Research Article

Cancer Epidemiology, Biomarkers & Prevention

Association between Vitamin D Deficiency and Antinuclear Antibodies in Middle-Aged and Older U.S. Adults

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Abstract

Background: Vitamin D deficiency is associated with cancer and autoimmune diseases, but little is known about the association between vitamin D and antinuclear antibodies (ANA), a biomarker of immune dysfunction in healthy populations. The objective of this study was to determine whether vitamin D deficiency is associated with ANA in middle-aged and older U.S. adults.

Methods: A cross-sectional analysis using the National Health and Nutrition Examination Survey (NHANES) 2001–2004 was conducted. Data were available for 1,012 adults aged 50 years and older. Serum 25-hydroxyvitamin D levels were measured by radioimmunoassay. ANA was measured in a 1:80 dilution of sera by immunofluorescence using HEp-2 cells (seropositive = 3 or 4+).

Results: Greater vitamin D deficiency was associated with higher ANA prevalence in the unadjusted (P_{trend} = 0.0002) logistic regression model and after adjustment for sex, age, education, race/ethnicity, season, and NHANES cycle (P_{trend} = 0.04). After adjustment, those with severe vitamin D deficiency (<10 ng/mL) had 2.99 (95% CI, 1.25–7.15) times the odds of ANA compared with having normal vitamin D levels (≥30 ng/mL), while deficient and insufficient individuals had twice the odds of ANA.

Conclusions: Among U.S. residents ages 50 and older, vitamin D deficiency was associated with higher prevalence of ANA. Vitamin D sufficiency may be important for preventing immune dysfunction in older populations.

Impact: Our findings support the growing evidence that vitamin D is an important immune modulator. Vitamin D deficiency in older adults may increase vulnerability to cancer by contributing to immune dysfunction. *Cancer Epidemiol Biomarkers Prev; 25(12); 1559–63. ©2016 AACR.*

Introduction

Vitamin D is inversely associated with cancer incidence, progression, and mortality in many observational studies, although results from randomized clinical trials are less clear (1–4). Vitamin D modulates innate and adaptive immune responses, and vitamin D deficiency, which is common in older adults, has been associated with a variety of autoimmune diseases (5–8). A hallmark of autoimmune disease is the presence of self-reactive autoantibodies, which are also of interest as immunologic biomarkers of cancer (9–11). Vitamin D deficiency may contribute to immune dysregulation, resulting in the production of autoantibodies, in particular antinuclear antibodies (ANA; refs. 6, 7). Elevated ANA is considered a marker of self-reactivity, seen across multiple autoimmune conditions that may precede the development of such conditions by several years, as has been observed for systemic lupus erythematosus (SLE; ref. 12). Elevated ANA is sometimes found in healthy individuals and has been consistently associated with female sex and older age (12–14).

ANA positivity has been associated with vitamin D deficiency in autoimmune disease patients (15–17), but little is known about vitamin D and ANA in healthy populations. One recent study examined this relationship in a small sample of clinical controls (7), but no population-based studies have been conducted. Therefore, we examined vitamin D deficiency in relation to ANA prevalence in the U.S. population aged 50 and older using data from the National Health and Nutrition Examination Survey (NHANES) 2001–2004. We hypothesized that middle-aged and older individuals with vitamin D deficiency would have a higher prevalence of ANA than those with vitamin D levels in the normal range.

Materials and Methods

Study population

The study sample was drawn from data collected by NHANES, a population-based, probability survey of the civilian, noninstitutionalized U.S. population (National Center for Health Statistics, Centers for Disease Control and Prevention). Data for this study are from the 2001 to 2002 and 2003 to 2004 cycles when both serum vitamin D levels and ANA (n = 3,041) were measured. The sample was limited to adults aged 50 years old and older (n = 1,130) to focus on an age range where age-associated elevations in ANA become apparent and to avoid complex interactions between vitamin D and hormones in premenopausal women

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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(14, 18). Of those, 118 participants with missing covariate information were excluded, resulting in a final sample of 1,012.

Measurement of serum vitamin D concentration: 25-hydroxyvitamin D [25(OH)D]

Frozen serum samples (−20°C) collected at mobile examination centers were shipped to the National Center for Environmental Health (Atlanta, GA) for testing. Serum 25(OH)D was measured with a radioimmunoassay kit (DiaSorin; refs. 19, 20). The coefficient of variation for the assay was calculated from blind QC pools and ranged from 6.5% to 11.3% for the 2001 to 2002 cycle and 4.4% to 13.2% for the 2003 to 2004 cycle (19, 20). Data from NHANES 2003 to 2004 cycle were adjusted for assay drift (21). Continuous serum 25(OH)D values were categorized as severe deficiency (<10 ng/mL), deficiency (10–19.9 ng/mL), insufficiency (20–29.9 ng/mL), and normal (≥30 ng/mL; ref. 22).

Measurement of ANA

Serum samples were tested for IgG autoantibodies to human cellular antigens using standard immunofluorescence methods described previously (14). HEp-2 cell slides (INOVA Diagnostics) with 1:80 dilution of sera were classified by intensities of immunofluorescence staining on a 0 to 4 scale based on comparison with a standard reference gallery (14). ANA intensity scores were confirmed independently by two experienced technicians who had an interrater agreement of greater than 95% (14). The weighted prevalence for each ANA immunofluorescence intensity score (range, 0–4) was as follows: 0 to 1, 19.3%; 2, 63.2%; 3, 16.8%; and 4, 0.7%. ANA fluorescence intensities of 3 or 4 were classified as seropositive for ANA, and intensities of 0 to 2 were classified as seronegative, consistent with previous studies in NHANES (14).

Covariates

Covariates included age, sex, education (less than high school, high school, greater than high school), race/ethnicity (non-Hispanic white, non-Hispanic black, or other), season of blood collection (summer or winter), body mass index (BMI; kg/m^2), self-report of at least 10 minutes of moderate or vigorous physical activity in the past 30 days (yes, no, unable), and NHANES cycle.

Statistical analyses

Analyses were performed using SAS version 9.3 (SAS Institute, Inc.), with PROC SURVEY procedures and Taylor series variance estimation to weight and adjust for strata and clustering of the complex survey design. Weights to account for subsampling in the present sample were applied as described previously (14). Bivariate relationships between ANA status, vitamin D category, and covariates were assessed using design-based Rao–Scott χ^2 and Wald F statistics. Potential confounders were selected on the basis of *a priori* hypotheses or if they were associated with the exposure and outcome. Several indicators of health status and comorbid conditions, including high blood pressure, high triglycerides, high cholesterol, and smoking status, were investigated as potential confounders, but they were not associated with the exposure or outcome. Nonetheless, we performed a sensitivity analysis, excluding hypertensives. We used multivariate logistic regression to estimate prevalence ORs (POR) and 95% confidence intervals (CI), modeling the association between vitamin D deficiency and ANA adjusted for age, sex, race/ethnicity, education, season, and NHANES cycle, and in a second model, additionally adjusting for BMI and physical activity.

Results

The estimated weighted prevalence of ANA positivity (score 3 or 4) was 17.5% in the U.S. population aged 50 and older. Prevalence varied by sex (20.7% of females and 13.9% of males were ANA positive) and by race/ethnicity (15.9% of non-Hispanic white, 26.7% of non-Hispanic black and 21.9% of other were ANA positive), but only the sex difference was statistically significant ($P = 0.02$; Table 1). When ANA prevalence was compared between non-Hispanic blacks and non-Hispanic whites, a statistically significant difference was observed ($P = 0.05$). ANA-positive participants were less likely or unable to engage in moderate or vigorous physical activity ($P = 0.01$). ANA was not associated with age, education, race/ethnicity, BMI, or NHANES cycle.

Individuals of non-Hispanic black race/ethnicity, those having a higher BMI, lower educational attainment, and those not performing or unable to perform moderate or vigorous physical activity had a higher prevalence of vitamin D deficiency than their respective comparison groups (Supplementary Table S1). Participants in the 2001 to 2002 cycle were more likely to be vitamin D deficient than the 2003 to 2004 cycle ($P = 0.02$). Vitamin D deficiency was not associated with age, sex, or collection season.

Greater vitamin D deficiency was associated with a higher prevalence of ANA ($P_{trend} = 0.0002$). This relationship did not appear to be driven by differences in vitamin D distributions by race/ethnicity, based on the consistent pattern of higher ANA prevalence for relatively lower vitamin D levels (categorized by a race/ethnic specific median split) across all race/ethnic categories (Fig. 1). PORs and 95% CIs for unadjusted and adjusted models are listed in Supplementary Table S2. Figure 2 shows the adjusted weighted POR and 95% CI of ANA by serum vitamin D level. Those with severe vitamin D deficiency had 2.99 (95% CI, 1.25–7.15) times the adjusted odds of ANA than those with vitamin D levels in the normal range. Vitamin D-deficient and insufficient individuals also had elevated odds of ANA (POR: 2.03; 95% CI, 1.16–3.55 and POR: 2.11; 95% CI, 1.15–3.88, respectively; $P_{trend} = 0.04$). Additional adjustment for BMI and physical activity had little impact on observed associations (severe deficiency POR: 2.64; 95% CI, 1.08–6.45; deficiency POR: 1.83; 95% CI, 1.01–3.30 and insufficiency POR: 2.01; 95% CI, 1.09–3.7; $P_{trend} = 0.05$).

Sensitivity analyses

We performed sensitivity analyses after excluding those with self-reported rheumatoid arthritis or thyroid problems, those unable to perform moderate physical activity, and premenopausal females (final analytic $N = 747$). NHANES did not collect self-reported diagnoses of SLE in 2001 to 2004. Severe deficiency and deficiency of vitamin D were combined due to small numbers. Vitamin D deficiency remained associated with ANA (POR: 1.90; 95% CI, 1.05–3.42), while the association between vitamin D insufficiency and ANA did not reach statistical significance in this smaller subsample (POR: 1.56; 95% CI, 0.78–3.11). In a separate sensitivity analysis excluding participants with hypertension, the POR for the association between vitamin D deficiency and ANA was strengthened (POR severe deficiency excluding hypertensives: 3.81; 95% CI, 1.33–10.89 versus 3.04; 95% CI, 1.25–7.40 including hypertensives), although less precise due to reduced sample size (final analytic $N = 619$).

Table 1. Demographic characteristics of ANA-positive and ANA-negative U.S. adults ages 50 years and older, NHANES 2001–2004, $N = 1{,}012$

	N	ANA+ (95% CI) *n* = 175	ANA− (95% CI) *n* = 837	*P*[a]
		Weighted mean		
Age	1,012	63.3 (60.8–65.8)	63.0 (62.1–64.0)	0.84
BMI (kg/m^2)	1,012	28.4 (27.4–29.4)	28.5 (28.1–29.0)	0.80
		Weighted percent		
Sex				**0.02**
Female	497	62.1 (53.8–70.4)	50.4 (46.5–54.3)	
Male	515	37.9 (29.6–46.2)	49.6 (45.7–53.5)	
Race/ethnicity				0.08
Non-Hispanic white	644	73.3 (64.2–82.4)	82.2 (77.2–87.1)	
Non-Hispanic black	147	13.3 (5.7–20.8)	7.7 (4.9–10.5)	
Other	221	13.4 (5.4–21.4)	10.1 (6.6–13.7)	
Education				0.62
<High school	345	25.2 (17.1–33.2)	21.9 (18.1–25.7)	
High school	232	21.3 (12.0–30.5)	25.1 (21.8–28.4)	
>High school	435	53.6 (43.8–63.3)	53.0 (48.8–57.1)	
Physical activity				**0.01**
Any moderate or vigorous	497	44.7 (38.2–51.3)	57.0 (51.9–62.1)	
No moderate or vigorous	434	43.6 (36.3–51.0)	37.1 (32.3–41.7)	
Unable	81	11.6 (5.6–17.6)	5.9 (4.3–7.5)	
Season of blood collection				0.67
Summer	590	64.7 (53.1–76.3)	66.7 (58.7–74.6)	
Winter	422	35.3 (23.7–46.9)	33.3 (25.4–41.3)	
NHANES cycle				0.41
2001–2002	489	47.8 (37.3–58.2)	43.3 (35.1–51.6)	
2003–2004	523	52.2 (41.8–62.7)	56.7 (48.4–64.9)	

NOTE: Bold text indicates statistical significance at $P = 0.05$.
[a]Rao–Scott χ^2 for categorical variables, Wald F statistic for continuous variables.

Discussion

This cross-sectional study of vitamin D deficiency and ANA in a middle-aged and older sample of the U.S. population found vitamin D deficiency to be associated with elevated odds of ANA compared with normal levels. These results are consistent with growing evidence that vitamin D plays a role in modulating immune function, in addition to regulating cellular processes important in cancer cell growth and differentiation (23), and may contribute to the development of autoimmunity as measured by antinuclear autoantibodies, a potential marker of immune dysfunction.

Vitamin D deficiency influences immune responses through several pathways, including regulation of dendritic cells, T cells, and B cells (6). Specifically, vitamin D influences the efficiency of regulatory T lymphocytes and activity of T helper lymphocytes (Th17), both thought to be important for mediating and regulating autoimmune responses (6, 24). Vitamin D deficiency also influences B-cell homeostasis directly, resulting in hyperactive B cells and increased immunoglobulin production (6, 7). Decreased T-cell regulation and increased B-cell activity may result in higher production of autoantibodies, including ANA (6).

In this sample restricted to middle-aged and older U.S. adults, ANA was not observed to increase with age in contrast to previous reports using NHANES data (14, 25). These prior analyses that showed ANA increasing with age included a broader age range covering the life course from 12 to 70+ years (14). Further investigation into age–ANA associations specifically among older healthy populations is needed.

This study has several strengths. As the first analysis conducted in a large, U.S. representative sample, this adds to a suggestive literature on vitamin D deficiency and ANA based on clinical studies of lupus patients and one small sample of clinical controls (7, 15). The observed association between vitamin D deficiency and ANA was unlikely due to including individuals who receive less vitamin D due to physical impairments or illness, as the association persisted in analyses restricted to those able to perform moderate or vigorous physical activity and free of rheumatoid arthritis or thyroid problems. This study also has limitations. Many autoimmune diseases were not assessed in NHANES, but disease-specific autoantibodies were uncommon in prior reports (14), and the onset of most autoimmune disease in older age is rare (26). ANA was measured using sera at a 1:80 dilution in NHANES. Higher dilutions may be useful to identify individuals with higher levels of ANA in clinical settings; however, research on ANA in this NHANES sample was designed to obtain an estimate of ANA prevalence in the general population, most of whom do not have a diagnosis of autoimmune disease. NHANES is a cross-sectional study, which limits the ability to determine causality of the association as temporality of exposure and outcome is not established. Comorbid conditions or medications could contribute to the association between ANA and vitamin D. Ability to perform moderate/vigorous physical activity is only a crude indication of possibly comorbidity. We were not able to evaluate use of specific medications due to small numbers, but several indicators of health status and comorbid conditions, including high blood pressure, high triglycerides, high cholesterol, and smoking status, that were investigated as potential confounders were not found to be associated with the exposure or outcome, and results were in fact strengthened in an analysis that excluded hypertensives. Finally, NHANES does not include the institutionalized elderly, who are particularly prone to vitamin D deficiency; therefore, our findings may underestimate the relationship between vitamin D deficiency and ANA prevalence at older ages (27).

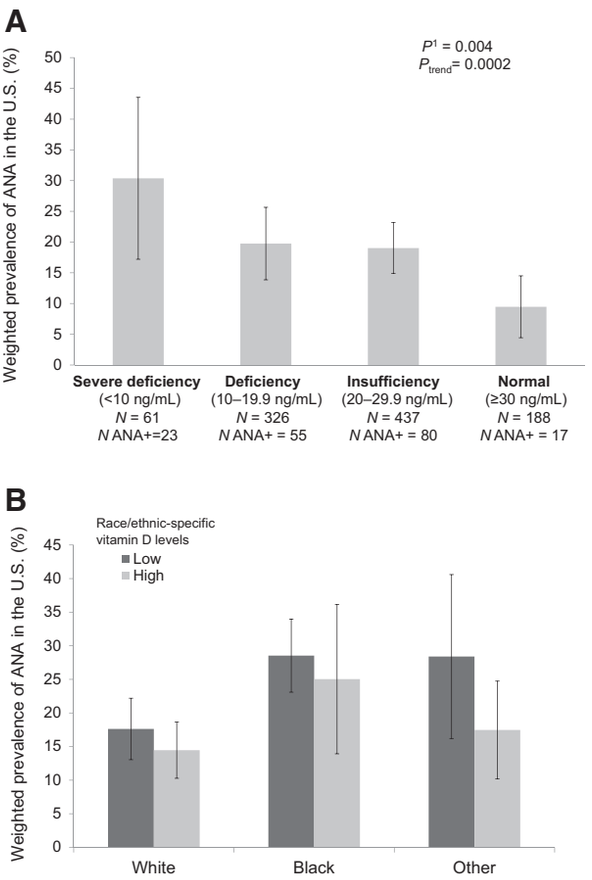

Figure 1.

A, Weighted prevalence (95% CI) of ANA by vitamin D status in the total U.S. population ages 50+, NHANES 2001–2004 ($N = 1{,}012$); [1]Rao–Scott χ^2. **B,** Weighted prevalence (95% CI) of ANA by race/ethnic-specific vitamin D levels in the U.S. population ages 50+, NHANES 2001–2004 ($N = 1{,}012$); low, <median; high, ≥median.

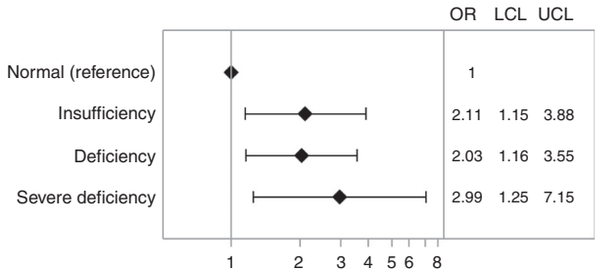

Figure 2.

Weighted PORs (95% CI) of ANA by serum vitamin D level in the U.S. population age 50+, NHANES 2001–2004 ($N = 1{,}012$) adjusted for gender, age, education, race/ethnicity, season, and NHANES cycle. $P_{trend} = 0.04$. LCL, lower 95% confidence limit; UCL, upper 95% confidence limit.

Conclusions

Among individuals in the U.S. population ages 50 and older, vitamin D deficiency was associated with higher prevalence of ANA. Prospective studies on ANA incidence in healthy aging individuals, including longitudinal data on vitamin D, are warranted and may reveal pathways by which vitamin D deficiency may contribute to the development of autoimmunity, a potential immunologic biomarker of cancer. Understanding the role of vitamin D in immune modulation, particularly in aging populations susceptible to vitamin D deficiency, may also help identify preventative or clinical opportunities to improve immune function and delay immunosenescence.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: H.C.S. Meier, D.P. Sandler, C.G. Parks
Development of methodology: C.G. Parks
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): H.C.S. Meier
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): H.C.S. Meier, D.P. Sandler, E.M. Simonsick, C.G. Parks
Writing, review, and/or revision of the manuscript: H.C.S. Meier, D.P. Sandler, E.M. Simonsick, C.G. Parks
Study supervision: D.P. Sandler

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