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Plasma 25, OH vitamin D Levels are not Associated with Rheumatoid Arthritis-Related Autoantibodies in Individuals at Elevated Risk for Rheumatoid Arthritis

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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 either anti-CCP antibodies or for at least two or more RFs (RF measured by nephelometry, RF-IgM, -IgG, -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 levels are not associated with RA-related autoimmunity in unaffected subjects at increased risk for RA.

Keywords

vitamin D; rheumatoid arthritis; autoantibodies

INTRODUCTION

Studies have reported that vitamin D insufficiency is common in patients with RA[1] and that RA patients have lower 25, OH vitamin D than healthy controls[2]. While this suggests that

vitamin D may be a protective factor in RA, it is necessary to study this prior to the 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 non-differential misclassification in vitamin D levels, potentially biasing the results towards the null.

Autoantibodies such as RF and 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. Therefore, we measured 25, OH vitamin D using a standardized sample handling protocol, in unaffected individuals with and without RA-related autoantibodies.

METHODS

The study population consisted of unaffected subjects in two 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 previously[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 (FDRs) of probands with RA was recruited via 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.

This study was approved by the 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 ACR criteria, as described previously[10]. Those with signs of arthritis were sent for x-rays 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 previously[10].

RF was measured by nephelometry (Dade Behring, Newark, DE). RF isotypes IgA, IgG, and IgM were measured by ELISA using QUANTA Lite™ kits (INOVA Diagnostics, Inc, San Diego, California). The 95th percentile of each autoantibody, based on tests of 491 adult blood bank donors from Denver was used as the cut-off for positivity. IgG antibodies to CCP (Diastat, Axis-Shield Diagnostics, Ltd., Dundee, Scotland, United Kingdom) were measured; a positive test was defined as > 5 units per mL.

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

Non-fasting plasma samples are processed immediately following the visit and stored in -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) that have been shown previously 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 (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 cut-off[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 levels 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 non-differential misclassification of vitamin D levels due to variation in handling of samples, nor to incomplete 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 inform as to what the levels were prior to appearance of autoantibodies. In addition, it is possible that vitamin D may affect progression of autoantibody positive individuals to clinical RA. Therefore, continued follow-up of our autoantibody-negative and autoantibody-positive subjects in SERA will help investigate these possibilities. Regardless of whether vitamin D influences RA risk, there is mounting evidence that lower vitamin D levels may be associated with increased disease severity or activity[13, 14]. Moreover, vitamin D receptors (VDR) polymorphisms have been associated with disease activity in individuals with RA[14,15].

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References

1. Kroger H, Penttila IM, Alhava EM. Low serum vitamin D metabolites in women with rheumatoid arthritis. *Scand J Rheumatol* 1993;22:172–177. [PubMed: 8356409]
2. Als OS, Riis B, Christiansen C. Serum concentration of vitamin D metabolites in rheumatoid arthritis. *Clin Rheumatol* 1987;6:238–43. [PubMed: 3621843]
3. Merlino LA, Curtis J, Mikuls TR, Cerhan JR, Criswell LA, Saag KG. Vitamin D intake is inversely associated with rheumatoid arthritis. *Arthritis Rheum* 2004;50:72–77. [PubMed: 14730601]
4. Costenbader KH, Feskanich D, Holmes M, Karlson EW, Benito-Garcia E. Vitamin D intake and risks of systemic lupus erythematosus and rheumatoid arthritis in women. *Ann Rheum Dis* 2008;67:530–5. [PubMed: 17666449]
5. Nielen MM, van Schaardenburg D, Lems WF, van de Stadt RJ, de Koning MH, Reesink HW, et al. Vitamin D deficiency does not increase the risk of rheumatoid arthritis: comment on the article by Merlino et al. *Arthritis Rheum* 2006;54:3719–24. [PubMed: 17075887]
6. Berglin E, Padyukov L, Sundin U, Hallmans G, Stenlund H, Van Venrooij WJ, Klareskog L, Dahlqvist SR. A combination of autoantibodies to cyclic citrullinated peptide (CCP) and HLA-DRB1 locus antigens is strongly associated with future onset of rheumatoid arthritis. *Arthritis Res Ther* 2004;6:R303–8. [PubMed: 15225365]
7. Majka DS, Deane KD, Parrish LA, Lazar AA, Baron AE, Walker CW, et al. The duration of pre-clinical rheumatoid arthritis-related autoantibody positivity increases in subjects with older age at time of disease diagnosis. *Ann Rheum Dis* 2008;67:801–7. [PubMed: 17974596]
8. Nielen MM, van Schaardenburg D, Reesink HW, van de Stadt RJ, van der Horst-Bruinsma IE, de Koning MH, et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum* 2004;50:380–86. [PubMed: 14872479]
9. Rantapaa-Dahlqvist S, de Jong BA, Berglin E, Hallmans G, Wadell G, Stenlund H, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum* 2003;48:2741–49. [PubMed: 14558078]
10. Bhatia SS, Majka DS, Kittelson JM, Parrish LA, Ferucci ED, Deane KD, et al. Rheumatoid factor seropositivity is inversely associated with oral contraceptive use in women without rheumatoid arthritis. *Ann Rheum Dis* 2007;66:267–9. [PubMed: 16868018]
11. Engelman CD, Fingerlin TE, Langefeld CD, Hicks PJ, Rich SS, Wagenknecht LE, Bowden DW, Norris JM. Genetic and environmental determinants of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels in Hispanic and African Americans: the IRAS Family Study. *J Clin Endocrinol Metab* 2008;93:3381–88. [PubMed: 18593774]
12. Holick MF. Vitamin D Deficiency. *N Engl J Med* 2007;357:266–281. [PubMed: 17634462]
13. Cutolo M, Otsa K, Lass K, Yprus M, Lehtme R, Secchi ME, et al. Circannual vitamin D serum levels and disease activity in rheumatoid arthritis: Northern versus Southern Europe. *Clin Exp Rheumatol* 2006;24:702–04. [PubMed: 17207389]
14. Cutolo M, Otsa K, Uprus M, Paolino S, Serio B. Vitamin D and Rheumatoid Arthritis. *Autoimmun Rev* 2007;7:59–64. [PubMed: 17967727]
15. Gomez-Vaquero C, Fiter J, Enjuanes A, Nogues X, Diez-Perex A, Nolla JM. Influence of the Bsm1 polymorphism of the vitamin D receptor gene on rheumatoid arthritis clinical activity. *J Rheumatol* 2007;34:1823–26. [PubMed: 17659758]

Table 1

Characteristics of Analysis Population By the Cohort From Which The Cases and Controls Were Selected for the SERA Case-Control Study, 2002–2007

	Cohort from which the Cases and Controls were Selected;		
	HLA-DR4-Enriched Cohort N=140	FDR Cohort N=90	Unadjusted p-value
Age in Years, 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%)	

* Education missing for 4 individuals

Table 2
Factors Associated with 25-OH vitamin D in the SERA Case-Control Study, 2002–07

	N	25-OH vitamin D (ng/ml) Mean (SD)	Unadjusted p-value	Adjusted p-value [†]
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[*]				
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-years	69 (30%)	24.93 (8.72)	0.10	0.08
20+ pack-years	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

* BMI was missing for 6 participants

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

SD, Standard Deviation

Table 3

Univariate Associations with RA-Related Autoimmunity in the SERA Case-Control Study, 2002–2007

	Cases (n=76)	Controls (n=154)	Unadjusted OR [*] (95% CI)	p-value
25-OH vitamin D level, mean (SD)	26.89 (10.04)	25.30 (9.01)	1.02 (0.99 – 1.05) [†]	0.22
Age in years, mean (SD)	42.80 (13.58)	41.90 (12.0)	1.01 (0.98 – 1.03)	0.52
Race/Ethnicity				
NHW	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⁺⁺				
HS	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				
Non-smoker	46 (61%)	105 (68%)	1.0	0.22
1–19 pack-years	28 (37%)	41 (27%)	1.56 (0.86 – 2.82)	
20+ pack-years	2 (3%)	8 (5%)	0.57 (0.08 – 2.39)	
Season of blood draw				
Warm Months (April – September)	40 (53%)	77 (50%)	1.0	0.71
Cold Months (October – March)	36 (47%)	77 (50%)	0.90 (0.52 – 1.56)	
Body Mass Index (BMI) Category				
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[§]				
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
FDR	30 (39%)	60 (39%)	0.98 (0.56 – 1.73)	

* OR calculated using univariate logistic regression

** included Asian and Native American Indian

⁺⁺ 4 declined to answer

^ψ 6 excluded for missing data

[§] 2 excluded for missing data

[†] 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 (0.93–1.63) for a 1 standard deviation difference in 25, OH vitamin D, p=0.15).