assay (ELISA).

Results: It was observed that the control group could not be differentiated from the study group by using phase angle values (P=0.925). Concerning the RANKL levels, although it shows a relative increase in the study group, it did not reach a significant level (P=0.116).

Conclusion: The phase angle values at 50 kHz and RANKL levels may not be used in predictive detection of knee OA. Additional studies with larger sample sizes are needed to interpret if these changes are consistent and clinically related.

Keywords: RANKL, Bioimpedance, Knee osteoarthritis

Öz

Amaç: Osteoartrit (OA), mekanik, genetik ve biyokimyasal faktörlerin etkilerinden kaynaklanan yaygın bir eklem hastalığıdır ve diz OA'i, hareket kısıtlaması ve engel nedenlerinden biridir. Bu çalışmada NF-κB ligandının (RANKL) reseptör aktivatörünün dolaşımdaki serum seviyelerini incelemeyi ve kontrolde ve diz OA'i olan hastalarda biyoelektrik empedansını değerlendirmeyi amaçladık.

Yöntemler: Çalışmamız bir vaka-kontrol çalışması olarak tasarlanmıştır. OA şiddeti Kellgren-Lawrence derecelendirme skalası ile değerlendirildi, gruplar 22 sağlıklı kontrol (derece 0 ve 1), ve çalışma grubu 11 erken (derece 2) ve 30 geç OA (derece 3 ve 4) olarak tanımlandı. 50kHz'te biyoimpedans faz açısı değerlendirildi. Serumdaki RANKL protein seviyeleri Enzyme Linked Immunosorbent Assay (ELISA) metodu ile ölçüldü.

Bulgular: Kontrol grubunun faz açısı değerleri kullanılarak diz OA grubundan ayırt edilemediği gözlendi (P=0,925). RANKL düzeyleri ile ilgili olarak, çalışma grubunda göreceli bir artış tespit edilse de, anlamlı bir seviyeye ulaşmadı (P=0,116).

Sonuç: 50 kHz faz açısı değerleri ve RANKL seviyeleri, diz OA'sının tespitinin öngörülmesinde kullanılamayabileceği belirlenmiştir. Bu değişikliklerin tutarlı ve klinik olarak ilişkili olup olmadığını yorumlamak için daha büyük örnek büyüklüklerine sahip ek araştırmalara ihtiyaç vardır.

Anahtar kelimeler: RANKL, Biyoimpedans, Diz osteoartiriti

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Introduction

Knee osteoarthritis (OA) is a common and disabling condition in middle-aged adults and the elderly, and its predominance has been growing with the advancing age of the population [1,2]. OA of the knee begins with the degeneration of articular cartilage and modifications to subchondral bone. As the disease progresses, destruction and deformation of cartilage occurs and is sometimes associated with reactive changes to bone and secondary synovitis [3].

Bioimpedance is characterized by the passive electrical components of biological tissues. It defines the capacity of biological structures to resist the flow of an alternating current. It is also incorporated with conductivity and permeability intracellular and extracellular electrolytes at different frequencies [4]. Hence, bioimpedance can be used for researching electrochemical processes that occur in biological tissues and examining physiological alterations associated with diseases [5]. Bioimpedance spectroscopy is a non-invasive, and inexpensive method that had been practiced in a high number of biomedical researches.

Osteoblastic lineage cells produce receptor activator of NF-κB ligand (RANKL), which is required to mediate bone resorption by regulating osteoclastogenesis. Osteoclastogenesis and osteoclast activity stimulates RANKL via connecting to the cell surface receptor RANK, which is located on precursor and mature osteoclasts [6-8]. Systemic changes like sex steroids, parathyroid hormone, and growth factors cause age-related bone degeneration, and can modulate RANKL in-vivo [9].

Magnetic resonance imaging and arthroscopy are essential methods to evaluate the severity of the OA. However, these methods have a limited role in diagnosis, Favero et al. [10] reported that knee articulation radiography could be normal in the initial stages of OA. Therefore, it is necessary to explore quantitative and sensitive methods for the examination of this disease. We aimed to study circulating serum levels of RANKL and assess knee bioelectric impedance in patients with different stages of knee OA, in addition to correlating these parameters with disease severity to combine all results and reveal if these parameters could be used as markers for the evaluation of knee OA.

Materials and methods

Study population

This clinical study was conducted at SANKO University, Department of Physiotherapy and Rehabilitation with the approval of the SANKO University Ethics Committee (2019/05-02). Written informed consent was acquired from all patients who agreed to participate in our study. Between May 2019 and February 2020, a total of 63 participants, including 41 knee OA patients diagnosed according to the American College of Rheumatology criteria [11] and 22 controls, were selected for the study. All participants included in the study were examined in terms of age, gender, BMI and any medications used. The patients with inflammatory rheumatic diseases, infectious/endocrine-related arthropathies, previous knee injuries, clinically unstable medical diseases, and those receiving chronic drug treatment that may alter body fluid balance were excluded. Blood samples were collected from the study and control groups at the time of diagnosis before any treatment was started. Bioimpedance measurements were performed on all volunteers in cooperation with the Physiotherapy and Rehabilitation Department. The OA severity was evaluated and classified by the Kellgren–Lawrence (KL) grading scale [12]. Grades were classified as 0 (normal), 1 (possible osteophyte), 2 (absolute osteophyte and possible joint space narrowing), 3 (mild osteophyte and/or absolute joint space narrowing), and 4 (dominant osteophyte, severe joint space narrowing and/or bone sclerosis). Patients with grade 0 and 1 were included in the control group (n=22), whereas the study group was classified as grade 2 (n=11) early knee OA, and grade 3 and 4 late knee OA (n=30).

Bioimpedance measurements

Bioimpedance analyzer (Quadscan 4000, Bodystat Inc.) was connected to the 1.0 cm disposable Ag/AgCl disc electrodes (3M, Brazil). The electrode placement protocol was set to maximize the current pathway in synovial fluid and minimize the variable's influence [13]. For this reason, two-disc electrodes were placed on the lateral and medial sides of the interarticular line of the knee, the volunteers were seated, and their knee was flexed 90 degrees. The purpose of electrode usage is to send an electrical signal to the synovial fluid and to collect its response [14]. The current was sent in multiple (5, 50, 100, and 200 kHz) frequencies for bioimpedance measurements. Many bioimpedance systems utilize 50 kHz as a frequency where the capacitor's reactance (X_C) becomes relatively small so that the current is defined mostly by resistance (R). The frequency of 50 kHz is one of the most essential and optimal frequencies. Besides, most published studies have been conducted using devices at a frequency of 50 kHz to differentiate the structures. Due to the logic of this reasoning, we have chosen to illustrate our results only for 50 kHz. We calculated the phase angle values by using arctan (X_C/R) formulas and investigated its values in this frequency [15]. Five phase angle measurements were obtained from each volunteer within 1-2 minutes, and their mean values were used for analysis.

ELISA for RANKL in serum

Total serum RANKL levels were quantified using a direct competitive chemiluminescent enzyme-linked immunosorbent assay (ELISA), RANKL kit (Cloud Clone Corp, TX, USA) considering the manufacturer's instructions. Absolute values were obtained based on a standard curve. The absorbance of the reaction mixture was measured at 450 nm. The sample concentrations (ng/ml) of RANKL were calculated from the standard curve.

Statistical analysis

IBM SPSS Statistics 23 was utilized for statistical analyses. Descriptive statistics were presented as mean and standard deviation or median and minimum-maximum values and percentages. For continuous variables, the Kolmogorov-Smirnov test was used to assess the normality of the data. Independent sample t-test and Mann Whitney U tests were used to compare normally and non-normally distributed two groups, respectively. Kruskal-Wallis test was utilized for comparing more than two groups with non-normally distributed data. Chi-

square test was used for comparison of categorical data. *P*-value <0.05 was considered statistically significant.

Results

This research was conducted on a total of 63 volunteers. The demographic data, RANKL levels and bioimpedance characteristics of the study and control groups are presented in Table 1. Age and BMI values were significantly lower in the control group than the study group (P<0.001). The phase angle values of the two groups were similar (P=0.925) (Figure 1). Concerning the RANKL levels, although it shows a relative increase in the study group, the difference was not significant (P=0.116). Kruskal-Wallis test was performed to compare RANKL levels and phase angle values between the control, early and late knee OA groups. There were no significant differences between these three groups, and the pairwise comparison was not done.

Table 1: Demographic and bioimpedance characteristics of the study and control groups

	Control group (n=22)	Study group (n=41)	*P*-values
Age[a] (years)	56.3 (6.41)	65.3 (8.66)	<0.001
Gender[b] (M/F)	36.4/63.6	17.1/82.9	0.160
BMI[a] (kg/m^2)	24.7 (4.61)	31.3 (4.65)	<0.001
RANKL[c] (ng/ml)	9.27[9.2-11.4]	9.52[9.2-13.6]	0.116
Phase angle[c] (degree)	14.8[4.8-41.3]	15.4[6.6-42.2]	0.925

[a] Measurements are presented as mean (SD), [b] Measurements are presented as percentage, [c] Measurements are presented as median [minimum-maximum]

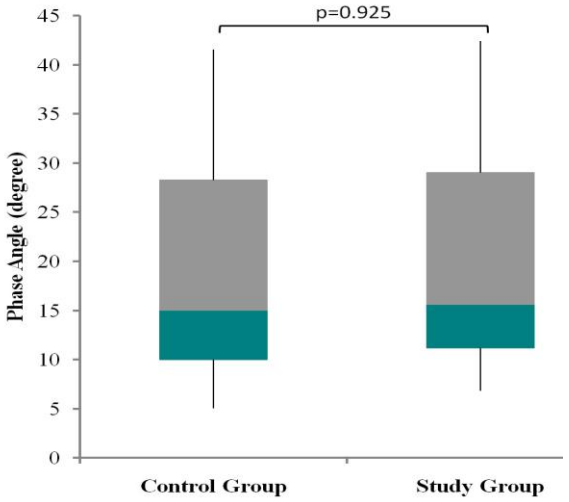

Figure 1: Box plot of phase angle results. The box border represents the interquartile range, the horizontal line in the box is the median. Insignificant differences between control and study groups of phase angle was shown.

Discussion

OA is a common form of degenerative joint disease that affects the western population. The knee is the main peripheral joint that is affected by age-related factors and OA in the knee results in progressive loss of function, pain, and stiffness [16]. Approximately one-tenth of the population over 50 years is estimated to be affected by this disease [17]. The effect of genetic components is evident in the prevalence of knee OA, but the responsible gene is not exactly known. Spector et al. [18] revealed that the effect of genetic factors on hand and knee radiographic OA is between 39% and 65% in women. Radiological evaluation of joint OA is based on plain films as gold standard research with high-resolution MRI evaluation [19]. These investigations are ineffective for the early detection of the condition, which is not always associated with patients' observing symptoms or progression of the disease [20-22]. Symptomatic OA patients often have significant and irreparable cartilage damage, resulting in knee arthroplasty as a final solution. It would be valuable for patients and health care systems if it were the potential to scan high-risk patients by using a biomarker examination. The success of the biomarkers utilized in this context will depend on their ability to identify the beginning of this biological process and respond to interventions in a timely manner [23].

RANKL originates from the surface of osteoblasts that bind to RANK, plays a crucial role in the improvement of osteoclastogenesis, osteoclast maturation, and activation and prevention of apoptosis of osteoclastic cells [24]. Cooperation between RANKL and RANK manages to improve bone loss and resorption [6]. The RANKL activity is the necessary factor in the improvement of bone damage in inflammatory joint diseases and defines the extent of bone destruction mediated by osteoclasts [25]. In our study, the BMI levels of the study group were higher compared to the control group. It is already known that BMI is a significant risk factor for OA [26]. Also, there was no significant differences between BMI categories and serum RANKL levels [27]. There are some studies in the literature concerning serum RANKL levels in knee OA patients. Pilichou et al. [28] evaluated 37 patients with primary knee OA and demonstrated that patients had higher serum RANKL levels compared to 20 controls. In another study, comprising 105 patients suffering from knee OA, transcript levels of the RANKL mRNA were measured using real-time quantitative RT-PCR, and it was shown that RANKL mRNA may be used to diagnose this disease at an early stage when radiological features do not reflect the degradation of articular cartilage [29]. In terms of this protein level, we did not find any significant differences between these two groups.

There are a lot of studies in the literature that use phase angle value to differentiate healthy and diseased states, according to which this value increases with improving clinical status [30,31]. In a study that used phase angle to differentiate structures, it was shown that low PA was associated with tumor, cell death or decreased cell integrity, but high PA was associated with the healthy cell or cell membrane [32]. We did not detect any significance to differentiate the study group from controls. Some limited studies investigated the application of a bioimpedance spectroscopy technique to diagnose knee OA. In a study that consisted of 14 knee OA patients and seven controls in which electrode placement was in the same pattern as ours, the authors suggested that bioelectrical impedance resistance evaluation is a valid method to determine the inflammatory pathological conditions of this joint [33].

Limitations

Electrode placement, electrode sizes and the position of the patient's knee may affect the penetration depth of the signal in bioimpedance measurements. To overcome these limitations electrode sizes are required to clarify whether these changes are clinically relevant and consistent. The placements of electrodes and the knee position with different angles should be considered.

Conclusions

Our results indicate that serum RANKL and phase angle values may not be a predictive marker of knee OA progression. Further controlled clinical researches with larger samples are needed.

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