Plasma 25,OH Vitamin D Concentrations Are Not Associated with Rheumatoid Arthritis (RA)-related Autoantibodies in Individuals at Elevated Risk for RA

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ABSTRACT. ***Objective.*** To evaluate the association between rheumatoid arthritis (RA)-related autoantibodies and plasma 25,OH vitamin D in subjects at risk for RA.

Methods. In 1210 subjects without RA, 76 were positive for anti-cyclic citrullinated peptide antibodies or for at least 2 rheumatoid factors (RF; by nephelometry: RF-IgM, RF-IgG, RF-IgA). 25,OH vitamin D was measured in these cases and 154 autoantibody-negative controls from this cohort.

Results. 25,OH vitamin D levels did not differ between cases and controls (adjusted OR 1.23, 95% CI 0.93–1.63).

Conclusion. Vitamin D concentrations are not associated with RA-related autoimmunity in unaffected subjects at increased risk for RA. (First Release March 15 2009; J Rheumatol 2009;36:943–6; doi:10.3899/jrheum.080764)

Key Indexing Terms:
VITAMIN D RHEUMATOID ARTHRITIS AUTOANTIBODIES

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Studies have reported that vitamin D insufficiency is common in patients with rheumatoid arthritis (RA)[1] and that patients with RA have lower 25,OH vitamin D concentrations than healthy controls[2]. While this suggests that vitamin D may be a protective factor in RA, it is necessary to study this before onset of RA to rule out the possibility that lower vitamin D levels are not simply an effect of disease.

Two cohort studies compared vitamin D intake to subsequent development of RA, and while one showed an inverse association between intake and RA risk[3] the other did not[4]. Nielen, *et al* used samples from a blood bank to compare 25,OH vitamin D between individuals with RA at 1 year, 2 years, and 5 years prior to diagnosis, and matched controls, observing no differences in vitamin D[5]. However, variation in sample handling, which may occur at a blood bank, may have resulted in nondifferential misclassification in vitamin D levels, potentially biasing the results toward the null.

Autoantibodies such as rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) precede the development of RA[6-9], suggesting that factors associated with the presence of autoantibodies may play an important early role in the pathogenesis of RA. We measured 25,OH vitamin D using a standardized sample-handling protocol, in unaffected individuals with and without RA-related autoantibodies.

MATERIALS AND METHODS

The study population consisted of unaffected subjects in 2 cohorts of the Studies of the Etiology of RA (SERA). The first cohort of 605 subjects was

recruited from parents of children at increased genetic risk for type 1 diabetes, as described[10]. This cohort is enriched with HLA-DR4 because this allele is a susceptibility marker for both type 1 diabetes and RA. A second cohort of 622 first-degree relatives of probands with RA was recruited through rheumatology clinics and community outreach efforts at the University of Colorado Denver, Cedars-Sinai Medical Center, The Feinstein Institute for Medical Research, the Rheumatoid Arthritis Investigational Network (RAIN) from the University of Nebraska Medical Center, and the Benaroya Research Institute at Virginia Mason Arthritis Center, Seattle, WA. The study was approved by institutional review boards at each site. Informed consent was obtained from all study subjects.

A standardized examination and interview was used to assess signs and symptoms consistent with RA according to the 1987 American College of Rheumatology criteria, as described[10]. Those with signs of arthritis were sent for radiographs for further evaluation. Two subjects found to have RA were excluded from the analysis cohort. Subjects were screened for HLA-DR4 subtypes that contain the shared epitope, as described[10].

RF was measured by nephelometry (Dade Behring, Newark, DE, USA). RF isotypes IgA, IgG, and IgM were measured by ELISA using QuantaLite™ kits (Inova Diagnostics, San Diego, CA, USA). The 95th percentile of each autoantibody, based on tests of 491 adult blood donors from Denver, was used as the cutoff for positivity. IgG antibodies to CCP (Diastat; Axis-Shield Diagnostics, Dundee, Scotland) were measured; a positive test was defined as > 5 units/ml.

We identified 76 subjects who met the case definition of being either positive for anti-CCP or positive for ≥ 2 of the 4 RF. Presence of anti-CCP or ≥ 2 RF has been found to be predictive of onset of RA in the future[8,9]. Power analysis, with $\alpha = 0.05$, $ß = 0.2$, indicated that we would be able to determine a mean difference in 25,OH vitamin D concentration of 3.4 ng/ml between the 2 groups using 2 controls per case. Autoantibody-negative controls were selected, frequency-matching by cohort (HLA-DR4-enriched, first-degree relatives) and recruitment site (n = 154). Table 1 describes the analysis population by the cohort from which cases and controls were selected.

Nonfasting plasma samples were processed immediately after donation and stored at –80°C. Mean storage time for samples was 3.3 years (range 4.5 months to 5.2 years). Samples were sent to the University of Colorado Pediatric Clinical Translational Research Center Core Laboratory for 25,OH vitamin D measurement by radioimmunoassay (DiaSorin Inc.). Quality control was assessed by testing 109 blinded duplicate samples; the intraclass correlation coefficient between pairs was 0.91 (unpublished data, 2007).

We examined correlates of 25,OH vitamin D using linear regression analysis. Logistic regression was used to examine the independent association of 25,OH vitamin D on case/control status. In the linear and logistic regression models, covariates were included if they were known risk factors for 25,OH vitamin D[11] or RA[6,10], respectively, regardless of significance of the univariate comparisons.

RESULTS

We assessed the internal validity of the 25,OH vitamin D measures by comparing them with known predictors (Table 2). 25,OH vitamin D levels were associated with several independent factors (Table 2) shown to affect 25,OH vitamin D[11], suggesting good internal validity of this measure.

The mean 25,OH vitamin D levels were similar in cases and controls (Table 3). Adjusting for age, shared epitope, and smoking status, 25,OH vitamin D levels were not associated with RA-related autoantibodies (adjusted OR 1.23, 95% CI 0.93–1.63, for a 1-standard deviation difference in 25,OH vitamin D; p = 0.15). We then categorized 25,OH vitamin D as sufficient/insufficient, using < 30 ng/ml as the cutoff[12]. The proportion of cases (67%) and controls (75%) who were vitamin D-insufficient was similar (adjusted OR 0.65, 95% CI 0.35–1.21).

DISCUSSION

In a healthy population at increased risk for RA, 25,OH vitamin D concentrations were not associated with the presence of RA-related autoantibodies. We found that the vitamin D levels in our population varied by factors that are typically associated with 25,OH vitamin D, suggesting internal validity of the exposure measure.

Our study is in agreement with Nielen, *et al*[5], and confirms that their negative findings were not likely explained by nondifferential misclassification of vitamin D levels due to variation in handling of samples, nor to incomplete

Table 1. Characteristics of the analysis population by the cohort from which the cases and controls were selected for the SERA case-control study, 2002–07.

Characteristic	Cohort from which Cases and Controls were Selected: HLA-DR4-Enriched Cohort, n = 140	FDR Cohort n = 90	Unadjusted p
Age, yrs, mean (SD)	38.56 (6.58)	47.38 (16.82)	0.001
Sex (%)			0.10
Male	47 (34)	21 (23)	
Female	93 (66)	69 (77)	
Race/ethnicity (%)			0.64
Non-Hispanic white	120 (86)	80 (89)	
Black	8 (6)	5 (6)	
Hispanic	10 (7)	3 (3)	
Other	2 (1)	2 (2)	
Education* (%)			0.62
High School	22 (16)	17 (18)	
College	77 (57)	45 (50)	
Graduate	37 (27)	28 (31)	

* Data missing for 4 individuals. FDR: first-degree relatives.

Table 2. Factors associated with 25,OH vitamin D in the SERA case-control study, 2002–07.

Characteristic	N (%)	25,OH Vitamin D, ng/ml, Mean (SD)	Unadjusted p	Adjusted p†
Sex				
Male	68 (30)	25.04 (8.91)	Referent	Referent
Female	162 (70)	26.21 (9.51)	0.37	0.31
Taking a supplement containing vitamin D				
Yes	157 (68)	26.51 (8.8)	Referent	Referent
No	73 (32)	24.47 (10.34)	0.14	0.15
Body mass index (BMI) category (missing for 6 participants)				
Normal (18.5–24.9)	100 (43)	26.89 (9.75)	Referent	Referent
Overweight (25–29.9)	70 (31)	26.39 (8.88)	0.57	0.52
Obese (30+)	44 (19)	21.47 (8.19)	0.001	0.003
Smoking				
None	151 (66)	26.88 (9.41)	Referent	Referent
1–19 pack-yrs	69 (30)	24.93 (8.72)	0.10	0.08
20+ pack-yrs	10 (4)	17.17 (8.16)	0.001	0.002
Season of blood draw (%)				
Warm months (Apr-Sep)	117 (51)	27.92 (9.51)	Referent	Referent
Cold months (Oct-Mar)	113 (49)	23.70 (8.69)	0.0005	0.0002
Race/ethnicity (%)				
Non-Hispanic white	200 (87)	26.31 (9.26)	Referent	Referent
Black	13 (6)	21.23 (9.91)	0.06	0.05
Hispanic	13 (6)	27.00 (7.82)	0.78	0.91
Other	4 (1)	14.45 (7.66)	0.01	0.03
Site of recruitment (%)				
Denver	185 (80)	26.06 (9.08)	Referent	Referent
Los Angeles	18 (8)	29.06 (11.74)	0.19	0.12
Nebraska	8 (2)	26.50 (10.65)	0.9	0.37
Seattle	19 (8)	19.94 (7.76)	0.007	0.04

† Adjusted for all other variables, in addition to case status. Vitamin D supplement included individuals taking a multivitamin supplement, vitamin D supplement, or vitamin D supplement with calcium.

adjustment for known risk factors for RA, such as smoking and shared epitope positivity. In our study, we followed a strict sample-processing protocol, which would minimize degradation of the sample, we examined a population that was at increased risk for RA, and we were also able to account for numerous potential risk factors for RA.

While vitamin D levels do not appear to be associated with the presence of autoantibodies in this population, our cross-sectional design does not reveal levels prior to the appearance of autoantibodies. In addition, it is possible that vitamin D may affect progression of autoantibody-positive individuals to clinical RA. Therefore, continued followup of our autoantibody-negative and autoantibody-positive subjects in the SERA will help investigate these possibilities. Regardless of whether vitamin D influences risk of RA, there is mounting evidence that lower vitamin D levels may be associated with increased disease severity or activity[13,14]. Moreover, vitamin D receptor polymorphisms have been associated with disease activity in individuals with RA[14,15].

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Table 3. Univariate associations with RA-related autoimmunity in the SERA case-control study, 2002–07.

Characteristic	Cases, n = 76	Controls, n = 154	Unadjusted OR*, (95% CI)	p
25,OH vitamin D level, mean (SD)	26.89 (10.04)	25.30 (9.01)	1.02 (0.99–1.05)†	0.22
Age, yrs, mean (SD)	42.80 (13.58)	41.90 (12.0)	1.01 (0.9–1.03)	0.52
Race/ethnicity (%)				
Non-Hispanic white	64 (84)	136 (89)	1.0	0.81
Hispanic	5 (7)	8 (5)	1.32 (0.39–4.14)	
Black	5 (7)	8 (5)	1.35 (0.42–4.28)	
Other**	2 (2)	2 (1)	2.13 (0.25–18.03)	
Sex (%)				
Female	52 (68)	110 (71)	1.0	0.64
Male	24 (32)	44 (29)	1.15 (0.65–2.15)	
Education (4 declined) (%)				
High school	14 (18)	25 (17)	1.0	0.94
College	40 (53)	82 (55)	0.87 (0.41–1.89)	
Graduate	22 (29)	43 (28)	0.91 (0.40–2.12)	
Cigarette smoking (%)				
Nonsmoker	46 (61)	105 (68)	1.0	0.22
1–19 pack-yrs	28 (37)	41 (27)	1.56 (0.86–2.82)	
20+ pack-yrs	2 (3)	8 (5)	0.57 (0.08–2.39)	
Season of blood draw				
Warm months (April–Sep)	40 (53)	77 (50)	1.0	0.71
Cold months (Oct–Mar)	36 (47)	77 (50)	0.90 (0.52–1.56)	
Body mass index (BMI) category (6 excluded for missing data) (%)				
Normal (18.5–24.9)	35 (47)	65 (43)	1.0	0.85
Overweight (25–29.9)	25 (19)	55 (20)	0.85 (0.45–1.60)	
Obese (30+)	14 (34)	30 (37)	0.96 (0.44–2.10)	
Taking a supplement containing vitamin D				
Yes	56 (74)	101 (66)	1.0	0.21
No	20 (26)	53 (34)	0.68 (0.36–1.24)	
Shared epitope (2 excluded for missing data) (%)				
No	32 (42)	78 (51)	1.0	0.19
Yes	44 (58)	74 (49)	1.45 (0.83–2.54)	
Cohort group (%)				
HLA-DR4-enriched	46 (61)	94 (61)	1.0	0.94
First-degree relatives	30 (39)	60 (39)	0.98 (0.56–1.73)	

* OR calculated using univariate logistic regression; ** included Asian and Native American Indian; † OR and 95% CI calculated based on standard deviation difference in 25,OH vitamin D level. Adjusting for age, shared epitope, and smoking status, 25,OH vitamin D levels were not associated with RA-related autoantibodies [adjusted OR 1.23 (95% CI 0.93–1.63) for a 1 standard deviation difference in 25,OH vitamin D, p = 0.15].

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