Serum Concentrations of Novel Vitamin D Biomarkers and Detection of Prevalent High-Risk HPV Infection

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Abstract

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Introduction: Vitamin D has potential immunomodulating benefits in infection. While one prior population-based cross-sectional study showed a protective association between serum concentrations of 25(OH)D and high-risk HPV (hrHPV) infection, additional biomarkers present at different stages along the vitamin D metabolic pathway may more completely characterize vitamin D status than 25(OH)D alone. We evaluated cross-sectional associations between 25(OH)D and four additional, novel biomarkers and prevalent hrHPV infection among mid-adult women in Seattle, Washington.

Methods: We identified stored sera from women 30-50 years old (N=404) enrolled into an HPV natural history study between 2011-2012. Sera were tested for 5 vitamin D biomarkers: 25(OH)D, 1,25(OH)D, 24,24(OH)D, free vitamin D, and vitamin D binding protein (DBP). Using multiple logistic regression, we estimated the cross-sectional association between serum concentrations of vitamin D and cervicovaginal hrHPV detection. Total vitamin D (25(OH)D) was modeled as both a continuous and categorical measure, using clinically-relevant cut-points to indicate sufficiency (≥20ng/mL or ≥30ng/mL). We controlled for biological, demographic, and behavioral characteristics using three stages of adjustment. Effect modification by race (White, non-White) was evaluated, and post hoc analyses examined likely vitamin D supplementation (25(OH)D ≥50ng/mL).

Findings: Mean serum concentrations of 25(OH)D were 31.3ng/mL (standard deviation 10.5). Overall prevalence of cervicovaginal hrHPV infection was 22%. 25(OH)D serum concentration was not associated with prevalent hrHPV; modeling the exposure as a categorical variable did not significantly change interpretation. Each 1ng/mL increase in 24,25(OH)D was borderline statistically significantly associated with a higher likelihood of hrHPV infection (aOR 1.23, 95% CI 0.97, 1.55). No significant associations were observed for other biomarkers. There was no evidence of effect modification by race. Women with serum concentrations of 25(OH)D ≥50ng/mL had a higher likelihood of prevalent hrHPV compared to women with levels <50ng/mL (aOR 2.82, 95% CI 1.12, 7.11).

Conclusion: In contrast to the prior population-based study, we did not find 25(OH)D serum concentrations were associated with prevalent hrHPV. Higher levels of one novel biomarker, 24,25(OH)D, were associated with increased odds of hrHPV, an unexpected finding. Post hoc analyses warrant further exploration into the relationship between vitamin D supplementation and hrHPV infection.

INTRODUCTION

Human Papillomavirus (HPV) is the most common sexually transmitted infection among adults in the United States, with 7 million annual incident cases and a lifetime cumulative risk of infection estimated between 80-90%.[1] Of 200 HPV genotypes, 19 are implicated in cancers of the cervix, anus, and oropharynx and thus considered high-risk HPV (hrHPV).[2] While approximately 90% of HPV infections clear within 1-2 years of detection, the minority that persist are associated with increased risk for cervical cancer.[3,4] Attempts to understand factors that contribute to acquisition and persistence of infection (i.e., vitamin D deficiency) are critical in the efforts to prevent HPV infections and HPV-related carcinogenesis.

Vitamin D sufficiency is associated with improved bone and intestinal health, reduced risk and severity of respiratory and influenza-like infections,[5] and lower incidence of certain cancers.[6] However, data collected by the National Health and Nutrition Examination Survey (NHANES) in 2005-2006 showed 42% of adults in the United States are vitamin D deficient or insufficient with 25(OH)D ≤20ng/mL.[7] Previous studies have investigated the potential biological pathways by which vitamin D acts as an immune modulator in the body.[8,9] Lower concentrations of serum vitamin D may lead to diminished immune function and increased susceptibility to and duration of infection; insufficient and deficient levels of vitamin D may limit the body's ability to produce and regulate the expression of antimicrobial peptides (AMP),[10] which respond to pathogens and prevent infection.[11] AMP are produced in the vagina and cervix, where they can specifically defend against sexually transmitted infections.[12]

Metabolism of vitamin D occurs in the bloodstream, kidney, and liver; this process generates several biomarkers indicative of an individual's vitamin D status.[13,14] The majority of epidemiologic studies estimate vitamin D status by measuring serum concentrations of total vitamin D, or 25(OH)D, but this biomarker is only one means (albeit a stable one[15]) of characterizing an individual's vitamin D status.[16] Additional biomarkers include 1) calcitriol (the hormonally-active metabolite of D_3 otherwise known as $1,25(OH)_2D$), 2) $24,25(OH)D_3$ (the most concentrated product of $25(OH)D_3$ catabolism), 3) vitamin D binding protein (DBP), and 4) free vitamin D (circulating unbound to DBP or albumin).[17,18] Several of these biomarkers, present at different stages along the vitamin D metabolic pathway, have been identified as potential means of characterizing one's vitamin D status more fully than 25(OH)D alone.[16] However, the absence of established cut-points of sufficiency for these additional biomarkers makes comparisons across biomarkers difficult. Additional vitamin D biomarkers have historically not been factored into recommendations.[19]

To date, there has been only one study to evaluate the association between serum 25(OH)D and HPV. Using data from the population-based National Health and Nutrition Examination Survey (NHANES), investigators concluded that lower concentrations of serum vitamin D were significantly associated with higher odds of HPV infection.[20] To expand on these findings, we present the cross-sectional associations between five different vitamin D biomarkers and prevalent hrHPV infection among mid-adult women in the pacific northwest.[21] Given previous literature suggesting differences in vitamin D metabolism and bioavailability of the micronutrient among racial groups,[22,23] we also evaluated whether this association varied by race.

METHODS

We used baseline data and stored specimens from a longitudinal cohort study of HPV infections in healthy mid-adult women in Seattle, Washington. Details of the parent study design were described previously.[21] Briefly, women between the ages of 30-50 years affiliated with the University of Washington (faculty, staff, or student) were enrolled from March 2011 to January 2012. Pregnant women were excluded, as were those with a history of hysterectomy or a current medical condition that prohibited participation. At their enrollment visit, participants self-collected vaginal swabs for HPV DNA testing and provided venous blood specimens; residual serum specimens were stored at -80° C for future testing. Demographic (age, race/ethnicity, education level, marital status), health (Body Mass Index (BMI), alcohol intake, smoking status, oral contraceptive use, HPV vaccination history), and sexual behavior characteristics (history of genital warts and/or other sexually-transmitted infections, history of pregnancy, and number and recency of male sex partners) were collected through an interview with a study coordinator or a self-administered online questionnaire. Information regarding vitamin D supplementation was not determined.

Laboratory Methods

HPV Testing

Self-collected vaginal swabs were genotyped as part of the parent study using the PCR-based Roche Linear Assay (Roche Diagnostics, Pleasanton, CA).[21] Our primary outcome of interest was infection with one or more of 19 hrHPV strains: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82, and IS39.[24]

Vitamin D Biomarker Testing

Vitamin D biomarker testing was conducted on stored sera at the University of Washington's Nutrition and Obesity Research Center Analytic Core (P30 DK035816). Sera specimens were thawed and 750 µL were aliquoted and transported on dry ice. Serum concentrations of albumin, calcium, and phosphate were determined using an automated clinical chemistry analyzer (Beckman AU5812). Concentrations of $1,25(OH)_2D_3$, $1,25(OH)_2D_2$, $24,25(OH)_2D_3$, $25(OH)D_3$, and $25(OH)D_2$ were determined using immunoaffinity enrichment-liquid chromatography-tandem mass spectrometry, as previously described.[25,26] Concentrations of vitamin D binding globulin and the haplotypes present in each sample were determined using trypsin digestion-liquid chromatography-tandem mass spectrometry, as previously described.[27,28]

Exposure Definitions

Specifically, we measured as exposures serum concentrations of 1) 25(OH)D, known as total vitamin D, 2) $1,25(OH)_2D$, 3) $24,25(OH)D_3$, 4) free/unbound vitamin D, and 5) total vitamin D adjusted for DBP, stratified by DBP haplotype. Total vitamin D and $1,25(OH)_2D$ are composite measures and are calculated as the sums of $25(OH)D_2$ and $25(OH)D_3$ and $1,25(OH)_2D_2$ and $1,25(OH)_2D_3$, respectively. We calculated free vitamin D using an established equation that factors in affinity constants for albumin and DBP.[29] Free vitamin D was measured in 1000*nmol/L given its presence in low concentrations.

Our primary exposure was total vitamin D, analyzed as a continuous increase of 10ng/mL of serum 25(OH)D. As a secondary analysis of total vitamin D, we generated two binary exposure variables using clinically meaningful cut-points of sufficiency and deficiency.[30,31] Sufficiency

was measured as ≥20ng/mL, and then again, as ≥30ng/mL. Determination of cut-points for serum 25(OH)D were based on recommendations from the Institute of Medicine,[30] the Endocrine Society,[31] and recent literature,[20] though clinical cut-points used to establish sufficiency remain a contested area of research.[32] The other four biomarkers were secondary exposures and were all analyzed as continuous variables.

Post Hoc 25(OH)D Analyses: Exposure Definitions

To better understand the potential implications of modeling total vitamin D linearly versus as a categorical measure in our population, we conducted a post hoc analysis using additional cut-points. First, we evaluated the prevalence of hrHPV among five subgroups of women categorized by 25(OH)D serum concentration: <12ng/mL, 12-19ng/mL, 20-29ng/mL, 30-49ng/mL, and ≥50ng/mL. While three of these cut-points (<12ng/mL, 12-19ng/mL, 20-29ng/mL) were utilized in the NHANES study,[20] the inclusion of a cut-point of 50ng/mL (serum concentrations at or above which typically indicate vitamin D supplementation[33]) was included due to potential clinical relevance (notably, approximately 5% of our study population measured at or above this cut-point). Second, we generated a binary exposure variable at 50ng/mL to compare women who likely supplement to those who likely do not with respect to odds of prevalent hrHPV.

Statistical Methods

Using logistic regression, we estimated odds ratios (OR), 95% confidence intervals (CI), and robust standard errors for associations between serum vitamin D and prevalent hrHPV detection. Because serum concentration of vitamin D fluctuate significantly with time of year, we adjusted

for season of enrollment using a B-spline with five degrees of freedom (to account for ten months of enrollment). We considered additional variables *a priori* for inclusion in our analyses. In an effort to present a reliable, precise estimate of an unconfounded relationship, we performed our modeling in a phased approach. For descriptive purposes, we produced a minimally-adjusted model adjusting only for age (continuous), self-reported race (White, Black, Asian, American Indian or Alaska Native, Native Hawaiian or Other Pacific Islander, other, or multiple races), and season. Our moderately-adjusted model is our primary model. This model incorporated additional biological and behavioral risk factors: oral contraceptive use (never, former, or current), highest level of education (less than college, bachelor's degree, or master's/doctoral degree), smoking status (never, former, or current smoker), BMI (underweight, normal weight, overweight, or obese), and serum concentrations of calcium (ng/mL) and phosphate (mg/dL). Finally, we ran a fully-adjusted model that also included sexual behavior risk factors evaluated as precision variables: lifetime number of male sex partners (continuous) and number of sex partners in the six months prior to enrollment (none, non-new only, one new, ≥2 new).

Based on previous studies that have found a difference in total vitamin D metabolism by race, we evaluated potential effect modification by race/ethnicity. The study population was predominantly White; comparisons were made between White and non-White women due to small sample sizes in non-White race groups. Non-White women, including Black, Asian, American Indian or Alaska Native, Native Hawaiian or Other Pacific Islander, women reporting other or multiple races, accounted for 21% of the sample.

Post Hoc 25(OH)D Analysis: Statistical Methods

We ran generalized additive models for each exposure to assess evidence of non-linearity in the relationship between prevalent hrHPV and our predictors. To examine a potential non-linear dose response between serum 25(OH)D and detection of hrHPV, we estimated prevalence of hrHPV and corresponding 95% CI among five subgroups of women defined by total vitamin D serum concentration

Using logistic regression to estimate OR, 95% CI, and robust standard errors, we measured an association between serum 25(OH)D and prevalent hrHPV among women who likely supplement (≥50ng/mL) versus all others. We conducted this analysis using the three stages of modeling outlined above to adjust for potential confounding.

All analyses were conducted in R version 3.4.3.[34]

RESULTS

Characteristics of the Study Population

One woman was excluded from analyses due to a specimen that was insufficient for HPV testing. Four women were excluded due to a suspicion of a specimen labeling error. The mean age of the remaining 404 participants was 38.3 years (SD 6.1 years) (Table 1). The study population was predominantly White, with a mean BMI of 25.9 (SD 5.8). One third of participants reported current hormonal contraceptive use, and 26% were current or former smokers. The majority of women reported no new partners or no partner in the six months prior to enrollment. Prevalence of cervicovaginal hrHPV infection was 22%.

Biomarker Summary Statistics

Median and interquartile ranges for all vitamin D biomarkers evaluated were within normal ranges[35] (Table 2). Mean serum concentrations of calcium[36] and phosphate[37] were normal as well, at 9.5ng/mL (SD 0.4ng/mL) and 3.5mg/dL (SD 0.5mg/dL), respectively.

Associations Between Serum Concentrations of Vitamin D and hrHPV Infection

Associations between serum concentrations of total vitamin D and hrHPV detection were inconsistent and not statistically significant (Table 3). Controlling for season of enrollment, age, race, BMI, smoking status, education level, and oral contraceptive use, each 10ng/mL increase in 25(OH)D was associated with a non-statistically significant increased odds of hrHPV infection (aOR 1.10, 95% 0.84, 1.45). Estimates produced using minimally- and fully-adjusted models were also not significantly different from 1.0.

Secondary analyses of categorical exposure variables designed to classify total vitamin D as sufficient or deficient also yielded non-significant results. After adjustment under our primary model, compared to women with deficient serum 25(OH)D levels below 20ng/mL, those with serum concentrations ≥20ng/mL (indicating sufficiency) had estimated odds of hrHPV infection of 0.85 (95% CI 0.37, 1.93). Under the same model, the estimated odds of infection for a higher 25(OH)D cut-point of 30ng/mL was also 0.85 (95% CI 0.50, 1.44).

Upon examination of $1,25(OH)_2D$, free vitamin D, and total vitamin D adjusted for DBP and DBP haplotype, we found no significant associations between serum concentrations of vitamin D

biomarkers and odds of hrHPV infection. However, in a minimally-adjusted model, serum 24,25(OH)D_3 was significantly associated with hrHPV infection. Each one ng/mL increase in 24,25(OH)D_3 was associated with higher odds of infection (aOR 1.31, 95% CI 1.05, 1.63), although the significance of this association waned upon further adjustment.

Evaluation of Effect Modification by Race

Analyses examining potential interaction between total vitamin D levels and race did not reveal significant differences in odds of hrHPV infection between White and non-White women (Table 5). After adjustment with our primary model, each 10ng/mL increase in total vitamin D suggested higher odds of infection among both White (aOR 1.08, 95% CI 0.79, 1.49) and non-White women (aOR 1.25, 95% CI 0.63, 2.48); however, these results were not statistically significant.

Post Hoc 25(OH)D Analysis

Although results of a generalized additive model did not provide strong evidence of a non-linear effect of continuous 25(OH)D (Figure 1), our post hoc analysis determined hrHPV prevalence varied by serum concentrations of total vitamin D (Table 4). Additionally, we evaluated the odds of prevalent hrHPV among women with 25(OH)D ≥50ng/mL (Table 3). Under our primary model, the odds of hrHPV infection among women who likely supplement were significantly higher than the odds among women in whom supplementation is unlikely (aOR 2.82, 95% CI 1.12, 7.11).

DISCUSSION

To our knowledge, this is the first study to analyze an association between serum vitamin D status and hrHPV prevalence using five different biomarkers of vitamin D status, four of which are novel in this context. We aimed to understand the relationship between serum concentrations of vitamin D and detection of hrHPV through evaluation of not only total vitamin D, but other forms of the micronutrient along the metabolic pathway as well. These biomarkers included byproducts of total vitamin D metabolism tasked with keeping cellular proliferation and differentiation in check (1,25(OH)D and 24,25(OH)D)[10,15] and a measure of circulating vitamin D unbound to binding proteins and other nutrients (free vitamin D). We did not observe significant associations between 25(OH)D and prevalent hrHPV in our analyses of total vitamin D as both a continuous and categorical variable, as defined *a priori*. Post hoc analysis of total vitamin D revealed women who likely supplement (25(OH)D ≥50ng/mL) had significantly higher odds of prevalent hrHPV compared to other women. Apart from our findings on 24,25(OH)D, we did not detect significant relationships between additional biomarkers and prevalence of hrHPV. Similarly, our analysis of race did not yield evidence of effect modification.

Higher serum concentrations of 24,25(OH)D were significantly associated with higher odds of hrHPV infection. Although statistical significance waned in the moderately and fully adjusted models, this hint of an association warrants closer examination. Low concentrations of 24,25(OH)D, the most plentiful byproduct of 25(OH)D metabolism by the enzyme CYP24A1 (found in most tissues throughout the body), have previously been associated with poor health outcomes.[38] Unsurprisingly, serum concentrations of 24,25(OH)D are positively correlated with

concentrations of total vitamin D.[39] Thus, the direction of the association we observed between increasing serum concentrations of 24,25(OH)D and detection of hrHPV is in the opposite direction than we would expect.

Our hypothesis of an inverse association between serum concentrations of 25(OH)D and detection of hrHPV was motivated by prior research of the relationship[20] and suggestions of vitamin D's immunomodulating properties in the context of chronic[40] and acute disease.[5] However, our selection of cut-points for our categorical analyses, though clinically-motivated, perhaps oversimplified the association and resulted in attenuated estimates. This was not apparent until we analyzed our primary exposure, in a post hoc analysis, as a more granular categorical variable rather than a binary measure. By grouping very deficient and deficient women (all with 25(OH)D <20ng/mL or <30ng/mL, depending on the analysis) and combining all subgroups of sufficiency (all whose serum concentrations were above the selected cut-point), our original categorical analyses generated comparison groups of women who looked very similar with regards to hrHPV prevalence. Thus, it was difficult to detect any difference between deficient and sufficient women despite the true variance of within-group hrHPV prevalence.

Only 2% of our study population were severely deficient in 25(OH)D, and none of these women had prevalent hrHPV infection. This absence of hrHPV among women with the lowest serum concentrations of vitamin D in our sample suggests a protective effect of severe deficiency. However, it is possible that women in the far left tail of the vitamin D distribution were experiencing medical conditions that predisposed them to be less likely to engage with new sexual partners (i.e. depression, which has been associated with low serum concentrations of

25(OH)D[41]) and thus less likely to acquire hrHPV (it is worth noting our study population included generally healthy women; few were immunocompromised). This correlation is born out in a post hoc analysis of total vitamin D by reported recent sexual behavior. Among severely deficient women, only 11% reported recent new sexual partners, while 25% with serum concentrations of total vitamin D ≥20ng/mL reported at least one new partner.

We observed this same influence of the far tails of the 25(OH)D distribution on prevalence estimates among women with the highest serum concentrations of total vitamin D. Among women whose 25(OH)D ≥30ng/mL, two distinct subgroups exist; those whose serum concentrations suggest supplementation (≥50ng/mL) had the highest odds of prevalent hrHPV in the study population, and this association was statistically significant. Unfortunately, the absence of data on supplement use in our study precludes definitive statements about supplementation. However, it is possible women who (likely) supplement were previously diagnosed as deficient, and an association between hrHPV status and 25(OH)D level *prior* to supplementation might better reflect a relationship between vitamin D status and detection of hrHPV. Additional data on supplementation in future studies would be instrumental in determining a potential association between artificially-elevated vitamin D and hrHPV infection.

In addition to the difference in the distribution of subjects' 25(OH)D serum concentrations, differences in age distribution may also explain the difference in our primary findings compared to the population-based NHANES sample. While Shim et al[20] studied the association between serum concentrations of vitamin D and hrHPV using a sample from NHANES of women ages 20-59 (over 20% of the sample was <30 years of age), we restricted our analyses to a narrower

age range of mid-adult women aged 30-50 years. In the NHANES study, younger women were the most likely, relative to all other age groups, to be severely vitamin D deficient (13% of subjects ages 20-24 years had total vitamin D <12ng/mL, compared to 10% of the overall sample) and to have prevalent hrHPV detected (35% of subjects ages 20-24 years had high-risk genotypes detected, compared to 22% of the overall sample). It is possible these younger women with low mean serum concentrations of total vitamin D and relatively high prevalence of hrHPV drive the association between 25(OH)D deficiency and detection of hrHPV observed in Shim's study population. However, because results of the NHANES study are not stratified by age, it is difficult to discern whether the association between serum 25(OH)D and detection of hrHPV varies between age groups.

Evaluation of race as a potential effect modifier in the association between continuous vitamin D and hrHPV infection yielded inconclusive results. Our study population was predominantly White, and additional research on more racially diverse populations would enable sufficiently-powered analyses of potential effect modification by race. In a study with a larger proportion of non-White women, we could further explore whether there are meaningful differences in the associations between vitamin D and hrHPV by race group, as previous literature on prostate cancer,[42] chronic kidney disease,[22] and colon cancer[6] has demonstrated.

Our study had several strengths, including secondary analyses with four novel vitamin D biomarkers. Further research into these biomarkers, particularly in longitudinal analyses, would be useful in better describing potential associations in less healthy populations. Additionally, our use of multiple stages of regression models allowed for transparent inclusion of confounders and

precision variables and enables comparison across studies. Finally, our findings speak to the merits of including measures of recent sexual behavior in models evaluating the relationship between vitamin D and hrHPV, as this information contributes to a better understanding of exposure.

Our study is not without limitations. Previous literature[43] suggests low serum concentrations of 25(OH)D may be a consequence of chronic infection (for example, with hrHPV), rather than a contributing factor in the development or persistence of disease. Our cross-sectional study design precludes causal inference and prevents understanding of whether serum vitamin D influences hrHPV acquisition or if concentrations of the micronutrient are a marker of inability to clear an existing infection. Data was collected for this study as part of a parent study[21] in which information on vitamin D supplementation was not collected. Future longitudinal studies would benefit from capturing information on supplementation at baseline and could evaluate whether supplementation affects acquisition or clearance of hrHPV. Finally, while NHANES data from 2005-2006 estimated 42% of US women were vitamin D deficient at <20ng/mL of serum 25(OH)D, our data revealed a healthy cohort in which only 14% of women had serum concentrations this low. Over half our study population was vitamin D sufficient at ≥30ng/mL and another third were sufficient at the cut-point of ≥20ng/mL, reducing power to detect associations between vitamin D insufficiency and hrHPV. Future studies would benefit from larger sample sizes in which detection of an association would be possible at smaller effect sizes.

In conclusion, evaluation of the association between vitamin D, as measured by five biomarkers measured along the micronutrient's metabolic pathway, and detection of prevalent hrHPV

yielded inconclusive results in a population of healthy mid-adult women in Seattle, WA. Findings from longitudinal studies in populations with both more variability in vitamin D status and data on supplementation may be useful in motivating clinical recommendations for subpopulations of women.

Figure 1. Assessment of a potential non-linear effect of continuous 25(OH)D.

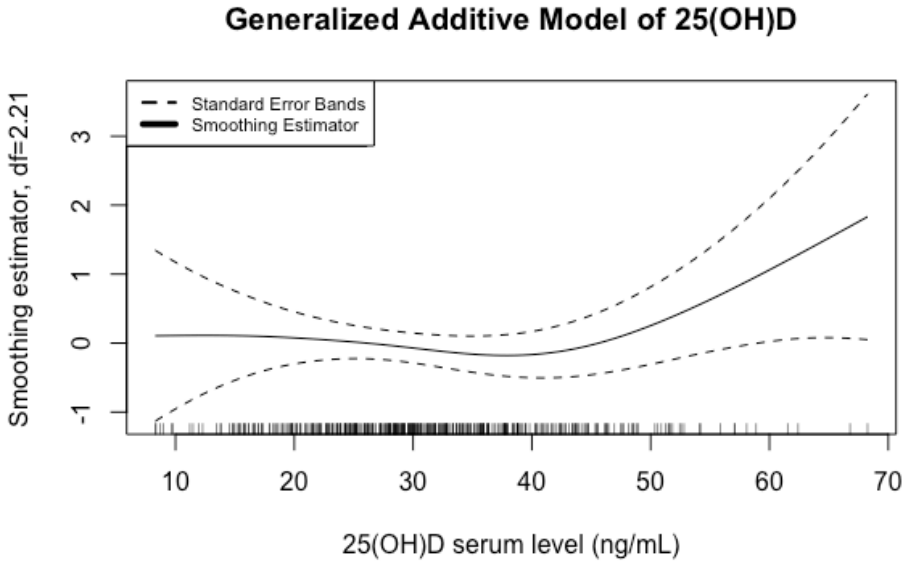

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Table 1. Characteristics of Study Population of Mid-Adult Women in Seattle, WA, 2011-2012 (N=404)

Demographic, health, and behavioral characteristics	*Mean (Standard Deviation)*
Age	38.3 (6.1)
	Median (Inner Quartile Range)
Lifetime number of male sex partners (n=404)[a]	7 (3-15)
	n (%)
Race	
White	319 (79.0)
African American	11 (2.7)
Asian	46 (11.3)
Other or Multiple Races[b]	28 (6.9)
Highest level of education	
Less than college	68 (16.8)
Bachelor's degree	150 (37.1)
Master's or Doctoral degree	186 (46.0)
Smoking status	
Never smoked	298 (73.9)
Former smoker	86 (21.3)
Current smoker	19 (4.7)
Missing	1
Hormonal Contraceptive Use	
Current use	137 (33.9)
Body Mass Index (BMI)	
Underweight (<18.5)	6 (1.5)
Normal (18.5-24.9)	217 (53.8)
Overweight (25-29.9)	112 (27.8)
Obese (30+)	68 (16.9)
Missing	1
Male sex partner(s) within 6 months prior to enrollment	
None	86 (21.7)
Non-new partners only	218 (54.9)
1 new partner	76 (19.1)
2+ new partners	17 (4.3)
Missing	7
High-risk HPV prevalence	89 (22.0)
>1 type detected	37 (46.1)[b]

[a]Four women excluded due to missing sexual history data

[b]Denominator is number of women with any high-risk HPV detected

[c]Includes women who self-reported as American Indian or Alaska Native, Native Hawaiian or Other Pacific Islander, other races, or multiple races

Table 2. Vitamin D biomarkers: descriptive statistics among the study population (N=404) and summary of biologic mechanisms.

Vitamin D Measure	Units	Mean	Standard Deviation	Median	Inner Quartile Range	Categorical definitions of sufficiency *n (%)*	Summary of Biomarker Mechanism in the Metabolic Pathway of Overall Vitamin D Synthesis
25(OH)D	ng/mL	31.32	10.52	30.18	24.32, 37.67	Institute of Medicine[30] <12ng/mL: 9 (2.2) ≥12ng/mL and <20ng/mL: 46 (11.3) ≥20ng/mL: 353 (86.5) Endocrine Society[31] <12ng/mL: 9 (2.2) ≥12ng/mL and <30ng/mL: 188 (46.1) ≥30ng/mL: 211 (51.7)	• Primary circulating form of vitamin D, the sum of D2 and D3 generated in the liver through metabolism[14] • Most commonly measured to assess deficiency[17] • Inversely associated with parathyroid hormone (PTH) levels[9] • Cut-points to establish sufficiency remain contested[32]
1,25(OH)D	pg/mL	53.03	18.32	51.45	39.48, 64.33	N/A	• Active form of vitamin D after metabolism of 25(OH)D in the kidney (a process regulated tightly by PTH, calcium, and phosphorous levels) • Higher levels improve efficiency of absorption of calcium and phosphorous • Induces expression of enzyme CYP24A1[15] • Charged with keeping cellular proliferation and differentiation in check[39] • Concentration varies by race/ethnicity[15]
24,25(OH)D	ng/mL	2.11	1.16	1.93	1.34, 2.72	N/A	• Biomarker of 25(OH)D and 1,25(OH)D catabolism • Levels may indicate local 1,25(OH)D activity[15]
Free Vitamin D	nmol/L x (1000)	19.13	6.23	18.77	14.91, 22.31	N/A	• Circulating vitamin D unbound to DBP or albumin • Calculated using equations that incorporate 25(OH)D, DBP, albumin, and genotypic differences in DBP (though the validity of these equations has been questioned)[18] • May be most relevant in populations showing variation in DBP levels (i.e. women using oral contraceptives)[23] • Accurate measurement difficult due to low concentration (<1%) of circulating vitamin D[29]
Vitamin D Binding Protein (DBP)	ug/ml	260.80	49.32	250.60	228.50, 279.20	N/A	• Binding protein that transports most (85-90%) of circulating vitamin D in blood[29] • Higher levels observed in pregnant women or women taking oral contraceptives[15]

Table 3. Associations between increasing levels of serum vitamin D biomarkers and high-risk HPV positivity at study enrollment among mid-adult women in Seattle, WA (2011-2012) (N=404)

	Minimally-adjusted[a]				Moderately-adjusted[b]				Fully-adjusted[c]			
Vitamin D Measure	*n*	*OR*	*95% CI*	*p-value*	*n*	*OR*	*95% CI*	*p-value*	*n*	*OR*	*95% CI*	*p-value*
25(OH)D, continuous serum level												
Per 10ng/mL increase	404	1.15	0.88, 1.50	0.30	402	1.10	0.84, 1.45	0.50	393	1.10	0.83, 1.47	0.50
25(OH)D, categorical (IOM)[30]												
<20ng/mL	55	1.00	--	--	54	1.00	--	--	53	1.00	--	--
≥20ng/mL	349	0.96	0.44, 2.10	0.92	348	0.85	0.37, 1.93	0.69	340	0.89	0.40, 2.03	0.79
25(OH)D, categorical (ES)[31]												
<30ng/mL	197	1.00	--	--	196	1.00	--	--	193	1.00	--	--
≥30ng/mL	207	0.88	0.53, 1.45	0.61	206	0.85	0.50, 1.44	0.54	200	0.78	0.44, 1.40	0.41
25(OH)D, categorical (post hoc analysis)												
<50ng/mL	382	1.00	--	--	380	1.00	--	--	371	1.00	--	--
≥50ng/mL	22	3.10	1.23, 7.82	0.01	22	2.82	1.12, 7.11	0.03	22	2.47	0.92, 6.64	0.07
1,25(OH)D		1.01	0.99, 1.02	0.47		1.01	0.99, 1.02	0.52		1.01	0.99, 1.02	0.58
24,25(OH)D		1.31	1.05, 1.63	0.02		1.23	0.97, 1.55	0.09		1.18	0.92, 1.52	0.19
free	404	1.02	0.97, 1.06	0.51	402	1.02	0.97, 1.07	0.37	393	1.03	0.98, 1.08	0.29
25(OH)D adjusted for Vitamin D Binding Protein (DBP), stratified by DBP haplotype[d]		1.01	0.98, 1.04	0.42		1.01	0.98, 1.05	0.36		1.02	0.99, 1.05	0.25

[a]Adjusted for race, age, and season of enrollment

[b]Adjusted for race, age, season of enrollment, Body Mass Index, highest education level, oral contraceptive use, smoking status, calcium (ng/mL) and phosphate (mg/dL) levels

[c]Adjusted for race, age, season of enrollment, Body Mass Index, highest education level, oral contraceptive use, smoking status, calcium (ng/mL) and phosphate (mg/dL) levels, number of lifetime sexual partners, and number of sexual partners in last 6 months

[d]Primary predictor was 25(OH)D, with additional adjustment for DBP levels and stratification by DBP haplotype

Table 4. Prevalence of high-risk HPV (hrHPV) by 25(OH)D levels used in a post hoc analysis of mid-adult women (N=404)

	Subgroup-specific hrHPV prevalence	
25(OH)D serum level, post hoc categorical variable	*Prevalence (%)*	*Exact 95% CI*
<12ng/mL	0.0	0.0, 33.6
12-19ng/mL	23.9	12.6, 38.8
20-29ng/mL	23.9	17.2, 31.8
30-49ng/mL	18.4	13.1, 24.7
≥50ng/mL	45.5	24.4, 67.8

Table 5. Association between increasing total vitamin D serum levels and high-risk HPV infection in mid-adult women in Seattle, WA, stratified by race (N=404).

	Minimally-adjusted[a] n=404				**Moderately-adjusted[b]** n=402				**Fully-adjusted[c]** n=393			
Self-identified race	*n*	*OR*	*95% CI*	*p-value*[d]	*n*	*OR*	*95% CI*	*p-value*[e]	*n*	*OR*	*95% CI*	*p-value*[f]
White												
Per 10ng/mL increase in serum 25(OH)D	319	1.14	0.85, 1.53	0.38	318	1.08	0.79, 1.49	0.62	312	1.08	0.77, 1.51	0.67
Non-White[g]												
Per 10ng/mL increase in serum 25(OH)D	85	1.13	0.67, 1.91	0.64	84	1.25	0.63, 2.48	0.53	81	1.56	0.51, 4.77	0.44

[a]Adjusted for age, and season of enrollment

[b]Adjusted for age, season of enrollment, Body Mass Index, highest education level, oral contraceptive use, smoking status, calcium (ng/mL), and phosphate (mg/dL) levels

[c]Adjusted for age, season of enrollment, Body Mass Index, highest education level, oral contraceptive use, smoking status, calcium (ng/mL) and phosphate (mg/dL) levels, number of lifetime sexual partners, and number of sexual partners in last 6 months

[d]p-value = 0.751 for interaction term comparing white and non-white race groups under minimal adjustment

[e]p-value = 0.792 for interaction term comparing white and non-white race groups under moderate adjustment

[f]p-value = 0.955 for interaction term comparing white and non-white race groups under full adjustment

[g]self-reported as Black or African American, Asian, American Indian or Alaska Native, Native Hawaiian or Other Pacific Islander, other, or multiple races