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□ ORIGINAL ARTICLE □

Decreased Plasma Vitamin D Levels in Patients with Undifferentiated Spondyloarthritis and Ankylosing Spondylitis

Sukran Erten[1], Orhan Kucuksahin[2], Ali Sahin[3], Alpaslan Altunoglu[4], Mesut Akyol[5] and Cemile Koca[6]

Abstract

Objective The aim of the present study was to evaluate the plasma vitamin D (vit D) levels and their association with the disease activity in patients with ankylosing spondylitis (AS) and undifferentiated spondyloarthritis (uSpA) compared with healthy populations.

Methods This study included 161 spondyloarthritis patients (113 uSpA patients and 48 AS patients) attending our rheumatology out-patient clinic, along with 92 controls.

Results The plasma vit D levels were 18 μg/L (8-38) in the AS group, 20 μg/L (4-92.3) in the uSpA group and 24.3 μg/L (7.2-76.8) in the control group. The plasma vit D levels of the AS patients were significantly lower than those of the patients in the control group (p=0.004). The men in the AS group had significanly lower vit D levels than those in the control group (p=0.005). On the other hand, the women in the uSpA group had significanly lower vit D levels than those in the control group (p=0.011). The vit D levels were inversely related to both erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) in the AS patients (p=0.002, R=-0.428; p<0.001, R=-0.592, respectively). This correlation was not demonstrated in the uSpA patients. The vit D levels were not found to correlate with the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) levels in either the AS or uSpA patients.

Conclusion 25-hydroxy-vit D deficiency is frequently observed in patients with SpAs. In this study, vit D deficiency was much more prominent in the male AS patients. On the other hand, among women, the uSpA patients exhibited much more prominent vit D deficiency than the control group subjects. The acute phase response may inversely affect the vit D levels in AS patients.

Key words: spondyloarthritis, ankylosing spondylitis, undifferentiated spondyloarthritis, 25-hydroxy vitamin D

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Introduction

Spondyloarthritis (SpA) is an inflammatory rheumatic disease affecting the axial spine (1). The SpA concept comprises ankylosing spondylitis (AS), psoriatic arthritis, arthritis/spondylitis with inflammatory bowel disease (IBD) and reactive arthritis (2). These diseases are characterized by the sharing of certain genetic and clinical features irrespective of the subtype of SpA, and all display variable onset, presentation and progression. Some common manifestations of this group include inflammatory back pain, peripheral arthritis, enthesitis, anterior uveitis, sacroiliitis and HLA B27 positivity (3).

AS is a subgroup of spondyloarthritis with predominant axial symptoms that occur as a result of sacroiliitis, spondy-

[1]Department of Rheumatology, Atatürk Education and Research Hospital, Turkey, [2]Department of Rheumatology, Ankara University School of Medicine, Turkey, [3]Division of Rheumatology, Şanliurfa Education and Research Hospital, Turkey, [4]Department of Nephrology, Atatürk Education and Research Hospital, Turkey, [5]Department of Biostatistics, Yıldırım Beyazıt University, Turkey and [6]Department of Biochemistry, Atatürk Education and Research Hospital, Turkey
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Correspondence to Dr. Sukran Erten, sukranerten@yahoo.com

litis, spondylodiscitis or entesitis (4). Patients with typical features of SpA who do not fulfill the criteria for one of the certain forms of SpA are classified as having undifferentiated spondyloarthritis (uSpA) (2). This disease is characterized by episodes of peripheral arthritis and enthesitis, axial symptoms and a lower incidence of HLA B27 compared to that observed in AS patients (5).

Vit D (cholecalciferol) is a secosteroid hormone that is produced in the skin under sunlight or obtained from the diet. In the liver, the inactive precursor vit D3 is converted to 25-hydroxy vit D (25(OH) D3) (6). 25(OH) D3 is the circulating compound denoting the vitamin status and it is usually measured for clinical testing (7). In the kidneys and extrarenal tissues, 25(OH) D3 is converted to the most active metabolite 1,25 dihydroxy vit D (1,25(OH)2 D3) (6). Vit D is necessary for intestinal calcium absorbtion, calcium and phosphorus regulation in the blood and skeletal and dental health. 1,25(OH)2 D3 works together with parathyroid hormone (PTH) and calcitonin to regulate the serum calcium and phosphorus levels. PTH is released in response to low serum calcium levels and it induces the production of 1,25 (OH)2 D3 (7).

Epidemiologic evidence shows a significant association between vit D deficiency and an increased incidence of autoimmune diseases, including systemic lupus erythematosus, type 1 diabetes mellitus, multiple sclerosis and rheumatoid arthritis (RA) (8). A decreased vit D intake has also been supposed to be a risk factor for the development of RA, and recent investigations have shown that low vit D levels are associated with increased disease activity and severity in patients with inflammatory arthritis (9).

Although some studies evaluating the vit D status of patients with AS (10-12) have shown decreased vitamin D levels and osteoprorosis in these patients, no studies have so far evaluated vit D status in patients with uSpA, which may be a different form of spondyloarthritis that may convert to AS. It is not known whether low vit D levels play a role in the conversion of uSpA to AS. The aim of the present study was to evaluate the plasma vit D levels in patients with AS and uSpA compared with those observed in healthy populations.

Materials and Methods

Patients and samples

This study included 161 consecutive patients with SpA (113 uSpA patients and 48 AS patients) attending the Atatürk Education and Research Hospital Rheumatology out-patient clinic. The control group comprised 92 healthy individuals. This study was conducted in the winter between December and March in both the patients and controls. The uSpA patients were diagnosed according to the ESSG criteria (13) and the AS patients were diagnosed according to the Modified NewYork criteria (14). Patients with associated inflammatory bowel disease, psoriasis, a preceding symptomatic infection of the urogenital or gastrointestinal tract in the four weeks before the onset of symptoms, Behçet's disease or familial Mediterrannean fever were excluded. In addition, patients with other known chronic diseases were excluded. The study protocol was approved by the local ethics committe and was in accordance with the Helsinki declaration 2008. Written informed consent was obtained from all subjects.

Blood samples from the patients with SpA and those in the control group were drawn. The plasma baseline 25(OH) D3 levels were measured according to the HPLC method using an Agilent 1,100 Liquid Chromatograph. Serum vit D levels <30 ng/mL were defined as reflecting vit D insufficiency, while those <10 ng/mL were defined as reflecting deficiency (9). The erythrocyte sedimentation rate (ESR) was measured automatically using the Kimased Auto 60-Vacutest Kima system, and the C-reactive protein (CRP) levels were measured using the BN II nephelometer, Dade Behring. The patients underwent peripheral and axial joint and entheses assessment, radiographic evaluation of the pelvic region to evaluate the sacroiliac joints and foot radiography to evaluate enthesopathy.

HLA B27 typing was determined using a polymerase chain reaction with sequence-specific primers (PCR-SSP). Low-resolution HLA-B genotyping was performed using commercial kits (Olerup SSP® HLA B*27, Saltsjöbaden, Sweden).

Power and sample size

The sample size of the study was calculated with G* Power (G*Power Ver. 3.0.10, Franz Faul, Üniversität Kiel, Germany, http://www.psycho.uni-duesseldorf.de/aap/projects/gpower/). The required sample size for 90% power, α=0.05 Type I error, β=0.10 Type II error and d=0.20 effect size was calculated to be a minimum of 253 total subjects.

Statistics

The one-sample Kolmogorov-Smirnov test was used to test the normality of continuous variables. Descriptive data are expressed as the mean ± SD. Skewed data are shown as the median and range. Univariate comparisons between nominal variables were performed using the chi-square test.

Comparisons of continuous variables between two groups were made using Student's t-test for parametric variables and the Mann-Whitney U test in the case of non-normal variables. To evaluate the correlation between two continuous variables, we used Spearman's correlation coefficients (Rho) test for non-parametric variables. The Kruskal Wallis non-parametric analysis of variance was used for comparisons of three groups. The level of significance was set at $p< 0.05$.

Results

Ten of the 48 AS patients were women (20.8%) and 38 were men (79.2%). Ninety-seven of the 113 uSpA patients

Table 1. Characteristics of the Patients with AS and uSpA

	AS(48) mean±SD, (median; min-max)	uSpA(113) mean±SD, (median; min-max)	p
Female/male, n(%)	10/38(20,8/79,2)	97/16(85,8/14,2)	<0,001
Age, yrs	35,5 ±10	46,2±10,5	<0,001
Disease duration, mo	110±84,6	79,1±60,6	<0,05
HLA B27 positive, n(%)	34(70,8)	13(14,9)	<0,001
Family history, n(%)	33(68,8)	37(32,7)	<0,001
IBP, n(%)	48(100)	82(72,6)	<0,001
Arthralgia/arthritis n(%)	33(68,8)	110(97,3)	<0,001
Enthesopathy, n(%)	32(66,7)	80(70,8)	NS
Eye involvement, n(%)	14(29,2)	5(3,7)	<0,001
BASDAI	6,4±1,1	4,9±2,4	<0,001
CRP, mg/L	22,8±18(20; 1-56)	8,7±12,4(3,5;3-104)	<0,001
ESR, mm/h	32,6±23,9(45; 3-90)	21,8±15,4(17,5;2-96)	<0,001

AS: ankylosing spondylitis, uSpA: undifferentiated spondyloarthritis, mo: months, IBP: inflammatory back pain, BASDAI: Bath Ankylosing Spondylitis Disease Activity Index, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate

Table 2. The Serum Ca, P, PTH and Plasma Vit D Levels of the SpA Patients and the Control Subjects

	AS patients (mean±sd) median(min-max)	uSpA patients (mean±sd) median(min-max)	control group (mean±sd) median(min-max)	P (AS-SPA, AS-C, SPA-C)
Ca (mg/dL) (8.6-10)	8,9±0,2	8,9±0,1	9,1±0.4	AS-uSpA=NS **AS-C=0,001** **uSpA-C<0,001**
P (mg/dL) (2.4-5.1)	3,2±0,3	3,2±0,3	3,4±0,6	AS-uSpA=NS AS-C=NS uSpA-C=NS
PTH (pg/mL) (11-67)	75(30-145)	66 (31-232)	60(13,7-172)	AS-uSpA=NS AS-C=NS uSpA-C=NS
Vit D (µg/L) (10-60)	18(8-38)	20 (4-92,3)	24,3(7,2-76,8)	AS-uSpA=NS **AS-C=0.004** uSpA-C=NS
Vit D (m)	16,5(8-38)	21,4 (88,8-59,5)	24(7,2-76,8)	AS-uSpA= NS **AS-C=0.005** uSpA-C=NS
Vit D (f)	20(8-31,4)	19,3(4-92,3)	28,7(7,8-70,3)	AS-uSpA=NS AS-C=NS **uSpA-C=0.011**

Ca: calcium, P: phosphorus, PTH: parathyroid hormone, Vit D: vitamin D, uSpA: undifferentiated spondyloarthritis, AS: ankylosing spondylitis, m: male, f: female

were women (85.8%) and 16 (14.2%) were men. Of the controls, 28 (30.4%) were women and 64 (69.6%) were men. The mean age of the control group was 39.4±13.04 (38, 18-65) years. Some of the clinical, laboratory and demographic parameters of the patients in the AS and uSpA groups are given in Table 1. The patients with AS were younger and predominantly male, with longer disease durations, higher levels of HLA B27 positivity, more frequent family histories for SpA, increased frequencies of inflammatory back pain (IBP) and eye involvement and higher ESR and CRP levels. On the other hand, peripheral arthralgia was more common in the uSpA patients, who were predominantly female.

The Ca levels were significantly lower in the AS and uSpA patients than in the control group subjects (p=0.001, p<0.001, respectively). Although not statistically significant, the parathyroid hormone levels were higher in the AS and uSpA groups than in the control group. The plasma vit D levels were significantly lower in the AS patients than in the control group subjects (p=0.004, Table 2).

When the male and female patients and the control group subjects were compared, the male AS and female uSpA patients were found to have significantly lower vit D levels than the control group subjects (p=0.005, p=0.011, respectively) (Table 2).

Thirty-eight (80%) patients in the AS group, eighty-eight (78%) patients in the uSpA group and fifty-seven (62%) subjects in the control group had vit D levels lower than 30 ng/mL (p<0.001, p<0.001 for AS control vs. SpA control). Eight (17%) patients in the AS group, nineteen (17%) pa-

Table 3. Comparison of Vit D Status in the AS, uSpA and Control Groups

	*Below 30 ng/mL N(%)	&Below 10 ng/mL N(%)
AS/f-m	38/9-29 (80%)	8/1-7 (17%)
Control /f-m	57/17-40 (62%)	4/1-3 (4%)
uSpA /f-m	88/72-16 (78%)	19/17-2 (17%)

*p<0.001 for AS-controls and uSpA-controls,
&p<0.001 for AS-controls and uSpA-controls

tients in the uSpA group and four (4%) subjects in the control group had vit D levels lower than 10 ng/mL (p<0.001, p<0.001 for AS control vs. SpA control) (Table 3).

The Spearman Rank correlation test demonstrated that the vit D levels were inversely related to the levels of ESR and CRP in the AS patients (p=0.002, r=-0.428; p<0.001, r= -0.592, respectively). This correlation was not demonstrated in the uSpA patients. The vit D levels were not found to correlate with the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) levels in either the AS or uSpA patients.

Discussion

Our study confirms that the plasma vit D levels are significantly lower in patients with AS and female uSpA patients.

Due to the very short half-life and tight regulation of 1,25 vit D by PTH, the serum 25 vit D level is the best indicator of true vit D status. The ideal blood levels of 25(OH) D3 are controversial, although the range of 30-60 ng/mL is widely accepted. The optimum levels of vit D may be accepted as adequate when the PTH levels are not elevated and vit D supplementation does not decrease the PTH levels (7). In the present study, although it was not statistically significant, the PTH levels were elevated in both patient groups. The Ca levels were significantly lower in the AS and uSpA patients compared with those observed in the controls.

The vit D receptor is a member of the nuclear hormone receptor superfamily and has been identified in mononuclear cells, dentritic cells and antigen presenting cells, as well as activated T-B lymphocytes (15). The concentrations of vit D are influenced by several factors, including age, diet, obesity, latitude, season, time spent outdoors, skin pigmentation, clothing, tanning habits and supplementation (16). In the present study, which was conducted during the winter, we found significanly lower 25(OH) D3 levels in the AS and female uSpA patients compared with those observed in the control group.

Secondary osteoporosis is a concomitant symptom of AS. It may be related to inflammatory activities, treatment modalities, decreased mobility or physical activity. Low 1,25 (OH)2 D3 levels may also contribute to osteoporosis in AS patients (12).

Previous studies have shown that the 25(OH) vit D levels should be kept above 30 ng/mL for optimal health and that adequate vit D levels decrease the risk of developing autoimmune diseases such as multiple sclerosis, inflammatory bowel disease, RA and type I diabetes mellitus (7, 9). There have so far been few studies evaluating the vit D levels in AS patients. In a study by Arends et al. (10), increased bone turnover, inflammation and low vit D levels were found to be important in the pathophysiology of AS-related osteoporosis in AS patients with active disease.

In a study from Turkey, the authors found low vit D levels in AS patients compared with those observed in controls without detecting a significant association with acute phase reactants and concluded that vit D deficiency in patients with AS may indirectly lead to osteoporosis by causing increases in inflammatory activity (11). Recently, we also found decreased vit D levels in patients with familial Mediterranean fever that were inversely related to the ESR, CRP and fibrinogen levels (17).

In this study, we found a negative correlation between disease activity (ESR, CRP) and the serum levels of 25(OH) D3 in the AS patients. Our results show that high disease activity is associated with alterations in vit D metabolism. There were no correlations between the vit D levels and the BASDAI levels among our patients. This may be related to confounding factors influencing the BASDAI levels, such as fibromyalgia, especially in the female patients with chronic rheumatic diseases. Ankylosing spondylitis disease activity score (ASDAS) may be a better measure for evaluating disease activity in SpA patients.

An interesting finding of the present study was the preponderance of women in the uSpA group. This finding is in accordance with the results of the study by Roussou et al. (18, 15). The uSpA patients were significantly older than the AS patients. Late onset of disease is more frequent in the subset of patients with uSpA than in those with AS and has a more equal distribution by sex (12, 19). Spondyloarthritis has been known to be more common in men; however, this study shows that the disease is also common in women, who usually have the undifferentiated form of the disease. Women may experience a less severe course of the disease and they are usually diagnosed at an older age.

The women in the uSpA group had lower vit D levels compared with those observed in the female controls. In one study, the 25(OH) D3 levels of 15,000 individuals were evaluated and lower levels were seen in women than in men (7). This may be related to the dressing habits and housebound status of women.

Another interesting finding of the present study is the lower HLA B27 status of the spondyloarthritis patients. In a recent study from Turkey, HLA B27 positivity was detected in 73.7% of 262 AS patients tested (20). This may be related to genetic factors.

Vit D deficiency in patients with undifferentiated connective tissue disease may play a role in subsequent progression to well-defined connective tissue disease, assuming that vit D plays a central role in the control of autoimmune proc-

esses (21). In the present study, the uSpA patients with low vit D levels might be regularly followed for the development of certain types of spondyloarthritis.

The serum vit D levels may be affected by several factors, including drugs. Anti-malarial drugs inhibit the 1α-hydroxylation of 25(OH)D, so that the active form of vit D decreases (9). On the contrary, the 25(OH)D levels may increase (9) or remain the same (22).

The limitations of the present study include the lack of assessment of dietary vit D intake, frequency and amount of sunlight exposure and assessment of mobility. Although none of the patients were using hydroxychloroquine, other drugs used by the patients, such as non-steroidal anti-inflammatory drugs or sulphasalazine, may have also affected the results.

Prospective trials are therefore needed to assess whether sustained low vit D levels in patients at an increased risk for the development of spondyloarthritis are associated with additional risks. In addition, in uSpA patients with low vit D levels, the future development of certain SpA subgroups should also be evaluated.

In summary, we found a high incidence of vit D deficiency in our AS and female uSpA patients. The vit D levels were found to be inversely related to the ESR and CRP levels in the AS patients. This correlation was not demonstrated in the uSpA patients. The vit D levels were not found to be correlated with the BASDAI levels in either the AS or uSpA patients. This may be related to the fact that AS is a more severe form of the disease and the acute phase response and inflammation are much more prominent in AS. Male predominance in this group may also be a cause. In patients with uSpA, however, acute inflammation is less prominent and some other factors such as sex may affect the results. Since the vit D levels are lower in women and there was a preponderance of women in the uSpA group, this factor may help to explain the results.

Treating vit D deficiency is also important in patients with SpA due to concomitant insults on tissues, including bone, in addition to the immunomodulatory effects exerted by vit D. It is therefore considered to be appropriate to look for vit D deficiency and to correct the vit D nutritional status in SpA patients.

The authors state that they have no Conflict of Interest (COI).

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