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ORIGINAL ARTICLE

# Vitamin D deficiency and low bone mineral density in native Chinese rheumatoid arthritis patients

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## Abstract

**Objective:** We aimed to examine the risk factors related to the development of osteoporosis in rheumatoid arthritis (RA) patients and whether there is an association among the changes in bone mineral density (BMD), disease activities (modified DAS28), serum 25-hydroxyvitamin D (25OHD) levels, and disease duration.

**Methods:** There were 110 patients with RA and 110 age- and sex-matched healthy controls who were concurrently studied. All of the patients underwent the following measurements: erythrocyte sedimentation rate, C-reactive protein, rheumatoid factor, and serum 25OHD. Dual-energy X-ray absorptiometry (DEXA) was also used to measure the BMD of the left femur at the time of recruitment. Patients taking vitamin D supplement or corticosteroids were excluded.

**Results:** The incidences of osteopenia (45.6% *vs*. 36.4%, $P = 0.170$) and osteoporosis (33.6% *vs*. 5.45%, $P = 0.000$) were higher in the RA patients than in the healthy controls. There was a significant negative correlation between vitamin D levels and DAS28 ($r = –0.325$, $P = 0.001$) and a significant positive correlation between vitamin D levels and BMD ($r = 0.422$, $P = 0.000$). The multiple regression analysis revealed that 25OHD levels were significantly correlated with disease activity and BMD ($F = 11.087$, $P = 0.000$). Stepwise multiple regression analysis showed that serum 25OHD levels were the significant predictors for low BMD and high disease activity (DAS28) in RA patients.

**Conclusion:** The incidences of osteoporosis and osteopenia were higher in RA patients compared to the age- and gender-matched healthy controls. Low serum 25OHD levels correlate with low BMD and high disease activity in RA patients.

**Key words:** native Chinese and rheumatoid arthritis, vitamin D deficiency.

## INTRODUCTION

Rheumatoid arthritis (RA) is an inflammatory joint disease characterized by bone complications, such as osteoporosis. In RA, periarticular bone loss, bone erosion and systemic osteoporosis are observed, along with an increased risk of bone fracture.[1] Age, the severity of the disease and the use of glucocorticoids are identified risk factors for osteoporosis in RA patients. However, bone loss can also occur in glucocorticoid-naïve patients, premenopausal women and those with early RA.[1]

In a previous study, Pye *et al.*[2] reported that increased disease activity and severity are associated with accelerated bone loss. However, a lower bone mineral density (BMD) failed to predict new occurrence of erosive disease. In addition, Merlino *et al.* found that an increased intake of vitamin D may be associated with a lowered

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risk of RA in older women, although the explanation for this finding remains unknown. Further studies need to corroborate their findings.[3] Vitamin D may act in a paracrine manner to decrease T-cell responsiveness through an inhibition of cellular proliferation and a reduction in lymphokine production. Therefore, vitamin D may have a beneficial effect on RA as an immunosuppressant.[4]

Vitamin D inadequacy is a global problem. Approximately 36% of healthy young adults and up to 57% of general medicine inpatients in the USA suffer from vitamin D inadequacy. These figures are even higher in Europe.[4,5] According to reports that have been published in China since 2000, vitamin D deficiency and insufficiency were widespread and prevalent. Vitamin D deficiency was highly prevalent in postmenopausal women in Beijing.[6] In recent years, vitamin D deficiency has also been linked to the pathogenesis and/or progression of several disorders, including cancer, hypertension, multiple sclerosis and diabetes. Multiple studies have reported that vitamin D levels are associated with BMD and RA disease activity.[7] The aim of our study was to investigate the serum vitamin D levels and their associations with BMD, disease activity, disease duration and age in native Chinese RA patients.

## MATERIAL AND METHODS

### Patients

We studied 110 RA patients. All of the patients fulfilled at least four criteria of the 1987 revised American College of Rheumatology (ACR) for RA diagnosis[8] and were treated at the Rheumatology Department at The First Afflicted Hospital of Xiamen University, China. Out of the total 110 study patients, 39 (35.4%) were postmenopausal women, 35 (31%) were premenopausal women and 37 (33.6%) were men. A similarly sized group of age- and sex-matched healthy volunteers were used as the concurrent controls.

A total of 110 patients (age 59.48 $\pm$ 11.41 years) with a mean disease duration of 6.51 $\pm$ 6.82 years were enlisted; none were receiving glucocorticoid treatment. At the time of the investigation, only six (18.3%) of the patients were receiving bisphosphonate therapy, and 21 (5.2%) were receiving disease-modifying anti-rheumatic drugs (DMARDs). The patients taking corticosteroids or vitamin D and those who presented renal insufficiency were excluded. The study was approved by the medical ethics committee of the First Afflicted Hospital of Xiamen University.

### Laboratory tests and analytical techniques

Dual-energy X-ray absorptiometry (DEXA, QDR-4500; Hologic, Inc., Bedford, MA, USA) was used to measure the BMD of the left femoral neck at the time of recruitment. The patients with T scores higher than –1.0 were considered to have normal BMD, those with a T score –1 to –2.5 were considered to have osteopenia and those with a T score lower than –2.5 were considered to have osteoporosis. In the BMD analysis, the patients who had prostheses in their hip joints were excluded from the BMD analysis.

Serum 25-hydroxyvitamin D (25OHD), N-amino terminal propeptide of type I collagen (P1NP), C-terminal telopeptide of type I collagen (A-CTX), osteocalcin (OC) and intact parathyroid hormone (iPTH) were measured using radioimmunoassays (Elecsys, Roche, Basel, Switzerland). Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were considered to be markers of inflammatory activity. The current disease activity was evaluated using the European League Against Rheumatism Disease Activity Score (DAS) and a modified DAS28, based on a 28-joint assessment.

### Statistical analysis

The data were analyzed using SPSS for Windows, version 13 (SPSS Inc., Chicago, IL, USA). Values are expressed as mean $\pm$ standard deviation (SD) to assess the differences in values between the two groups and then analyzed using the unpaired *t*-test for numeric variables. The correlations were evaluated using the Spearman correlation. To compare the frequency of osteoporosis between the groups, a Chi-square test was used. The differences were considered not significant if $P > 0.05$ and significant if $P < 0.05$. A multiple linear regression analysis was performed using vitamin D as the response variable and age, disease duration, gender, disease activity (modified DAS28), and BMD as the independent variables.

## RESULTS

The demographic data, 25OHD levels, DAS28 results, BMD levels, disease durations, swollen joint counts and tender joint counts for the study subjects are summarized in Table 1.

Osteopenia was observed in 45.5% (50/110) and osteoporosis was observed in 33.6% (37/110) of the RA patients. In comparison, the frequency of osteopenia was 36.4% (40/110) and that of osteoporosis was 5.45% (6/110) in the healthy controls.

**Table 1** Clinical and laboratory characteristics of the study groups

| Variables | Rheumatoid arthritis ($n$ = 110) | Control ($n$ = 110) | χ/T | P | Significance |
|---|---|---|---|---|---|
| Age (mean ± SD), years | 59.48 ± 11.41 | 56.92 ± 10.51 | 1.762 | 0.08 | NS |
| Gender | | | | | |
| Male ($n$ = 73) | 34 (30.9%) | 39 (35.5%) | 1.498 | 0.504 | NS |
| Female ($n$ = 146) | 75 (68.2%) | 71 (64.5%) | | | |
| Bone mineral density | | | | | |
| Normal ($n$ = 87) | 23 (20.9%) | 64 (58.2%) | 47.782 | 0.000 | HS |
| Osteopenia ($n$ = 90) | 50 (45.6%) | 40 (36.4%) | | | |
| Osteoporosis ($n$ = 43) | 37 (33.6%) | 6 (5.4%) | | | |
| 25OHD (mean ± SD), ng/mL | 14.27 ± 6.92 | 20.85 ± 7.35 | 6.834 | 0.000 | HS |
| DAS28 (mean ± SD) | 4.43 ± 0.74 | | | | |
| Swollen joint count (mean ± SD) | 1.68 ± 2.02 | | | | |
| Tender joint count (mean ± SD) | 4.39 ± 1.70 | | | | |
| Disease duration (mean ± SD), months | 6.51 ± 6.82 | | | | |

NS, not significant; HS, highly significant.

No significant correlations were found between vitamin D levels and age, gender and disease duration ($P > 0.05$). However, there was a significant negative correlation between vitamin D levels and DAS28 ($r = -0.325$, $P < 0.01$). In addition, we found a significant positive correlation between vitamin D levels and BMD ($r = 0.422$, $P < 0.01$, Table 2).

The analysis of variance (ANOVA) of the multiple regression analysis revealed a significant linear correlation between 25OHD levels, disease activity and BMD ($F = 11.087$, $P = 0.000$). A stepwise multiple regression analysis using 25OHD as a dependent variable indicated that age, DAS28, BMD and disease duration were independent variables. The serum 25OHD level was a significant predictor of reduced BMD and increased disease activity (DAS28) in RA patients (Table 3).

## DISCUSSION

One of the main findings of our study is that vitamin D deficiency and low mineral density can be observed in RA patients compared to healthy controls. Low serum 25OHD levels were associated with a low BMD and high disease activity (DAS28) in RA patients. There was a significant negative correlation between vitamin D levels and DAS28 and a significant positive correlation between vitamin D levels and BMD. Another finding is the fact that osteopenia was observed in 45.5% and osteoporosis in 33.6% of the RA patients. In comparison, these conditions were observed in only 36.4% and 5.45% of the healthy controls, respectively.

Previous studies showed that vitamin D may act in a paracrine manner to decrease T-cell responsiveness by inhibiting cellular proliferation and reducing lymphokine production. Therefore, vitamin D has a beneficial effect as an immunosuppressant.[4] A murine model of human RA revealed that active vitamin D treatment decreased both the incidence and severity of the disease in mice. Interestingly, vitamin D from supplements had a stronger influence on RA development than did dietary vitamin D.[4] Vitamin D has modulatory effects on B lymphocytes and immunoglobulin production, and recent reports have demonstrated that $1,25(OH)_2D_3$ does indeed exert direct effects on B-cell homeostasis.[9] A circannual rhythm has been observed for vitamin D, with low levels during the winter and peaks during the summer; these seasonal changes showed negative correlation with clinical status, at least in RA and systemic lupus erythematosus (SLE) patients. Recently, the onset of symptoms of early arthritis during winter or spring has been associated with stronger radiographic evidence of disease progression at 12 months, a result that is possibly also related to seasonal decreases in vitamin D serum levels.[10]

**Table 2** Correlation between vitamin D and BMD, DAS28, age, gender and disease duration

| Variables | $r$ | $P$ | Significance |
|---|---|---|---|
| BMD | 0.422 | 0.000 | HS |
| DAS28 | −0.325 | 0.001 | HS |
| Age | −0.067 | 0.487 | NS |
| Gender | −0.010 | 0.915 | NS |
| Disease duration | −0.103 | 0.283 | NS |

BMD, bone mineral density; DAS28, disease activity score of 28 joints; HS, highly significant; NS, not significant.

**Table 3** Estimation and testing results of the regression coefficient of serum 25(OH)D with BMD and DAS28 in RA patients

| Variables | Unstandardized coefficients | Standardized coefficients | | | |
|---|---|---|---|---|---|
| | B | SE | Beta | *t* | Significance |
| (Constant) | 23.852 | 3.397 | 0 | 7.020 | 0.000 |
| DAS28 | −2.175 | 0.785 | −0.254 | −2.772 | 0.007 |
| BMD | 1.358 | 0.462 | 0.269 | 2.937 | 0.004 |

BMD, bone mineral density; DAS28, disease activity score of 28 joints.

Cloin *et al.*[11] found that the presence of 1,25 $(OH)_2D_3$ reduced interleukin-17A (IL-17A) and interferon-γ (INFγ) levels and increased IL-4 levels in the stimulated peripheral blood mononuclear cells (PBMCs) of treatment-naïve patients with early RA. Th17 cells and Th17 cytokines may play important roles in the development and onset of RA. Furthermore, supplementary 1,25$(OH)_2D_3$ may serve to prevent Th1 and especially Th17 polarization during the very early stage of RA and may protect against the development of this disabling disease.[11]

Tumor necrosis factor (TNF) is a key molecule in the pathogenesis of RA, and the over-expression of TNF can trigger systemic bone loss. Receptor activator of nuclear factor kappa-B ligand (RANKL) has a permissive effect on the osteoclastogenic effect of TNF, and osteoprotegerin protects against TNF-induced bone loss.[1] Successful DMARD treatment can trigger an increase in osteoprotegerin expression and a decrease in RANKL expression at the synovial tissue level, which correlates with a reduction in erosion scores.[1] Many processes contribute toward the pathology of RA-associated osteoporosis; however, increased osteoclast activation and subsequent bone resorption, mediated by IL-6 and other inflammatory cytokines, as well as by disturbances in the RANK/RANKL/OPG (osteoprotegerin) system, are thought to play key roles.[12]

Therapeutic blockade of TNFα produces clinical responses in the majority of RA patients.[13] Furthermore, inhibiting TNFα production prevents periarticular and generalized bone loss in RA patients. The effects of 1,25 $(OH)_2D_3$ on TNFα are complex, with both stimulation and inhibition of TNFα in various T-cell subgroups. 1,25$(OH)_2D_3$ was shown to inhibit the production of TNFα, IL-17, and IL-22.[11] The combination of neutralizing TNF activity and increasing 1,25$(OH)_2D_3$ controls human Th17 activity and additively inhibits synovial inflammation. This effect indicates the valuable therapeutic potential of activating vitamin D receptor signaling over current TNF neutralization strategies in patients with RA and potentially other Th17-mediated inflammatory diseases.[14]

The negative correlation between serum 25OHD levels and disease activity in RA patients suggests the involvement of vitamin D in the pathogenesis of RA.[9] Whether 1,25$(OH)_2D_3$ and 1,25$(OH)_2D_3$ analogs can prevent periarticular bone loss in RA patients is currently unknown.[11] In a study by Colin *et al.*,[15] there was a low incidence of vitamin D deficiency in healthy volunteers and in patients with early RA. An increased incidence of vitamin D deficiency has been observed in patients with established RA. Lower serum levels of 25OHD have been reported to be associated with higher disease activity.[9] Our study confirmed this observation, as the 25OHD levels significantly increased after RA treatment with DMARDs and/or TNFα inhibitors (data not shown).

A number of studies found that 25OHD levels were positively correlated with BMD in a healthy population,[16,17] whereas other studies demonstrated this association only under low 25OHD concentrations.[18,19] These conflicting results could be due to various reasons.[6] One study in Chicago measured the serum 25OHD levels and BMD in 104 young physicians and found that vitamin D insufficiency and a low BMD could be important contributors to future osteoporotic fractures.[5] The present study showed that low serum 25OHD levels were associated with low BMD and high disease activity in RA. Vitamin D may play an important role in the pathogenesis of osteoporosis in RA patients.

The effect of vitamin D supplementation on disease activity is unknown. In collagen-induced arthritis (CIA), supplementation with active vitamin D or vitamin D analogs can diminish disease activity.[20] Greater intake of vitamin D may be associated with a lower risk of RA in older women.[3] Further study on supplementation with active vitamin D and vitamin D analogs to diminish disease activity in RA should be undertaken.

## CONCLUSION

Osteoporosis and osteopenia were observed in 33.6% and 45.5% of RA patients, respectively, compared to 5.45% and 36.4% of healthy controls. Vitamin D deficiency is widespread and prevalent in native Chinese RA patients. Low serum 25OHD levels correlate with low BMD and high disease activity in RA patients. Future investigations of the role of vitamin D in active RA are warranted.

## AUTHOR CONTRIBUTIONS

Study conception and design: Juan Chen. Acquisition of data: Juan Chen, Wen Liu, Qingyan Lin, Liying Chen. Analysis and interpretation of data: Huiping Huang, Junping Yin.

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