Original Article

Relationship between bone mineral density and anti-citrullinated protein antibody and rheumatoid factor in patients with rheumatoid arthritis

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Abstract

Objective: Rheumatoid arthritis (RA) is one of the causes of osteoporosis, and it leads to systemic bone loss. The anti-citrullinated protein antibody (ACPA) and rheumatoid factor (RF) are associated with local and systemic low bone mineral density and osteoclast-mediated bone resorption independently of inflammation in patients with RA. In this article, we aimed to evaluate the relationship between the ACPA, RF, and systemic bone mineral density in patients with RA.

Methods: Ninety-three patients (6 male, 87 female) with RA were included in the study. The disease activity score 28-erythrocyte sedimentation rate and titers of RF, ACPA, and bone mineral density of the total hip, femoral neck, and lumbar areas were evaluated. The independent samples t-test, Mann-Whitney U-test, Spearman's correlation, and multivariable regression analysis were used for the statistical analysis.

Results: The RF and ACPA were positive in 40.9% and 48.4% of patients with RA, respectively. Disease activity was negatively correlated with the T- and Z-scores. The T- and Z-scores were lower in the seropositive group than in the seronegative group. The ACPA was negatively correlated with the T- and Z-scores of the femoral neck. There was a significant difference for the Z-score of the femoral neck in patients with ACPA and RF-positive patients compared to seronegative patients with RA.

Conclusion: A low bone mineral density, especially in the femoral neck, is associated with the presence of ACPA and RF. It would be a more appropriate approach to carefully monitor osteoporosis in seropositive RA patients.

Keywords: Rheumatoid arthritis, bone mineral density, anti-citrullinated protein antibody, rheumatoid factor

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Introduction

Rheumatoid arthritis (RA) is a chronic, autoimmune, and inflammatory disease that affects many systems, including the lungs and the heart, and it is characterized by the inflammation of small joints and local and systemic bone loss. The local bone loss in RA begins in the early stage of the disease, and if the disease activity cannot be controlled, localized bone erosion, periarticular osteopenia, and osteoporosis develop in the areas of inflammation. There is also an increase in the incidence of generalized osteoporosis due to systemic bone loss and destruction. Bone destruction is associated with autoantibodies such as the anti-citrullinated protein antibody (ACPA), rheumatoid factor (RF), and the degree of inflammation in RA (1-3). Inflammatory cytokines such as tumor necrosis factor-alpha, interleukin (IL)-1β, and IL-6 increase bone resorption by enhancing the differentiation of osteoclasts (3). Inactivity, the female gender, smoking, alcohol, uncontrolled disease, high disease activity, and corticosteroid therapy are among the risk factors of generalized osteoporosis (2-4). The use of corticosteroids is an independent risk factor for the bone mineral loss in the total hip and spine (1). A high number of swollen joints, a moderate-to-high disease activity according to the validated composite measures, a positive RF and/or ACPA, and an early erosive disease are poor prognostic factors in patients with RA (5). High acute-phase reactant levels and autoantibody positivity, especially the high levels of RF and/or ACPA, cause the skeletal injury and erosions (3-5).

The ACPA is a predictor of bone resorption, erosion, and severe disease in RA. It has been reported to be strongly associated with osteoclast-mediated bone resorption, independently of inflammation (6, 7). Specific N-terminal citrullination of vimentin is induced during osteoclast differentiation, and also osteoclasts se-

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crete enzymes providing protein citrullination (6). Local and systemic bone loss in patients with ACPA positivity has been shown to begin even before the onset of clinical disease (3). The ACPA positivity is associated with a lower systemic bone mineral density in patients with early arthritis. It has been reported that the RA patients with ACPA had a lower lumbar and hip bone mineral density compared to AC-PA-negative RA patients (8). In addition, in the multivariate analysis for adjusting confounding factors such as gender, age, and menopause, anti-CCP remained as independent factors. Our study differs from others due to determining the relationship between the systemic bone mineral density and RF as another risk factor. We found the association between the steroid dose and disease activity and the femur neck Z-score, unlike Llorente et al. (8) study. Also, our study was conducted on Turkish patients with RA. In another study, it was reported that the RF positivity and oral steroid dose are negatively associated with the femoral neck bone mineral density in female patients with RA (9).

In the present study, we aimed to evaluate the relationship between the systemic bone mineral density and ACPA and RF in patients with RA. Another aim was to determine the effect of steroid dose and disease activity score (DAS28-ESR) on systemic bone mineral density in patients with RA. This study differs from others because it aims to determine the relationship between the systemic bone mineral density and RF as another risk factor, and it is also evaluated in Turkish patients with RA.

Methods

A total of 93 patients (6 male, 87 female, with the mean age of 60.0±8.9 years) diagnosed with RA were included in the cross-sectional study. Patients with the history of any rheumatic disease other than RA or younger than 18 years were excluded from the study. This study was approved by the Adnan Menderes University Ethical Board of Clinical Research (approval no. 2018/1419) and conducted in accordance with the guidelines of the Declaration of Helsinki.

Clinical information was obtained from patients' records. Gender, age, disease duration, bone mineral density, DAS28-ESR, and the titers of RF and ACPA were evaluated. The diagnosis of RA was made based on the criteria outlined by the American College of Rheumatology and the European League Against Rheumatism in 2010 (10). The patients with the RF values <18 IU/mL and ACPA <4.99 U/mL were considered to be negative. Low titers of RF or ACPA refer as the values 1-3 times the upper limit of the normal and high titers as values exceeding >3 upper limits of normal. The bone mineral density was measured systematically in all patients of the cohort on a single machine using dual X-ray absorptiometry (DEXA). The total hip, femoral neck, and lumbar areas were measured to determine the bone mineral density. We diagnosed osteoporosis with the T-score. The Z-score was evaluated for premenopausal females, males younger than 50 years, and differential diagnosis of secondary osteoporosis. A reduced bone mineral density was defined as a Z-score ≤-1 standard deviation (SD). All patients whose scores were within the osteoporotic range were treated according to the guidelines after the diagnosis of osteoporosis.

Statistical analysis

All statistical analyses were performed with the Statistical Package for Social Sciences version 17.0 (SPSS Inc.; Chicago, IL, USA) program for Windows. The results were expressed as the number of patients (n), percentages (%) for categorical variables, and mean±SD or median (25%-75%) for continuous variables according to the distribution of normality determined by the Kolmogorov-Smirnov test. The independent sample t-test and Mann-Whitney U-test were used to compare binary groups. Spearman's correlation test was used for the statistical analysis and was assessed by Spearman's rho correlation coefficient. The multivariable regression analysis was performed to correlate demographic data with bone mineral density. For multivariate analysis, a generalized linear model was used for possible confounders. Regression analyses were adjusted for possible confounders, including the age, gender, and disease duration. The factors for the model were determined based on the results of previous studies. The results were assessed at the 95% confidence interval, and the p-value <0.05 was regarded as statistically significant.

Results

Most of the patients included in the study were female (6.5% male, 93.5% female). The mean age of female patients with RA was 59.7±8.8 years, and for male patients, it was 64±9.4 years. The mean disease duration of RA

Table 1. Demographic and clinical features of Turkish patients with rheumatoid arthritis

	Rheumatoid Arthritis (n=93)
Age (years)	60.0±8.9
Gender (male/female)	6/87
Biological Therapy	34.4%
Disease Duration (months)	55.8±28.0
Steroid Dose (mg)	8.3±8.0
DAS28-ESR	3.9±1.0
Rheumatoid Factor (RF)	
• RF positivity n (%)	38 (40.9%)
• RF titer*	74.1 (2.2-66.2)
• RF titer in positive patients*	174.7 (53.2-208.5)
ACPA	
• ACPA positivity n (%)	45 (48.4%)
• ACPA titer*	49.9 (0.5-84.3)
• ACPA titer in positive patients*	102.3 (25-200)
T-score	
• Lumbar	-2.4±0.9
• Femoral neck	1.8±1.0
• Total hip	-0.9±1.2
Z-score	
• Lumbar	-1.0±1.1
• Total femur	-0.9±1.2
• Femoral neck	-0.3±1.0

*median (25%-75%)

DAS28-ESR: disease activity score 28-erythrocyte sedimentation rate; ACPA: anti-citrullinated protein antibody

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Table 2. The distribution of T- and Z-scores in Turkish patients with rheumatoid arthritis with and without anti-citrullinated protein antibody

	ACPA (+) n=45	ACPA (-) n=48	p
Age (years)	61.8±8.7	58.4±8.8	NS
Gender (male/female)	4/41	2/46	NS
Disease Duration (months)	61.6±28.6	50.4±26.6	NS
Steroid Dose (mg)	8.3±8.2	8.3±7.8	NS
DAS28-ESR	3.8±1.1	3.9±0.9	NS
Rheumatoid Factor Positivity n (%)	29 (64.4)	9 (18.8)	<0.001*
T-score			
• Lumbar	-2.3±1.0	-2.4±0.9	
• Femoral neck	-2.0±0.9	-1.7±1.1	NS
• Total hip	-1.1±1.2	-0.8±1.2	
Z-score			
• Lumbar	-1.1±1.0	-0.8±1.2	NS
• Femoral neck	-0.6±0.7	-0.1±1.1	0.03*
• Total hip	-0.8±1.1	-0.1±1.2	NS

*p<0.05

NS: not significant; DAS28-ESR: disease activity score 28-erythrocyte sedimentation rate; ACPA: anti-citrullinated protein antibody

Table 3. The correlation between femoral neck bone mineral density and other variables

Variables	Z-femoral Neck Score
Age (year)	r=-0.13, p=0.20
Gender	r=0.11, p=0.27
Disease duration (months)	r=-0.14, p=0.17
Steroid dose (mg)	r=-0.30, p=0.003
DAS28-ESR	r=-0.26, p=0.01
Rheumatoid factor titer	r=-0.24, p=0.01
ACPA titer	r=-0.25, p=0.005

r: correlation coefficient; DAS28-ESR: disease activity score 28-erythrocyte sedimentation rate; ACPA: anti-citrullinated protein antibody

was 55.8±28.0 months. Each patient received at least one disease-modifying antirheumatic drug, including hydroxychloroquine, sulfasalazine, methotrexate, leflunomide, and a corticosteroid. None of the patients with RA were receiving any selective estrogen receptor modulators, calcitonin, or bisphosphonates before the diagnosis of osteoporosis by DEXA, and all of them were non-smokers.

The demographic and clinical features of the patients included in the study are summarized in Table 1. The RF was positive in 40.9% of patients. The median RF titer in positive patients was 174.7 (53.2-208.5). A total of 48.4% of the patients were ACPA positive, and the median ACPA titer in positive patients was 102.3 U/mL (25-200). The mean lumbar T-score was -2.4±0.9, -1.8±1.0 in the femoral neck, and -0.9±1.2 for the total hip. The Z-scores were -1.0±1.1, -0.3±1.0, and -0.3±1.2, respectively. The mean DAS28-ESR score was 3.9±1.0, and the steroid dose was 8.3±8.0 mg/day at the time of the evaluation of bone mineral density by DEXA. The positive correlation was found between the DAS28-ESR score and steroid dose (p<0.001, r=0.686). The disease activity (DAS28-ESR) was significantly negatively correlated with the T- and Z-scores.

There were no significant differences in age, gender, body mass index, disease duration, and the mean steroid dose between seropositive and seronegative RA patients. The T- and Z-scores were lower in the RF-positive group than in the RF-negative group. No significant difference was found for the T- and Z- scores in patients between low and high RF titers. The RF titer was negatively correlated with the Z-femoral neck score (p=0.01, r=0.248). In regression analyses, RF titers were independent risk factors for lower bone mineral density of the femoral neck. There was a statistically significant difference between both groups only in terms of the Z-femoral neck score.

The distribution of the T- and Z- scores in patients with and without ACPA are shown in Table 2. The femoral neck Z-score was statistically significantly lower in the ACPA positive group than the ACPA negative group (p=0.03). There were no differences for the T- and Z- scores between high and low ACPA titers. Ninety RA patients with the lumbar Z-score below -1 were detected. It was 54 and 24 for the total femur and femur neck, respectively. The ACPA negatively correlated with the T- and Z-score of the femoral neck (respectively, p=0.02, r=0.226; p=0.01, r=0.259). A multiple regression analysis showed that the steroid dose, DAS28-ESR, ACPA, and RF were the risk factors for lower bone mineral density of the femoral neck. The ACPA positivity was independently associated with a lower BMD at the femoral neck Z-score, but not at the total femur and lumbar Z-score. Furthermore, there was a significant difference for the Z-score of the femoral neck in patients with ACPA and who were RF positive compared to seronegative patients with RA.

Discussion

In the present study, the T- and Z-scores were all found to be lower in seropositive patients with RA compared to the seronegative group. Although the scores were lower, a statistically significant difference was only detected for the femoral Z-score. We wanted to investigate the effects of secondary risk factors related to RA on the bone mineral densitometry other than general risk factors in our patients; hence, we used the Z-score, which is adjusted in accordance with sex and age. We found that the disease activity (DAS28-ESR) was significantly negatively correlated with the T- and Z-scores in patients with RA. These findings suggest that the presence of RF and ACPA is important to be considered in determining the low systemic bone mineral density in RA.

The pro-inflammatory cytokines lead to periarticular osteopenia and systemic bone loss by providing the osteoclast activation (11-13). However, it has been reported that osteoporosis may not be only associated with inflammation, but also with autoantibodies such as ACPA and RF in RA (8, 14). A significant increase in the receptor activator of nuclear factor kappa-B ligand levels in serum and synovial fluid was found in untreated patients with early RA with ACPA than in those with negative. It is thought to be a pathogenic mechanism that suggests that the ACPA leads to initial localized bone loss and erosions and that increases the number of osteoclasts (3, 12, 15). The antibodies against citrullinated vimentin, which is targeted by ACPA bind to osteoclast precursors, and it leads to the TNF-α release and osteoclast differentiation. The presence of ACPA induce damage of the bone microstructure and cortical bone even in healthy individuals without

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any clinical signs of arthritis (16). In addition, the ACPA remained as an independent factor for the bone loss at the lumbar spine and hip in the multivariate analysis for adjusting confounding factors such as gender, age, and menopause (8).

The femoral neck is mainly constituted by the cortical bone similar to the MCP joints. The vertebral bone mainly consists of trabecular bone and may be affected by many factors other than ACPA due to its more metabolic activity (14). Lower lumbar and femoral Z-scores in ACPA-positive patients were reported (14). There was no difference in the Z-score between the high and low ACPA titers, and both groups showed a significantly lower systemic bone mineral density than the one that was negative. In our study, the T- and Z-scores were found to be lower in the ACPA-positive patients compared to the ACPA-negative patients. There was a statistically significant difference in terms of the femoral neck Z-score between both groups. According to Llorente et al. (8) study, the bone mineral density scores of the lumbar spine, femoral neck, and total hip were significantly lower in the ACPA-positive patients compared to the APCA-negative patients. Bugatti et al. (17) reported a decreased Z-score in the lumbar spine in patients with a positive ACPA compared to patients with a negative APCA. However, it did not reach a statistically significant difference in the total hip Z-score. In a retrospective cross-sectional study by Kweon et al. (18), male RA patients aged over 50 years revealed the total hip bone mineral density to be significantly lower than healthy controls. They also found that the high titers of ACPA were related to a low bone mineral density in L1-L4, and DAS28-ESR was found to be associated with osteoporosis, but no association was found with cumulative glucocorticoid doses. In our study, there was a significant difference in the Z-score of the femoral neck in the seropositive patients, and the ACPA was negatively correlated with the T- and Z-scores of the femoral neck. Like Kweon et et al. (18) study, the axial skeleton seems to be preserved, and therefore, in RA, which is the cause of secondary osteoporosis, it is suggested that the factors affecting the sites of the reduced bone mineral density may be related to RA-specific risk factors, such as the RA-related autoimmunity other than traditional risk factors.

High titers of ACPA and RF are often associated with extraarticular manifestations and erosive disease. The RF is present in only 50%-60% of those with established RA, but less than 50% of patients with early RA (19). The RF was found to be associated with a bone mineral density reduction in both the lumbar and hip regions, and only at high titers. Both in the presence of RF and ACPA, the ACPA affected negatively the lumbar Z-score even at low RF titrations (17). We found a negative correlation between the RF titer and Z-femoral neck score, and also the T- and Z-score of the femoral neck and ACPA titer. Consequently, the RF has titer-dependent effects on the bone mineral density, and it is more prominent when in combination with ACPA due to providing a subclinical inflammatory milieu with its increased immune complex activity (20).

Although the glucocorticoid use is an independent risk factor for osteoporosis, it may have beneficial effects on bone mineral density in the ultradistal and medial region of the forearm when used for short periods to migitate inflammation (21). In a study, the negative correlation was found between the femur Z-score and cumulative glucocorticoid dose (14). In another study, no association was found between both the systemic and juxta-articular bone mass and glucocorticoid use and DAS28 (8). We found a positive correlation between the DAS28-ESR score and steroid dose. Also, the DAS28-ESR score was significantly negatively correlated with the T- and Z-scores. There were no significant differences in the steroid dose between the seropositive and seronegative patients. The limitation of our study is that it is cross-sectional in nature, and we were not able to discriminate whether the BMD decreases were associated with autoimmunity. Nonetheless, it may be considered as an autoimmunity effect because there is no difference between the seropositive and seronegative group in terms of disease duration. In multivariable linear regression analyses, there was no independent association between the disease duration and bone mineral density. Another limitation of our study is that we were not able to evaluate the cumulative disease activity, inflammation, the radiographic bone erosions of the patients, and vitamin D levels.

In conclusion, a low systemic bone mineral density, especially in the femoral neck, is associated with the presence of ACPA and RF. A more appropriate approach could be careful monitoring of osteoporosis in seropositive patients with RA.

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