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Serum 25-hydroxyvitamin D concentrations in mid-adulthood and Parkinson's disease risk

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Abstract

Background—Low vitamin D levels are common among patients with Parkinson's disease (PD). Experimental evidence further suggests that vitamin D may be protective against PD.

Objectives—We prospectively assessed the association between serum 25-hydroxyvitamin D and PD among 12,762 participants of the Atherosclerosis Risk in Communities Study cohort.

Methods—Serum samples were collected in 1990–1992 and 25-hydroxyvitamin D was measured by liquid chromatography mass spectrometry. A total of 67 incident PD cases were

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identified through December 31, 2008. The median length of follow-up was 17 years. We used Cox proportional hazards models to obtain hazard ratios and 95% confidence intervals, adjusting for age, sex, and race. We did not find any association between serum 25-hydroxyvitamin D concentrations and PD risk, regardless of how serum 25-hydroxyvitamin D was modeled. Compared to participants with serum 25-hydroxyvitamin D < 20 ng/mL, the hazards ratio for PD was 1.05 (95% confidence interval = 0.58, 1.90) for 20–30 ng/mL and 1.14 (95% confidence interval = 0.59, 2.23) for ≥ 30 ng/mL. Similar results were obtained in sensitivity analyses that included white participants only and that were stratified by the length of follow-up.

Conclusion—This prospective study lends no support to the hypothesis that vitamin D may reduce the risk of PD.

Keywords

Parkinson's disease; vitamin D; cohort study

Introduction

Parkinson's disease (PD) is a common neurodegenerative disorder that affects approximately 10 in 1000 United States (U.S.) adults aged 65 years or older.¹ Although both genetic and environmental factors have been implicated, its etiological factors are mostly unknown.² The disease is associated with substantial public health and economic burdens,³ which is expected to increase in the future with a rapidly growing older population.⁴ Identification of modifiable risk factors may therefore have important public health implications.

Vitamin D is a fat-soluble vitamin that has been suggested to have numerous biological effects beyond the maintenance of bone health.⁵ Experimental evidence suggests that vitamin D may also protect against PD via anti-oxidative and neurotrophic mechanisms.^{6–9} For instance, studies have demonstrated that administration of 1,25-dihydroxyvitamin D₃ attenuates the toxic effects of 6-hydroxydopamine on nigrostriatal dopaminergic neurons in rats.^{6, 7, 9} Multiple epidemiological studies have shown that relative to controls, PD cases have lower serum/plasma levels of 25-hydroxyvitamin D (25(OH)D), a measure of vitamin D sufficiency status.^{10–13} However, interpretation of these cross-sectional comparisons is not straightforward due to concerns about reverse causation, since sun exposure influences 25(OH)D levels and PD patients may be less likely to spend time outdoors. To the best of our knowledge, only one prospective study has examined the association between serum vitamin D and risk of PD.¹⁴ This previous study, which included 50 incident PD cases identified from a nationwide drug-reimbursement database in Finland, found a lower risk of PD among individuals with higher serum 25(OH)D concentrations. We therefore examined serum levels of 25(OH)D in relation to PD risk, using data from the Atherosclerosis Risk in Communities (ARIC) Study.

Material and Methods

Study population

The ARIC study is a prospective cohort that was established in late 1980s to examine risk factors for cardiovascular diseases in the general U.S. population. Details of the study design

have been published elsewhere.¹⁵ Briefly, 15,792 men and women, aged 45 to 64 years, were recruited between 1987 and 1989 (visit 1) from four U.S. communities: Washington County, Maryland; suburbs of Minneapolis, Minnesota; Jackson, Mississippi; and Forsyth County, North Carolina. At enrollment participants underwent a clinical examination, provided a blood sample, and answered a comprehensive survey on demographics, diet, lifestyle, medication use, and cardiovascular disease risk factors. Similar follow-up clinical examinations were conducted in 1990–1992 (visit 2), 1993–1995 (visit 3), 1996–1998 (visit 4), and 2011–2013 (visit 5). In addition to the clinical visits, the cohort was followed up by annual telephone interviews and community-wide surveillance including the search of hospitalizations and deaths. Causes of deaths were identified via search of the National Death Index. Both hospitalization diagnosis codes and causes of death were coded according to the International Classification of Diseases (ICD). At visit 4, participants were asked if and when they had been diagnosed with PD. At all clinical visits and the 2006–2008 annual calls, participants were asked to report all medications used in the past two weeks. Of the 14,348 participants who attended visit 2, 12,762 were included in the primary analyses. Figure 1 provides details on study eligibility and exclusions. The ARIC study was approved by the Institutional Review Boards from all study sites. All participants provided written informed consent.

Parkinson's disease case identification and confirmation

Details on PD ascertainment have been published elsewhere.^{16, 17} Briefly, in 2010, we searched ARIC data to identify potential PD cases with the following criteria: (i) use of typical antiparkinsonian medications at any of the four clinical visits between 1987 and 1998, or at the annual calls between 2006 and 2008; (ii) self-reports of PD diagnosis at visit 4; (iii) PD as hospitalization discharge diagnosis (ICD-9 code 332.0); or (iv) cause of death (ICD-9 code 332.0 or ICD-10 code G20). A total of 293 potential PD cases were identified and underwent a two-stage confirmation process. First, we contacted these individuals or their proxies to confirm the diagnosis and conducted a structured interview that asked for age at diagnosis, whether the diagnosis was made by a neurologist or movement disorder specialist, major complaints that led to the diagnosis, and PD medications used and subsequent therapeutic response. For participants who confirmed PD, we further contacted their treating physicians to obtain additional information regarding disease diagnosis and treatment, and a copy of relevant medical records. The study movement disorder specialist (X.H.) reviewed all data from the participants or their proxies and the treating physicians and adjudicated PD diagnosis to 106 participants. Details of this confirmation process, including numbers in each confirmation step, were published previously.¹⁷ PD diagnosis was considered confirmed if: (1) participants self-confirmed the PD diagnosis, and their treating physicians or medical record review by the study movement disorder specialist further confirmed having PD ($n = 42$); (2) participants self-confirmed the diagnosis and the study movement disorder specialist concurred with participants' report after reviewing medical history provided by them, in the absence of information from a treating physician ($n = 31$); (3) two or more aforementioned sources indicated PD diagnosis ($n = 21$); or (4) two or more hospitalizations occurred with PD listed as a discharge diagnosis ($n = 10$), or PD medication use was reported at any of the first four visits and also at the 2006–2008 phone

calls ($n = 2$). Of these, 88 provided blood samples at visit 2, including 67 incident cases and 21 prevalent cases in reference to visit 2.

Serum 25(OH)D measurements

Concentrations of 25(OH)D₂ (i.e., 25-hydroxy form of vitamin D₂ or ergocalciferol) and 25(OH)D₃ (i.e., 25-hydroxy form of vitamin D₃ or cholecalciferol) were measured using serum samples that were collected at visit 2 (1990–1992). The serum samples were archived at -70°C until analyzed in 2012–2013. The analysis was conducted at the University of Minnesota Molecular Epidemiology and Biomarker Research Laboratory, using an AB SCIEX Triple Quad 5500 Liquid Chromatography-Mass Spectrometer/Mass Spectrometer (LC-MS/MS) system. The laboratory is Clinical Laboratory Improvement Amendments certified, and participates in the College of American Pathologists proficiency program. The detailed analytical procedure can be found elsewhere.¹⁸ In addition, blind duplicate split specimens collected from single participant blood draws were analyzed. The blind duplicate coefficients of variation and Pearson correlation coefficients were 20.8% and 0.98 for 25(OH)D₂, and 6.9% and 0.97 for 25(OH)D₃, respectively.¹⁸

Serum 25(OH)D was calculated as the sum of 25(OH)D₂ and 25(OH)D₃. It was adjusted for month of the blood draw to account for seasonal variation.¹⁸ This was accomplished by using linear regression models, with month of the blood draw as the predictor variables (modeled using 11 dummy variables) and serum 25(OH)D as the dependent variable; the analysis was conducted separately for whites and blacks. The residuals obtained from the linear regression models were added to the model generated grand mean to obtain a season-adjusted serum 25(OH)D concentration for each individual.

Statistical analysis

We conducted Chi-square tests, Wilcoxon tests, and Spearman correlation to assess bivariate associations among serum 25(OH)D concentrations, PD and covariates. We used Cox proportional hazards model to evaluate the association between serum 25(OH)D and incident PD, adjusting for age at visit 2, sex, and race, and report hazard ratios (HR) and 95% confidence intervals (CI). Follow-up time was defined as the time since visit 2 to PD diagnosis, loss-to-follow up, death, or December 31, 2008, whichever occurred first. Serum 25(OH)D was modeled as a continuous variable as well as a categorical variable using established clinical cutoffs (i.e., < 20 ng/mL for deficiency, 20–30 ng/mL for insufficiency, and ≥ 30 ng/mL for sufficiency).¹⁹ Analyses were repeated adjusting for additional covariates: smoking status and daily caffeine intake. We evaluated the proportional hazards assumption by including a product term between time and a covariate in the models and examining log (–log) survival curves and cumulative martingale residual plots. We conducted various sensitivity analyses: (1) restricting to whites; (2) stratified by sex; (3) stratified by follow-up period (i.e., the first 10 years and the last 9 years) to evaluate whether possible long-term consequences were different from short-term effects; (4) accounting for death as a competing risk;²⁰ and (5) including the 57 potential cases who did not respond to our case confirmation effort and there was no sufficient information to make an adjudication by the movement disorder specialist (Figure 1). We also evaluated the association between serum 25(OH)D and prevalent PD using a logistic regression model. All the statistical tests

were two-tailed with $\alpha = 0.05$. Statistical analyses were performed using SAS software version 9.3 (SAS Institute Inc., Cary, NC) and R (R Core Team, Vienna, Austria).

Results

After a median of 17 years of the follow-up (maximum 19 years), a total of 67 participants developed PD. The median age at diagnosis was 69 years. Compared to those who remained free of PD, participants who developed the disease were older (mean age at visit 2 (standard deviation (SD)): 59.2 (5.4) vs. 57.0 (5.7) years), and were more likely to be men and whites, but less likely to be current smokers (Table 1).

The mean (SD) serum concentrations of 25(OH)D at visit 2 were similar between those who developed PD (25.6 (8.4) ng/mL) and those who did not (24.2 (8.5) ng/mL, $p = 0.24$). They were also comparable in the proportions with vitamin D deficiency (25(OH)D concentration < 20 ng/mL: 26.9% vs. 32.6% among participants with and without PD respectively) and insufficiency (20–30 ng/mL: 46.2% vs. 44.5%).

No significant association was detected between serum 25(OH)D concentrations and PD incidence in the multivariate analysis adjusting for age, sex, and race (Table 2). The HR associated with one interquartile range (i.e. 11.2 ng/mL) increase in serum 25(OH)D was 1.11 (95% CI = 0.81, 1.52). Using participants with 25(OH)D < 20 ng/mL as the reference, the HR was 1.05 (95% CI = 0.58, 1.90) for 20–30 ng/mL and 1.14 (95% CI = 0.59, 2.23) 30 ng/mL. The results were similar after adjusting for smoking status and daily caffeine intake, restricting to whites only, or after accounting for death as a competing risk (data not shown). We also did not detect any associations in the analyses that were stratified by follow-up time (Table 3), sex (data not shown), or by the median age at diagnosis (data not shown), or in the sensitivity analysis that included 57 non-respondents as potential cases (data not shown). Finally, despite the small number of prevalent PD cases ($n = 21$), we compared their vitamin D status at visit 2 with incident PD and those remained PD free. Only 9.5% of prevalent PD patients had 25(OH)D ≥ 30 ng/mL as compared to 26.9% among those who later developed PD or 22.9% who remained PD free (Supplemental Table 1).

Discussion

Recently there has been a growing interest in investigating the role of vitamin D in neurological diseases, including PD.^{21–23} The potential importance of vitamin D in PD pathogenesis came to light after a study demonstrated widespread distribution of vitamin D receptor and 1 α -hydroxylase, the enzyme responsible for the conversion of 25(OH)D into bioactive 1,25-dihydroxyvitamin D, in the substantia nigra of the adult human brain.²⁴ It has been hypothesized that the substantia nigra is highly dependent on local production of 1,25-dihydroxyvitamin D, and therefore, chronic inadequacy of 25(OH)D may lead to neuronal damage and also may make neurons more susceptible to toxins.²⁵ Further support regarding a protective role of vitamin D for PD comes from experimental animal studies. For instance, 1,25-dihydroxyvitamin D₃ has been shown to attenuate 6-hydroxydopamine –induced toxicity in nigrostriatal dopaminergic neurons in rats, potentially via upregulation of glial cell derived neurotrophic factor.^{6–9, 26} In addition, *in vitro* study has shown that 1,25-

dihydroxyvitamin D₃ attenuates rotenone-induced neurotoxicity by augmenting autophagy signaling pathways and reducing oxidative stress.²⁷

Several epidemiological studies have evaluated the potential association between serum vitamin D concentrations and PD. Four US-based case-control studies have reported a higher prevalence of vitamin D deficiency in PD patients than in controls, or a dose-response relationship between serum 25(OH)D concentrations and the odds of PD.^{10–13} One study conducted in the Faroese population did not find any association.²⁸ Caution should be exercised when interpreting case-control results as reverse causation may explain some of these findings. For instance, reduced mobility may limit adequate sunlight exposure in PD patients, leading to low serum vitamin D concentrations. Also, PD patients may alter dietary intake due to PD-associated non-motor conditions such as gastrointestinal dysfunction and depression. To the best of our knowledge, only one prospective cohort has evaluated serum 25(OH)D in relation to incident PD.¹⁴ The study followed 3,173 Finnish individuals from 1978–1980 to 2007 and identified a total of 50 incident cases via linkage to their nationwide Drug Imbursement Register. The study reported a dose-response relationship between higher serum 25(OH)D and lower risk of PD.

Our study is prospective in design, and is larger than the Finnish study. We however did not find association between serum 25(OH)D and PD risk in the overall analysis or in several preplanned sensitivity analyses. There are a few notable differences between the current study and the Finnish study. In our study, we identified PD patients via multiple sources and conducted case confirmation, whereas the Finnish study identified cases via a nationwide administrative database without any diagnostic validation. Further, in the Finnish cohort, 25(OH)D levels were significantly lower than among the ARIC participants.

Strengths of our study include the prospective design, long-term follow up and availability of data on potential confounders. Additionally, we used the gold standard LC-MS/MS assay for serum 25(OH)D measurements which is superior to immunoassay techniques, including the Diasorin radioimmunoassay that was used in the Finnish study.^{29,30} Our study also has several limitations. First, we identified PD cases from multiple sources at various time points without a systematic clinical examination. Despite our effort of diagnostic validation, we may have missed or misdiagnosed some cases. Further, approximately one-third of the potential cases (or their proxies) did not respond to our confirmation request, which is not surprising for a study with 20 years of follow-up. Nevertheless, we were able to replicate the known associations for PD with age, sex, and smoking in the ARIC cohort, which indirectly supports the internal validity of case identification. We only had 67 incident PD cases which may have limited the power to detect weak to moderate associations. Our power calculation shows that we have 80% power to detect a relative risk of 0.33 or stronger which was suggested by the Finnish study. More importantly, our risk estimates were very close to null, suggesting no association between 25(OH)D level and PD risk. Additionally, the few PD cases in blacks (n = 8) prohibited us from analyzing the association separately for blacks. It would have been interesting to look at race-specific associations since blacks are more susceptible to vitamin D deficiency³¹ and yet have lower PD incidence than whites,^{32,33} which is seemingly contradictory to the vitamin D hypothesis of PD. Further, vitamin D status may have been misclassified as one time-measurement may not capture an

individual's true vitamin D status. If early-life exposure or exposure during early adulthood is important in PD pathogenesis, then vitamin D status in late adulthood may not be an appropriate measure. Additionally, we used serum that had been stored for about 20 years before 25(OH)D measurements. However, several studies have consistently shown that serum 25(OH)D is extremely stable under a variety of preanalytic conditions^{34,35} and with long-term storage.³⁶

In conclusion, in this population based study with 19 years of follow-up, we did not detect any association between serum 25(OH)D concentrations and PD incidence. The results lend no support to the hypothesis that vitamin D may protect against PD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Flowchart depicting study participants selection for serum 25-hydroxyvitamin D and Parkinson's disease analysis, Atherosclerosis Risk in Communities Study, 1990-1992. Abbreviations: PD, Parkinson's disease; CVD, Cardiovascular disease.

Table 1

Baseline characteristics by incident Parkinson's disease status, Atherosclerosis Risk in Communities Study, 1990–1992 (n = 12,762).

Characteristics	No PD (n = 12,695)	PD (n = 67)	p ^a
Serum 25(OH)D in ng/mL			0.24
Mean (SD), Range	24.2 (8.5), 0.6–109.1	25.6 (8.4), 5.9–47.7	
Median, Q1 – Q3	23.7, 18.3–29.5	24.5, 19.6–30.8	
Serum 25(OH)D (n (%))			0.56
< 20 ng/mL	4133 (32.6)	18 (26.9)	
20–30 ng/mL	5656 (44.5)	31 (46.2)	
30 ng/mL	2906 (22.9)	18 (26.9)	
Age in years			0.001
Mean (SD), Range	57 (5.7), 46–70	59.2 (5.4), 50–68	
Daily caffeine intake in mg ^b			0.74
Mean (SD), Range	287 (292), 0–1427	281 (309), 0.1–1003	
Sex (n (%))			0.002
Women	7121 (56.1)	25 (37.3)	
Men	5574 (43.9)	42 (62.7)	
Race (n (%))			0.01
Black	3305 (26.0)	8 (11.9)	
White	9390 (74.0)	59 (88.1)	
Smoking Status (n (%)) ^c			0.005
Current Smoker	2855 (22.5)	4 (6.0)	
Former Smoker	4741 (37.4)	29 (43.3)	
Never Smoker	5077 (40.1)	34 (50.7)	

Abbreviations: 25(OH)D, 25-hydroxyvitamin D (adjusted for month of blood collection); PD, Parkinson's Disease; Q1, Quartile 1; Q3, Quartile 3; Range, Minimum – Maximum; SD, Standard Deviation.

^aP-value obtained from Wilcoxon Test, or Chi-Square Test.

^bMeasured at visit 1 (1987–1989), and n = 284 missing;

^cn = 22 missing.

Table 2

Association between serum 25(OH)D (ng/mL) and incident Parkinson's disease, Atherosclerosis Risk in Communities Study, 1990 – 2008 (n = 12,762).

Model: Adjusted for Covariate Set 1 ^a							Model: Adjusted for Covariate Set 2 ^b		
Overall	n (PD)	Total PY	HR (95% CI)	p	p ^T		HR (95% CI)	p	p ^T
Serum 25(OH)D ^c			1.11 (0.81, 1.52)	0.53			1.12 (0.81, 1.54)	0.50	
Serum 25(OH)D									
< 20 ng/mL	18	64,317	Ref	-	0.69	Ref	-	-	0.66
20 – 30 ng/mL	31	89,118	1.05 (0.58, 1.90)	0.86		1.05 (0.58, 1.89)		0.88	
30 ng/mL	18	46,022	1.14 (0.59, 2.23)	0.69		1.16 (0.60, 2.26)		0.66	
Among Whites (n = 9449)									
Serum 25(OH)D ^c			1.09 (0.78, 1.53)	0.61		1.10 (0.79, 1.55)		0.58	
Serum 25(OH)D									
< 20 ng/mL	15	37,256	Ref	-	0.75	Ref	-	-	0.72
20 – 30 ng/mL	26	71,220	0.90 (0.48, 1.71)	0.76		0.89 (0.47, 1.70)		0.73	
30 ng/mL	18	40,655	1.12 (0.56, 2.22)	0.75		1.13 (0.57, 2.25)		0.72	

Abbreviations: 25(OH)D, 25-hydroxyvitamin D (adjusted for month of blood collection); CI, Confidence Interval; HR, Hazard Ratio; PD, Parkinson's Disease; PY, Person Year; Ref, Reference.

^aCovariate Set 1: Adjusted for age, sex, and race (for the models restricted to whites only, race was not included as a covariate).

^bCovariate Set 2: Adjusted for age, sex, race, daily caffeine intake at visit 1 (mg), and smoking status (never, former, current); for the models restricted to whites only, race was not included as a covariate.

^cContinuous serum 25(OH)D, HR expressed per one interquartile range increase (i.e., 11.2 ng/mL).

p^T: p-value from trend test.

Association^a between serum 25(OH)D (ng/mL) and incident Parkinson’s disease stratified by follow-up time, Atherosclerosis Risk in Communities Study, 1990 – 2008 (n = 12,762).

Table 3

First 10 years of follow-up (n = 12,762)					Follow-up beyond 10 years (n = 11,335)				
n (PD)	Total PY	HR (95% CI)	p	p ^T	n (PD)	Total PY	HR (95% CI)	p	p ^T
Serum 25(OH)D ^b									
		1.06 (0.66, 1.68)	0.81				1.15 (0.75, 1.78)	0.52	
Serum 25(OH)D									
< 20 ng/mL	8	39,395	Ref	-	0.74	10	61,567	Ref	-
20 – 30 ng/mL	15	54,166	1.06 (0.45, 2.53)	0.89	16	85,495	1.05 (0.47, 2.34)	0.91	0.82
30 ng/mL	9	27,866	1.18 (0.45, 3.09)	0.74	9	44,303	1.12 (0.44, 2.81)	0.82	

Abbreviations: 25(OH)D, 25-hydroxyvitamin D (adjusted for month of blood collection); CI, Confidence Interval; HR, Hazard Ratio; PD, Parkinson’s Disease; PY, Person Year; Ref, Reference.

^a Adjusted for age, sex, and race.

^b Continuous 25(OH)D, HR expressed per one interquartile range increase (i.e., 11.2 ng/mL).

p^T: p-value from trend test.