The relation between disease activity, vitamin D levels and bone mineral density in men patients with ankylosing spondylitis

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Abstract

The aim of this study was to assess the vitamin D status in patients with ankylosing spondylitis (AS), and to investigate the relation between vitamin D levels, bone mineral density (BMD) and disease activity in men with ankylosing spondylitis. Seventy patients with AS and 140 healthy individuals were included in the study. BMD of femur and lumbar spine was measured by DXA. Serum 250H vitamin D, parathormone, serum calcium, C-reactive protein levels of all participants were also measured. The disease activity was evaluated by Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), and functional status by Bath Ankylosing Spondylitis Functional Index (BASFI). The mean 25(OH)D level was 17.5±9.7 ng/mL in AS patients and 21.9±7.7 ng/mL in controls (P<0.001). In comparison with the control group, AS patients showed significantly higher CRP, and a significant reduction of vitamin D. In AS group, 62 patients (88.6%) had Vitamin D deficiency, and 35 patients (50%) were osteoporotic. Vitamin D was negatively correlated to BASDAI without any changes after adjustment for age, duration of disease, sunlight exposure, and total taking steroids (r=-0.32, P<0.001). We found a high incidence of vitamin D deficiency in our patients. Our study suggests that vitamin D deficiency in male AS may indirectly lead to osteoporosis by causing an increase in the inflammatory activity. Monitoring vitamin D levels would be useful in order to determine the patients under osteoporosis risk.

Introduction

Ankylosing spondylitis (AS) is a chronic, systemic and inflammatory disease that characteristically involves the axial skeleton, enthesis regions, and in some patients the peripheral joints.2 Osteoporosis is a well-recognized complication of ankylosing spondylitis (AS), strongly correlated with disease activity. Several studies have shown involvement of inflammatory processes in the pathophysiological of AS-related osteoporosis.2-7 Furthermore, various other factors, such as drug intake and decreased mobility in relation to pain and stiffness, may contribute to the development of osteoporosis in AS patients.8 In addition, recent studies on AS have suggested that alterations in vitamin D metabolism are associated with inflammatory activity and bone mineral density (BMD).9,13

In fact, vitamin D is a crucial factor in the regulation of calcium homeostasis and maintenance of skeletal health. It also plays an important role in the modulation of the immune system. The expression of vitamin D receptor, constitutively or after immune stimulation, on antigen presenting cells, dendritic cells, T and B cells, further suggests an immunoregulatory role of vitamin D.14,15 The aim of this study was to assess the vitamin D status in male patients with ankylosing spondylitis, and to investigate the relation between vitamin D levels, bone mineral density and disease activity in men with ankylosing spondylitis.

Materials and Methods

Subjects

This study was approved by the ethics committee of our hospital (El Ayachi University Hospital, Morocco) and all patients provided written informed consent to participate in this study.

Seventy men patients with the diagnosis of AS according to the Modified New York Criteria and 140 healthy individuals were included in the study as the control group.21 We decided to include only men with age less than 65 years in order to eliminate potential confounding factors on bone, such as age and menopause. No patient used alcohol. Fifty-eight patients had received intermittent non-steroidal anti-inflammatory drugs (NSAID) in the previous 12 months. No patient was receiving glucocorticoid medication at the time of the study; only 5 patients had received such medication for a short time in the past (5-10 mg/day for 1 to 3 months during the disease duration). Eight patients were receiving infliximab.

Exclusion criteria for the study were: concomitant presence of inflammatory bowel disease, chronic renal or hepatic disease, diabetes mellitus, parathyroid or thyroid disease, recent fractures, malnutrition, or taking medications in the past two years known to influence bone metabolism such as vitamin D, calcium and bisphosphonate.

Data collection and measurements

Demographic and clinical variables were recorded by anamnesis and clinical examination. In order to determine the level of disease activity, Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) was used.22 Physical function was assessed using Bath Ankylosing Spondylitis Functional Index (BASFI; on a scale of 0-10).23 The validity and reliability of the Moroccan versions of these forms have been approved.24

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Contributions: FA and NHH conceived the study and supervised its design, execution, and analysis and participated in the drafting and critical review of the manuscript. IH, FA and RA did data management and statistical analyses. All other authors’ enrolled patients, participated in data acquisition and critical revision of the manuscript. IH wrote the paper with input from all investigators. All authors read and approved the final manuscript.

Conflict of interests: the authors declare no potential conflict of interests.

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Anthropometric data
Weight and height were measured without clothes or shoes at the time of bone densitometry measurements. The body mass index (BMI) was calculated as body weight/height (kg/m²).

Physical performance measures
The short form of the international physical activity questionnaire (IPAQ) was used to evaluate physical performance. The items of IPAQ were structured to provide separate scores on walking, moderate intensity and vigorous intensity activity. Computation of total score requires summation of the duration (in minutes) and frequency (days) of walking, moderate intensity and vigorous intensity activities.25

Dietary calcium questionnaire
Dietary calcium intake was assessed with the frequentual self-questionnaire of Fareedollie,26 which has been modified, simplified and adjusted to the Moroccan food habits.

Biochemical measurements
Laboratory assessment included C-reactive protein (CRP), alkaline Phosphatase, serum levels of calcium, phosphor, 25-OH vitamin D and parathormon (PTH). DiaSorin 25(OH)D \textsuperscript{125I} radioimmunooassay kit was used for quantitative determination of 25(OH)D in plasma or serum. The intra-and interassay CV were 5 and 11%, respectively and the normal range was 30-60 ng/mL. Immunoassay Elecsys PTH Test System was used for quantitative determination of intact PTH in plasma or serum. Intra-and interassay variances were 5 and 7% and the normal range was 15-65 pg/mL. Vitamin D deficiency was defined as 25(OH)D serum level less than 20 ng/mL, vitamin D insufficiency as 25(OH)D levels of 20-30 ng/mL and vitamin D sufficiency as levels greater than 30 ng/mL.27 We measured 25(OH)D during summer season which could have been the highest level of 25(OH)D.

Bone mineral density assessments
Lumbar spine, trochanter, femoral neck and total hip BMD were measured by dual-energy X-ray absorptiometry with a Lunar prodigy densitometer. Daily quality control was carried out by measurement of a Lunar phantom. At the time of the study, phantom measurements showed stable results. The phantom precision expressed as the CV(%) was 0.07. Both T and Z scores were obtained. In the T-score calculations, the manufacturer’s ranges for European population reference were used because of the absence of a Moroccan database. Osteoporosis was defined as a T-score <-2.5, according to the World Health Organisation study group definition.28

Statistical analysis
Statistical analysis was performed with the Windows 13.0 version of SPSS software (SPSS Inc., Chicago, IL, USA). With 70 cases and 140 controls, this study had 80% power to detect the difference in the prevalence of hypovitaminosis D in AS patients and healthy subjects using a 95% confidence interval (95% CI). For the calculation of the target sample size, we have assumed that difference between 25 hydroxyvitamin D levels in AS patients and in controls is 10 ng/mL. Values are expressed mean ± S.D or percentages. Normality of the data was tested with a one-sample Kolmogorov Smirnov test to indicate the appropriateness of parametric testing. With the aim of evaluating the differences between groups, Student’s t test for the variables with a normal distribution was used. Proportions were compared between groups by using chi-squared test or Fisher’s exact test. While studying the relations between the variables in the patient group, Pearson correlation test was used. Then, we tried to conduct a multivariate analysis to detect the factors associated with the low vitamin D like BMI, disease duration, disease activity, CRP, BMI and physical activity. However, this approach did not result in any relevant association. Finally, we used the correlation between vitamin D levels and BASDAI after adjustment for age, duration of disease, sunlight exposure, and total taking steroids agreement. A P value of <0.05 was accepted as statistically significant.

Results
Vitamin D status, clinical, and laboratory characteristics of patients with ankylosing spondylitis and controls
The clinical and laboratory characteristics of patients with AS and controls are summarised in Table 1.

The mean age of patients was 40±12 years old and the mean age of the control group was 42±11 years old. The mean BMI of the patients and control group were respectively 23.1±4.4 kg/m² and 24±4.1 kg/m² (P<0.000). In comparison with the control group, AS patients showed significantly increased CRP and a significant reduction in vitamin D (P<0.0001). No differences in serum calcium, phosphorus and parathormon were detected between groups.

In patients group, 62 patients (88.6%) had vitamin D deficiency and only eight of subjects (11.4%) had concentration of vitamin D >30 ng/mL. No patient has shown severe vitamin D deficiency 25(OH)D <5 ng/mL.

Bone mineral density values of ankylosing spondylitis patients and controls
The lumbar spine BMD, femoral neck BMD, trochanter BMD and total hip BMD were significantly lower in men with AS compared to controls (P<0.001; Table 2).

According to the WHO classification, 35 patients (50%) were osteoporotic and 16 (22.9%) were osteopenia (compared to controls P<0.0001).

Correlations between bone mineral density values, biochemical and clinical assessments
In AS patients, the serum level of vitamin D was negatively correlated with BASFI, serum calcium and PTH (r=−0.22, P<0.05; r=−0.32, P<0.001, and r=−0.41, P<0.01; respectively). Disease duration was not correlated with clinical parameters, BMD or biochemical assessments (data not shown, all P>0.05). CRP was negatively correlated with PTH (r=−0.25, P<0.05). BASFI was positively correlated with CRP and ESR (r=0.39, P<0.05 and r=0.36, P<0.05; respectively).

A negative correlation was found between lumbar spine, BASDAI and BASFI (P<0.001). We found positive correlation between BMD at lumbar spine and femoral total and serum vitamin D levels (r=0.55, P<0.001 and r=0.38, P<0.001, respectively; Figures 1 and 2).

We found no correlation between IPAQ or total calcium intake and parameters of disease activity. Also, We found no correlation between Vitamin D levels and BMD T-scores (data not shown, all P>0.05) (Table 3).

Correlation between vitamin D levels and Bath Ankylosing Spondylitis Disease Activity Index after adjustment for confounding variables
Vitamin D was negatively correlated to BASDAI without any changes after adjustment for age, duration of disease, sunlight exposure, and total taking steroids (r=−0.32, P<0.001) (Figure 3).

Discussion
In our study, 62 male AS patients (88.6%) had Vitamin D deficiency, and 35 patients (50%) were osteoporotic. Vitamin D was negatively correlated to BASDAI without any changes after adjustment for age, duration of disease, sunlight exposure, and total taking steroids.

We found that male AS patients had a lower
lumbar spine and total hip BMD than controls. According with our results, previous studies have documented osteoporosis in AS.3,11,29-32 Thirty-five patients were osteoporotic. These data are consistent with previous reported prevalence of osteoporosis in AS patients that vary from 18.7% to 62%.11,30-32 The etiology of osteoporosis in AS has not been completely clarified and in various studies, it was suggested that different mechanisms such as immobilization caused by pain and spinal restriction, daily physical activity, inflammatory cytokines, genetic factors, glucocorticoids used for treatment, and NSAIDs have played a role.4,5,34 Longitudinal studies had demonstrated a clear relationship between bone loss and markers of disease activity in AS.5,9-11,35 In our study, we find correlations between femoral and lumbar BMD and disease activity parameters and serum vitamin D levels. The lack of correlation between BMD and disease duration observed in our cohort agrees with some previous studies and suggests that bone loss occurs early in the disease.35,36 However, several other studies found a positive correlation between disease duration and lumbar spine BMD, indicating that overestimation of the lumbar spine BMD (measured by DXA) occurred in patients with advanced AS.5,11

In comparison with the control group, male AS patients showed significantly decreased vitamin D. Vitamin D was negatively correlated to BASDAI without any changes after adjustment for age, duration of disease, sunlight exposure, and total taking steroids. Clinical studies have reported the impact of vitamin D in AS as an endogenous immune modulator, suppressing activated T cells and cell proliferation that may accelerate the inflammation process.14-19 In a recent study Lange et al. observed that high disease activity in AS is associated with an alteration in vitamin D metabolism, increased bone resorption and that AS patients with osteoporosis had significantly lower vitamin D levels compared to AS patients with normal BMD.7,11 Obermayer et al. suggested a close association of BMD, bone metabolism, and inflammatory activity with Fok1 polymorphisms of the vitamin D receptor gene in male AS patients.13 Also, it was stated that with the lack of vitamin D, which is an endogenous immunomodulator, proliferation of the T cells and activation cannot be prevented and that the severity of inflammatory process may increase.5

In our study a surprisingly high incidence of vitamin D deficiency was found in Moroccan patients and controls. Even Morocco is a sunny country where the exposure to sunlight might be considered to be sufficient to keep adequate vitamin D status, vitamin D deficiency is often observed. Previous study has shown that vitamin D deficiency is common among Moroccan

Table 1. The demographic and laboratory parameters of ankylosing spondylitis patients and controls.

<table>
<thead>
<tr>
<th></th>
<th>AS patients (n=70)</th>
<th>Controls (n=140)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>40±12</td>
<td>42±11</td>
<td>0.21</td>
</tr>
<tr>
<td>Body mass index</td>
<td>23.1±4.4</td>
<td>24.8±4.1</td>
<td>0.008</td>
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<td>25 hydroxyvitamin D (ng/mL)</td>
<td>17.5±9.7</td>
<td>21.9±7.7</td>
<td>&lt;0.001</td>
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<td>CRP (mg/L)</td>
<td>24.1±19.6</td>
<td>1.6±1.2</td>
<td>&lt;0.001</td>
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<td>Total calcium (mg/L)</td>
<td>93.4±4.9</td>
<td>93.1±3.6</td>
<td>0.58</td>
</tr>
<tr>
<td>Phosphorus (mg/L)</td>
<td>29.6±5.1</td>
<td>30.6±4.1</td>
<td>0.13</td>
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<tr>
<td>Parathormon (pg/mL)</td>
<td>64.5±47.6</td>
<td>56.9±24.3</td>
<td>0.13</td>
</tr>
<tr>
<td>Total calcium intake (mg/m²)</td>
<td>589±232</td>
<td>664±344</td>
<td>0.09</td>
</tr>
<tr>
<td>Total physical activity (min/wk)</td>
<td>3712±2648</td>
<td>3582±4672</td>
<td>&lt;0.001</td>
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<tr>
<td>Vitamin D deficiency</td>
<td>62 (88.6)</td>
<td>57 (40.7)</td>
<td>&lt;0.001</td>
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<tr>
<td>BASDAI</td>
<td>4.1±1.9</td>
<td></td>
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<tr>
<td>BABSFI</td>
<td>4.5±2.7</td>
<td></td>
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<td>Duration of disease (years)</td>
<td>12.1±7.2</td>
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Table 2. Bone mineral density values of ankylosing spondylitis patients and controls.

<table>
<thead>
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<th>Controls (n=140)</th>
<th>P</th>
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<tbody>
<tr>
<td>Lumbar spine (g/cm²)</td>
<td>0.930±0.301</td>
<td>1.120±0.148</td>
<td>&lt;0.001</td>
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<td>Femur neck (g/cm²)</td>
<td>0.990±0.268</td>
<td>1.195±0.180</td>
<td>&lt;0.001</td>
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<td>Trochanter (g/cm²)</td>
<td>0.909±0.445</td>
<td>0.822±0.145</td>
<td>&lt;0.001</td>
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<td>Femur total (g/cm²)</td>
<td>0.862±0.175</td>
<td>1.038±0.163</td>
<td>&lt;0.001</td>
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Table 3. Correlations between clinical and biochemical assessments in ankylosing spondylitis patients.

<table>
<thead>
<tr>
<th></th>
<th>25OH D</th>
<th>Age</th>
<th>BASFI</th>
<th>BASDAI</th>
<th>CRP</th>
<th>ESR</th>
<th>Ca</th>
<th>PTH</th>
<th>IPAQ</th>
<th>BMD L</th>
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<tbody>
<tr>
<td>Age</td>
<td>NS</td>
<td></td>
<td>-0.22*</td>
<td>0.34*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>BASDAI</td>
<td>0.32**</td>
<td>NS</td>
<td>0.62**</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td>NS</td>
<td>NS</td>
<td>0.39**</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>ESR</td>
<td>NS</td>
<td>NS</td>
<td>0.36*</td>
<td>NS</td>
<td>0.48**</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Ca</td>
<td>-0.41**</td>
<td>NS</td>
<td>-0.03*</td>
<td>NS</td>
<td>-0.26*</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTH</td>
<td>-0.41**</td>
<td>NS</td>
<td>-0.29*</td>
<td>NS</td>
<td>-0.25*</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>IPAQ</td>
<td>NS</td>
<td>NS</td>
<td>-0.24*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
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<tr>
<td>BMD LS</td>
<td>0.31**</td>
<td>NS</td>
<td>-0.31**</td>
<td>-0.32**</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>BMD Fem</td>
<td>0.32**</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.59**</td>
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women and this was influenced by a lack of sun exposure and veiled clothing style. The ethnicity factor was highly correlated with low levels of vitamin D. Another factor of the high incidence of vitamin D deficiency among Arab populations may be a genetic polymorphism of VDRs, an issue that has to be investigated.

Our study had few limitations and a number of strengths. First, BMD was monitored with dual-energy x-ray absorptiometry (DXA). However, previous studies have shown that the lumbar spine BMD in AS can be overestimated by the presence of syndesmophytes, ligament calcifications, and fusion of facet joints. Currently, quantitative computed tomography (QCT) is considered to be the best technique to measure spinal BMD in patients with advanced AS. However, QCT is expensive and has a high radiation dose compared to DXA. Therefore, an alternative method to monitor bone loss in AS patients is desirable.

Second, it would be useful if blood samples were collected at different times of the year to study the seasonal variation of vitamin D serum level. Also, vitamin D metabolism may be influenced by age. However, we found significantly lower vitamin D levels in AS patients compared with age-matched controls. In addition, we found no significant correlation between age and vitamin D serum levels.

Also, The IPAQ questionnaire has not been validated for use in AS. But The IPAQ has reasonable measurement properties for monitoring population levels of physical activity among 18- to 65-yr-old adults in diverse settings. The short IPAQ form last 7 d recall is recommended for national monitoring and the long form for research requiring more detailed assessment.

Conclusions

We found a high incidence of vitamin D deficiency in our patients. Our study suggests that vitamin D deficiency in male AS may indirectly lead to osteoporosis by causing an increase in the inflammatory activity. Monitoring vitamin D levels would be useful in order to determine the patients under osteoporosis risk.

References

2. Franck H, Meurer T, Hofbauer LC. Evaluation of bone mineral density, hormones, biochemical markers of bone...


