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Clinical correlation and determination of Dkk-1 and sclerostin levels in patients with rheumatoid arthritis

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Abstract

The aim of this study is to compare serum Sclerostin and Dickkopf-1 (Dkk-1) levels in patients with rheumatoid artrithis (RA) and healty controls to determine their clinical significance in patients with RA. Sixty with RA according to American Collage of Rhematology criteria and at least one year follow up, enrolled in this study and compared thırty healty controls. To evaluate disease activity score 28 (DAS28) was calculated. Physical function capasity (disability) was asssessed with Health Assesment Quastionnarie (HAQ) and Nottingham Health Profile (NHP). Erythrocyte Sedimentation Rate, C Reactive Protein, Rheumatoid Factor and anticyclic citrullinated peptide levels were determined by routine laboratory methods. Serum sclerostin and Dkk-1 levels of the patients with RA and healty controls were measured by ELISA. Radiographic assesment of hands joints was evaluated according to the modified Larsen score. Between patients with RA and healty controls, there was significant difference with respect to the age (respectively p=0.00) and significant difference with respect to gender (p=0.033). Serum sclerostin and dickkopf-1 levels were significantly higher (p=0.002, p=0.049) in patients with RA compared to healty controls. Serum sclerostin and dickkopf-1 levels were doesnt correlated with clinical and laboratory parameters of disease activity. There was no significant correlation between radiological scoring of joint damage and serum sclerostin and dickkopf-1levels (p 0.05). This study shows that Serum sclerostin and dickkopf-1 levels were increased in RA patients in comparison to control group but there was no significant correlation with the disease activity and joint damage. The sample of our study can be enlarged and further studies are required.

Keywords: Rheumatoid artrithis, sclerostin, dickkopf-1

Introduction

Rheumatoid arthritis (RA) is a progressive, chronic systemic disease and afflicts several organs, however, damage to joints is the most dramatic feature. In particular, wrist and small hand joints are the most common and the first affected areas [1]. According to the studies conducted, the prevalence of RA is between 0.3% and 1.5% [2]. The disease has a close relationship with age and gender. The female / male incidence rate is accepted as an average of 3/1. 80% of the patients are between the ages of 35 and 50 [3]. It has been suggested that many factors play a role in the etiopathogenesis of RA. Many mechanisms such as genetic factors, infectious agents and the pathogenic and immune inflammatory responses triggered by them, disorders of autoimmunity against articular cartilage and synovia, and disorders in the regulation of proinflammatory cytokines have been blamed for the disease [4]. Although joints are the most affected area in rheumatoid arthritis, the disease has extra-articular involvement. In addition to systemic bone loss, local bone loss, characterized by periarticular osteopenia and focal bone erosions, is also observed in RA [5].

There are many factors that affect the pathogenesis of osteoporosis in rheumatoid arthritis. It has been shown that increase in osteoclast activity during the erosion process and inhibition of the new bone formation by WNT pathway take an important role in pathogenesis. High disease activity, drugs used in treatment such as glucocortoids, age, body mass index, gender and physical inactivity are risk factors for osteoporosis. The optimal treatment for the rudimentary disease is important to regulate the lifestyle and prevent and treat OP [6]. Recently, studies have been conducted on the increasing levels of sclerostine and dickkopf-1, two inhibitors of the WNT signaling pathway, in patients with rheumatoid arthritis and the disease is associated with damage to the joint and bone.

WNT proteins are synthesized by hematopoietic cells, basal cells at the bottom of the epithelial tissue, blood vessels, adult stem cells

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found in tissues and organs such as the brain, liver, lung, prostate. The synthesized WNT protein is released into the extracellular matrix by these cells through various post-translational modifications. [7]. In the studies conducted, it was concluded that the WNT signaling pathway increases the osteoblast transformation from mesenchymal cells and thus reduces bone loss. Genetic studies show that in invitro conditions, the WNT signal path extends the lifespan of osteoblasts [8].

In the present study; it was aimed to define Dkk-1 (dickopf-1) and sclerostin levels and to correlate the disease with other clinical and radiological parameters in rheumatoid arthritis patients.

Materials and Methods

In this study; A total of 60 RA patients were admitted to the FTR-Rheumatologist outpatient clinic, diagnosed as RA according to the 1987 ACR criteria, 30 patients treated with biological agents, 30 patients treated with DMARD. In addition, a control group consisting of 30 healthy individuals who were matched with the age and gender of the patients in the patient groups and without any autoimmune or inflammatory disease was created. Patients with advanced age group, patients with malignancy, patients with endocrine disorder that could perform secondary osteoporosis, and patients with metal implants in their body, as Dual energy x - ray absorptiometry (DEXA) would be performed, were not included in the study.

The demographic characteristics, disease duration, pain and global assessment (with VAS) of all patients were made, and the patients filled NHP (Nottingham Health Profile), HAQ-Stanford Health Assessment Questionnaire. In addition, DAS-28 score was estimated for patients with RA.

All cases included in the study were first informed about their diseases. Then, our study aim was explained both verbally and written, and an informed consent form was signed for the patients who were willing to participate in the study.

Our study was found and approved ethically by the Ethics Committee of Fırat University. The financial support of the study was provided by Fırat University Scientific Research Projects Coordination Unit (FÜBAP).

Clinical Evaluations

The basic features of each patient involved in the study, such as age and gender, were first questioned. Patients' height, weight measurements were made and body mass index (BMI: Weight (kg) / Height (m^2)) was calculated from these measurements. The menopausal status of the patients was questioned and the duration of menopause was specified as the year. Disease duration and current medical treatments were questioned in RA patients and patients were classified into DMARD group and biological agent group.

The patients were evaluated in terms of disease activity indicators used in RA follow-up. The pain intensity experienced by the patients in the last week was evaluated with visual analog scale (VAS) (0 = no pain, 10 = the most severe pain). The overall health of the patients was assessed by the patient and physician separately with VAS (0 = best possible condition, 10 = possible worst condition) Similarly, the severity of fatigue and fatigue was assessed by the patient with VAS. The duration of the morning stiffness was determined in minutes.

The number of swollen joints and sensitive joints was determined over a total of 28 joints determined for the assessment of disease activity [9]. Disease activity score 28 (DAS 28) was then calculated using the number of swollen and tender joints, erythrocyte sedimentation rate (ESR), and the patient's overall health status with VAS. This score can range from 9.4 (highest disease activity) to 0 (lowest disease activity) depending on disease activity. According to this system, patients with a DAS28 score of> 5.1 are classified as intense disease activity, patients with> 3.2 and ≤ 5.1 are assorted as temperate disease activity, and patients with ≤ 3.2 are classified as basic disease activity. In accordance with these criteria, we divided the patients in our study into 2 groups according to the DAS28 score, and evaluated the patients with DAS28 ≤ 3.2 as a low illness activity group, and those with DAS28> 3.2 as a medium / high disease activity group [9]. The general condition of the patients (functional disability) was assessed with the Turkish version of the health assessment questionnaire (HAQ) and Nottingham Health Profile (NHP).

The Health Assessment Questionnaire evaluates 8 basic areas (dressing and self-care, righting, eating, walking, hygiene, reaching, comprehending, activities) related to daily life activities. There are 2 or 3 questions in each area. By summing the highest scores in these 8 areas, the result is divided into 8 and the HAQ score ranging from 0 (best possible condition) to 3 (worst possible condition) is obtained [10].

Nottigham's health profile is a 38-item questionnaire with 6 main topics. The subjects related to sleep (5 items), pain (8 items), energy level (3 items), emotional reactions (9 items), physical mobility (8 items) and social isolation (5 items) are answered as yes or no. The score that can be obtained from each parameter varies between 0-100. The maximum total score that can be taken from the survey is 600 [11].

Laboratory Evaluations

Biochemistry, complete blood count, ESR, CRP, RF results were recorded for routine controls. In addition, the patients' Ca, P, ALP, PTH, vitamin D, TSH, anti-CCP values were examined. Similar examinations were made to healthy volunteers in the control group, and the results were recorded. Blood was drawn into 6 mL straight biochemistry tubes from patients and healthy volunteers. Then, the blood was centrifuged at 3000 rpm for 5 minutes and 2 mL serum was separated and then stored at -80 ° C. After reaching the sufficient number of cases for the study, the stored samples were studied according to the manual of the commercial kit (YH Biosearch Laboratory human ELISA kit) used by ELISA method in the Department of Medical Biochemistry of Fırat University. Results are given in ng / mL.

Radiographic Evaluation

Bone mineral density of all patients included in our study was determined by applying DEXA. BMD measurement is the most valuable and easily applicable quantitation method for investigating

the risk of fractures and determining the skeletal response to different treatments [12, 13]. Bone mineral content is quantified in grams, bone mineral density (BMD) in gr / cm2 (area) or gr / cm3 (volume). DEXA is the most advanced, reliable and frequently used method for today. It was developed in the 1980s and has been widely used since 1988. The skeleton zone is subjected to two X-ray beams of different intensity and the BMD is calculated from the amount of radiation by computer programs. By making two separate measurements, the effect of soft tissue (varying amounts of muscle and adipose tissue) is calculated and subtracted from the measured value. Central (hip and spine), peripheral (forearm) and even whole body screening can be done with DEXA [12, 13].

The International Clinical Densitometry Association recommends measuring from two different sites, if possible, the diagnose should be grounded on the lowest T-Score. It recommends averaging the L2-L4 measurement instead of a single vertebra. In hip assesments, total hip or femoral neck measurements are used (whichever standard deviation is lower). T and Z scores are used to demonsrate the outcomes of DEXA measurements. SD values are given for both. SD refers to the normal variability of assesment in a population. The difference between the 5 and 95 percentiles of a group is about 4 SD. An SD in the hip or spine corresponds to about 10-15% of the average value. The Z - Score shows how many SDs below (- SD) or above (+ SD) the average BMD of people of the same age. The T-Score shows how many SDs below (-SD) or above (+ SD) the average BMD of young adults (20-30 years old). Since BMD decreases with age in all bones, after 30 years of age, T - Scores are smaller than Z - Scores and this difference increases as we get older. By definition, the diagnosis of OP is made when the T - score is <2.5 SD [12].

Radiographic evaluation of patients with rheumatoid arthritis was done using the standard hand-to-wrist direct radiographs taken in the last 6 months, with the Larsen score modified in 1995 and the Sharp score modified by van der Heijde. In Modified Larsen scoring, a total score is obtained by scoring a total of 24 joint regions between 0 and 5 in both hands (minimum score 0, maximum score 120) [14]. It is 2-5 in this scoring system. Metacarpal joints (total 2x4 = 8 joints in both hands) are 2-5. Proximal interphalangeal joints (total 2x4 = 8 joints in both hands) and finally the wrist is divided into 4 regions (total 2x4 = 8 joints in both hands) and evaluated [15].

The main problem with the Sharp method is that the feet are excluded in the scoring table, even if they are often kept. So, in 1989, the Sharp method was modified by van der Heijde and feet were added to the score as well [16].

Some areas were removed due to erosions in the hands and narrowing of the joint space. Reason for removal; The fact that these regions are difficult to see and often scored in many radiographs and therefore leads to incompatibility between observers. It is scored as 0 if normal and 1 if erosions are observed. Wide erosions are scored as 2 or 3 according to the surface area of the joint. Erosions that affect more than half of the bone are scored as 4. In carpal bones, it is generally impossible to score erosions separately when the bone is completely collapse. In this situation, the collapse area is scored in accordance with the affected joint surface and the full collapse of the bone is scored as 5. Every erosion is scored. Any comment should be avoided if erosions are caused by rheumatoid process or osteoarthritic lesions. Joint space narrowing is combined with a (sub) luxation score and; 0 = normal; 1 = focal or suspicious; 2 = generalized, the remaining joint gap is more than 50% of the original joint gap; 3 = generalized, remaining joint gap less than 50% of the original joint gap or subluxation; 4 = bone ankylosis or full luxation.

As a result, it was concluded that the maximum score of erosions in each joint area should be 10 for the feet. The maximum erosion score is 160 on the hands and 120 on the feet. The maximum score for the narrowing of the joint range is 120 in the hands, 48 in the feet. Collecting all scores of hands and feet (0-448) adds more value to the foot joints. But since more joints are scored on the hands, the score of the hands is still of the gratest importance [17].

Statistical analysis

All statistical evaluations were made with 'Statistical Packages for Social Sciences Version 21.0 for MS Windows' program. Correlations between the two numerical variables were evaluated with Spearman and Pearson correlation tests. To show comparisons found between groups, Mann-Whitney U test was used for two groups. Comparisons between the three groups were evaluated with the Post Hoc test after applying variance analysis with the Kruskal Wallis test. In multiple comparisons, the limit of significance was taken as 0.05 / Number of comparisons (3 comparisons) = 0.016. In other comparisons, 0.05 was accepted as the limit of significance.

Results

The study included 60 patients with rheumatoid arthritis (46 women, 14 men) and 30 healthy controls (15 women, 15 men). The average disease duration of patients with RA was 12.5 ± 5.8 years. Table 1 shows the demographic and clinical features between patients with rheumatoid arthritis and the healthy control group.

Serum ESH mean of the patient group was 27.5 ± 15.2 mm / hour, and that of control group was 14.8 ± 10.3 mm / hour. Serum CRP mean was 12.4 ± 10.2 mg / dl in the patient group and 5.6 ± 4.4 mg / dl in the control group.

The mean DAS28 score of the patient group was 4.1 ± 1.2. The mean of the modified larsen score was 52.5 ± 22.4, and the mean of the modified Sharp score was 142.4 ± 47.1.

Serum RF mean of the patient group was 88.2 ± 110.8 mm / hour, mean of anti CCP was 310.8 ± 332.5 U / ml, RF mean of the control group was 11.99 ± 0.7 mm / hour. Serum ESH, CRP, RF levels were significantly higher in the patient group compared to the control group, and there was a statistically significant difference ($p < 0.001$). (Table 2)

Vitamin D levels were 22.7 ± 12 in the control group and 21.5 ± 14.3 in the patient group. There was no statistically significant difference between two groups ($p = 0.4$).

The number of patients using only DMARD in the rheumatoid arthritis group was 14, the number of patients using at least one different DMARD together with methotrexate was 16, and the number of patients using biological agent was 30. (Table 3)

Table 1. Demographic and clinical features in patients with rheumatoid arthritis and healthy control group

		RA (n:60)	Control Group	P
Age		48.6±6.7	43.8±4.2	0.000
Gender	**Female**	46 (77.9%)	15 (50%)	0.033
	Male	14 (23.3)	15 (50%)	
Disease Duration (Years)		13.5±58 (1-30)	-	-
Plow arrest period		111.3±8.75 (0-360)	-	-
Severity of pain (0-10 VAS)		5.73±1.8 (1-10)	1.60±0.96 (0-3)	0.000
Tiredness-fatigue (0-10 VAS)		5.8±1.44 (1-9)	1.43±0.96 (0-3)	0.000
GA of the patient		5.8±1.55 (1-10)	1.60±0.96 (0-5)	0.000
Physician's GA		5.82±1.45 (1-7)	1.47±0.86 (1-4)	0.007
Number of sensitive joints (0-25)		6.5±4.9 (0-18)	-	-
Swollen joint pain		4.06±2.3 (0-14)	-	-
HAQ score (0-3)		1.5±0.21 (0-3)	0.25±0.3 (0-1.1)	0.002
NHP sleep		69.5±40.58 (0-100)	10±16.4 (0-40)	0.000
NHP social isolation		20.6±6.6 (0-100)	6.6±10.9 (0-40)	0.003
NHP emotional reaction		12.4±8.5 (0-100)	4.4±8.5 (0-22.2)	0.000
NHP physical activity		62.0±19.5 (0-100)	14.1±10.7 (0-37.5)	0.000
NHP fatique		70.0±42.2 (0-100)	21.1±4.0 (0-100)	0.020
BMI		30.4±4.75 (19.5-38.10)	30.2±4.01 (21-40)	0.045
Menopausal status	**Yes**	29 (63.4%)	7(46.7%)	0.193
	No	17(37.6%)	8(53.7%)	
Menopause duration		7.01±4.1 (1-16)	3.14±1.4 (1-5)	0.033

VAS: Visual Analogue Scale, HAQ:Healt Assessment Questionnaire, NHP: Nottingham Health Profile, BMI: Body Mass İndex, GA: Global Assessment

Table 2. Various laboratory features in patients with rheumatoid arthritis and healthy control group

	RA (n:60)	Control Group	P
ESR (mm / hour)	27.5±15.2 (5-73)	14.8±10.3 (5-40)	0.000
CRP(g/dl)	12.8±10.2 (2-56)	5.6±4.04 (3-18)	<0.001
RF	90.5±115.8 (10.712)	11.0±0.7 (1-11)	<0.001
Anti CCP	315.8±335.2 (3-1000)	-	-
DAS28/0-9.4	4.0±1.27 (0.8-6.11)	-	-
Ca	9.5±3.9 (8-9.8)	9.09±0.75 (8-10.8)	0.491
P	3.8±0.4 (2.5-4.2)	3.5±0.3 (3.20-4.20)	0.0751
PTH	65.3±23.7 (20-135)	62.1±16.3 (35-82)	0.970
Vitamin D	21.5±14.3 (5-71)	22.7±12.8 (5-53)	0.481

ESR: Erythrocyte Sedimentation Rate, CRP: C Reactive Protein, Anti CCP: Anticyclic Citrullinated Peptide,
RF: Rheumatoid Factor, DAS28: Disease Activity Score 28, PTH:Parathormone

Serum sclerostin and dickkopf-1 levels were found statistically dramatically higher in the RA group in comparison to the control group. (p = 0.02), (p = 0.049). (Table 4)

Serum sclerostin and dickkopf-1 levels were similar in patients receiving anti-TNF and in patients using other disease modifying drugs. There was no statistically meaningful difference between these two treatment groups in terms of ESR and CRP, RF, Anti-CCP.

The modified-Larsen and modified-Sharp scores were statistically dramatically lower in the group receiving anti-TNF (p = 0.02, p = 0.03, respectively). There was no significant difference between DEXA measurements between the two groups. (Table 5)

Serum sclerostin and dickkopf-1 levels were found statistically significantly higher in the RA group receiving DMARD in comparison to the control group (p = 0.001, p = 0.011, respectively), while only the sclerostin level was found to be remarkably higher in the anti TNF receiving group (p = 0.008).

There was no correlation between sclerostin and Dkk-1 levels and the activity parameters of the disease, demographic, laboratory and radiological parameters in the rheumatoid arthritis group. Only positive correlation was found between Sclerostin and Dkk-1 (p = 0.003, r = 0.382).

Table 3. Distribution of patients with rheumatoid arthritis according to treatment protocols

Tectai Prolaxy	N(%)
DMARD only	14(23.3%)
Metotreksat+other DMARD	16(26.7%)
Metotreksat+Anti TNF	30(50%)
DMARD: Disease Modifying Anti-Rheumatic Drugs	

Table 4. Comparison of serum sclerost and dipckopf-1 levels in patients with rheumatoid arthritis and the control group.

	RA (n:60)	Control Group	P
Skreostin	21.5±7.7 (0.80-33.9)	16.3±5.5 (2.4-27.4)	0.02
Dkk-1	39.5±29 (14.3-102.9)	24.6±12.8 (3.10-79.5)	0.049
Dkk-1: Dickopf-1			

Table 5. Radiologic parameters of patients receiving anti-TNF and patients using DMARD

	Anti INF (n:30)	DMARD	P
Modified larsen (0-120)	45.8±21.1 (20-80)	64.2±22.6 (24-100)	0.02
Modified Sharp	125±47.0 (56-200)	159±43 (68-240)	0.037
DEXA L1 BMD	0.7±0.18 (0.4-1.10)	0.72±0.18 (0.4-1.0)	0.271
L1 Z score	-0.8±1.03 (-3.20-1.2)	-1.03±1.35 (-3.5-1.2)	0.599
L1 T score	-1.0±1.2 (-3.20-1.20)	-1.1±1.3 (-3.8-1.2)	0.578
DEXA L2 BMD	0.7±0.14 (0.5-1.10)	+0.9±0.12 (0.4-1.10)	0.267
DEXA L2 Z score	-0.9±1.0 (-3.10-1.20)	-1.1±1.2 (-3.3-1.0)	0.437
DEXA L2 T score	-1.05±1.3 (-3.4-1.6)	-1.2±1.4 (-3.70-1.20)	0.319
Femur total BMD	0.7±0.36 (-0.5-1.1)	0.78±0.18 (0.4-1.10)	0.319
Z score	-0.6 ±1.04 (-3.2-1.5)	-0.7±1.07 (-3.5-0.5)	0.684
T score	-0.7±1.2 (-3.5-1.6)	-0.8±1.2 (-3.80-0.7)	0.790
DEXA: Dual energy x - ray absorptiometry BMD: Bone mineral density			

Discussion

Rheumatoid Arthritis is a chronic disease with an important disability. Disability is seen more intensely in the hand and wrist joints, where the disease is most seen and the most active involvement. RA, one of the most familiar autoimmune diseases affecting roughly 1% of the world population, is a common disease that causes disability as a result of being late in diagnosis and treatment.

In our study, when the RA group was compared with the healthy controls, the L1-L4 and L2-L4 DEXA measurements were found to be remarkably lower than the control group. There was no important difference between the patient group and the control group regarding vitamin D levels. This finding shows us that the inflammatory process in RA may have triggered osteoporosis.

Osteoporosis, focal bone erosion, periarticular OP and generalized OP are seen in 3 types in rheumatoid arthritis [18, 19). Many factors are effective in the pathogenesis of osteoporosis in these patients. The concept of osteoimmunology gained importance as the relations between the immune system and bone became increasingly understandable. Osteoclast, macrophage colony stimulating factor and proinflammatory cytokines explain the relationship between inflammation and osteoporosis. Control of inflammation in rheumatic diseases reduces structural bone damage and bone loss. In inflammatory rheumatic diseases such as rheumatoid arthritis (RA), ankylosing spondylitis (AS) and systemic lupus erythematosus (SLE); The link between osteoclast, macrophage colony stimulating factor (M-CSF) stimulating factor, and especially proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-alpha) and interleukin-1 (IL-1) shows the relationship between inflammation and osteoporosis. Periarticular trabecular bone loss is characterized by a reduction in bone formation as a result of inhibition of Wnt signaling. Two inhibitors of the Wnt signal pathway play an important role in dickopf-1 (Dkk-1) and sclerostin RA [5].

Rossını et al. [20] compared 154 postmenopausal women with RA and 125 healthy control groups and found Dkk-1 level significantly higher in RA patients. Pınzone et al. [21] found Dkk-1 levels high in RA patients and accused Dkk-1 of making erosive

arthritis. They stated that Dkk-1 neutralization could be a new option in the treatment of RA and osteoporosis. Marenzena et al. stated that in inflammatory diseases, bone loss increases and so the risk of fracture increases. They stated that anti-inflammatory treatments reduce bone loss but are ineffective on bone formation. In animal experiments with inflammatory arthritis, anti-sclerost treatment has been reported to reduce bone loss, but is ineffective on systemic inflammation [22].

In studies conducted, it is stated that the treatment of existing osteoporosis in RA patients should be done with new treatment agents such as sclerostin and Dkk-1 antagonists. Daoussis and Andonopoulos [23] reported that low level of Dkk-1 was associated with high bone mineral density and increased remodeling. This supports the sclerostin and Dkk-1 release by inflammatory cells, and high levels of serum sclerostin and Dkk-1 in patients with intense inflammation and high inflammatory cell load. In the literature, it is thought that sclerostin and Dkk-1 are produced from inflamed synovium and strongly reflects joint inflammation in elevated serum sclerostin and Dkk-1 levels not only in RA but also in mixed connective tissue disease, SLE and psoriatic arthritis [24]. Studies show that sclerostin levels in synovial fluid and serum are high in RA patients and inflammatory cytokines disrupt the balance between bone formation and destruction on these molecules. Wehmeyer et al. [25], the sclerostin level in the synovium of RA and osteoarthritis patients was compared and found significantly higher in RA patients. In parallel with these studies, sclerostin levels and serum Dkk-1 were found to be remarkably higher in RA patients in comparison to the healthy control group.

TNF, which has a prominent role in the pathogenesis of rheumatoid arthritis, can trigger systemic bone loss. Studies have shown that IL-1 is required for TNF-mediated bone loss, the results obtained in these studies may explain that the severity of joint destruction and bone involvement is different in clinical observations.

However, Vis et al. found that [26], in patients receiving infliximab therapy, there was a dramatic decrease in serum type 1 collagen cross-linked C telopeptide (CTX) and RANKL levels in parallel with the increase in BMD. This finding was supported in our study too. BMD values were higher in the group receiving anti-TNF. TNF blockade has been shown to inhibit structural bone damage independent of its anti-inflammatory effect [27]. In the study conducted Wang et al. [28], between 100 RA patients, 100 other inflammatory diseases and 40 healthy controls, Dkk-1 level was found to be dramatically higher in the RA group. When the RA group was compared to the group receiving infliximab and the group receiving other agents, the group receiving infliximab showed a decrease in Dkk-1 level. Lim et al. [29] stated in their study that, 33 active RA patients were treated with DMARD group drugs and compared with healthy control group who received no treatment, received etanercept treatment. Sclerostin levels were lower in the group receiving etanercept and correlation with DAS28 was detected. Agnes et al. [24] performed etanercept treatment for 12 months in 35 RA and 27 psoriatic arthritis patients and there was no significant decrease in sclerostin and Dkk-1 levels in both groups.

In our study, the patients receiving anti-TNF and the patients receiving DMARD were compared among themselves and individually healthy controls. There was no important difference in serum sclerostin and Dkk-1 levels between the group receiving anti-TNF and the group receiving DMARD. While there was a significant difference between sclerostin levels between the group receiving anti-TNF and healthy control, there was no significant difference between Dkk-1. This finding showed us that Dkk-1 was suppressed in the group receiving anti-TNF in parallel with other studies. Compared to healthy controls, the group receiving DMARD showed a significant difference between sclerostin and Dkk-1 levels.

As a result, we can say that Anti-TNF treatment shows its mechanism of action mostly through Dkk-1. As the two groups were compared in terms of radiological evaluations, Modified Larsen and Modified Sharp scores were found to be remarkably lower in the group receiving anti-TNF. When DEXA was evaluated, anti-TNF group had significantly higher bone mass especially in femoral neck and total results. Although our patients received DMARD treatment for a long time and preferred anti-TNF treatments in the late period, this group had lower radiological erosion scores and higher DEXA values. Suppressed Dkk-1 in the anti-TNF group may be associated with decreased joint and bone erosion and increased bone formation. In our study, patients were seperated into groups that received anti-TNF and other DMARDs, and there was no remarkable difference between the two groups in terms of sclerostin and Dkk-1 levels. This may be because of the restricted number of patients and the fact that many of our patients started anti-TNF treatment recently. Today, when the patient is diagnosed with RA, initiation of DMARD therapy has become a standard method. For this reason, the effect of serum sclerostin and Dkk-1 levels on the use of corticostreoid, biological agent, methotrexate and other disease-modifying drugs may be the reason for no significant difference in serum sclerostin and Dkk-1 levels between our treatment groups.

In patients who use glucocorticoids, it is generally considered that bone formation is affected, however, in patients with early RA who do not use glucocorticoids, it has been stated that increased bone resoption associated with osteoclast is in the foreground [30]. Although it is known that glucocorticoids are linked with bone loss and fracture risk, in some studies, it has been reported that glucocorticoid use decreases bone turnover in patients with early RA, and prevents loss of hip and hand BMD [31, 32]. It has been reported that when low-dose glucocorticoid use is required, bone loss can be stopped by optimal control of inflammation [32]. RA increases the risk of hip and vertebral fractures two-fold regardless of glucocorticoid use [33]. In the study, Gifre et al. conducted [34], 25 RA patients received glucocorticoid therapy for 12 months and sclerostin and Dkk-1 levels were compared with healthy controls. As a result, it was observed that there was no decrease in the level of sclerostin while initially increased Dkk-1 level was decreased.

Serror et al. [35] found that, 813 patients with arthritis were followed for 2 years and 694 of these patients were diagnosed with RA. Dkk-1 levels of patients diagnosed with RA were higher than the other group. Among 694 RA patients, high Dkk-1 levels correlated with radiological progression. In conclusion, it was emphasized that the investigation of Dkk-1 in the early phases of rheumatoid arthritis can help predict the prognosis and response to treatment in patients with aggressive destructive disease. Juarez et al. [36] found out in their study that, a synovial biopsy of 12 patients with early RA, 8 remissions of RA and 9 arthritis patients

was found to be higher in RA patients than in healthy controls. There was not any difference between early RA and late RA. In our study, serum sclerostin and Dkk-1 rates did not show any correlation with the duration of the disease in RA patients. The reason for not finding a significant difference in serum sclerostin and Dkk-1 levels in the early RA and late RA groups may be that all patients were using drugs that would affect serum sclerostin and Dkk-1 levels such as corticosteroid, MTX and Anti TNF. Another factor may be that the number of patients forming the early-late RA group is not homogeneous.

In our study, no correlation was found between the activation parameters of the disease, the CRP values, the questionnaires used in clinical evaluation, and serum sclerostin and Dkk-1 levels. In the literature, some studies did not correlate with these values. Serum levels of sclerostin and Dkk-1 are high, but more comprehensive studies are needed to elucidate the correlation between sediments, CRP and DAS28.

In our study, serum sclerostin and Dkk-1 levels were not correlated with radiological scores in the RA group, but there was a remarkable statistical difference between the DMARD group and the anti-TNF group in terms of radiological scores. Erosion scores were found to be lower in the anti-TNF group. This may be due to the fact that many of our patients are in the late RA group and their erosion scores are high. Serum sclerostin and Dkk-1 levels and DEXA values did not correlate with rheumatoid arthritis group. The reason for this may be that our patients mostly have osteopenia. So as to clearly consider the relationship between serum sclerostin and Dkk-1 levels with RA, we preferred our patients to be osteopenic. As the increasing information about the pathophysiology of osteoporosis observed in RA, new therapeutic concepts, including anti-erosive treatments, are expected to emerge.

There are studies indicating that serum sclerostin and Dkk-1 levels correlate with age, menapozole status and gender levels in the healthy population and RA group. In our study, sclerostin and Dkk-1 levels in the healthy group and RA group were not correlated with bone markers such as age, gender, menopausal status, menopause duration. Rossini et al. [37] stated in their study that, 154 postmenopausal RA patients were compared with healthy controls and Dkk-1 level was found to be significantly higher in serum RA patients and its correlation with PTH and DEXA was found.

There are studies in the literature that indicate that serum sclerostin level correlates with vitamin D. Garnero et al. [38] followed 572 postmenopausal women for 6 years and found that those with high serum sclerostin levels had lower bone mineral density measurements determined by DEXA. There was no correlation between sclerostin level and vitamin D. Dawson et al. [39] in their study found that, the initial serum sclerostin and Dkk-1 levels of 270 male and 279 female patients were measured and vitamin D supplementation and placebo treatment were applied to these patients for 2 years. In the measurements at the end of 2 years, there was no remarkable difference between the group taking vitamin D and the levels of Dkk-1 and sclerostin compared to the placebo group. Ahmed et al. [40] conducted a study over 60 postmenopausal women, in which they were divided into 3 as osteoporosis, osteopenia and normal bone mineral density. Sclerostin and Dkk-1 levels of patients were measured and correlated with vitamin D. Sclerostin and Dkk-1 levels were found high in women in the postmenopausal period, but no correlation was found with vitamin D in any group. In our study, serum sclerostin and Dkk-1 levels were not correlated with vitamin D in both RA and healthy groups. In the patient group, all our RA patients received daily treatment of vitamin Ca / D in the routine, and this colud be the reason for no correlation. Also, the mean vitamin D level in both groups was below normal, that is, the average vitamin D level of patients and the healthy group was within the limits of osteomalacia. This may be because patients cannot be grouped in terms of vitamin D.

An ideal 'disease indicator' should be able to reflect ongoing active inflammation, even in patients who take medication that can change the course of the disease. Therefore, we think that sclerostin and Dkk-1 can give more accurate results, especially in patients with early RA that have not yet received any treatment. It may reflect disease activity, radiographic progression of sclerostin and Dkk-1, and may be a useful marker for predicting aggressive destructive disease and osteoporosis. In addition, new treatment approaches for osteoporosis can be developed and thus an increased bone formation can be achieved in RA with sclerostin and Dkk-1 antibodies.

The goal of this case-control study was to investigate serum sclerostin and Dkk-1 levels in RA patients and evaluate the association between sclerostin and Dkk-1 with other disease activation parameters. The patients were seperated into two groups receiving anti-TNF and DMARD, and the effect of anti-TNF treatment on sclerostin and Dkk-1 level was investigated.

Based on the findings of our study, the following conclusions can be drawn. Serum sclerostin and Dkk-1 level is higher in RA patients than healthy controls. There is no significant difference in sclerostin and Dkk-1 levels between the group receiving DMARD and anti-TNF. Erosion scores are lower and BMD measurements are higher in the group receiving anti-TNF. Dkk-1 levels were suppressed in the group receiving anti-TNF. As a result, Sclerost and Dkk-1 levels play an important role in the etiopathogenesis and joint damage of osteoporosis in RA, and their neutralization may be a new approach to stop joint damage and osteoporosis in RA.

Conflict of interests

The authors declare that they have no competing interests.

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Ethical approval

Our study was found and approved ethically by the Ethics Committee of Fırat University.

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