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Research Article

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Vitamin and Mineral Supplement Use and Anti-Hepatitis A Virus Seropositivity: NHANES 2013–2018 Cross-Sectional Association

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Abstract

Dietary vitamins and minerals play important roles in maintaining normal immune function. However, the relationship between short-term dietary supplement intake and hepatitis A virus (HAV) serostatus remains insufficiently characterized. This study aimed to investigate the association between vitamin and mineral supplement intake during a 24-hour period and anti-HAV seropositivity among participants in the National Health and Nutrition Examination Survey (NHANES) from 2013 to 2018. This cross-sectional study used data from three NHANES cycles: 2013–2014, 2015–2016, and 2017–2018. Data from participants aged 6 years and older with available information on anti-HAV antibody and dietary supplement intake based on two 24-hour recall periods were combined. The final analytical sample included 23,003 participants. Anti-HAV serostatus was classified as positive or negative. Vitamin and mineral supplement intake were evaluated in relation to anti-HAV serostatus using logistic regression analysis. Odds ratios (ORs), 95% confidence intervals (CIs), and p values were used to assess the associations. Overall, the investigated dietary supplemental nutrients were not significantly associated with anti-HAV serostatus. However, vitamin D intake showed a statistically significant exploratory association with anti-HAV seropositivity (OR = 1.000, 95% CI: 1.000–1.001; p = 0.004). Although the association reached statistical significance, the estimated effect size was very small. Furthermore, the analysis involved multiple nutrient comparisons, increasing the possibility of chance findings. Therefore, the vitamin D results should be interpreted cautiously and should not be considered evidence of a causal relationship. In this NHANES-based cross-sectional study, dietary supplement intake was generally not significantly associated with anti-HAV serostatus. The exploratory association observed for vitamin D warrants further investigation. Longitudinal studies with detailed assessments of supplement use, vaccination history, and potential confounding factors are needed to determine whether this association is reproducible and clinically meaningful.

Keywords: Hepatitis; Anti-HAV antibodies; Viral infection; Cross-sectional study; Dietary supplements; Vitamin D; Hepatitis A antibodies.

1. Introduction

Hepatitis A virus (HAV) is considered one of the most infectious etiologies of acute hepatitis worldwide. HAV infection can be transmitted through the fecal-oral route by contaminated food, water, or close physical contact with an infectious person. Fulminant hepatitis is also rare compared with HCV; the opposite is true for HBV.[1] Notably, the risk of severe or fulminant HAV increases with age and chronic liver disease. Typical symptoms of acute infection include nausea, vomiting, abdominal pain, fatigue, malaise, poor appetite, and fever; management is supportive care.[2]

Previous studies reported the importance of HAV vaccination for children 12 months or older, adults at risk of exposure, and patients with chronic liver disease.[3] After routine hepatitis A vaccination started in 1999, the incidence of hepatitis A has declined by 90%.[4] In contrast to Mexico, which has a high prevalence of individuals with anti-HAV antibodies, the United States has a low prevalence. Globally, the rates of HAV have decreased because of improvements in public healthcare policies, sanitation, and education, but infection rates of other hepatitis viruses appear to be increasing.[5]

Different studies have reported the importance of immunomodulators such as vitamins A, C, D3, E, and β-carotene, as well as microelements such as zinc, selenium, iron, omega-3 fatty acids, and live active probiotic bacteria, for their contribution to a stronger cellular response by increasing the number of monocytes that produce MHC II molecules.[6-8] Therefore, in this study, we investigated the association between anti-HAV antibodies and the use of some minerals and vitamin supplements using a 2-day recall.

2. Methods

2.1 Study design and data collection

The cross-sectional analysis used publicly available data merged using the participant identifier (SEQN) from the U.S. National Health and Nutrition Examination Survey (NHANES). Keywords included demographic characteristics, hepatitis A serology, vaccination history, and body mass index (BMI) data, which were obtained from the corresponding NHANES demographic, laboratory, questionnaire, and examination datasets. Dietary intake (DS1TOT_J, DS2TOT_J): 2-day dietary recall for minerals and some vitamins. Laboratory examination (HEPA_J) revealed hepatitis A antibodies. Examination Survey (NHANES) spanning three continuous cycles (cycle 1; 2013–2014; cycle 2; 2015–2016; and cycle 3; 2017–2018) with suffixes H, I, and J, respectively. A total of 29,400 participants were enrolled across the three cycles (10,175; 9,971; and 9,254, respectively). Data from three NHANES cycles (2013–2014, 2015–2016, and 2017–2018) were combined following the NHANES Analytic Guidelines. Data were obtained from three consecutive NHANES survey cycles (2013–2014, 2015–2016, and 2017–2018). Demographic characteristics were extracted from the demographic datasets, hepatitis A serostatus from the laboratory datasets, vaccination history from the questionnaire datasets, body mass index (BMI) from the examination datasets, and dietary supplement nutrient intake from the Dietary Supplement Total Nutrient Intake files corresponding to each survey cycle (suffixes H, I, and J for 2013–2014, 2015–2016, and 2017–2018, respectively). Participants were linked across datasets using the unique participant identifier (SEQN). We excluded participants who lacked anti-HAV results (n = 6397) and those with missing covariate data for age, sex, race, education, and BMI. After these exclusions, participants whose dietary supplements were missing for more than 2 days were also excluded. The final analytic sample included 23003 participants, as shown in Fig. 1. The dietary supplement nutrient intake data were obtained from the NHANES Dietary Supplement Total Nutrient Intake datasets for the 2013–2014, 2015–2016, and 2017–2018 survey cycles. Nutrient intake estimates were derived using information from both day 1 and day 2 dietary supplement recalls when available. Therefore, the exposure variables represent two-day dietary supplement nutrient intake rather than a single 24-hour assessment.

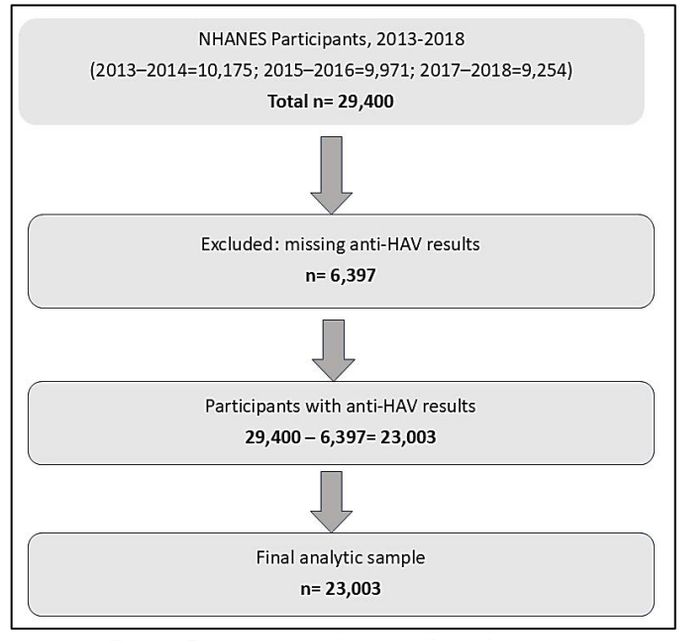


Fig. 1: Participant selection flow diagram.

2.2 Nutrient variables from individual dietary supplements

Dietary supplement nutrient intake was assessed using the NHANES Dietary Supplement Total Nutrient Intake datasets. Nutrient variables included vitamins (B1, B2, B6, B12, folate, and vitamins C, D, and K) and minerals (calcium, phosphorus, magnesium, iron, zinc, copper, sodium, and selenium). The methods used to derive nutrient intake variables were based on the NHANES dietary supplement nutrient intake files. The term "dietary supplement nutrient intake" is used throughout

the manuscript to reflect the nature of the exposure variables.

2.3 Anti-HAV immunoassay

Anti-HAV immunoassay was estimated using a competitive immunoassay technique that involves preincubation of anti-HAV in the sample with HAV antigen in the assay reagent. This was followed by incubation with a conjugate reagent that contained biotinylated mouse monoclonal anti-HAV antibody and horseradish peroxidase (HRP)-labeled mouse monoclonal anti-HAV antibody. The streptavidin in the wells captures the immune complex, and then the unbound materials are removed by washing. [9,10] After that, a luminescent reaction is used to measure the bound HRP. A reagent containing luminogenic substrates (a luminol derivative and a peracid salt) and an electron transfer agent (increases the level of light produced and prolongs its emission) is added to the wells. If the HRP conjugate binds to the reagent antigen, it produces light by catalyzing the oxidation of the luminol derivative. The light signals are read by the VITROS ECi/ECiQ or VITROS 3600 immunodiagnostic system. The binding of HRP is indicative of the absence of anti-HAV antibody.[11]

2.4 Statistical Analysis

Anti-HAV serostatus was used as the dependent variable in binary logistic regression analyses. The outcome variable was coded according to NHANES laboratory results, and odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to estimate associations between dietary supplement nutrient intake and anti-HAV serostatus. Statistical significance was defined as a two-sided p value < 0.05. The dependent variable was anti-HAV antibody status, which was categorized as positive or negative according to the NHANES laboratory results. The regression models included age, sex, race/ethnicity, education, body mass index (BMI), and vaccination history as covariates. Nutrient intake variables were entered as continuous variables. For each regression model, regression coefficients (B), standard errors (SE), odds ratios (ORs), 95% confidence intervals (CIs), and p values were obtained. The regression coefficient represents the change in the log odds of the modeled outcome associated with a one-unit increase in the corresponding predictor. Because the analyses were performed using standard binary logistic regression rather than the NHANES Complex Samples procedure, the models did not incorporate the NHANES sampling strata, primary sampling units, or survey weights. Accordingly, the regression estimates should be interpreted as associations within the analyzed study sample rather than as design-based population estimates for the U.S. population. A p value ≤0.05 was considered to indicate statistical significance. Because multiple vitamin and mineral nutrients were examined, statistically significant findings were interpreted as exploratory and were considered in the context of the number of comparisons performed.

The outcome variable was anti-HAV serostatus, coded as positive or negative according to the NHANES laboratory data. Binary logistic regression models were used to examine the association between dietary supplement nutrient intake and anti-HAV serostatus. The coding scheme and reference category for the outcome variable were verified and explicitly specified to ensure accurate interpretation of the estimated odds ratios.

2.5 Measurement

- Dependent variable: Anti-HAV antibody status (positive/negative) was used as the dependent variable. The variable was obtained from the NHANES laboratory data file (HEPA_J) and measured by a chemiluminescence immunoassay. The results were dichotomized as seropositive (≥ cutoff value) or seronegative (< cutoff value) according to NHANES laboratory procedures.
- Main exposure variables: The main exposures included daily intakes of vitamins (B1, B2, B6, B12, C, D, K, folic acid) and minerals (Ca, P, Mg, Fe, Zn, Cu, Na, K, Se) derived from the 24-hour dietary recall of dietary supplement use (DS1TOT_J and DS2TOT_J). Each variable represents the total amount (mg or μg) of nutrient intake within the 24-hour recall period for regression analysis; nutrient intake was treated as a continuous variable, and extreme outliers (values >99th percentile) were winsorized to minimize the influence of implausible reporting.
- Covariates: Covariates were selected on the basis of prior literature and biological relevance.[12] These included demographic factors such as age (years, continuous), sex (male/female), and race/ethnicity (non-Hispanic White, non-Hispanic Black, Mexican American, other Hispanic, non-Hispanic Asian, other/multiracial) from the DEMO_J file. Socioeconomic status: education level (five categories as provided in the NHANES). Health behavior factors: BMI and vaccination history.
- Variable handling and weighting: All analyses applied the NHANES complex sampling design and used the combined 6-year sample weights (WTMEC6YR) calculated according to the NHANES analytic guidelines.[13]

  Continuous variables are summarized as the means ± standard errors, and categorical variables are expressed as weighted percentages.

3. Results

The results in Table 1 show the demographic characteristics, sex, race/ethnicity, and education of U.S. participants from the NHANES across three cycles (2013-2018): Cycle 1 (2013-2014), Cycle 2 (2015-2016), and Cycle 3 (2017-2018).

Table 2 shows the characteristics of U.S. participants

by mineral supplement use over 24 hours over three cycles (2013-2018): cycle 1 (2013-2014); cycle 2 (2015-2016); cycle 3 (2017-2018), as well as the concentration of mineral supplement use over 24 hours. Additionally, Table 3 indicates the same data but for mineral supplement use over 24 hours.

Table 1: Demographic characteristics of U.S. participants (NHANES 2013-2018), cycle 1 (2013-2014); cycle 2 (2015-2016); cycle 3 (2017-2018). A total of 23,003 participants were examined for anti-HAV antibodies

Ethnicity Cycle	Cycle 1(total 10175 participants) (%)	Cycle 2 (total 9971 participants) (%)	Cycle 3 (total 9254 participants) (%)	p-value
Sex				0.958
Male	5088 (50)	4892 (49.06)	4557 (49.24)	
Female	5087 (50)	5079 (50.93)	4697 (50.75)	
Race/ethnicity				<0.001
Non-Hispanic White	3674 (36.10)	3066 (30.47)	3150 (34.04)	
Non-Hispanic Black	2267 (22.28)	2129(21.53)	2115(22.58)	
Mexican American	1730 (17)	1921(19.62)	1367(14.77)	
Other Hispanic	960 (9.43)	1308 (13.11)	820 (8.68)	
Non-Hispanic Asian	1074 (10.55)	1042 (10.54)	1168 (12.62)	
Other/Multiracial	470 (4.62)	505 (5.06)	634 (6.58)	
Education				<0.001
<9th grade	455 (4.47)	688 (6.9)	479 (5.02)	
9–11th grade (Includes 12th grade with no diploma)	791 (7.77)	676 (6.77)	638 (6.98)	
High school/GED	1303 (12.8)	1236 (12.39)	1325(14.13)	
Some college/AA	1770 (17.93)	1692 (16.96)	1778 (19.21)	
College graduate	1443 (14.18)	1422 (14.62)	1336 (14.34)	

Table 2: Characteristics of U.S. participants by mineral supplement use: 24 hours (NHANES 2013-2018), cycle 1 (2013-2014); cycle 2 (2015-2016); cycle 3 (2017-2018)

No. of Participants	Cycle 1 participants (%)	Cycle 2 participants (%)	Cycle 3 participants (%)
Minerals supplements			
Zinc (0.0001 to 450 mg)	1600 (15.72)	1316 (13.19)	1283 (13.86)
Calcium (0.18 to 3750 mg)	1579 (15.52)	1302 (13.06)	1267 (13.69)
Phosphorus (0.67 to 1200 g)	647 (6.36)	521 (5.22)	502 (5.42)
Magnesium (0.08 to 4635 mg)	1205 (11.84)	877 (8.79)	883 (9.54)
Iron (0.002 to500 mg)	872 (8.57)	682 (6.83)	657 (7.09)
Copper (0.0002 to 6.1 mg)	1144 (11.24)	877 (8.97)	866 (9.35)
Sodium (0.1 to 1500 mg)	414 (4.06)	378 (3.79)	377(4.07)
Potassium (0.01 to 6750 mg)	722 (7.09)	638 (6.39)	627 (6.77)
Selenium (0.1 to 566 mcg)	1001 (9.83)	806 (8.08)	820 (8.86)

Table 3: Characteristics of U.S. participants by vitamin supplement use: 24 hours (NHANES 2013-2018), cycle 1 (2013-2014); cycle 2 (2015-2016); cycle 3 (2017-2018)

No. of Participants	Cycle 1 participants (%)	Cycle 2 participants (%)	Cycle 3 participants (%)
Vitamins supplements			
Thiamin (Vitamin B1) (0.001 to1500 mg)	1046 (10.28)	1071 (10.74)	1302 (14.06)
Riboflavin (Vitamin B2) (0.001 to 401.7 mg)	1040 (10.22)	1674 (16.78)	1304 (14.09)
Vitamin B6 (0.02 to 10010 mg)	1449 (14.24)	1064 (10.67)	1723 (18.62)
Folic acid (1 to 20400 mcg)	1412 (13.87)	1450 (15.44)	1671 (18.06)
Vitamin B12 (0.04 to 60000 mcg)	1553 (15.8)	1562 (15.66)	1760 (19.02)
Vitamin C (0.33 to 18000 mg)	1607 (15.79)	1635 (16.39)	1876 (20.72)
Vitamin K (0.1 to 600 mcg)	787 (7.73)	788 (7.8)	932 (10.07)
Vitamin D (D2 + D3) (0.004 to 2570 mcg)	1803 (17.72)	1810 (18.15)	2044 (22.09)

As shown in Table 4, sex, age, vaccination status, education status, race, BMI, and all minerals were not significantly different at the 0.05 level. These findings indicate that there was no direct association between mineral intake and anti-HAV antibody production.

As shown in Table 5, significant interactions across combined cycles were observed for phosphorus ($p = 0.002$), iron ($p = 0.001$), zinc ($p < 0.001$), copper ($p=.001$), potassium ($p < 0.001$), and selenium ($p < 0.001$), indicating variation in these associations across cycles.

Table 4: Variables in the equation showing binary logistic regression analysis for mineral supplement use over 24 hours and anti-HAV antibodies

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	Gender (1)	-0.111	0.644	0.030	1	0.863	0.895	0.253	3.159
	Age	-0.023	0.013	2.921	1	0.087	0.978	0.953	1.003
	Vaccination	0.271	0.143	3.587	1	0.058	1.311	0.991	1.736
	Race			3.293	4	0.510			
	Education			3.149	12	0.994			
	BMI			0.080	3	0.994			
	Ca	0.001	0.001	3.133	1	0.077	1.001	1.000	1.002
	P	0.001	0.005	0.073	1	0.788	1.001	0.992	1.011
	Mg	-0.001	0.002	0.131	1	0.718	0.999	0.996	1.003
	Fe	0.013	0.019	0.441	1	0.507	1.013	0.976	1.051
	Zn	-0.016	0.017	0.878	1	0.349	0.984	0.951	1.018
	Cu	0.050	0.027	3.387	1	0.066	1.051	0.997	1.108
	Na	-0.006	0.005	1.468	1	0.226	0.994	0.983	1.004
	K	0.001	0.004	0.122	1	0.727	1.001	0.994	1.009
	Se	-0.010	0.008	1.580	1	0.209	0.990	0.974	1.006
	Constant	2.792	3.085	0.819	1	0.365	16.314		

a. Variables entered on step 1: gender, age, vaccine, race, education, BMI, Ca, P, mg, Fe, Zn, Cu, Na, K, Se.

Table 5: Test of heterogeneity of minerals across cycles

	Cycle 1	Cycle 2	Cycle 3	Combined cycle
	Sig.	Sig.	Sig.	Sig.
Ca	0.820	0.333	0.166	0.163
P	0.244	0.000*	0.000*	0.000*
mg	0.314	0.634	0.926	0.882
Fe	0.965	0.010*	0.008*	0.001*
Zn	0.060	0.195	0.002*	0.000*
Cu	0.051	0.001*	0.626	0.001*
Na	0.643	0.130	0.648	0.100
K	0.239	0.000*	0.000*	0.000*
Se	0.225	0.250	0.000*	0.000*

No significant heterogeneity was detected for calcium, magnesium, sodium, or potassium ($p > 0.05$), indicating the consistency of these predictors across cycles.

Binary logistic regression models were used to examine the associations between vitamin and mineral supplement nutrient intake and anti-HAV serostatus after adjustment for the selected demographic and clinical covariates.

In the mineral model as shown in Table 6, none of the examined mineral intake variables showed a statistically significant association with anti-HAV serostatus at the 0.05 level. The adjusted model also included sex, age, vaccination history, education, race/ethnicity, and BMI.

In the vitamin model, vitamin D demonstrated an exploratory association with anti-HAV serostatus (OR = 1.000, 95% CI: 1.000–1.001; $p = 0.004$). Given the small effect size and the multiple comparisons performed, these findings should be interpreted cautiously. However, the estimated effect size was very small, with a reported odds ratio of approximately 1.000 per unit of vitamin D intake. Because the nutrient was modeled using its original measurement unit, the small numerical values of the coefficient and odds ratio should not be interpreted as evidence of a strong association.

The statistical significance of the vitamin D association should also be interpreted cautiously because multiple nutrient variables were examined. In particular, 17 nutrient-related tests were considered in the analyses; therefore, the vitamin D findings should be regarded as exploratory rather than as definitive evidence of an association.

The analyses of differences across NHANES cycles were also interpreted as exploratory because the standard binary logistic regression models did not incorporate the complex survey design.

The results in Table 7 show significant interactions

Table 6: Variables in the equation showing binary logistic regression analysis for vitamin supplement use, 24-hour, and anti-HAV antibodies

		B	S.E.	Wald	Df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	Gender (1)	-0.089	0.097	0.839	1	0.360	0.915	0.756	1.107
	Age	-0.001	0.003	0.082	1	0.775	0.999	0.994	1.004
	Vaccination	0.010	0.018	0.323	1	0.570	1.010	0.975	1.047
	Race			8.084	4	0.089			
	Education			23.494	17	0.134			
	BMI			2.327	3	0.507			
	Vitamin B1	0.003	0.003	0.989	1	0.320	1.003	0.997	1.008
	Vitamin B2	0.003	0.002	1.776	1	0.183	1.003	0.999	1.007
	Vitamin B6	0.000	0.000	1.377	1	0.241	1.000	0.999	1.000
	Vitamin B12	0.000	0.000	1.268	1	0.260	1.000	1.000	1.000
	Folic acid	0.000	0.000	2.119	1	0.146	1.000	0.999	1.000
	Vitamin C	0.000	0.000	0.034	1	0.855	1.000	1.000	1.000
	Vitamin K	0.001	0.001	0.604	1	0.437	1.001	0.999	1.003
	Vitamin D	0.000	0.000	8.391	1	0.004*	1.000	1.000	1.001
	Constant	-1.089	1.265	0.741	1	0.389	0.337		

a. Variables entered on step 1: gender, age, vaccination, race, education, BMI, vitamin B1, vitamin B2, vitamin B6, vitamin B12, folic acid, vitamin C, vitamin K, vitamin D.

Table 7: Test of heterogeneity of vitamins across cycles

	Cycle 1	Cycle 2	Cycle 3	Combined Cycles
	Sig.	Sig.	Sig.	Sig.
Vitamin B1	0.571	0.492	0.374	0.492
Vitamin B2	0.009*	0.014*	0.327	0.014
Vitamin B6	0.078	0.275	0.637	0.275
Vitamin B12	0.484	0.812	0.511	0.812
Folic acid	0.484	0.328	0.250	0.328
Vitamin C	0.470	0.490	0.307	0.490
Vitamin K	0.156	0.038*	0.008*	0.038*
Vitamin D	0.092	0.267	0.381	0.267

across the combined cycles. The results were observed for vitamin B2 (p = 0.009, 0.014) and vitamin K (p = 0.038, 0.008), indicating variation in these associations across cycles. No significant heterogeneity was detected for vitamin B1, vitamin B6, vitamin B12, folic acid, vitamin C, or vitamin D (p > 0.05), indicating the consistency of these predictors across cycles.

4. Discussion

In this cross-sectional analysis of NHANES participants, we examined the association between vitamin and mineral supplement nutrient intake and anti-HAV serostatus. The analyses did not identify statistically significant associations between the examined mineral supplements and the modeled anti-HAV outcome. Among the vitamins examined, vitamin D intake showed a statistically significant association in the regression model. None of the minerals tested had a significant effect on anti-HAV antibodies, indicating that mineral intake for 24 hours could not be used as a predictor of HAV antibodies, which suggests that the relationship between micronutrient status and the immune response to HAV may be more complex and influenced by other factors, such as nutritional status, infection history, vaccination, or genetic differences in immune function. However, some studies have reported that some minerals, such as zinc and selenium, have a well-documented immune system effect against viral infections.[14,15]

The vitamin D findings should, however, be interpreted cautiously. The magnitude of the estimated association was very small, with an odds ratio close to 1.000 per unit of vitamin D intake. In addition, the exposure information was derived from dietary supplement recall data, whereas anti-HAV serostatus reflects immune exposure accumulated over a substantially longer period, including previous infection and/or vaccination. Therefore, the temporal relationship required to infer a causal association cannot be established from these data. The observed association may also reflect residual confounding or other sources of bias. Individuals who use dietary supplements may differ from non-users in health-related behaviors, healthcare utilization, vaccination history, socioeconomic characteristics, or other factors that may also be associated with anti-HAV seropositivity. Some of these factors may not have been fully captured by the variables included in the present models.

These findings should additionally be considered in the context of multiple statistical comparisons. Seventeen

nutrient-related associations were examined, and the vitamin D p value of 0.004 was close to the conventional significance threshold. Therefore, these findings should be considered exploratory and should not be interpreted as evidence that vitamin D supplementation increases anti-HAV antibodies. These results strongly support the importance of calcitriol in stimulating the antimicrobial activities of macrophages and monocytes through VDR-RXR signaling, which triggers the production of cathelicidins that directly influence various respiratory viruses by disrupting viral envelopes.[16-18] Additionally, vitamin D affects the immune system, as its calcitriol active metabolite shifts the production of cytokines from Th1 to Th2.[19-22]

Other studies suggest that vitamin D sufficiency may support optimal antibody-mediated immunity. However, such effects occur over weeks to months rather than hours or days and are typically observed in relation to overall vitamin D status (serum 25(OH)D), not acute short-term supplement consumption. Thus, while vitamin D may plausibly play a long-term role in shaping immune responses, the 2-day exposure measured here cannot directly explain the elevations in anti-HAV IgG levels.[20,23,24] Therefore, further longitudinal or mechanistic studies are needed to confirm the direct association effect of vitamin D on anti-HAV antibodies.

No significant heterogeneity across cycles was detected in the logistic regression of all vitamins except vitamin B1 and vitamin K, which showed significant heterogeneity, indicating consistency in results across different time periods or cycles, and there was no statistically meaningful variation in how the independent variables affected the outcome variable across those cycles.

The absence of statistically significant associations for the other nutrients should likewise not be interpreted as evidence that these nutrients have no biological relationship with immune responses. Rather, the present analysis did not detect statistically significant associations under the specific exposure definitions, covariate adjustments, or statistical models used.

Another important consideration is that the NHANES anti-HAV measurement represents total anti-HAV serostatus and does not establish whether seropositivity resulted from previous natural infection or vaccination. Consequently, the observed associations cannot be interpreted as associations with a specific mechanism of immunity. Overall, the findings provide an exploratory assessment of the relationship between reported supplemental nutrient intake and anti-HAV serostatus. Longitudinal studies and studies incorporating more appropriate measures of habitual nutrient exposure and immune response are needed to clarify whether the observed vitamin D findings represent a reproducible association.

5. Limitations

Several limitations should be considered when interpreting these findings. First, the cross-sectional design of the NHANES precludes causal inference. Second, multiple nutrients were evaluated simultaneously, increasing the possibility of chance findings due to multiple comparisons. Third, the observed association for vitamin D was characterized by a very small effect size; therefore, its clinical significance remains uncertain. Finally, the analyses were performed using standard binary logistic regression, and the complex NHANES sampling design was not explicitly incorporated into variance estimation. Finally, some nutrient variables were highly skewed and were analyzed as continuous variables. The interpretation of odds ratios close to 1.000 is therefore dependent on the measurement unit of the nutrient and should not be taken to indicate a clinically meaningful effect without further investigation.

6. Conclusion

In this cross-sectional analysis, no statistically significant associations were observed between the examined mineral supplement nutrients and anti-HAV serostatus. Vitamin D intake showed a statistically significant association in the binary logistic regression model; however, the magnitude of the association was very small, and the findings should be interpreted cautiously because of the cross-sectional design, the short-term nature of the supplement recall measure, the inability of total anti-HAV testing to distinguish previous infection from vaccination, and the number of nutrient comparisons examined. These findings should therefore be considered exploratory and do not establish a causal relationship between vitamin D supplementation and anti-HAV immunity. Further studies using longitudinal designs and more comprehensive measures of habitual nutrient exposure and immune status are warranted.

CRediT Author Contribution Statement

Hussein S. Salama: Conceptualization, Data curation, Methodology, Formal analysis, Writing-Original draft preparation, Writing- Review & editing**. Ling Yang:** Conceptualization, Data curation, Validation, Investigation, Supervision, Writing- Review & editing. **Hadeer M. Nageeb:** Conceptualization, Data curation, Methodology, Writing-Original draft preparation, Writing- Review & editing. **Mostafa I. Abdelglil:** Investigation, Writing- Review & editing. **Mingxuan Hou:** Data curation, Investigation, Writing- Review & editing. **Mohamed T. Shaaban:** Writing-Original draft preparation. All authors have read and agreed to the published version of the manuscript.

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Ethics Statement

This study is a secondary analysis of publicly available, de-identified data from the National Health and Nutrition Examination Survey (NHANES). The NHANES protocol was approved by the NCHS Research Ethics Review Board, and informed consent was obtained from all participants by the survey organizers. As this analysis used anonymized data with no direct participant contact, additional institutional review board approval or informed consent was not needed, in accordance with the applicable legal and institutional requirements for studies involving secondary data. The authors accessed and used the data in compliance with the NCHS data use guidelines and in line with the principles of the Declaration of Helsinki.

Data Availability Statement

The datasets and supporting analytical results generated and/or analyzed during this study are available from the corresponding author upon reasonable request.

Conflict of Interest

The authors received no specific financial support for the research, authorship, or publication of this work.

Artificial Intelligence (AI) Use Disclosure

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript, and no images were manipulated using AI.

Supporting Information

Not applicable

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