The Journal of Infectious Diseases
MAJOR ARTICLE

Association Between Serum 25-Hydroxyvitamin D Level and Human Papillomavirus Cervicovaginal Infection in Women in the United States

Jinhee Shim,[1] Adriana Pérez,[2] Elaine Symanski,[3] and Alan G. Nyitray[3]

[1]Department of Business Intelligence and Analytics, Texas Children's Health Plan, Houston, [2]Department of Biostatistics, University of Texas School of Public Health, Austin, and [3]Department of Epidemiology, Human Genetics, and Environmental Sciences, University of Texas School of Public Health, Houston

Background. A sufficient level of vitamin D enhances protection against several infectious diseases; however, its association with cervicovaginal human papillomavirus (HPV) infection has not been studied.

Methods. Data for this cross-sectional study were from National Health and Nutrition Examination Survey 2003–2006. A total of 2353 sexually active women for whom cervicovaginal HPV infection status and serum 25-hydroxyvitamin D (25[OH]D) level were known were studied. Associations between serum 25(OH)D levels (continuous and categorical forms) and cervicovaginal HPV infection (due to high-risk HPV or vaccine-type HPV) were estimated using weighted logistic regression.

Results. After adjustment for age, race/ethnicity, and marital status, the odds of high-risk HPV infection were increased per each 10 ng/mL decrease in serum 25(OH)D level (adjusted odds ratio [aOR], 1.14; 95% confidence interval [CI], 1.02–1.27). Similarly, the odds of vaccine-type HPV infection were increased in women with vitamin D levels that were severely deficient (serum 25[OH]D level, <12 ng/mL; aOR, 2.90; 95% CI, 1.32–6.38), deficient (12–19 ng/mL; aOR, 2.19; 95% CI, 1.08–4.45), and insufficient (20–29 ng/mL; aOR, 2.19; 95% CI, 1.22–3.93), compared with those with vitamin D levels that were sufficient (≥30 ng/mL).

Conclusions. Cervicovaginal HPV prevalence is associated with less-than-optimal levels of serum vitamin D.

Keywords. human papillomavirus; HPV; vitamin D; 25-hydroxyvitamin D; cervix; NHANES.

Human papillomavirus (HPV) is the most common sexually transmitted virus in the United States. According to a nationwide survey, 39.9 million US women (42.5%) aged 14–59 years were estimated to have at least 1 type of HPV in 2003–2006 [1]. While a majority of HPV infections are spontaneously cleared by natural immune responses, a portion of HPV infections become persistent, which can lead to disease [2]. The risk of acquiring and clearing HPV infection varies from one person to another, and the underlying reasons for this variability are poorly understood [3, 4]. Approximately 40 HPV types, classified as high-risk HPV (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) or low-risk HPV (HPV 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 73, 81, 82, 83, 84, 89, and IS39) according to their carcinogenic potential at the cervix, can infect the cervicovaginal region [5].

About 11 000 incident cases of cervical cancer and 800 incident cases of vaginal cancer occur annually in the United States [6]. Almost all cervical malignancies (99.7%) and approximately 40% of vaginal cancers are caused by HPV [7, 8]. Although cervical cancer incidence in the United States has declined with regular screening, cervical cancer is still one of the 10 most common cancers among black and Hispanic women in the United States [1]. Also, cervical cancer is the leading cancer among women in resource-poor countries, where HPV-associated Papanicolaou cytology and colposcopy are prohibitively expensive [9].

There are vaccines to prevent HPV infection and related morbidity. A prophylactic vaccine that covers HPV 6, 11, 16, and 18 has been approved for use in US women since late 2006 [10]. These 4 HPV genotypes are responsible for the majority of HPV-associated disease. HPV types 16 and 18 are responsible for 70% of cervical cancers, and HPV types 6 and 11 are responsible for 90% of genital warts [11, 12]. A new vaccine was approved for protection against 9 HPV types in December 2014 [13].

Vitamin D is well known for its essential role in bone homeostasis, with a major source of this micronutrient being endogenous synthesis after sun exposure [14]. Generally, serum levels of 25-hydroxyvitamin D (25[OH]D) are used as a standard indicator of vitamin D [15]. It reflects levels of active vitamin D (1,25-dihydroxyvitamin D), which controls gene expression [16]. Levels of serum 25(OH)D vary by age, race/ethnicity, diet, duration, and quantity of sun exposure [17–19]. Generally, serum 25(OH)D levels are lower in higher latitudes and in winter, owing to less sun exposure [17]. Also, older people and

Received 3 November 2015; accepted 5 February 2016; published online 15 February 2016.
Presented in part: 30th International Papillomavirus Conference and Clinical and Public Health Workshops, Lisbon, Portugal, 18 September 2015. Abstract HPV15-0021.
Correspondence: A. G. Nyitray, 1200 Pressler, RAS-E707, Houston, TX 77030 (alan.g.nyitray@uth.tmc.edu).

The Journal of Infectious Diseases® 2016;213:1886–92

DOI: 10.1093/infdis/jiw065

people with darker skin tend to have lower serum 25(OH)D levels [18, 19].

Over the last decade, the role of vitamin D in supporting the immune system has drawn significant attention [20]. Sufficient levels of vitamin D offer protection against certain infectious diseases, while suboptimal levels of vitamin D correlate with an increased risk and severity of disease. For example, upper and lower respiratory tract infections (eg, influenza and respiratory syncytial virus infection) are inversely correlated with serum 25(OH)D levels and peak during the winter, when vitamin deficiency is more prevalent [21, 22]. Also, a high level of serum 25(OH)D was found to be associated with a 2-fold reduction in acute respiratory tract infections, as well as reduced infection duration [23]. In addition, serum 25(OH)D levels were found to be inversely associated with the risk of active tuberculosis, as well as positively associated with faster recovery from tuberculosis [24]. These phenomena occur because vitamin D strengthens innate and adaptive immunity against infection [20].

Considering the relationship between serum 25(OH)D levels and other infections, we postulated that serum 25(OH)D might play a protective role in cervicovaginal HPV infection. The objective of the current analyses was to explore the association between serum 25(OH)D levels and the prevalence of cervicovaginal HPV infection. This study focused on oncogenic HPV types and types in the quadrivalent HPV vaccine, which are responsible for a majority of HPV-associated diseases [11, 12].

METHODS AND MATERIALS

Study Design and Study Population

We used National Health and Nutrition Examination Survey (NHANES) 2003–2006 data to investigate the association between serum 25(OH)D levels and the prevalence of HPV infection. NHANES is a nationwide survey conducted to evaluate the health conditions of people in the United States, and it uses a complex, multistage, probability sampling design to obtain a nationally representative sample. This survey is released in 2-year cycles by the National Center for Health Statistics. NHANES has been approved by the National Health Statistics Institutional Review Board.

We selected women who provided a self-collected cervicovaginal swab specimen for an HPV DNA assay, had available data on serum 25(OH)D level, and reported a history of vaginal, anal, or oral sex in a sexual behavior interview. Women aged 14–59 years provided cervicovaginal swab specimens and sexual behavior information. The age range of the study population was limited to 20–59 years because sexual behavior information for women age <20 years was not publicly available. Women who provided an inadequate cervicovaginal swab specimen (n = 41), or reported 0 sex partners during their lifetime (n = 7) were excluded; thus, among 2401 women initially included, our final analysis sample comprised 2353 women. There were demographic and behavioral differences between women who submitted an adequate cervicovaginal swab specimen and women who refused to or did not submit an adequate cervicovaginal swab specimen [25].

Cervicovaginal HPV Infection

NHANES performed Roche Linear Array HPV genotyping tests for self-collected vaginal swab specimens and reported the results of HPV DNA detection tests for 37 types of HPV. We determined the high-risk HPV infection status and quadrivalent vaccine-type HPV infection status of each woman. A specimen was labeled as high-risk HPV infection if it had at least 1 of 13 oncogenic HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, or 68), regardless of the presence of any other HPV genotype [5]. A specimen was labeled as vaccine-type HPV infection if it had at least 1 of 4 HPV types (6, 11, 16, or 18), regardless of the presence of any other HPV genotype [10]. Given increased potential for disease, the current analysis focuses on 13 high-risk types and HPV-6 and HPV-11.

Serum 25(OH)D Levels

The serum 25(OH)D level was used as the indicator of an individual's vitamin D level [15]. Serum for vitamin D analysis was collected in the winter (November–April) in the southern United States and in the summer (May–October) in the northern United States [26]. NHANES used the DiaSorin radioimmunoassay kit (DiaSorin, Stillwater, Minnesota) to measure serum 25(OH)D levels in women's blood samples. The serum 25(OH)D level was modeled and analyzed in continuous and categorical forms. A variable was created by dividing the serum 25(OH)D values in the original NHANES data set by 10, to evaluate the effect of a continuous 10-ng/mL decrease in serum 25(OH)D level on the prevalence of cervicovaginal HPV infection. In addition, we categorized serum 25(OH)D levels according to prior clinical conventions, as follows: <12 ng/mL, indicative of severe vitamin D deficiency; 12–19 ng/mL, indicative of vitamin D deficiency; 20–29 ng/mL, indicative of vitamin D insufficiency; and ≥30 ng/mL, indicative of vitamin D sufficiency [27, 28].

Other Variables

Using prior literature, directed acyclic graphs [29–31], and a 10% change in estimate method, we assessed variables, specified as follows, that might potentially confound the association between serum 25(OH)D levels and cervicovaginal HPV infection: age (20–59 years), race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, or other), marital status (married, widowed/divorced/separated, living with partner, or never married), poverty level (below the poverty level or at or above the poverty level), smoking status (former, current, or never), season of examination (November–April or May–October), lifetime number of sex partners (range, 1–2003), and number of sex partners in the past 12 months (range, 0–43).

Statistical Analysis

All analyses were conducted using SAS Survey Procedure (SAS Institute, version 9.3; Cary, North Carolina). Responses coded as

"don't know," "refused," or "missing" in the original NHANES data were treated as missing. We used the Medical Examination Center (MEC) examination sampling weights provided by the National Center for Health Statistics to take into account the unequal probability of selection and nonresponse. All estimates shown were weighted by these sampling weights except when reporting the sample size by demographic characteristics.

Descriptive statistics (sample sizes and weighted proportions) were computed along with mean serum 25(OH)D levels, the weighted prevalence of categorical serum 25(OH)D levels, and the weighted prevalence of high-risk HPV and vaccine-type HPV infection for the following characteristics of the study population: age, race/ethnicity, marital status, poverty level, smoking status, season of the examination, lifetime number of sex partners, and number of sex partners in the past 12 months. Additionally, the weighted means and standard deviations of age were calculated. The Rao-Scott χ^2 test was used to assess whether categorical serum 25(OH)D levels or the prevalence of HPV infection were different between categories of each characteristic. A type I error level of 0.05 was used. In addition, the prevalence of high-risk HPV and vaccine-type HPV was plotted by 10-ng/mL levels of serum 25(OH)D.

We estimated crude odds ratios (ORs) and 95% confidence intervals (CI) between serum 25(OH)D levels and HPV infection, using weighted logistic regression. Taylor series linearization was used for robust variance estimation [32]. Model 1 described the association between high-risk HPV infection and a 10-ng/mL decrease in serum 25(OH)D level, while model 2 described the association between vaccine-type HPV infection and a 10-ng/mL decrease in serum 25(OH)D level. Model 3 described the association between high-risk HPV infection and categorical serum 25(OH)D levels, and model 4 described the association between vaccine-type HPV infection and categorical serum 25(OH)D levels.

We developed a multivariable model, using a change-in-estimate method. This variable selection procedure was conducted for all 4 models. Variables substantially affecting any model were considered as confounders in each model. Adjusted ORs (aORs) and their 95% CIs were reported. According to the results of confounding analyses, the 4 models were adjusted for age, race/ethnicity, and marital status. Other variables, including number of sex partners, did not substantially affect ORs. We did not consider statistical interactions in this investigation.

RESULTS

Summary statistics of the study population are described in Table 1. More than half of the sexually active women were ≥40 years old. The majority of women (72.0%) were non-Hispanic white. Most women (58.9%) were married, and 12.6% were living below the poverty line. More than half (55.7%) never smoked, but 25.2% were current smokers. Over 70% of women had ≥3 sex partners in their lifetime.

Serum 25(OH)D levels ranged from 3 to 76 ng/mL (data not shown). Approximately 25.3% of women had vitamin D deficiency (serum 25[OH]D level, 12–19 ng/mL), while 10.2% of women had severe vitamin D deficiency (serum 25[OH]D level, <12 ng/mL; Table 1). About 39.7% of women had an insufficient vitamin D level (serum 25[OH]D level, 20–29 ng/mL). Categorical serum 25(OH)D levels were different by age, race/ethnicity, marital status, poverty level, smoking status, and season at examination. The prevalence of high-risk HPV infection and vaccine-type HPV infection in the total study population was 21.7% and 8.2%, respectively. For both high-risk and vaccine-type HPV infection, prevalence was different by age, race/ethnicity, marital status, poverty level, smoking status, number of sex partners in lifetime, and number of sex partners in the past 12 months. Figure 1 shows the prevalence of high-risk HPV and vaccine-type HPV infection by serum 25(OH)D level. In each group, prevalence declined as serum 25(OH)D level increased.

In unadjusted analyses using weighted logistic regression, both continuous and categorical serum 25(OH)D levels were inversely associated with cervicovaginal HPV infection (Table 2). An OR of >1 means the exposure is associated with a higher odds of the outcome, and a 95% CI excluding 1 implies the presence of statistical significance. Each 10-ng/mL decrease in serum 25(OH)D level was significantly associated with an increased odds of high-risk HPV infection (OR, 1.15; 95% CI, 1.03–1.28). When categorical serum 25(OH)D levels were used as the exposure, the odds of HPV infection continued to be inversely associated with serum 25(OH)D levels. For example, compared with women with vitamin D sufficiency, women with severe vitamin D deficiency had an increased odds of high-risk HPV infection (OR, 1.67; 95% CI, 1.15–2.41) and vaccine-type HPV infection (OR, 2.37; 95% CI, 1.22–4.60).

After adjustment for confounders, each 10-ng/mL decrease in serum 25(OH)D level was associated with an increased odds of high-risk HPV infection (aOR, 1.14; 95% CI, 1.02–1.27; Table 3). All categories of serum 25(OH)D levels below sufficiency (<30 ng/mL; ie, severe deficiency, deficiency, or insufficiency) were associated with an increased prevalence of vaccine-type HPV infection. The odds of vaccine-type HPV infection were more than double in women with severe vitamin D deficiency (aOR, 2.90; 95% CI, 1.32–6.38), vitamin D deficiency (aOR, 2.19; 95% CI, 1.08–4.45), and vitamin D insufficiency (aOR, 2.19; 95% CI, 1.22–3.93), compared with women with sufficient levels of vitamin D.

DISCUSSION

We analyzed HPV prevalence prior to the introduction of HPV vaccination among a subpopulation of sexually active US women. To the best of our knowledge, this is the first study to explore an association between serum 25(OH)D level and cervicovaginal HPV infection. As we hypothesized, a lower level of

Table 1. Summary Statistics of the Study Population, by Overall Serum 25-Hydroxyvitamin D (25[OH]D) Level, Categorical Serum 25(OH)D Level, and Human Papillomavirus (HPV) Prevalence

Characteristic	Subjects, No. (%)	Overall 25(OH)D Level, ng/mL, Mean	Categorical 25(OH)D Level, Subjects, %[a]				HPV Prevalence, Subjects, %[b]	
			<12 ng/mL	12–19 ng/mL	20–29 ng/mL	≥30 ng/mL	High-Risk Genotypes	Vaccine Genotypes
Overall	2353 (100.0)	23.8	10.2	25.3	39.7	24.8	21.7	8.2
Age, y								
Mean ± SD	39.9 ± 11.0	. . .	. . .	. . .	. . .	. . .	. . .	. . .
20–24	344 (10.9)	24.7	13.4	24.5	32.2	29.9	35.2	18.3
25–29	332 (10.5)	25.7	5.1	24.3	39.8	30.7	27.0	12.1
30–39	601 (25.3)	24.6	10.4	24.1	36.9	28.6	23.5	8.8
40–49	602 (29.1)	22.6	12.0	25.0	44.0	19.0	17.8	5.4
50–59	474 (24.2)	23.3	8.4	27.9	40.9	22.9	16.2	4.6
Race/ethnicity								
Non-Hispanic white	1184 (72.0)	26.3	4.8	19.4	44.4	31.4	20.7	8.5
Non-Hispanic black	530 (11.9)	14.4	39.5	41.5	16.6	2.4	29.8	11.1
Mexican American	466 (7.7)	19.2	16.7	39.3	34.3	9.7	22.9	6.3
Other[c]	173 (8.5)	20.6	8.9	40.2	36.7	14.2	18.2	3.4
Marital status								
Married	1290 (58.9)	24.7	6.8	23.9	42.6	26.6	14.6	4.9
Widowed/divorced/ separated	407 (17.3)	22.1	13.4	28.2	39.2	19.2	27.3	6.9
Living with partner	213 (7.9)	24.6	10.3	26.5	36.8	26.4	34.1	19.9
Never married	442 (16.0)	22.0	18.9	27.1	31.0	23.0	35.4	15.9
Missing[d]	1 (0.0)	. . .	. . .	. . .	. . .	. . .	ND	ND
Poverty income ratio[e]								
<1	443 (12.6)	21.1	15.4	32.3	35.2	17.1	32.9	15.0
≥1	1826 (84.0)	24.3	9.3	23.8	41.0	25.8	19.8	7.4
Missing[d]	84 (3.4)	. . .	. . .	. . .	. . .	. . .	ND	ND
Smoking status								
Former smoker	423 (19.0)	24.3	8.0	22.5	44.1	25.5	16.6	8.5
Current smoker	532 (25.2)	23.7	13.2	27.4	32.2	27.2	31.3	12.6
Never smoker	1396 (55.7)	23.7	9.5	25.3	41.7	23.5	19.1	6.1
Missing[d]	2 (0.0)	. . .	. . .	. . .	. . .	. . .	ND	ND
Season at examination								
November–April	1081 (41.1)	21.4	14.7	32.6	34.1	18.5	23.8	8.5
May–October	1272 (58.9)	25.5	7.0	20.2	43.6	29.2	20.3	8.0
Sex partners, no.								
In lifetime								
1	430 (17.5)	23.9	6.7	29.5	41.2	22.6	7.0	2.8
2	242 (9.9)	23.2	12.1	23.5	44.3	20.0	16.8	4.6
3–5	661 (27.3)	23.2	11.4	27.0	38.3	23.4	23.0	7.9
≥6	992 (44.3)	24.3	10.4	22.8	39.1	27.7	28.2	11.5
Missing[d]	28 (1.0)	. . .	. . .	. . .	. . .	. . .	ND	ND
In past 12 mo, no.								
0	279 (12.8)	21.6	13.3	29.1	42.2	15.5	14.3	4.1
1	1720 (73.4)	24.3	9.4	24.7	39.2	26.7	19.3	7.2
2	180 (7.3)	23.3	11.5	25.5	39.6	23.5	39.1	14.5
≥3	156 (6.0)	23.9	11.3	22.3	41.0	25.3	47.4	22.0
Missing[d]	18 (0.5)	. . .	. . .	. . .	. . .	. . .	ND	ND

All percentages are weighted.

[a] Severe vitamin D deficiency was defined as a serum 25[OH]D level of <12 ng/mL; deficiency, as a level of 12–19 ng/mL; insufficiency, as a level of 20–29 ng/mL; and sufficiency, as a level of ≥30 ng/mL.

[b] The Rao-Scott χ^2 test was used to compare categorical serum 25(OH)D level and HPV prevalence between categories of each population characteristic. All *P* values were <.01, except categorical serum 25(OH)D levels and number of sex partners in lifetime (*P* = .09), categorical serum 25(OH)D levels and number of sex partners in past 12 months (*P* = .09), high-risk genotypes by season at examination (*P*=.11), vaccine genotype by race/ethnicity (*P* = .02), and vaccine genotype by season at examination (*P* = .67).

[c] "Other Hispanic" and "other race" in the National Health and Nutrition Examination Survey 2003–2006 data set were collapsed into "other" in the current study.

[d] HPV prevalence was not determined (ND) for individuals with missing data on serum 25(OH)D level.

[e] Calculated as the ratio of family income to the poverty threshold defined by the Department of Health and Human Services.

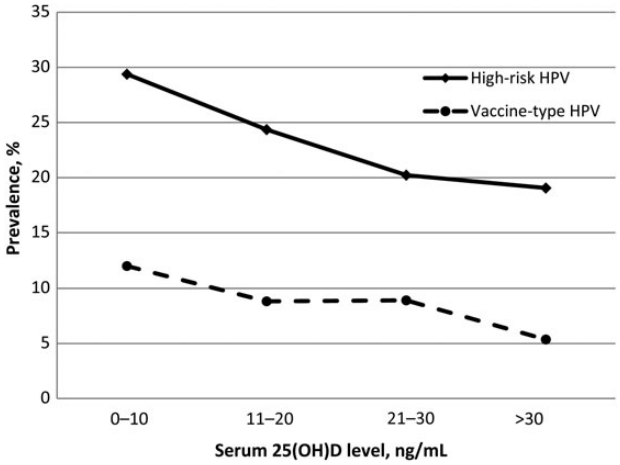

Figure 1. High-risk human papillomavirus (HPV) and vaccine-type HPV prevalence, by serum 25-hydroxyvitamin D (25[OH]D) level.

Table 3. Adjusted Analysis of the Association Between Serum 25-Hydroxyvitamin D (25[OH]D) Level and Cervicovaginal Human Papillomavirus (HPV) Infection Among 2353 Participants in the National Health and Nutrition Examination Survey 2003–2006

	aOR (95% CI), by HPV Genotype	
Variable	High Risk	Vaccine Based
Continuous serum 25(OH)D level	Model 1	Model 2
Per 10 ng/mL decrease	1.14 (1.02–1.27)	1.25 (.97–1.60)
Categorical serum 25(OH)D level[a]	Model 3	Model 4
<12 ng/mL	1.46 (.96–2.23)	2.90 (1.32–6.38)
12–19 ng/mL	1.41 (1.00–1.99)	2.19 (1.08–4.45)
20–29 ng/mL	1.19 (.87–1.62)	2.19 (1.22–3.93)
≥30 ng/mL	1.0	1.0

The models were adjusted for age, race/ethnicity, and marital status.

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval.

[a] Severe vitamin D deficiency was defined as a serum 25[OH]D level of <12 ng/mL; deficiency, as a level of 12–19 ng/mL; insufficiency, as a level of 20–29 ng/mL; and sufficiency, as a level of ≥30 ng/mL.

serum 25(OH)D was generally associated with an increased odds of high-risk HPV and vaccine-type HPV infection, after adjustment for confounders.

Although the association between serum 25(OH)D level and HPV infection has not been studied until now, other infections of the cervicovaginal region have been reported as being correlated with vitamin D levels in both humans and animals. Hensel et al reported that low serum 25(OH)D levels (<20 ng/mL) were associated with bacterial vaginosis, although this result was limited to pregnant women [33]. He et al observed that vitamin D receptor knockout mice were more likely to be infected with *Chlamydia trachomatis* and cleared the infection more slowly [34]. Also, they reported that replication of *Chlamydia* was decreased in epithelial cells that were pretreated with $1,25(OH)_2D$, compared with nonpretreated epithelial cells, supporting a role of vitamin D in preventing *Chlamydia* infection.

Table 2. Unadjusted Analysis of the Association Between Serum 25-Hydroxyvitamin D (25[OH]D) Level and Cervicovaginal Human Papillomavirus (HPV) Infection Among 2353 Participants in the National Health and Nutrition Examination Survey 2003–2006

	Crude OR (95% CI), by HPV Genotype	
Variable	High Risk	Vaccine Based
Continuous serum 25(OH)D level	Model 1	Model 2
Per 10 ng/mL decrease	1.15 (1.03–1.28)	1.14 (.93–1.41)
Categorical serum 25(OH)D level[a]	Model 3	Model 4
<12 ng/mL	1.67 (1.15–2.41)	2.37 (1.22–4.60)
12–19 ng/mL	1.35 (.97–1.87)	1.62 (.83–3.19)
20–29 ng/mL	1.08 (.82–1.42)	1.73 (.98–3.06)
≥30 ng/mL	1.0	1.0

Abbreviations: CI, confidence interval; OR, odds ratio.

[a] Severe vitamin D deficiency was defined as a serum 25[OH]D level of <12 ng/mL; deficiency, as a level of 12–19 ng/mL; insufficiency, as a level of 20–29 ng/mL; and sufficiency, as a level of ≥30 ng/mL.

The role of serum 25(OH)D on innate immunity could explain the inverse associations observed in this study. Serum 25(OH)D is involved in the regulation of expression of antimicrobial peptides (AMPs), which nonspecifically protect a host from invading pathogens [35]. Therefore, if serum 25(OH)D levels are suboptimal, AMP production would be restricted [36]. AMPs directly inactivate a pathogen and also attract other immune cells such as phagocytes, resulting in enhanced innate immune responses [14]. For many other infections that are protectively associated with serum 25(OH)D, this AMP regulation of serum 25(OH)D has been suggested as the mechanism behind the associations [37–39]. Impaired production of AMPs is linked to increased susceptibility to and severity of certain infections [40].

Endothelial cells at the vagina and cervix produce AMPs in response to a pathogen [41, 42]. AMPs may block the initial phase of HPV infection by eliminating the invading virus [43]. Buck et al reported that certain AMPs protect against HPV infection, even before the virus establishes infection in targeted cells [44]. Since HPV suppresses inflammatory responses and avoids systemic adaptive immune responses, this kind of nonspecific innate defense before the establishment of the infection may be critical for protection from HPV [45]. AMP levels can be measured but are not available data from NHANES.

In addition, vitamin D makes the physical barriers, including the skin, respiratory tract, and genitourinary tract, more resistant to bacteria and viruses by upregulating proteins that promote tight junctions, gap junctions, and adhere junctions [46]. Therefore, sufficient vitamin D may interfere with HPV penetration into the basal layer by making the mucosal barriers stronger [45, 47]. Conversely, insufficient levels of vitamin D may lead to a higher prevalence of HPV infection by increasing vulnerability to HPV penetration and reducing the host's ability to clear the virus.

As expected, the number of sex partners was strongly associated with HPV prevalence in these data; however, the number of partners was not associated with the level of vitamin D (Table 1). Therefore, the number of sex partners was not considered as a confounder. Similarly, seasonality was associated with categorical serum 25(OH)D levels, but it was not associated with HPV prevalence in our data. Therefore, season at examination was also not considered as a confounder. In addition, we tested whether the number of sex partners in the past 12 months was associated with the season of vitamin D measurement but found no association ($\chi^2 = 6.34$, $P = .10$).

While vitamin D levels are generally higher in lower latitudes, an inverse association between vitamin D level and HPV prevalence does not imply lower cervical cancer rates in lower latitudes, since other factors also affect progression to cervical cancer [8]. Higher cervical cancer rates in developing countries at lower latitudes are primarily a result of lack of cervical cytology and colposcopy screening. In addition, global HPV infection patterns are inconsistent, with high HPV infection prevalence found at both equatorial and temperate latitudes [48].

There are different opinions on optimal vitamin D levels for health and the classification of sufficient versus nonsufficient vitamin D levels; however, <12 ng/mL of serum 25(OH)D is generally considered to indicate a severe deficiency in vitamin D, since this level can cause rickets in children and osteomalacia in adults [27]. The National Institutes of Health defines a serum 25(OH)D level of ≥20 ng/mL as adequate; however, some experts believe that 20–29 ng/mL is still not sufficient, owing to interference with intestinal calcium absorption and, instead, regard ≥30 ng/mL as sufficient [27, 28].

In addition, there is no professional consensus on a cutoff of serum 25(OH)D that is associated with a lower risk of infections in general (ie, due to HPV or other pathogens), although 25(OH)D deficiency has been reported as increasing the risk of a number of infections [23, 24]. We found that categorical serum 25(OH)D levels indicative of vitamin D insufficiency were significantly associated with a higher odds of having vaccine-type HPV infection. Given our finding, levels of serum 25(OH)D indicative of vitamin D sufficiency should be investigated further as an appropriate clinical goal to prevent HPV infection. Unfortunately, a significant percentage of the US population has suboptimal serum 25(OH)D levels. For example, 1 of 3 persons aged ≥1 year in the United States had a serum 25(OH)D level of <20 ng/mL according to NHANES 2001–2006 data [49]. In our analysis, 35% of women had a serum 25(OH)D level of <20 ng/mL, and 75% had a serum level of <30 ng/mL.

There are a number of strengths of our study. First, the study population was large in this analysis and is representative of the US population. Therefore, the results are reliable and generalizable for noninstitutionalized, sexually active US women aged 20–59 years who are willing to provide their sexual history and serum and cervicovaginal specimens for a national survey. Second, we used a comprehensive approach evaluating serum 25(OH)D levels both as continuous and categorical variables. As a result, we not only detected trends in a continuous model but also found supporting evidence of the clinical importance of the categorical classification. Third, we analyzed HPV infection by 2 outcome types, which were detected by a highly sensitive polymerase chain reaction–based test. Fourth, all variables in the study were assessed as potential confounders, with age, race/ethnicity, and marital status the only variables associated with both exposure and outcome in the study which also modified ORs by more than 10%; thus, the association of vitamin D and HPV infection was independent of the effects of these 3 variables.

The study also has limitations. First, the cross-sectional design does not allow us to infer temporality. Second, the season-at-examination variable used in this analysis was binary, although season and sun exposure are critical to serum 25(OH)D levels. However, that binary variable was the only available temporal information, since NHANES does not provide the date of examination. Third, cervicovaginal samples were self-collected; therefore, there is the possibility that sampling was not accurately performed, resulting in potentially incorrect HPV DNA results; however, self-collected samples have been shown to be highly comparable to physician-collected samples [50]. Fourth, there was a potential for selection bias since women who provided an adequate cervicovaginal swab specimens were different (eg, age and race/ethnicity) from women who refused to or did not provide a swab specimen [25]. Nonresponders to sexual behavior questions may also have different characteristics than responders, resulting in biased estimates. Finally, current HPV DNA detection methods for large epidemiological studies are not able to distinguish whether HPV is from the participant or their partner or whether it indicates active infection [25].

In conclusion, results from this US nationally representative sample support the hypothesis that vitamin D level, accessed by measurement of serum 25(OH)D levels, is inversely associated with prevalence of cervicovaginal HPV infection in sexually active women. This adds to the rapidly growing literature about vitamin D's association with infection. Future studies are warranted to assess the association between vitamin D and HPV persistence and to clarify the underlying mechanism for the associations.

Note

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Hariri S, Unger ER, Sternberg M, et al. Prevalence of genital human papillomavirus among females in the United States, the National Health And Nutrition Examination Survey, 2003–2006. J Infect Dis **2011**; 204:566–73.
2. Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. N Engl J Med **1998**; 338:423–8.

3. Nyitray AG, Menezes L, Lu B, et al. Genital human papillomavirus (HPV) concordance in heterosexual couples. J Infect Dis **2012**; 206:202–11.
4. Scott M, Nakagawa M, Moscicki AB. Cell-mediated immune response to human papillomavirus infection. Clin Diagn Lab Immunol **2001**; 8:209–20.
5. Bouvard V, Baan R, Straif K, et al. A review of human carcinogens–Part B: biological agents. Lancet Oncology **2009**; 10:321–2.
6. Watson M, Saraiya M, Ahmed F, et al. Using population-based cancer registry data to assess the burden of human papillomavirus-associated cancers in the United States: overview of methods. Cancer **2008**; 113(suppl 10):2841–54.
7. Parkin DM, Bray F. Chapter 2: The burden of HPV-related cancers. Vaccine **2006**; 24(suppl 3):11–25.
8. Walboomers JM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol **1999**; 189:12–9.
9. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin **2011**; 61:69–90.
10. US Food and Drug Administration. Approved product: Gardasil. http://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM094042. Accessed 8 May 2015.
11. Bosch FX, Burchell AN, Schiffman M, et al. Epidemiology and natural history of human papillomavirus infections and type-specific implications in cervical neoplasia. Vaccine **2008**; 26(suppl 10):K1–16.
12. Giuliano AR, Tortolero-Luna G, Ferrer E, et al. Epidemiology of human papillomavirus infection in men, cancers other than cervical and benign conditions. Vaccine **2008**; 26(suppl 10):K17–28.
13. US Food and Drug Administration. Approved product: Gardasil 9. http://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm426445.htm. Accessed 8 May 2015.
14. Bartley J. Vitamin D: emerging roles in infection and immunity. Expert Rev Anti Infect Ther **2010**; 8:1359–69.
15. Heaney RP. Serum 25-hydroxyvitamin D is a reliable indicator of vitamin D status. Am J Clin Nutr **2011**; 94:619–20.
16. Chawla A, Repa JJ, Evans RM, Mangelsdorf DJ. Nuclear receptors and lipid physiology: opening the X-files. Science **2001**; 294:1866–70.
17. Lips P. Worldwide status of vitamin D nutrition. J Steroid Biochem Mol Biol **2010**; 121:297–300.
18. Wallace TC, Reider C, Fulgoni VL. Calcium and vitamin D disparities are related to gender, age, race, household income level, and weight classification but not vegetarian status in the United States: Analysis of the NHANES 2001–2008 data set. J Am Coll Nutr **2013**; 32:321–30.
19. Christensen MH, Lien EA, Hustad S, Almas B. Seasonal and age-related differences in serum 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D and parathyroid hormone in patients from Western Norway. Scand J Clin Lab Invest **2010**; 70:281–6.
20. Di Rosa M, Malaguarnera M, Nicoletti F, Malaguarnera L. Vitamin D3: a helpful immuno-modulator. Immunology **2011**; 134:123–39.
21. Juzeniene A, Ma LW, Kwitniewski M, et al. The seasonality of pandemic and non-pandemic influenzas: the roles of solar radiation and vitamin D. Int J Infect Dis **2010**; 14:e1099–5.
22. Sacco RE, Nonnecke BJ, Palmer MV, Waters WR, Lippolis JD, Reinhardt TA. Differential expression of cytokines in response to respiratory syncytial virus infection of calves with high or low circulating 25-hydroxyvitamin D3. PLoS One **2012**; 7: e33074.
23. Sabetta JR, DePetrillo P, Cipriani RJ, Smardin J, Burns LA, Landry ML. Serum 25-hydroxyvitamin d and the incidence of acute viral respiratory tract infections in healthy adults. PLoS One **2010**; 5:e11088.
24. Sita-Lumsden A, Lapthorn G, Swaminathan R, Milburn HJ. Reactivation of tuberculosis and vitamin D deficiency: the contribution of diet and exposure to sunlight. Thorax **2007**; 62:1003–7.
25. Dunne EF, Unger ER, Sternberg M, et al. Prevalence of HPV infection among females in the United States. JAMA **2007**; 297:813–9.
26. Centers of Disease Control and Prevention (CDC). CDC's second nutrition report. http://www.cdc.gov/nutritionreport/pdf/Introduction.pdf. Accessed 25 January 2016.
27. Institute of Medicine, Food and Nutrition Board. Dietary reference intakes for calcium and vitamin D. Washington, DC: National Academy Press, **2010**.
28. Holick MF. Vitamin D deficiency. N Engl J Med **2007**; 357:266–81.
29. Shrier I, Platt RW. Reducing bias through directed acyclic graphs. BMC Med Res Methodol **2008**; 8:70.
30. Greenland S, Neutra R. Control of confounding in the assessment of medical technology. Int J Epidemiol **1980**; 9:361–7.
31. Greenland S. Invited commentary: variable selection versus shrinkage in the control of multiple confounders. Am J Epidemiol **2008**; 167:1142.
32. Korn E, Graubard B. Analysis of Health Surveys. New York: Wiley, **1999**. 389p.
33. Hensel KJ, Randis TM, Gelber SE, Ratner AJ. Pregnancy-specific association of vitamin D deficiency and bacterial vaginosis. Am J Obstet Gynecol **2011**; 204:41.
34. He Q, Ananaba GA, Patrickson J, et al. Chlamydial infection in vitamin D receptor knockout mice is more intense and prolonged than in wild-type mice. J Steroid Biochem Mol Biol **2013**; 135:7–14.
35. Guani-Guerra E, Santos-Mendoza T, Lugo-Reyes SO, Teran LM. Antimicrobial peptides: general overview and clinical implications in human health and disease. Clin Immunol **2010**; 135:1–11.
36. Liu PT, Stenger S, Li H, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science **2006**; 311:1770–3.
37. Barlow PG, Svoboda P, Mackellar A, et al. Antiviral activity and increased host defense against influenza infection elicited by the human cathelicidin LL-37. PLoS One **2011**; 6:e25333.
38. Liu PT, Stenger S, Tang DH, Modlin RL. Cutting edge: vitamin D-mediated human antimicrobial activity against Mycobacterium tuberculosis is dependent on the induction of cathelicidin. J Immunol **2007**; 179:2060–3.
39. Nseir W, Taha M, Nemarny H, Mograbi J. The association between serum levels of vitamin D and recurrent urinary tract infections in premenopausal women. Int J Infect Dis **2013**; 17:e1121–4.
40. Bals R, Weiner DJ, Moscioni AD, Meegalla RL, Wilson JM. Augmentation of innate host defense by expression of a cathelicidin antimicrobial peptide. Infect Immun **1999**; 67:6084–9.
41. Frew L, Makieva S, McKinlay AT, et al. Human cathelicidin production by the cervix. PLoS One **2014**; 9:e103434.
42. Horne AW, Stock SJ, King AE. Innate immunity and disorders of the female reproductive tract. Reproduction **2008**; 135:739–49.
43. Iwasaki A. Antiviral immune responses in the genital tract: clues for vaccines. Nat Rev Immunol **2010**; 10:699–711.
44. Buck CB, Day PM, Thompson CD, et al. Human alpha-defensins block papillomavirus infection. Proc Natl Acad Sci U S A **2006**; 103:1516–21.
45. Stanley MA. Immune responses to human papilloma viruses. Indian J Med Res **2009**; 130:266–76.
46. Hewison M, Zehnder D, Chakraverty R, Adams JS. Vitamin D and barrier function: a novel role for extra-renal 1 alpha-hydroxylase. Mol Cell Endocrinol **2004**; 215:31–8.
47. Herfs M, Hubert P, Moutschen M, Delvenne P. Mucosal junctions: open doors to HPV and HIV infections? Trends Microbiol **2011**; 19:114–20.
48. Bruni L, Diaz M, Castellsague X, Ferrer E, Bosch FX, de Sanjose S. Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. J Infect Dis **2010**; 202:1789–99.
49. Looker AC, Johnson CL, Lacher DA, Pfeiffer CM, Schleicher RL, Sempos CT. Vitamin D status: United States, 2001–2006. NCHS data brief. No 59. Hyattsville, MD: National Center for Health Statistics, **2011**.
50. Karwalajtys T, Howard M, Sellors JW, Kaczorowski J. Vaginal self sampling versus physician cervical sampling for HPV among younger and older women. Sex Transm Infect **2006**; 82:337–9.