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Association between serum vitamin D levels and *Helicobacter pylori* cytotoxic-associated gene A seropositivity: a cross-sectional study in US adults from NHANES III

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ABSTRACT

Objective To assess the association of serum vitamin D (VD) levels and *Helicobacter pylori* (*H. pylori*) cytotoxic-associated gene A (CagA) seropositivity, and further explore potential effect modifiers in this association.

Design Cross-sectional study.

Setting Data from phase I of the National Health and Nutrition Examination Survey (NHANES III, 1988–1991) led by the Center for Disease Control and Prevention.

Participants A total of 3512 US adults (≥20 years) with both serum VD levels and *H. pylori* CagA antibody data from NHANES III were included in the analysis.

Methods VD deficiency was defined as serum 25(OH) D concentrations<20 ng/mL. Logistic regression models were used to assess the association of serum VD levels and *H. pylori* CagA seropositivity (VD–*Hp* CagA+), and stratification analyses were used to explore potential effect modifiers.

Results There was no significant association of VD–*Hp* CagA+ in the general population. But serum 25(OH)D concentrations were associated with *H. pylori* CagA+ in non-Hispanic whites (adjusted OR=1.02, 95% CI: 1.00 to 1.03), other races/ethnicities (adjusted OR=1.08, 95% CI: 1.01 to 1.06), populations born in other countries (adjusted OR=1.09, 95% CI: 1.04 to 1.15) or occasional drinkers (adjusted OR=0.93, 95% CI: 0.88 to 0.99). VD deficiency was associated with *H. pylori* CagA+ in non-Hispanic whites (adjusted OR=0.69, 95% CI: 0.53 to 0.92), populations born in other countries (adjusted OR=0.47, 95% CI: 0.25 to 0.89), non-drinkers (adjusted OR=0.80, 95% CI: 0.65 to 0.99), occasional drinkers (adjusted OR=2.53, 95% CI: 1.06 to 6.05), population with first quartile level of serum ferritin (adjusted OR=0.70, 95% CI: 0.51 to 0.96) or fourth quartile level of serum folate (adjusted OR=0.63, 95% CI: 0.46 to 0.87).

Conclusions Racial/ethnic differences and different serum ferritin or serum folate levels may be effect modifiers for the association of VD–*Hp* CagA+.

Strengths and limitations of this study

- ► To our best knowledge, this is the first study to explore the association of serum vitamin D (VD) levels with *Helicobacter pylori* cytotoxic-associated gene A (CagA) seropositivity using nationally representative data from the National Health and Nutrition Examination Survey.
- ► We used a method based on statistical considerations to screen potential confounding factors and a comprehensive stratification analysis to explore potential effect modifiers in the association of serum VD levels with *H. pylori* CagA seropositivity.
- ► This cross-sectional study did not allow us to determine the temporality and the causality between serum VD levels and *H. pylori* CagA seropositivity.
- ► The lack of international, unequivocal threshold values for diagnosing VD deficiency might affect our study's intensity.
- ► We lacked data on participants' time spent on sun exposure behaviours that could affect their serum VD levels.

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a spiral-shaped gram-negative bacterium that colonises the human gastric mucosa.[1 2] *H. pylori* infection, with the prevalence being approximately 44.3% worldwide, is believed to trigger several gastrointestinal diseases, including chronic gastritis, atrophic gastritis, peptic ulcer disease and mucosa-associated lymphoid tissue lymphoma.[1–4] In addition, over 75% of all gastric cancer cases are associated with *H. pylori* infection.[5] *H. pylori* strains may become more virulent when they are able to produce and secrete cytotoxin-associated gene A (cagA) protein.[6] CagA gene, a part of the cag pathogenicity island that cagA is injected into the host cell and then it tethered with the inner surface of the cytoplasmic membrane during *H. pylori* attaching host cells, promotes the epithelial–mesenchymal transition, contributing to carcinogenesis.[5–10] Clinically, it is verified

that individuals with CagA seropositivity (CagA+) have an increased risk of gastric cancer.[11 12] Therefore, in view of CagA's significance for *H. pylori* pathogenicity, further exploring factors that influence CagA expression are needed.

Vitamin D (VD) is functionally a hormone rather than a vitamin and is an essential regulator of cell proliferation, differentiation, apoptosis and angiogenesis.[13 14] In addition to osteoporosis and rickets, low serum VD levels also contribute to the increased risk of infections, chronic diseases and even cancers.[15–19] Previous studies have indicated that lower serum VD levels could contribute to *H. pylori* infection in adults.[1 17 20–25] Currently, a study showed that *H. pylori* CagA might be involved in inhibiting the MCOLN3 protein expression of the Ca^{2+} channel, leading to Ca^{2+} accumulation and impaired lysosomal acidification, which further inhibited autolysosomal degradation functions and promoted *H. pylori* infection.[26] In contrast, VD_3 could reverse the downregulated MCOLN3 protein expression and reactivate autolysosomal degradation functions to remove *H. pylori* in host cells.[26] Based on the above studies, it is suggested that serum VD levels may also be associated with *H. pylori* CagA seropositivity. However, observational studies on their association are scarce. Therefore, using publicly available data from a national representative sample of the US adults, we assessed the association of serum VD levels with *H. pylori* CagA seropositivity (VD–*Hp* CagA+) and further explored potential effect modifiers in this association.

METHODS

Study design and participants

The data of this cross-sectional study were from the National Health and Nutrition Examination Survey (NHANES). The NHANES, a series of surveys led by the Center for Disease Control and Prevention (CDC), provides multistage, national representative nutrition and health data of the civilian, non-institutionalised US population, which is used to assess the health and nutritional status of US adults and children since 1960s.[27] NHANES includes in-person household interviews and health examinations from a mobile examination centre.

Data were selected from phase I of NHANES III (1988–1991) because serum 25(OH)D concentrations and *H. pylori* CagA antibody were only measured in this cycle. Participants aged 20 years or older were eligible to measure *H. pylori* CagA antibody from collected blood samples. Final samples were those with both serum 25(OH)D concentrations and *H. pylori* CagA antibody measurements.

Additionally, we excluded participants who were *H. pylori* seronegative because *H. pylori* CagA status was shown only in those with *H. pylori* seropositivity. Participants who reported taking VD supplements within the last month were also excluded in the present study because their serum VD levels might be inflated (figure 1).

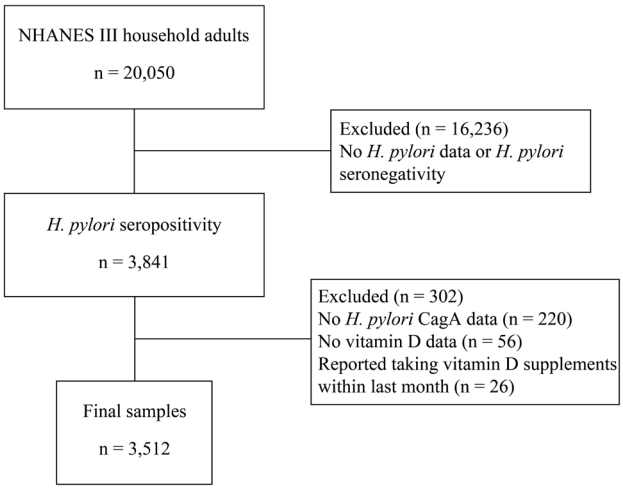

Figure 1 Flowchart of the study population. CagA, cytotoxic-associated gene A; *H. pylori, Helicobacter pylori*; NHANES, National Health and Nutrition Examination Survey.

Measures of serum 25(OH)D concentrations

Serum 25(OH)D concentrations were measured at the National Center for Environmental Health, CDC, Atlanta, Georgia, USA, using a radioimmunoassay kit (Diasorin, Stillwater, Minnesota, USA) and the coefficients of variations were 13%–19% in the NHANES III.[28] VD deficiency was defined as serum 25(OH)D concentrations <20 ng/mL.[14]

Measures of *H. pylori* CagA antibody

H. pylori CagA antibody was measured on participants 20 years or older from phase I of NHANES III by an immunoglobulin G enzyme-linked immunosorbent assay (Wampole Laboratories, Cranbury, New Jersey, USA) with sensitivity and specificity of 96%.[29 30]

Covariates

First, covariates in this study were selected based on previous studies, including age, sex, race/ethnicity, family income to poverty ratio, place of birth, marital status, education level, smoking status, alcohol consumption, physical exercise, source of drinking water, peptic ulcer, cardiovascular disease (eg, congestive heart failure, hypertension, heart attack and stroke), respiratory disease (eg, asthma, chronic bronchitis and emphysema), diabetes, osteoporosis, body mass index, haemoglobin, serum cholesterol, serum triglycerides, serum ferritin, serum folate, serum vitamin A, serum vitamin E, serum vitamin C, serum α-carotene, serum β-carotene, plasma glucose, glycated haemoglobin, serum C reactive protein, serum creatinine, serum thyroid stim hormone and serum thyroxine.[1 14 20 21 31]

Second, the above covariates with an estimated variance inflation factor (VIF) value of more than 5 would be excluded because of leading to multicollinearity.[32] There was no multicollinearity among covariates showed by VIF tests (online supplemental table S1).

Third, we selected the above covariates as potential confounders if they changed the estimates of serum 25(OH)D concentrations on *H. pylori* CagA+ more than 10%, or were significantly associated with *H. pylori* CagA+ (significant association was defined as p value<0.10) (online supplemental tables S2 and S3).[33 34] Finally, the following covariates were selected as potential confounders in this study: age, sex (male, female), race/ethnicity (non-Hispanic white/black, Mexican American, other), place of birth (50 United States, Mexico, other), education level (less than high school, high school, high school above), alcohol consumption (never, <1 time/month, 2–4 times/month, >4 times/month), haemoglobin (g/dL), serum ferritin (ng/mL), serum folate (ng/mL), serum vitamin A (µg/dL), serum vitamin E (µg/dL) and serum β-carotene (µg/dL) (online supplemental table S4).

Statistical analysis

The descriptive analysis of population characteristics was performed to examine the distribution of each variable, including count and weighted proportion, weighted means and SD. χ^2 test or Fisher's exact test was used to analyse categorical variables, and Mann-Whitney U test or Kruskal-Wallis test was used to compare continuous variables.

Logistic regression models were used to assess the association of continuous serum 25(OH)D concentrations and VD deficiency with *H. pylori* CagA seropositivity. In model 1, we adjusted no factors. In model 2, we adjusted potential confounders that changed the estimates of serum 25(OH)D concentrations on *H. pylori* CagA seropositivity more than 10%, including age, sex, race/ethnicity, education level, serum folate, serum vitamin A, serum vitamin E and serum β-carotene. In model 3, we further adjusted potential confounders significantly associated with *H. pylori* CagA seropositivity (p value < 0.10), including place of birth, alcohol consumption, haemoglobin and serum ferritin. Finally, we stratified the above analyses by potential effect modifiers, including age groups, sex, race/ethnicity, place of birth, education level, alcohol consumption, haemoglobin, ferritin, folate, vitamin A, vitamin E and β-carotene. In addition, based on the Analytic and Reporting Guidelines of NHANES III,[35] age was categorised into age groups with 10 years in each group. Serum concentrations of haemoglobin, ferritin, folate, vitamin A, vitamin E and β-carotene were categorised into quartiles to maximise the sample size in each stratum. The regression analyses were reported as ORs and 95% CIs.

Besides, to maximise statistical power and minimise bias that might occur if participants with missing data were excluded from analyses, we used multiple imputations (MI), based on five replications and a chained equation approach method in the MI procedure of R statistical software, to account for missing data.[36 37] The MI data were only used for regression analyses. We repeated regression analyses using complete data for the comparison of primary outcomes (online supplemental tables S5 and S6).

All analyses used appropriate statistical weights provided in NHANES, and all tests were two sided with a p value < 0.05 considered statistically significant. All analyses were conducted with R's statistical software packages (https://www.R-project.org, The R Foundation) and EmpowerStats (https://www.empowerstats.net/en/, X&Y Solution, Boston, Massachusetts, USA).

Patient and public involvement

There was no patient or public involvement in this study.

RESULTS

A total of 3512 participants were included in our study, and the weighted prevalence of *H. pylori* CagA+ and VD deficiency was 53.69% and 37.81%, respectively (table 1). A majority reported non-Hispanic whites (64.89%), born in the USA (78.24%), high school education (52.14%) and non-drinkers (53.60%). The prevalence of *H. pylori* CagA seropositivity was higher with 20–49 years old (eg, 60.72% in 40–49 age group), race/ethnicity other than non-Hispanic white (eg, 77.63% in non-Hispanic black), populations born in other countries (64.77%), population with education level other than high school (eg, 56.63% in high school above) and drinkers (eg, 63.37% in occasional drinkers who had alcohol consumption<1 time/month). Significantly lower serum 25(OH)D concentrations with higher VD deficiency prevalence were shown in females and race/ethnicity other than non-Hispanic white.

The association of serum 25(OH)D concentrations with *H. pylori* CagA+ was shown in table 2. In the general population, *H. pylori* CagA+ was negatively associated with serum 25(OH)D concentrations (OR=0.98, 95% CI: 0.97 to 0.99). While the direction of the association was reversed after adjusting for potential confounders in model 2 and model 3, the association was neither statistically significant (models 2 and 3: OR=1.02, 95% CI: 0.99 to 1.04). In stratified analyses, the adjusted association of serum 25(OH)D concentrations with *H. pylori* CagA+ was statistically significant in non-Hispanic white (adjusted OR=1.02, 95% CI: 1.00 to 1.03), other race/ethnicity (adjusted OR=1.08, 95% CI: 1.01 to 1.06), populations born in other countries (adjusted OR=1.09, 95% CI: 1.04 to 1.15) and occasional drinkers (adjusted OR=0.93, 95% CI: 0.88 to 0.99). No statistically significant association was shown in any other strata.

The association of VD deficiency with *H. pylori* CagA+ was shown in table 3. Similarly, the adjusted association of VD deficiency with *H. pylori* CagA+ in the general population was neither statistically significant (models 2 and 3: adjusted OR=0.90, 95% CI: 0.77 to 1.05). In stratified analyses, the adjusted association was statistically significant in non-Hispanic white (adjusted OR=0.69, 95% CI: 0.53 to 0.92), populations born in other countries (adjusted OR=0.47, 95% CI: 0.25 to

Table 1 Distribution characteristics of *Helicobacter pylori* CagA seropositive prevalence and vitamin D levels among 3512 US adults from the NHANES III

Characteristic	N (weighted %)	*H. pylori* CagA+ (N, %)*	25(OH)D concentrations†	VD deficiency (N, %)*
Age group (years)				
20–29	517 (11.63)	333 (54.91)	22.66 (8.71)	228 (40.69)
30–39	592 (20.66)	397 (57.15)	24.14 (9.04)	307 (37.43)
40–49	555 (16.47)	339 (60.72)	21.80 (7.70)	312 (41.37)
50–59	430 (15.21)	252 (51.78)	24.44 (8.37)	184 (36.94)
60–69	617 (17.52)	348 (45.58)	23.38 (7.74)	280 (37.60)
70–79	461 (12.91)	268 (54.47)	23.71 (7.43)	168 (31.28)
≥80	340 (5.54)	163 (46.36)	21.94 (7.42)	132 (40.86)
Sex				
Male	1878 (48.95)	1131 (55.46)	25.03 (8.00)	709 (23.59)
Female	1634 (51.05)	969 (51.99)	21.67 (8.11)	902 (42.49)
Race/ethnicity				
Non-Hispanic white	1184 (64.89)	521 (44.67)	25.54 (7.85)	329 (26.65)
Non-Hispanic black	980 (15.94)	751 (77.63)	17.70 (6.96)	648 (68.81)
Mexican American	1220 (8.57)	736 (59.96)	21.22 (6.90)	571 (46.01)
Other‡	128 (10.60)	92 (67.82)	19.86 (7.57)	63 (52.83)
Place of birth				
50 United States	2571 (78.24)	1507 (50.86)	23.90 (8.39)	1185 (35.74)
Mexico	669 (5.08)	406 (60.61)	21.52 (6.69)	301 (43.98)
Other§	267 (16.60)	183 (64.77)	21.15 (7.41)	121 (45.46)
Education level				
Less than high school	1229 (21.72)	752 (56.57)	23.34 (7.72)	532 (36.48)
High school	1592 (52.14)	922 (51.26)	23.42 (8.49)	759 (37.71)
High school above	665 (25.65)	410 (56.63)	23.10 (8.18)	311 (39.54)
Alcohol consumption				
Never	1946 (53.60)	1126 (51.19)	23.09 (8.23)	883 (39.47)
<1 time/month	180 (5.41)	123 (63.37)	22.26 (7.51)	74 (41.58)
2–4 times/month	588 (17.21)	363 (54.41)	22.72 (7.85)	291 (38.83)
>4 times/month	789 (23.44)	485 (56.99)	24.42 (8.50)	361 (32.83)
H. pylori CagA+				
No	1412 (46.31)	–	23.71 (8.27)	598 (36.49)
Yes	2100 (53.69)	–	22.98 (8.18)	1013 (38.94)
Serum VD level				
Normal	1901 (62.19)	1087 (52.70)	28.38 (5.84)	–
Deficiency	1611 (37.81)	1013 (55.30)	14.99 (3.40)	–

Weighted percentages that do not add up to 100% are attributable to missing data.
*Weighted population prevalence.
†Values were reported as weighted mean±SD. (ng/mL).
‡Other race/ethnicity includes all race/ethnicity other than Mexico-American, non-Hispanic white and black.
§Other countries include all countries other than USA and Mexico.
CagA, cytotoxic-associated gene A; VD, vitamin D.

0.89), non-drinkers (OR=0.80, 95% CI: 0.65 to 0.99), occasional drinkers (adjusted OR=2.53, 95% CI: 1.06 to 6.05), population with quartile 1 level (ranging from 2 to 48 ng/mL, weighted mean±SD: 26.58±13.69 ng/mL) of serum ferritin (adjusted OR=0.70, 95% CI: 0.51 to 0.96) or quartile 4 level (ranging from 6.9 to 60.7 ng/mL, weighted mean±SD: 11.57±4.76 ng/mL) of serum folate (adjusted OR=0.63, 95% CI: 0.46 to 0.87). No statistically significant association was shown in any other strata.

Table 2 OR (95% CI) for the association of serum 25(OH)D concentrations with *Helicobacter pylori* cytotoxic-associated gene A seropositivity

Effect modifier	Model 1	Model 2	Model 3
Overall	0.98 (0.97 to 0.99)*	1.02 (0.99 to1.04)	1.02 (0.99 to1.04)
Age group (years)			
20–29	0.99 (0.96 to 1.01)	1.00 (0.98 to 1.03)	1.00 (0.97 to 1.03)
30–39	0.98 (0.96 to 0.99)*	1.02 (0.99 to 1.05)	1.03 (0.99 to 1.06)
40–49	0.98 (0.96 to 1.01)	1.00 (0.97 to 1.02)	0.99 (0.97 to 1.03)
50–59	0.98 (0.96 to 1.00)	1.00 (0.98 to 1.04)	1.01 (0.98 to 1.04)
60–69	0.97 (0.95 to 0.99)*	0.99 (0.97 to 1.01)	0.99 (0.97 to 1.02)
70–79	0.99 (0.97 to 1.01)	1.01 (0.99 to 1.02)	1.02 (0.99 to 1.05)
≥80	0.99 (0.97 to 1.01)	1.02 (0.99 to 1.05)	1.02 (0.99 to 1.05)
Sex			
Male	0.98 (0.96 to 0.99)*	1.00 (0.99 to 1.01)	1.00 (0.99 to 1.01)
Female	0.98 (0.97 to 0.99)*	1.01 (0.99 to 1.03)	1.01 (0.99 to 1.03)
Race/ethnicity			
Non-Hispanic white	1.01 (0.98 to 1.03)	1.02 (1.00 to 1.03)*	1.02 (1.00 to 1.03)*
Non-Hispanic black	1.00 (0.98 to 1.02)	0.99 (0.97 to 1.01)	0.99 (0.97 to 1.02)
Mexican American	0.99 (0.98 to 1.01)	0.99 (0.98 to 1.01)	0.99 (0.97 to 1.01)
Other	1.05 (0.99 to 1.11)	1.08 (1.01 to 1.15)*	1.08 (1.01 to 1.16)*
Place of birth			
50 United States	0.97 (0.96 to 0.98)*	1.00 (0.99 to 1.01)	1.00 (0.99 to 1.01)
Mexico	1.00 (0.98 to 1.02)	–†	–†
Other	1.05 (1.01 to 1.09)*	1.09 (1.04 to 1.14)*	1.09 (1.04 to 1.15)*
Education level			
Less than high school	0.99 (0.98 to 1.01)	1.01 (0.99 to 1.03)	1.01 (0.99 to 1.03)
High school	0.98 (0.96 to 0.99)*	1.00 (0.98 to 1.01)	1.00 (0.98 to 1.01)
High school above	0.97 (0.95 to 0.99)*	1.01 (0.99 to 1.04)	1.02 (0.99 to 1.04)
Alcohol consumption			
Never	0.98 (0.97 to 0.99)*	1.01 (0.99 to 1.02)	1.01 (0.99 to 1.02)
< 1 time/month	0.96 (0.92 to 0.99)*	0.93 (0.88 to 0.99)*	0.93 (0.88 to 0.99)*
2~4 times/month	0.97 (0.95 to 0.99)*	1.00 (0.98 to 1.03)	1.00 (0.98 to 1.03)
> 4 times/month	0.99 (0.97 to 1.00)	1.01 (0.99 to 1.03)	1.01 (0.99 to 1.03)
Hemoglobin level			
Quartile 1	0.98 (0.96 to 0.99)*	1.01 (0.99 to 1.03)	1.01 (0.99 to 1.03)
Quartile 2	0.98 (0.96 to 1.00)	1.00 (0.98 to 1.02)	1.00 (0.98 to 1.02)
Quartile 3	0.99 (0.97 to 1.01)	1.02 (0.99 to 1.04)	1.02 (0.99 to 1.04)
Quartile 4	0.98 (0.96 to 1.00)	1.00 (0.99 to 1.01)	1.01 (0.99 to 1.02)
Serum ferritin level			
Quartile 1	0.99 (0.97 to 1.00)	1.01 (0.99 to 1.03)	1.01 (0.99 to 1.03)
Quartile 2	0.98 (0.96 to 1.00)	1.00 (0.98 to 1.02)	1.00 (0.98 to 1.02)
Quartile 3	0.99 (0.97 to 1.00)	1.01 (0.99 to 1.03)	1.01 (0.99 to 1.03)
Quartile 4	0.97 (0.95 to 0.99)*	1.00 (0.98 to 1.02)	1.00 (0.98 to 1.02)
Serum folate level			
Quartile 1	0.99 (0.97 to 1.00)	1.02 (0.99 to 1.04)	1.02 (0.99 to 1.04)
Quartile 2	0.98 (0.96 to 0.99)*	1.00 (0.98 to 1.02)	1.00 (0.98 to 1.02)
Quartile 3	0.97 (0.95 to 0.98)*	0.99 (0.97 to 1.01)	0.99 (0.97 to 1.01)

Continued

Table 2 Continued

Effect modifier	Model 1	Model 2	Model 3
Quartile 4	1.00 (0.98 to 1.01)	1.02 (0.99 to 1.04)	1.02 (0.99 to 1.04)
Serum vitamin E level			
Quartile 1	0.98 (0.97 to 1.00)	1.01 (0.99 to 1.03)	1.01 (0.99 to 1.03)
Quartile 2	0.98 (0.96 to 0.99)*	1.00 (0.98 to 1.02)	1.00 (0.98 to 1.03)
Quartile 3	0.99 (0.97 to 1.01)	1.02 (0.99 to 1.04)	1.02 (0.99 to 1.04)
Quartile 4	0.98 (0.96 to 0.99)*	0.99 (0.97 to 1.01)	0.99 (0.97 to 1.01)
Serum β-carotene level			
Quartile 1	0.97 (0.95 to 0.99)	1.00 (0.98 to 1.02)	1.00 (0.98 to 1.02)
Quartile 2	0.97 (0.95 to 0.99)	0.99 (0.98 to 1.01)	0.99 (0.98 to 1.01)
Quartile 3	0.99 (0.97 to 1.01)	1.01 (0.99 to 1.03)	1.01 (0.99 to 1.03)
Quartile 4	0.99 (0.97 to 1.01)	1.02 (0.99 to 1.04)	1.02 (0.99 to 1.04)

Factors were not adjusted when they were used as effect modifiers.
*Statistically significant associations (p value<0.05).
†The model failed because of the small sample size of other covariates in this stratum.

DISCUSSION

Previous studies have found that serum VD levels are associated with *H. pylori* infection, while the association of VD–*Hp* CagA+ is unclear.[1 17 20–25] Therefore, using a national representative sample of US adults, we assessed the association of VD–*Hp* CagA+ and further explored potential effect modifiers in this association. The present study showed that serum VD levels trended to be positively associated with *Hp* CagA+ in the general population after adjusting potential confounders, although this association was not statistically significant. While in stratified analyses, we found that serum VD levels were significantly positively associated with *Hp* CagA+ in non-Hispanic white, other race/ethnicity, populations born in other countries, non-drinkers, populations with first quartile level of serum ferritin or fourth quartile level of serum folate. Possible explanations are as follows:

First, racial/ethnic differences in VD levels and the prevalence of *H. pylori* CagA+ are significant. In the US cross-sectional study of VD metabolism, white populations had higher baseline serum 25(OH)D and lower parathyroid hormone concentrations compared with black populations and other races/ethnicity.[38] The other cohort study showed that the prevalence of *H. pylori* CagA+ among black populations were approximately two times higher than that among white populations.[39] In comparison, almost all isolated *H. pylori* strains were CagA positive among East Asian populations.[6] Such differences may vary VD–*Hp* CagA+ in different races/ethnicities, although we only observed significant VD–*Hp* CagA+ in non-Hispanic whites and other races/ethnicities. The present study showed that VD–*Hp* CagA+ was positively correlated among non-Hispanic whites with higher baseline serum 25(OH)D concentrations and lower *Hp* CagA+ prevalence. In comparison, a negative correlation of VD–*Hp* CagA+ was shown among non-Hispanic blacks with lower baseline serum 25(OH)D concentrations and higher *Hp* CagA+ prevalence. Besides, we also found a significant association of VD–*Hp* CagA+ in populations born in other countries. However, this association was more likely to go along with racial/ethnic differences. For instance, 75.43% of populations born in 50 United States were non-Hispanic white, and almost all of the populations born in Mexico were Mexican American (data not shown).

Second, the effect of alcohol intake on serum VD levels or *H. pylori* infection is still controversial. A recent review summarised that research before 2000 mainly was carried out among specific populations with small sample sizes. The results tended to show no correlation or negative correlation between alcohol intake and serum VD levels. In comparison, after 2000, research that had larger sample sizes and more extended follow-up periods trended to show a positive correlation.[40] Additionally, although alcohol intake was found to be linked to the increased risk of gastric cancer, studies only demonstrated this association in individuals with heavy alcohol intake or *H. pylori* seronegativity.[41] A recent systematic review further showed that no significant association could be found between *H. pylori* infection and alcohol consumption.[42] The present study's descriptive analysis of population characteristics showed significant differences in serum 25(OH)D as well as *Hp* CagA+ prevalence between occasional drinkers and populations with other alcohol intakes. Moreover, we found a significantly positive association of VD–*Hp* CagA+ among non-drinkers but a significantly negative association of VD–*Hp* CagA+ among occasional drinkers. In contrast, the association of VD–*Hp* CagA+ tended to be positive among populations with other alcohol intakes. It seems that occasional drinkers could be regarded as an effect modifier in the association of VD–*Hp* CagA+. However, we noted that the weighted proportion of occasional drinkers was deficient (5.41%) in the present study. Hence its association's

Table 3 OR (95% CI) for the association of vitamin D deficiency with *Helicobacter pylori* cytotoxic-associated gene A seropositivity

Effect modifier	Model 1	Model 2	Model 3
Overall	1.27 (1.11 to 1.45)*	0.90 (0.77 to 1.05)	0.90 (0.77 to 1.05)
Age group (years)			
20–29	1.19 (0.83 to 1.72)	0.93 (0.61 to 1.41)	0.96 (0.63 to 1.46)
30–39	1.45 (1.03 to 2.05)*	0.79 (0.53 to 1.20)	0.78 (0.52 to 1.20)
40–49	1.18 (0.84 to 1.67)	0.96 (0.65 to 1.42)	0.95 (0.64 to 1.41)
50–59	1.23 (0.83 to 1.81)	0.83 (0.53 to 1.32)	0.82 (0.51 to 1.31)
60–69	1.31 (0.95 to 1.80)	0.94 (0.65 to 1.35)	0.87 (0.60 to 1.27)
70–79	1.38 (0.94 to 2.04)	0.97 (0.62 to 1.52)	0.96 (0.61 to 1.52)
≥80	0.85 (0.55 to 1.32)	0.71 (0.44 1.14)	0.70 (0.43 to 1.13)
Sex			
Male	1.44 (1.19 to 1.75)*	0.99 (0.80 to 1.23)	1.02 (0.82 to 1.27)
Female	1.14 (0.94 to 1.39)	0.81 (0.65 to 1.02)	0.80 (0.64 to 1.00)
Race/ethnicity			
Non-Hispanic white	0.74 (0.57 to 0.95)*	0.71 (0.54 to 0.94)*	0.69 (0.53 to 0.92)*
Non-Hispanic black	1.12 (0.82 to 1.52)	1.14 (0.82 to 1.58)	1.12 (0.80 to 1.56)
Mexican American	0.97 (0.77 to 1.22)	0.98 (0.77 to 1.25)	1.01 (0.79 to 1.29)
Other	0.70 (0.32 to 1.52)	0.58 (0.24 to 1.41)	0.57 (0.23 to 1.43)
Place of birth			
50 United States	1.48 (1.26 to 1.73)*	0.98 (0.81 to 1.77)	0.95 (0.79 to 1.15)
Mexico	0.87 (0.63 to 1.18)	–†	–†
Other	0.72 (0.43 to 1.20)	0.49 (0.26 to 0.90)*	0.47 (0.25 to 0.89)*
Education level			
Less than high school	0.96 (0.76 to 1.20)	0.77 (0.59 to 1.00)	0.78 (0.60 to 1.00)
High school	1.58 (1.30 to 1.94)*	1.18 (0.93 to 1.48)	1.17 (0.93 to 1.47)
High school above	1.27 (0.93 to 1.74)	0.63 (0.43 to 1.00)	0.63 (0.42 to 1.00)
Alcohol consumption			
Never	1.16 (0.97 to 1.39)	0.81 (0.66 to 0.99)*	0.80 (0.65 to 0.99)*
< 1 time/month	1.62 (0.84 to 3.12)	2.53 (1.01 to 5.48)*	2.53 (1.06 to 6.05)*
2–4 times/month	1.53 (1.10 to 2.13)*	0.97 (0.66 to 1.43)	0.97 (0.66 to 1.42)
>4 times/month	1.34 (0.99 to 1.78)	0.98 (0.71 to 1.37)	0.98 (0.71 to 1.37)
Hemoglobin level			
Quartile 1	1.32 (0.99 to 1.75)	0.90 (0.65 to 1.25)	0.87 (0.63 to 1.21)
Quartile 2	1.26 (0.93 to 1.65)	0.96 (0.71 to 1.31)	0.93 (0.68 to 1.26)
Quartile 3	1.07 (0.82 to 1.41)	0.73 (0.54 to 1.00)	0.74 (0.54 to 1.01)
Quartile 4	1.28 (0.97 to 1.70)	1.09 (0.80 to 1.49)	1.09 (0.80 to 1.50)
Serum ferritin level			
Quartile 1	1.03 (0.78 to 1.36)	0.71 (0.52 to 0.97)*	0.70 (0.51 to 0.96)*
Quartile 2	1.19 (0.90 to 1.57)	0.87 (0.63 to 1.19)	0.86 (0.63 to 1.19)
Quartile 3	1.31 (0.99 to 1.72)	0.91 (0.66 to 1.24)	0.90 (0.66 to 1.24)
Quartile 4	1.57 (1.20 to 2.06)*	1.12 (0.82 to 1.52)	1.09 (0.80 to 1.48)
Serum folate level			
Quartile 1	1.23 (0.93 to 1.62)	0.83 (0.60 to 1.14)	0.82 (0.59 to 1.13)
Quartile 2	1.27 (0.97 to 1.66)	0.94 (0.70 to 1.28)	0.95 (0.70 to 1.29)
Quartile 3	1.57 (1.19 to 2.08)*	1.21 (0.89 to 1.65)	1.22 (0.90 to 1.68)

Continued

Table 3 Continued

Effect modifier	Model 1	Model 2	Model 3
Quartile 4	0.91 (0.69 to 1.21)	0.64 (0.47 to 0.88)*	0.63 (0.46 to 0.87)*
Serum vitamin A level			
Quartile 1	1.27 (0.95 to 1.70)	0.92 (0.66 to 1.28)	0.90 (0.65 to 1.26)
Quartile 2	1.13 (0.86 to 1.47)	0.95 (0.71 to 1.27)	1.00 (0.74 to 1.36)
Quartile 3	1.17 (0.90 to 1.54)	0.81 (0.60 to 1.10)	0.78 (0.57 to 1.06)
Quartile 4	1.36 (0.99 to 1.75)	0.91 (0.70 to 1.25)	0.90 (0.65 to 1.27)
Serum vitamin E level			
Quartile 1	1.25 (0.94 to 1.65)	0.89 (0.64 to 1.23)	0.89 (0.64 to 1.24)
Quartile 2	1.22 (0.93 to 1.61)	0.89 (0.65 to 1.23)	0.86 (0.62 to 1.81)
Quartile 3	1.16 (0.89 to 1.52)	0.86 (0.63 to 1.17)	0.88 (0.64 to 1.19)
Quartile 4	1.19 (0.90 to 1.56)	0.98 (0.72 to 1.32)	0.98 (0.72 to 1.33)
Serum β-carotene level			
Quartile 1	1.59 (1.14 to 2.10)*	1.01 (0.71 to 1.44)	0.98 (0.69 to 1.41)
Quartile 2	1.41 (1.10 to 1.80)*	1.00 (0.76 to 1.33)	1.00 (0.76 to 1.33)
Quartile 3	1.13 (0.86 to 1.48)	0.84 (0.61 to 1.15)	0.85 (0.62 to 1.16)
Quartile 4	1.10 (0.83 to 1.44)	0.75 (0.55 to 1.02)	0.76 (0.55 to 1.05)

Factors were not adjusted when they were used as effect modifiers.
*Statistically significant associations (p<0.05).
†The model failed because of the small sample size of other covariates in this stratum.

statistical significance might be inflated by stratified analyses. Besides, the significant association of VD–*Hp* CagA+ among non-drinkers could not indicate the effect of alcohol intake on the association of VD–*Hp* CagA+. Therefore, we considered that the effect of alcohol intake on the association of VD–*Hp* CagA+ was still limited.

Third, *Hp* CagA+ may be associated with iron status and one-carbon metabolites. A recent study found that one-carbon metabolism (involving serum folate, vitamin B12 and homocysteine levels) was mainly mediated by iron status (involving serum ferritin levels and transferrin saturation), while *H. pylori* infection could increase reactive oxygen species (ROS) by weakening iron status or indirectly reducing one-carbon metabolism levels.[43] Protein expression levels of CagA were subsequently upregulated in response to oxidative stress.[44] It is suggested that lower levels of iron status or one-carbon metabolism may contribute to *H. pylori* CagA+. We found some evidence in the present study, which showed a significantly positive association of VD–*Hp* CagA+ among populations with first quartile level of serum ferritin and fourth quartile level of serum folate. But there are contradictions in our result: based on the outcomes of the above studies, populations with first quartile level may have lower serum folate levels and higher ROS levels, which should be more likely to result in *H. pylori* CagA+. However, respectively only 32.03% and 23.54% of this subgroup populations had their serum ferritin and folate levels lower than minimum cutoffs (data not shown), which might result from the study sample from national representative populations, rather than hospital inpatients or other specific people. Therefore, we inferred that their antioxidant status might still be equal to inhibit the protein expression of CagA and lead to decreased *H. pylori* CagA+. More observational and mechanistic studies on the role of iron status and one-carbon metabolites in the relationship between VD levels and *H. pylori* CagA+ are needed.

Not fully understanding the influencing factors of *H. pylori* CagA+ is a crucial research gap. To our knowledge, this is the first study to explore the association of VD–*Hp* CagA+ using nationally representative data. Based on previous studies on the relationship between VD levels and *H. pylori* infection, we further used a method based on statistical considerations to screen potential confounding factors. Moreover, we used a comprehensive stratification analysis to explore potential effect modifiers in this association, finding that racial/ethnic differences and different serum ferritin or folate levels may affect the association of VD–*Hp* CagA+. Future randomised controlled trials of VD–*Hp* CagA+ may need to fully consider the effects of race/ethnicity, serum ferritin and serum folate levels.

However, there are limitations to our studies. First, the data used in the present study are not current because the date of serum VD levels and *H. pylori* CagA antibody is only provided in NHANES III. Second, this study was designed as a cross-sectional study, which did not allow us to determine the temporality and the causality between VD levels and *H. pylori* CagA+. Third, the lack of international, unequivocal threshold values for diagnosing VD deficiency might affect our study's intensity. Fourth, we also lacked data on participants' time spent on sun exposure behaviours that could affect their VD levels. At last,

the lack of data on quantity of alcohol or times of alcohol intake, which will affect the results.

CONCLUSIONS

In a nationally representative sample, VD levels may be not associated with *H. pylori* CagA seropositivity in the general population. Still, racial/ethnic differences and different serum ferritin or serum folate levels may be effect modifiers for the association of serum VD levels with *H. pylori* CagA seropositivity.

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Ethics approval This study involves human participants but was not approved by Data used for analysis in this study is publicly available on the NHANES website. The National Center for Health Statistics Research Ethics Review Board provided approval for the NHANES data collection. Use of the public use data sets requires neither Institutional Review Board review nor an exempt determination. Participants gave informed consent to participate in the study before taking part.

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REFERENCES

1 Pellicano R, Ribaldone DG, Fagoonee S, *et al*. A 2016 Panorama of Helicobacter pylori infection: key messages for clinicians. *Panminerva Med* 2016;58:304–17.
2 Alexander SM, Retnakumar RJ, Chouhan D, *et al*. *Helicobacter pylori* in Human Stomach: The Inconsistencies in Clinical Outcomes and the Probable Causes. *Front Microbiol* 2021;12:713955.
3 Smith S, Fowora M, Pellicano R. Infections with *Helicobacter pylori* and challenges encountered in Africa. *World J Gastroenterol* 2019;25:3183–95.
4 Tsipotis E, Leontiadis GI. Editorial: the prevalence of Helicobacter pylori infection worldwide-knowns and unknowns. *Aliment Pharmacol Ther* 2018;47:1330–1.
5 Mentis A-FA, Boziki M, Grigoriadis N, *et al*. Helicobacter pylori infection and gastric cancer biology: tempering a double-edged sword. *Cell Mol Life Sci* 2019;76:2477–86.
6 Hatakeyama M. Helicobacter pylori CagA and gastric cancer: a paradigm for Hit-and-run carcinogenesis. *Cell Host Microbe* 2014;15:306–16.
7 Sougleri IS, Papadakos KS, Zadik MP, *et al*. Helicobacter pylori CagA protein induces factors involved in the epithelial to mesenchymal transition (EMT) in infected gastric epithelial cells in an EPIYA- phosphorylation-dependent manner. *Febs J* 2016;283:206–20.
8 Mani SA, Guo W, Liao M-J, *et al*. The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell* 2008;133:704–15.
9 Brabletz T, Kalluri R, Nieto MA, *et al*. Emt in cancer. *Nat Rev Cancer* 2018;18:128–34.
10 Mentis A-FA, Kararizou E. Metabolism and cancer: an up-to-date review of a mutual connection. *Asian Pac J Cancer Prev* 2010;11:1437–44.
11 Matos JI, de Sousa HAC, Marcos-Pinto R, *et al*. Helicobacter pylori CagA and vacA genotypes and gastric phenotype: a meta-analysis. *Eur J Gastroenterol Hepatol* 2013;25:1431–41.
12 Ekström AM, Held M, Hansson LE, *et al*. Helicobacter pylori in gastric cancer established by CagA immunoblot as a marker of past infection. *Gastroenterology* 2001;121:784–91.
13 Nagpal S, Na S, Rathnachalam R. Noncalcemic actions of vitamin D receptor ligands. *Endocr Rev* 2005;26:662–87.
14 Mut Surmeli D, Surmeli ZG, Bahsi R, *et al*. Vitamin D deficiency and risk of Helicobacter pylori infection in older adults: a cross-sectional study. *Aging Clin Exp Res* 2019;31:985–91.
15 Wang L, Song Y, Manson JE, *et al*. Circulating 25-hydroxy-vitamin D and risk of cardiovascular disease: a meta-analysis of prospective studies. *Circ Cardiovasc Qual Outcomes* 2012;5:819–29.
16 Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. *Am J Clin Nutr* 2008;87:1080S–6.
17 Yang L, He X, Li L, *et al*. Effect of vitamin D on Helicobacter pylori infection and eradication: a meta-analysis. *Helicobacter* 2019;24:e12655.
18 Meier HCS, Miller FW, Dinse GE, *et al*. *Helicobacter pylori* seropositivity is associated with antinuclear antibodies in US adults, NHANES 1999-2000. *Epidemiol Infect* 2020;148:e20.
19 Charoenngam N, Holick MF. Immunologic effects of vitamin D on human health and disease. *Nutrients* 2020;12:2097.
20 Salem-Sokhn E, Salami A, Fawaz M, *et al*. Helicobacter pylori interacts with serum vitamin D to influence hypertension. *Curr Aging Sci* 2021;14:26–31.
21 Han C, Ni Z, Yuan T, *et al*. Influence of serum vitamin D level on Helicobacter pylori eradication: a multi-center, observational, prospective and cohort study. *J Dig Dis* 2019;20:421–6.
22 Gao T, Zhao M, Zhang C, *et al*. Association of *Helicobacter pylori* Infection with Vitamin D Deficiency in Infants and Toddlers. *Am J Trop Med Hyg* 2020;102:541–6.
23 Assaad S, Costanian C, Jaffal L, *et al*. Association of TLR4 polymorphisms, expression, and vitamin D with Helicobacter pylori infection. *J Pers Med* 2019;9:2.
24 Yildirim O, Yildirim T, Seckin Y, *et al*. The influence of vitamin D deficiency on eradication rates of Helicobacter pylori. *Adv Clin Exp Med* 2017;26:1377–81.
25 Kawaura A, Takeda E, Tanida N, *et al*. Inhibitory effect of long term 1.ALPHA.-Hydroxyvitamin D3 administration on Helicobacter pylori infection. *J Clin Biochem Nutr* 2006;38:103–6.
26 Hu W, Zhang L, Li MX, *et al*. Vitamin D3 activates the autolysosomal degradation function against Helicobacter pylori through the Pdia3 receptor in gastric epithelial cells. *Autophagy* 2019;15:707–25.
27 Prevention CfDCa. National health and nutrition examination survey: centers for disease control and prevention, 2020. Available: https://www.cdc.gov/nchs/nhanes/index.htm [Accessed 05 Apr 2021].
28 Deng X, Song Y, Manson JE, *et al*. Magnesium, vitamin D status and mortality: results from US National health and nutrition examination survey (NHANES) 2001 to 2006 and NHANES III. *BMC Med* 2013;11:187.
29 Cárdenas VM, Boller F, Román GC. *Helicobacter pylori*, Vascular Risk Factors and Cognition in U.S. Older Adults. *Brain Sci* 2019;9:370.
30 Everhart JE, Kruszon-Moran D, Perez-Perez G. Reliability of Helicobacter pylori and cagA serological assays. *Clin Diagn Lab Immunol* 2002;9:412–6.
31 Muzaheed. Helicobacter pylori oncogenicity: mechanism, prevention, and risk factors. *ScientificWorldJournal* 2020;2020:1–10.
32 Kim JH. Multicollinearity and misleading statistical results. *Korean J Anesthesiol* 2019;72:558–69.

33 Jaddoe VWV, de Jonge LL, Hofman A, *et al*. First trimester fetal growth restriction and cardiovascular risk factors in school age children: population based cohort study. *BMJ* 2014;348:g14.
34 Bjerregaard LG, Pedersen DC, Mortensen EL, *et al*. Breastfeeding duration in infancy and adult risks of type 2 diabetes in a high-income country. *Matern Child Nutr* 2019;15:e12869.
35 Prevention CfDCa. Analytic and reporting guidelines: the third National health and nutrition examination survey, NHANES III (1988-94). Available: https://wwwn.cdc.gov/nchs/nhanes/analyticguidelines.aspx [Accessed 05 Apr 2021].
36 Park S-Y, Freedman ND, Haiman CA, *et al*. Association of coffee consumption with total and cause-specific mortality among Nonwhite populations. *Ann Intern Med* 2017;167:228–35.
37 Farrar D, Fairley L, Santorelli G, *et al*. Association between hyperglycaemia and adverse perinatal outcomes in South Asian and white British women: analysis of data from the born in Bradford cohort. *Lancet Diabetes Endocrinol* 2015;3:795–804.
38 Hsu S, Hoofnagle AN, Gupta DK, *et al*. Race, ancestry, and vitamin D metabolism: the multi-ethnic study of atherosclerosis. *J Clin Endocrinol Metab* 2020;105:e4337–50.
39 Varga MG, Butt J, Blot WJ, *et al*. Racial Differences in *Helicobacter pylori* CagA Sero-prevalence in a Consortium of Adult Cohorts in the United States. *Cancer Epidemiol Biomarkers Prev* 2020;29:2084–92.
40 Tardelli VS, Lago MPPdo, Silveira DXda, *et al*. Vitamin D and alcohol: a review of the current literature. *Psychiatry Res* 2017;248:83–6.
41 Ma S-H, Jung W, Weiderpass E, *et al*. Impact of alcohol drinking on gastric cancer development according to Helicobacter pylori infection status. *Br J Cancer* 2015;113:1381–8.
42 Venneman K, Huybrechts I, Gunter MJ, *et al*. The epidemiology of Helicobacter pylori infection in Europe and the impact of lifestyle on its natural evolution toward stomach cancer after infection: a systematic review. *Helicobacter* 2018;23:e12483.
43 Beydoun MA, Dore GA, Canas JA, *et al*. Helicobacter pylori seropositivity's association with markers of iron, 1-carbon metabolism, and antioxidant status among US adults: a structural equations modeling approach. *PLoS One* 2015;10:e0121390.
44 Huang C-H, Chiou S-H. Proteomic analysis of upregulated proteins in Helicobacter pylori under oxidative stress induced by hydrogen peroxide. *Kaohsiung J Med Sci* 2011;27:544–53.