Association of Plasma 25-Hydroxyvitamin D Concentrations and Pathogenic Oral Bacteria in Postmenopausal Females

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Background: Previous findings of an association between 25-hydroxyvitamin D [25(OH)D] concentrations and periodontal disease may be partially explained by the antimicrobial properties of vitamin D. To the best of the authors' knowledge, no study has investigated the association between 25(OH)D and pathogenic oral bacteria, a putative cause of periodontal disease.

Methods: The association between plasma 25(OH)D concentrations and pathogenic oral bacteria was examined among postmenopausal females in the Buffalo Osteoporosis and Periodontal Disease Study (1997 to 2000), an ancillary study of the Women's Health Initiative Observational Study. Subgingival plaque samples were assessed using immunofluorescence for the presence of *Porphyromonas gingivalis*, *Tannerella forsythia*, *Fusobacterium nucleatum*, *Prevotella intermedia*, and *Campylobacter rectus*. Logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for prevalent bacteria by quintile (Q) of 25(OH)D concentrations, adjusting for age and body mass index.

Results: Of the 855 participants, 288 (34%) had deficient/inadequate (<50 nmol/L) 25(OH)D concentrations, and 496 (58%) had at least one species of pathogenic bacteria. No significant association was found between 25(OH)D and presence of any of these bacteria (adjusted OR for high [Q5] compared to low [Q1] 25(OH)D = 0.96; 95% CI: 0.61 to 1.50; *P* for trend = 0.50). Inverse, although not statistically significant, associations were found between 25(OH)D and more than one species of pathogenic bacteria (adjusted OR for adequate compared to deficient/inadequate 25(OH)D = 0.85; 95% CI: 0.60 to 1.19).

Conclusions: No association was observed between pathogenic oral bacteria and 25(OH)D concentrations in postmenopausal females. This may be attributable to the species of bacteria assessed, small effect size, or a true absence of an association. *J Periodontol 2014;85:944-955.*

KEY WORDS

Dental plaque; periodontal diseases; periodontitis; postmenopause; 25-hydroxyvitamin D; vitamin D.

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Inflammation of the oral gingiva found in persons with periodontal diseases is thought to be caused by the response of the immune system to bacterial infection.[1,2] Gingival inflammation (gingivitis), assessed by the degree of gingival bleeding, is the least severe form of periodontal disease but can also be present in people with more severe periodontal disease. Vitamin D has been hypothesized to protect against gingival bleeding by two potential mechanisms: 1) suppression of inflammation[2,3] and/or 2) upregulation of antimicrobial peptide transcription.[4,5]

Limited data have been published on the association between vitamin D status and gingivitis. Using data from the Third National Health and Nutrition Examination Survey (1988 to 1994), Dietrich et al.[6] observed an inverse dose–response association between serum 25-hydroxyvitamin D [25(OH)D] concentrations and gingival bleeding on probing. In a cross-sectional study of postmenopausal females, Millen et al.[7] found that plasma 25(OH)D concentrations were inversely associated with the percentage of gingival sites that bleed on assessment. Because of the established etiologic role subgingival bacteria have in periodontal disease, and because 25(OH)D is thought to have antimicrobial properties, it is possible that the observed inverse association between vitamin D status and gingival bleeding was, in part, by way of an association with oral bacteria. To the best of the authors' knowledge, there have been no published studies to date that have directly tested this hypothesis.

The purpose of this study is to evaluate whether the association between plasma 25(OH)D concentrations and gingival bleeding reported previously[6,7] could be explained, at least in part, by the postulated antimicrobial properties of vitamin D. To test this hypothesis, the present study examines whether there was a cross-sectional association between plasma 25(OH)D concentrations and the presence of pathogenic oral bacteria among 855 postmenopausal females with both plasma 25(OH)D concentrations and five pathogenic oral bacteria (determined by immunofluorescence) measured from samples collected at a single study visit conducted from 1997 to 2000.

MATERIALS AND METHODS

Study Design

The present study uses information collected from the Buffalo Osteoporosis and Periodontal Disease Study (OsteoPerio Study), an ancillary study of the Women's Health Initiative (WHI) Observational Study (OS) conducted among females enrolled at the Buffalo, New York center. The WHI was designed to examine common health concerns of postmenopausal females, including cancer, heart disease, and osteoporosis, as well as other health outcomes.[8] The study included both a series of randomized clinical trials and an observational study. The OsteoPerio Study was timed to coincide with participants' year-3 clinic visit in the WHI OS conducted from 1997 to 2000. The primary purpose of the OsteoPerio Study was to examine the association between osteoporosis, oral bone loss, and periodontal disease in a well-characterized cohort of postmenopausal females.

Study Population

The Buffalo, New York clinical center of the WHI OS enrolled 2,249 female participants (aged 53 to 83 years). These females were subsequently invited by mail to take part in the OsteoPerio Study around the time of their year-3 clinic visit in the WHI OS. As described previously by Wactawski-Wende et al.,[9] of those invited to participate in the study, 115 could not be reached, 343 stated that they were not interested in participating, 52 were deceased, 27 canceled their appointments, and 12 were deemed temporarily ineligible as a result of recent oral x-rays or radioluminescent dye tests. Of those screened for eligibility, 338 females did not meet the study criteria because of the following: 1) had <6 teeth (162 females); 2) had been diagnosed with cancer in the previous 10 years (106 females); 3) had a serious illness at the time of the year-3 visit (52 females); 4) had bilateral hip replacement (16 females); or 5) had been diagnosed with bone disease (two females).[9] This left 1,362 eligible females who participated in the study. All participants signed informed consent. The study protocol was approved by the University at Buffalo Health Sciences Institutional Review Board, and the study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000.

Of these 1,362 participants, 16 had inadequate study x-rays and five either did not complete or did not return study questionnaires. These females were excluded from this analytic dataset.[9] Blood collection was added to the study protocol after the study initiation. As such, stored plasma for assays of 25(OH)D were available for 934 of the 1,341 females enrolled. Of those females with plasma available, 78 were missing measures of oral bacteria, and one woman was excluded because of her extreme value for plasma 25(OH)D (530 nmol/L), leaving a sample size of 855 for the current analyses.

Plasma 25(OH)D Concentrations

Morning fasting blood samples were collected from participants during their OsteoPerio Study visit. Plasma from these samples was separated according to standard protocols into 0.5 mL cryogenic straws,

frozen, and stored at –196°C in liquid nitrogen at the time of collection. Samples were taken from liquid nitrogen and placed into a –80°C freezer where they were later thawed, aliquoted into cryovials, refrozen, and shipped on dry ice to the laboratory,§ as described previously.[10] Once received by the laboratory, samples were rethawed and assayed in batches over a 4-month period. Assays‖ were performed to quantify plasma 25(OH)D concentrations. Masked duplicate quality-controlled samples were included in each batch and gave a within-pair coefficient of variation of 4.9%.

Collection and Assessment of Periodontal Bacteria

Before conducting any oral probing measures, subgingival plaque samples were taken from 12 index teeth or their substitutes using the paper point technique described previously by Brennan et al.[11] The paper points containing the plaque were placed directly in Ringer's solution as they were removed from the mouth. These were sent to the laboratory for processing, where one half of the solution was frozen. The other half was fixed, using 10% neutral buffered formalin, and remained at room temperature until used for the bacterial analysis. For bacterial analysis, samples of this plaque suspension were vortexed and then heat fixed to a slide. The resulting smears were then reacted with species-specific antisera or monoclonal antibodies, washed and incubated with fluorescein-coagulated immunoglobulin G, and washed again.[11] The stained smears were examined with a specially equipped microscope and fluorescence graded as 1+ through 4+. For the purpose of this study, any fluorescence graded as 3+ or 4+ was considered serologically positive. Plaque samples were tested for the presence of eight species of bacteria.[12] Five of these bacterial species were chosen because Socransky et al. had classified them as "red complex" (*Porphyromonas gingivalis* and *Tannerella forsythia*) or "orange complex" (*Fusobacterium nucleatum*, *Prevotella intermedia*, and *Campylobacter rectus*), signifying that they were thought to be periodontal pathogens.[12-14] Red-complex bacteria are found in the gingiva of people with the most severe periodontal disease, and orange-complex bacteria are found in those with less severe periodontal disease.[13,14] Both *Capnocytophaga* sp. and *Streptococcus saburreum* were collected as controls because they were not thought to be associated with periodontal disease, and it was unknown whether *Eubacterium saburreum* had any association with periodontal disease.[12]

Oral Health Exam

After collection of subgingival plaque samples, each participant in the OsteoPerio Study was given a clinical oral exam. This comprehensive exam included radiographs for assessment of alveolar crestal height and probing measurements, including periodontal probing depth (PD) and clinical attachment level (as described previously by Brennan et al.[11]). Briefly, PD was assessed for six sites of each tooth present, when possible, using a constant-force electronic periodontal probing system¶ that measures the distance from the gingival margin to the bottom of the periodontal pocket.[11] Whole-mouth mean PD was determined by averaging all PD measures across all sites in a participant's mouth.

Questionnaire Data and Physical Measurements

Data used in this study were collected from questionnaires administered during the participants' WHI OS baseline and third visits. These questionnaires, along with questions specific to the OsteoPerio Study, inquired about demographics, lifestyle, and oral health habits, as well as systemic and oral health history. The OsteoPerio Study participants were also asked to bring in all medications and supplements used in the past 30 days, and the dose, frequency, and duration of medications and supplements were recorded. Participants' height (meters) and weight (kilograms) were assessed with a wall-fixed stadiometer and a calibrated balance beam, respectively. From these data, body mass index (BMI) (kilograms per square meters) was calculated.

Statistical Methods

If *P. gingivalis*, *T. forsythia*, *F. nucleatum*, *P. intermedia*, or *C. rectus* were detected, the participant was considered to have pathogenic oral bacteria present, which is the primary outcome in the present analysis. For the primary analyses, quintiles (Q) of 25(OH)D concentrations were created, with Q1 having the lowest 25(OH)D concentrations. The distribution of participant characteristics and potential risk factors in the study sample was examined, both according to quintile of the exposure, plasma 25(OH)D, and presence of any of these pathogenic oral bacteria. Several individual characteristics and risk factors were investigated that have been shown to be associated with gingival bleeding or periodontal disease in previously published literature as follows: 1) age; 2) race; 3) BMI; 4) frequency of toothbrushing; 5) frequency of flossing; 6) frequency of dental visits; 7) days since last dental cleaning; 8) whole-mouth mean PD; 9) self-reported history of diabetes, smoking, alcohol consumption, antibiotic use in the 30 days before the study visit; 10) current use of hormone therapy drugs; and 11) self-reported total recreational physical activity. Analysis of the

§ Heartland Assays, Ames, IA.
‖ LIAISON 25-OH Vitamin D TOTAL Assay, DiaSorin, Stillwater, MN.
¶ The Florida Probe System, Florida Probe, Gainesville, FL.

distribution of these characteristics and risk factors within the study population by exposure category or outcome status was performed using χ^2 tests for categorical variables and *t* tests or analyses of variance (ANOVAs) for continuous variables. Two-sided *P* values ≤0.05 were considered statistically significant.

Next, the association between 25(OH)D concentrations and presence of pathogenic oral bacteria was evaluated using logistic regression. Unadjusted odds ratios (ORs) and 95% confidence intervals (CIs) for presence of oral pathogenic bacteria (yes/no) were estimated for participants in Q2 through Q5 compared to Q1 (reference group) of 25(OH)D. Then, an adjusted model was constructed by forward selection in which variables univariately associated with both the exposure and outcome at a *P* value ≤0.20 were considered as potential confounders. Each potential confounder was added to the crude model separately. The variable that influenced the OR for the association between 25(OH)D and pathogenic bacteria to the greatest extent was included in the logistic regression model provided it changed the OR by ≥10%. Next, additional potential confounders were added sequentially to determine whether they further influenced the OR by ≥10%. This stepwise process continued until addition of no other potential confounders influenced the OR ≥10%.

Opinions vary concerning what indicates an adequate concentration of 25(OH)D. The Institute of Medicine uses a cutoff point of ≥50 nmol/L as adequate with respect to bone-related outcomes.[15] However, ideal concentrations of 25(OH)D may depend on the condition being studied.[16] For this reason, an additional analysis was conducted using clinically defined cut points: 1) deficient (<30 nmol/L); 2) inadequate (30 to <50 nmol/L); 3) adequate (50 to 75 nmol/L); and 4) adequate (>75 nmol/L).[15] Whether 25(OH)D concentrations >75 nmol/L impart any additional benefit on health outcomes has not been determined and therefore was investigated in these analyses.[15]

Additionally, in a restricted analysis, participants who did not have the full possible 12 teeth sampled for plaque were removed to examine whether potential outcome misclassification from limited teeth sampling may have influenced the present results. Additional exploratory analyses were conducted to estimate the ORs of having more than one pathogenic, all orange-complex, or all red-complex bacterial species among participants with adequate (≥50 nmol/L) compared to inadequate (<50 nmol/L) 25(OH)D concentrations.[17]

It was hypothesized that females with low levels of oral hygiene may benefit more from high concentrations of vitamin D than those with high levels of oral hygiene, and therefore an exploratory analysis was conducted to assess the interaction between plasma 25(OH)D concentrations and oral hygiene level. Frequency of flossing was chosen to represent level of oral hygiene because it was the dental hygiene variable most strongly associated with oral bacteria. Interactions between 25(OH)D concentrations and whole-mouth mean PD, smoking status, current use of antibiotics, current hormone therapy use, and self-reported history of diabetes were also explored. A *P* value for interaction <0.10 was considered statistically significant.

RESULTS

Mean 25(OH)D concentrations within quintile exposure groups ranged from 29.4 ± 7.8 nmol/L in Q1 to 95.0 ± 36.5 nmol/L in Q5 (Table 1). Females in Q5 were, on average, slightly younger, had more teeth, and had a lesser proportion of teeth that bled on assessment than females in Q1. Oral hygiene practices were significantly different between females according to quintile of 25(OH)D. A greater proportion of participants in Q5 than Q1 reported more frequent toothbrushing, flossing, and dental visits. A greater proportion of participants in Q5 than Q1 had lower BMIs, used hormone therapy, did not self-report a history of diagnosed diabetes, and, on average, reported greater levels of total recreational physical activity.

There were 496 (58%) females in whom the presence of at least one of the five species of pathogenic bacteria was detected (Table 2). Although 25(OH)D concentrations were, on average, lower in those with versus without any pathogenic bacteria (59.3 versus 61.2 nmol/L), this difference was of a small magnitude and not statistically significant. Females with pathogenic bacteria had, on average, a greater proportion of teeth that bled on assessment and a higher whole-mouth mean PD compared to females with no pathogenic bacteria. A greater proportion of females with pathogenic bacteria flossed infrequently, had higher BMIs, were never-smokers, and did not use hormone therapy compared to those without pathogenic bacteria.

In the crude analysis, high compared to low 25(OH)D concentrations were inversely, but not significantly, associated with the presence of pathogenic oral bacteria (OR for Q5 compared to Q1: 0.78; 95% CI: 0.51 to 1.20; *P* for trend = 0.18) (Table 3). Only BMI changed the crude association between 25(OH)D and pathogenic bacteria by >10%. Age was also added to the adjusted model because it has been shown to be a strong predictor of periodontal disease in the literature.[18] After adjustment for age and BMI, the association was

Table 1.

Baseline (1997 to 2000) Characteristics of Females in the Osteoporosis and Periodontal Disease Study With Baseline Measurements of Both Plasma 25(OH)D and Pathogenic Oral Bacteria According to Qs of Plasma 25(OH)D Concentrations (N = 855)

Characteristic	Q1 (n = 172)	Q2 (n = 170)	Q3 (n = 173)	Q4 (n = 171)	Q5 (n = 169)	*P* Values*
Plasma 25(OH)D, nmol/L						
Q range	5.9 to 40.3	40.4 to 53.9	54.0 to 64.1	64.2 to 77.7	77.9 to 154.4	
Mean ± SD	29.4 ± 7.8	47.2 ± 4.1	59.4 ± 3.0	69.9 ± 4.0	95.0 ± 36.5	<0.001
Demographic characteristics						
Age (years), mean ± SD	66.6 ± 7.2	67.6 ± 6.8	67.3 ± 7.1	66.2 ± 7.1	64.8 ± 7.1	<0.01
Race, n (%)						0.24
White	164 (95.4)	165 (97.1)	169 (97.7)	168 (98.3)	166 (98.2)	
Other	8 (4.7)	5 (2.9)	4 (2.3)	3 (1.75)	3 (1.8)	
Education§, n (%)						0.48
High school	38 (22.4)	33 (19.4)	39 (22.7)	33 (19.5)	28 (17.1)	
College	84 (49.4)	69 (40.6)	74 (43.0)	81 (47.9)	79 (48.2)	
Post college	48 (28.2)	68 (40.0)	59 (34.3)	55 (32.5)	57 (34.8)	
Measure of oral health						
Number of teeth present, mean ± SD	22.1 ± 6.3	23.8 ± 5.1	23.6 ± 5.1	23.8 ± 5.2	24.1 ± 3.6	<0.003
Proportion of teeth that bled on assessment, mean ± SD	0.42 ± 0.3	0.42 ± 0.3	0.34 ± 0.2	0.31 ± 0.2	0.36 ± 0.2	<0.001
Whole-mouth mean PD§, mean ± SD	2.2 ± 0.38	2.2 ± 0.38	2.2 ± 0.4	2.2 ± 0.4	2.2 ± 0.4	0.80
Oral hygiene practice						
Frequency of toothbrushing, n (%)						0.009
Once a day or less	54 (31.4)	43 (25.3)	37 (21.4)	26 (15.2)	43 (25.4)	
Twice a day	85 (49.4)	98 (57.7)	94 (54.3)	101 (59.1)	85 (50.3)	
More than twice a day	33 (19.2)	29 (17.1)	42 (24.3)	44 (25.7)	41 (24.3)	
Frequency of flossing§, n (%)						0.05
Not every week	38 (22.1)	34 (20.0)	29 (16.8)	23 (13.5)	36 (21.4)	
Once a week	12 (7.0)	17 (10.0)	16 (9.3)	23 (13.5)	17 (10.1)	
More than once a week	62 (36.1)	45 (26.5)	60 (34.7)	42 (24.6)	39 (23.2)	
Every day	60 (34.9)	74 (43.5)	68 (39.3)	83 (48.5)	76 (45.2)	
Frequency of dental visits, n (%)						0.04
Never/only with a problem	27 (15.7)	12 (7.1)	17 (9.8)	10 (5.9)	11 (6.5)	
Once a year	24 (14.0)	27 (15.9)	14 (8.1)	28 (16.4)	23 (13.6)	
More than once a year	121(70.4)	131 (77.1)	142 (82.1)	133 (77.8)	135 (79.9)	
Other health and lifestyle characteristics						
BMI (kg/m^2), n (%)						<0.001
<25	48 (27.9)	64 (37.7)	68 (39.3)	92 (53.8)	103 (61.0)	
25 to ≤30	60 (34.9)	63 (37.1)	72 (41.6)	55 (32.2)	53 (31.4)	
>30	64 (37.2)	43 (25.3)	33 (19.1)	24 (14.0)	13 (7.7)	
Current smoking status, n (%)						0.09
Never	93 (54.1)	94 (55.3)	92 (53.2)	88 (51.5)	89 (52.7)	
Former	67 (39.0)	72 (42.4)	79 (45.7)	80 (46.8)	75 (44.4)	
Current	12 (7.0)	4 (2.4)	2 (1.2)	3 (1.8)	5 (3.0)	

attenuated (OR for Q5 compared to Q1 = 0.96; 95% CI: 0.61 to 1.50; *P* for trend = 0.50). Results were similar when using clinically defined cut points of 25(OH)D and when the analysis was restricted to include only females who had a complete set of 12 teeth sampled (adjusted OR for Q5 compared to Q1: 0.98; 95% CI: 0.54 to 1.50; *P* for trend = 0.50). Because the cut point of 75 nmol/L 25(OH)D may not have been high enough to show an association between 25(OH)D and oral bacteria, a sensitivity analysis was also conducted using a higher cut point of 100 nmol/L and found an inverse association (adjusted OR = 0.56 comparing females with ≥100 nmol/L of 25(OH)D to those with <30 nmol/L),

Table 1. (continued)

Baseline (1997 to 2000) Characteristics of Females in the Osteoporosis and Periodontal Disease Study With Baseline Measurements of Both Plasma 25(OH)D and Pathogenic Oral Bacteria According to Qs of Plasma 25(OH)D Concentrations (N = 855)

Characteristic	Q1 (n = 172)	Q2 (n = 170)	Q3 (n = 173)	Q4 (n = 171)	Q5 (n = 169)	*P* Values*
Alcoholic beverage consumption§† (ounces/day), mean ± SD	0.35 ± 0.7	0.42 ± 0.7	0.47 ± 0.7	0.46 ± 0.6	0.55 ± 0.8	0.10
Recent antibiotic use (yes), n (%)	10 (5.8)	15 (8.8)	16 (9.3)	11 (6.4)	18 (10.7)	0.45
Hormone therapy use§ (yes), n (%)	67 (39.2)	80 (47.3)	84 (48.8)	88 (52.4)	98 (58.3)	0.009
Self-reported diagnosis of diabetes (yes), n (%)	11 (6.4)	15 (8.8)	6 (3.5)	5 (2.9)	2 (1.2)	<0.006
Total recreational physical activity§ (MET-hours‡/week), mean ± SD	10.5 ± 13.1	12.4 ± 14.5	14.1 ± 13.3	16.6 ± 13.7	16.7 ± 15.4	<0.001

MET = metabolic equivalent.
* *P* value from ANOVAs for continuous variables and χ^2 tests for categorical variables; participants missing information on a variable were not included in ANOVA or χ^2 calculation for that variable.
† Average ounces of ethanol from wine, beer, and liquor per day in the past year.
‡ MET-hour = duration of activity (hours) × 3.5 mL of O_2/kg/min.
§ Totals (n values) for these variables do not add up to 855 due to missing data. The totals for these n variables were: education (n = 845); whole mouth mean PD (n = 849); frequency of flossing (n = 854); hormone therapy use (n = 848); alcoholic beverage consumption (n = 845); and total recreational physical activity (n = 848).

although this was not statistically significant (95% CI: 0.28 to 1.11).

Associations between 25(OH)D and females with more than one pathogenic bacteria, all orange-complex bacteria, or all red-complex bacteria were further examined. Although the results were not statistically significant, a stronger, inverse association was found between 25(OH)D concentrations and the presence of more than one pathogenic bacteria (adjusted OR for adequate compared to deficient/inadequate 25(OH)D concentrations = 0.85; 95% CI: 0.60 to 1.19), all orange-complex bacteria (adjusted OR = 0.68; 95% CI: 0.37 to 1.32), and all red-complex bacteria (adjusted OR = 0.80; 95% CI: 0.48 to 1.33) than in the primary analysis.

In exploratory analyses, it was examined whether associations between 25(OH)D concentrations and pathogenic oral bacteria were modified by frequency of flossing, whole-mouth mean PD, smoking status, recent use of antibiotics, hormone therapy use, and self-reported history of diabetes (Table 4). A slightly stronger association was observed in females who flossed no more than once a week (adjusted OR = 0.61; 95% CI: 0.35 to 1.09) compared to those who flossed more than once a week (adjusted OR = 1.11; 95% CI: 0.65 to 1.89) or every day (adjusted OR = 1.13; 95% CI: 0.69 to 1.85). However, the *P* value for interaction was not significant (*P* for interaction = 0.22). A statistically significant interaction by smoking status was observed. OR and 95% CI comparing ≥50 with <50 nmol/L 25(OH)D was 0.25 (0.02 to 3.34) in females who reported being current smokers and 1.00 (0.74 to 1.35) in females who reported being non-smokers at the time of the study (*P* for interaction = 0.03). However, the number of current smokers in this study cohort was very small, limiting interpretation of the findings.

DISCUSSION

The cross-sectional association was examined between plasma 25(OH)D concentrations and pathogenic oral bacteria in postmenopausal females participating in the OsteoPerio Study. To the best of the authors' knowledge, the present study was the first to examine the relationship between vitamin D status and pathogenic oral bacteria. The odds of the presence of at least one species of pathogenic oral bacteria in females did not differ by quintile of 25(OH)D concentrations, and these results did not vary greatly when clinical cut points for 25(OH)D were used. However, inverse, although not statistically significant, associations were found between 25(OH)D concentrations and pathogenic oral bacteria when analyses were limited to females with more than one pathogenic, all orange-complex, or all red-complex bacteria species. This may suggest that vitamin D status is associated with more severe oral bacteria composition profiles, i.e., having multiple species of pathogenic oral bacteria as opposed to at least one.

Although no association was observed between vitamin D status and any pathogenic oral bacteria in the present study, there is a strong biologic rationale to believe that one might exist. Previous research in cross-sectional studies[6,7] has shown inverse associations between 25(OH)D concentrations and

Table 2.

Baseline (1997 to 2000) Characteristics of Females in the Osteoporosis and Periodontal Disease Study With Baseline Measurements of Plasma 25(OH)D Concentrations and Pathogenic Oral Bacteria According to the Presence of Pathogenic Bacteria (N = 855)

Characteristic	Pathogenic Oral Bacteria		*P* Values†
	None,* n = 359 (42%)	At Least One,* n = 496 (58%)	
Plasma 25(OH)D (nmol/L), mean ± SD	61.2 ± 23.7	59.3 ± 30.3	0.30
Demographic characteristics			
Age (years), mean ± SD	66.1 ± 7.2	66.8 ± 7.0	0.20
Race, n (%)			0.26
White	352 (98.1)	480 (96.8)	
Other	7 (2.0)	16 (3.2)	
Education‖, n (%)			0.67
High school	70 (19.7)	101 (20.7)	
College	159 (44.7)	228 (46.6)	
Post college	127 (35.7)	160 (32.7)	
Measures of oral health			
Number of teeth present, mean ± SD	23.2 ± 5.7	23.6 ± 4.8	0.24
Proportion of teeth that bled on assessment, mean ± SD	0.31 ± 0.2	0.41 ± 0.2	<0.001
Whole-mouth mean PD‖, mean ± SD	2.1 ± 0.4	2.3 ± 0.4	<0.001
Oral hygiene practices			
Frequency of toothbrushing, n (%)			0.22
Once a day or less	76 (21.2)	127 (25.6)	
Twice a day	196 (54.6)	267 (53.8)	
More than twice a day	87 (24.2)	102 (20.6)	
Frequency of flossing‖, n (%)			0.04
Not every week	52 (14.5)	108 (21.8)	
Once a week	39 (10.9)	46 (9.1)	
More than once a week	103 (28.7)	145 (29.3)	
Every day	165 (46.0)	196 (39.6)	
Frequency of dental visits, n (%)			0.14
Never or only with a problem	27 (7.5)	50 (10.1)	
Once a year	42 (11.7)	74 (14.9)	
Less than once a year	290 (80.8)	372 (75.0)	
Other health and lifestyle characteristics			
BMI (kg/m^2), n (%)			<0.01
<25	183 (50.1)	192 (38.7)	
25 to 30	112 (31.2)	191 (38.5)	
>30	64 (17.8)	113 (22.8)	
Current smoking status, n (%)			<0.008
Never	209 (58.2)	247 (49.8)	
Former	145 (40.4)	228 (46.0)	
Current	5 (1.4)	21 (4.2)	

gingival bleeding, a marker of gingival inflammation. Because vitamin D has been shown to have both antimicrobial and anti-inflammatory properties,[3-5,18] it has been hypothesized that vitamin D would decrease gingival bleeding or gingivitis through reduction of oral bacteria, inflammation, or a combination of the two.

The antimicrobial properties of vitamin D have been characterized. In the presence of pathogens, Toll-like receptors on monocytes and macrophages activate genes in the vitamin D pathways, including *CYP27B1* (which encodes for 1,25-α hydroxylase) and the vitamin D receptor.[4] Increased production of 1,25-α hydroxylase has been shown to increase

Table 2. (continued)

Baseline (1997 to 2000) Characteristics of Females in the Osteoporosis and Periodontal Disease Study With Baseline Measurements of Plasma 25(OH)D Concentrations and Pathogenic Oral Bacteria According to the Presence of Pathogenic Bacteria (N = 855)

Characteristic	Pathogenic Oral Bacteria		*P* Values†
	None,* n = 359 (42%)	At Least One,* n = 496 (58%)	
Alcoholic beverage consumption‡‖ (ounces/day), mean ± SD	0.46 ± 0.7	0.44 ± 0.7	0.58
Recent antibiotic use (yes), n (%)	32 (8.9)	38 (7.7)	0.51
Hormone therapy use‖ (yes), n (%)	203 (57.0)	214 (43.5)	<0.001
Self-reported diagnosis of diabetes (yes), n (%)	17 (4.7)	22 (4.4)	0.84
Total recreational physical activity‖ (MET-hours§/week), mean ± SD	14.5 ± 13.7	13.7 ± 14.5	0.39

MET = metabolic equivalent.
* Of the pathogenic bacteria measured.
† *P* value from ANOVAs for continuous variables and χ^2 tests for categorical variables; participants missing information on a variable were not included in ANOVA or χ^2 calculation for that variable.
‡ Average ounces of ethanol from wine, beer, and liquor per day in the past year.
§ MET-hour = duration of activity (hours) × 3.5 mL of $O_2 \cdot kg^{-1} \cdot min^{-1}$.
‖ Totals for these n variables do not add up to 855 due to missing data. The totals for these n variables were: education (n = 845); whole mouth mean PD (n = 849); frequency of flossing (n = 854); hormone therapy use (n = 848); alcoholic beverage consumption (n = 845); and total recreational physical activity (n = 848).

production of cathelicidin, an antimicrobial peptide.[4,5] Data supporting the proposed antimicrobial effect of vitamin D have been reported in both animal[19] and human studies.[20,21]

Several factors that could potentially modify the association between 25(OH)D concentrations and the presence of pathogenic oral bacteria were also investigated. It was hypothesized that the association between vitamin D and oral bacteria would be greater in those with poor, compared to those with better, dental hygiene practices because those with poor dental hygiene would have a greater load of oral bacteria. A more inverse, but not statistically significant, association was observed in females with less frequent flossing, but the *P* value for interaction was 0.22. It is probable that a wider variation in dental hygiene practices, or a larger sample size, may have demonstrated an association between vitamin D and oral bacteria in some strata of the population. A statistically significant interaction was observed between vitamin D status and current smoking status, with an inverse association between vitamin D status and oral bacteria in smokers. Although this observation suggests that smokers may be a population subgroup in which sufficient levels of plasma vitamin D may confer a protective effect, the small relative frequency of smokers and the wide CI for the stratum-specific OR require that this observation be interpreted and generalized cautiously.

The present findings may indicate an actual absence of an association between 25(OH)D concentrations and pathogenic bacteria, but this study is a secondary analysis of data from the OsteoPerio Study, which was not initially designed to examine the relationship between vitamin D and bacteria. It is possible that 25(OH)D concentrations have an effect on pathogenic oral bacteria that is too small for this study to detect. It is also conceivable that higher concentrations of 25(OH)D are associated with fewer pathogenic bacteria. A sensitivity analysis was conducted that found an inverse yet not statistically significant association between 25(OH)D concentrations and prevalence of pathogenic bacteria, but it is difficult to draw conclusions from this analysis because only 37 of 855 participants had 25(OH)D concentrations >100 nmol/L.

It is also hard to know whether adjustment for BMI led to overadjustment of the present association, because anthropometric measures of body fatness have been shown to be a strong predictor of vitamin D status in this cohort of postmenopausal females.[22] It is also possible that the present assay was not able to detect bacteria in those participants who had less than the full 12 paper point subgingival samples taken. However, restricting the analyses to include only participants with a complete set of 12 paper points sampled did not alter the results.

Additionally, some (≈24%) of the participants in the New York clinical center of the WHI OS were not available for screening into the OsteoPerio Study, and others (≈15%) did not meet eligibility criteria as a result of having <6 teeth or certain health conditions. The present study does not have any information on

Table 3.

Crude and Adjusted ORs and 95% CIs for Prevalent Pathogenic Oral Bacteria Among Females in Q2 Through Q5 Compared to Q1 for Plasma 25(OH)D: The Osteoporosis and Periodontal Disease Study (1997 to 2000) (N = 855)

Association	No. With Outcome/Total No.	Crude		Adjusted*	
		OR	95% CI	OR	95% CI
Any pathogenic bacteria (n = 855)					
Q of plasma 25(OH)D, nmol/L (Q range)					
Q1 (5.9 to 40.3)	104/172	1.00	Referent	1.00	Referent
Q2 (40.4 to 53.9)	107/170	1.11	(0.72 to 1.72)	1.16	(0.75 to 1.81)
Q3 (54.0 to 64.1)	92/173	0.74	(0.48 to 1.14)	0.78	(0.51 to 1.21)
Q4 (64.2 to 77.7)	101/171	0.94	(0.61 to 1.45)	1.09	(0.70 to 1.70)
Q5 (77.9 to 154.4)	92/169	0.78	(0.51 to 1.20)	0.96	(0.61 to 1.50)
P for trend			0.18		0.50
Clinical cut points of plasma 25(OH)D, nmol/L					
Deficient (<30)	47/80	1.09	(0.67 to 1.78)	1.00	(0.61 to 1.65)
Inadequate (30 to <50)	130/210	1.24	(0.88 to 1.76)	1.15	(0.81 to 1.64)
Adequate (50 to <75)	209/369	1.00	Referent	1.00	Referent
Adequate (≥75)	110/196	0.98	(0.69 to 1.39)	1.12	(0.76 to 1.55)
P for trend			0.74		0.65
Deficient/inadequate (<50)	177/290	1.00	Referent	1.00	Referent
Adequate (≥50)	319/565	0.83	(0.62 to 1.11)	0.93	(0.69 to 1.25)
More than one pathogenic bacteria (n = 630)					
Deficient/inadequate (<50 nmol/L)	104/217	1.00	Referent	1.00	Referent
Adequate (≥50 nmol/L)	167/413	0.74	(0.53 to 1.03)	0.85	(0.60 to 1.19)
All orange-complex bacteria† (n = 400)					
Deficient/inadequate (<50 nmol/L)	17/130	1.00	Referent	1.00	Referent
Adequate (≥50 nmol/L)	24/270	0.61	(0.32 to 1.17)	0.68	(0.37 to 1.32)
All red-complex bacteria† (n = 441)					
Deficient/inadequate (<50 nmol/L)	33/146	1.00	Referent	1.00	Referent
Adequate (≥50 nmol/L)	49/295	0.68	(0.42 to 1.12)	0.80	(0.48 to 1.33)

* Adjusted for BMI categorized as <25, 25-30, and >30 kg/m^2 and age.
† Orange-complex bacteria are *F. nucleatum*, *P. intermedia*, and *C. rectus*. Red-complex bacteria are *P. gingivalis* and *T. forsythia*.

the 25(OH)D or oral bacteria status of these participants. The present results may have been different if these females were included.

Because quantities of each bacteria species present were not measured, it was not possible to investigate whether 25(OH)D concentrations were associated with quantity of pathogenic oral bacteria. It is possible that 25(OH)D has an antimicrobial effect sufficient enough to lower the quantity of bacteria present to less than the infectious dose but still above the limit of detection of the assay. If this is the case, an inverse association of 25(OH)D concentrations and gingival bleeding might have been seen, as described by Millen et al.,[7] but not an inverse association with the presence and absence of oral bacteria. This is supported by the exploratory analyses which suggest that vitamin D status may be more strongly associated with oral bacterial load (e.g., presence of more than one pathogenic bacterium).

Although the species of bacteria measured have been shown to be associated with periodontal disease,[14] the oral cavity can contain >500 species of bacteria, both pathogenic and non-pathogenic.[23] Assays were conducted to detect just five pathogenic species of bacteria. Although the antimicrobial effect of vitamin D on bacteria may not be species specific, it is possible that some bacteria may be more prone to its effects. DNA sequencing of bacteria and other pathogens from the oral cavity would provide a more comprehensive description of the species of bacteria present in study participants. The association between vitamin D status and oral bacteria should be further investigated within these studies.

These data may not be generalizable to other populations because the present participants were

Table 4.

Adjusted ORs and 95% CIs for Prevalent Pathogenic Oral Bacteria Among Females With Adequate (≥50 nmol/L) Compared to Deficient/Inadequate (<50 nmol/L) Plasma 25(OH)D Concentrations Stratified by Potential Effect Modifiers: The Osteoporosis and Periodontal Disease Study (1997 to 2000) (N = 855)

Strata of Potential Effect Modifier	No. With Outcome/Total No.	Adjusted OR*	95% CI
Frequency of flossing†			
Once a week or less	154/248	0.61	0.35 to 1.09
More than once a week	146/247	1.11	0.65 to 1.89
Every day	195/361	1.13	0.69 to 1.85
P for interaction			0.22
Whole-mouth mean PD†			
<2 mm	129/254	0.91	0.53 to 1.57
2 to <3 mm	348/562	0.91	0.62 to 1.31
≥3 mm	19/33	1.84	0.34 to 9.91
P for interaction			0.34
Current smoker			
No	475/829	1.00	0.74 to 1.35
Yes	21/26	0.25	0.02 to 3.34
P for interaction			0.03
Recent antibiotic use			
No	458/785	0.97	0.71 to 1.32
Yes	38/70	0.59	0.19 to 1.86
P for interaction			0.54
Current hormone therapy use†			
No	278/431	0.97	0.65 to 1.45
Yes	214/417	0.81	0.53 to 1.24
P for interaction			0.61
Self-reported diagnosis of diabetes			
No	475/816	0.92	0.68 to 1.26
Yes	21/39	0.63	0.16 to 2.52
P for interaction			0.69

* Adjusted for BMI categorized as <25, 25-30, and >30 kg/m^2 and age.
† Totals for these n variables do not add up to 855 due to missing data. The totals for these n variables were: whole mouth mean PD (n = 849); frequency of flossing (n = 854); hormone therapy use (n = 848).

predominately white, well-educated, in good health, and reported high levels of oral hygiene practices. The present study was also cross-sectional in nature, measuring both vitamin D concentrations and presence of oral bacteria at the same point in time. It is possible that past levels, rather than current levels, of 25(OH)D are related to oral pathogenic bacteria. However, this seems unlikely because gingivitis, for which bacteria is a necessary cause, is an acute disease often appearing just days after cessation of oral hygiene.[24] Even so, previous analyses of these data show that plasma 25(OH)D concentrations in females remain relatively stable over an ≈5-year period.[10]

Despite some noted limitations, the present study has a number of strengths. The OsteoPerio Study was conducted within a large, well-established cohort and collected data on a large number of covariates that were investigated as potential confounders. This study appears to be the first to investigate the association between vitamin D status and laboratory-identified oral pathogenic bacteria. Intriguing exploratory analyses suggest that vitamin D status may be associated with oral bacteria composition profiles (e.g., those with more than one pathogenic species of bacteria) or within certain subgroups of the population (those with poor oral hygiene and smokers). The present study is not confined to using less precise measures of vitamin D (e.g., dietary intake) but had measures of 25(OH)D concentrations reflecting intake from all oral sources of vitamin D and sunlight exposure over an

≈3-week time period before the blood draw.[25] Furthermore, both blood and gingival plaque samples were collected at the same time. This was a strength because it was hypothesized that recent plasma 25(OH)D concentrations were more relevant to the presence of pathogenic oral bacteria than past concentrations.

CONCLUSIONS

A lack of association was observed between five species of pathogenic oral bacteria and 25(OH)D concentrations in postmenopausal females in the OsteoPerio Study. These findings may be attributable to the species of bacteria assessed, small effect size, or a true absence of an association. Additional studies are needed to specifically investigate this association using techniques that can better identify and quantify oral bacteria.

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