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ORIGINAL ARTICLE

Lower Vitamin D Levels are Associated with Higher Seroprevalence of *Toxoplasma gondii*—a US National Survey Study

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Abstract

Objective: Vitamin D deficiency is associated with high susceptibility to infections. The present study aimed at exploring the relationship between vitamin D levels and *Toxoplasma gondii* (*T. gondii*) infection, on the basis of a nationally representative database.

Methods: The study data came from the National Health and Nutrition Examination Surveys (NHANES) 2001–2004. Participants underwent both *Toxoplasma* IgG antibody testing and serum vitamin D testing. Vitamin D deficiency was defined by a serum 25-hydroxyvitamin D level <20 ng/mL. Multivariate logistic regression and propensity score matching were used to adjust for potential confounders. All analyses were conducted in R software.

Results: A total of 10613 participants were included. Among these, 3973 (37.4%) were vitamin D deficient, and 2070 (19.5%) were seropositive for *T. gondii* IgG antibody. Vitamin D deficiency was found in 42.3% of the seropositive population, compared with 36.3% of the seronegative population (P<0.001). After adjustment for sex, age, body mass index, smoking history, drinking history and testing season, vitamin D deficiency was associated with an elevated risk of *T. gondii* infection (OR=1.303, 95% CI=1.136–1.495, P<0.001). This effect persisted in the propensity matching cohort.

Conclusions: Low vitamin D levels are associated with high seroprevalence of *T. gondii*.

Keywords: Vitamin D, *Toxoplasma gondii*, NHANES, infection

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INTRODUCTION

Zoonoses remain major public health challenges [1]. *Toxoplasma gondii* (*T. gondii*) is an intracellular protozoan parasite that can infect nearly all mammalian cells. It is among the most common parasitic zoonoses worldwide, affecting approximately 30% of the world's population [2]. As a food-borne parasite, *T. gondii* is usually transmitted by ingestion of contaminated food or water. Other transmission routes include congenital transmission, blood transfusion and organ transplantation [3]. The clinical manifestations of *T. gondii* infection are usually subclinical and benign; however, some patients may have overt clinical symptoms, such as lymphadenopathy, hepatitis, ophthalmitis and schizophrenia [4–8].

Vitamin D is the key steroid hormone in bone metabolism. Additionally, vitamin D may play a role in immune regulation [9]. Vitamin D deficiency, which is common in the general population [10,11], has been found to increase susceptibility to some infections, such as

human papillomavirus, latent tuberculosis, COVID-19 and acquired immune deficiency syndrome [12-15]. *In vitro* and *in vivo* studies have demonstrated a protective role of vitamin D against *T. gondii* infection [16,17]; however, the results regarding the relationship between vitamin D status and *T. gondii* infection in humans have been contradictory [18-20]. The present study aimed at exploring the relationship between vitamin D levels and *T. gondii* infection, on the basis of a nationally representative database.

METHODS

Study population

The study data came from the National Health and Nutrition Examination Surveys (NHANES) 2001–2004, periodic surveys conducted by the Centers for Disease Control and Prevention of the United States. All datasets are available online. Participants who received both serum vitamin D testing and *Toxoplasma* IgG antibody testing were selected for this study. All datasets were publicly accessible and anonymous; thus, ethics approval was not required for this study. The dataset can be retrieved freely from the NHANES website (https://www.cdc.gov/nchs/nhanes/about_nhanes.htm).

Assessment of *Toxoplasma* antibody and vitamin D levels

In NHANES 2001–2004, participants 6–49 years of age received *T. gondii* IgG antibody testing through the indirect enzyme immunoassay method. A result of 0 IU/mL (undetectable) was defined as no infection. Participants with detectable antibody levels were diagnosed with *T. gondii* infection. The active form of vitamin D in the human body is 25-hydroxyvitamin D (25(OH)D). Serum vitamin D status is usually evaluated with serum 25(OH)D levels [21]. Vitamin D deficiency is defined as a serum 25(OH)D level < 20 ng/mL [22].

Statistical analysis

Categorical variables are expressed as percentages and were compared with chi-squared tests. Continuous variables are expressed as means with standard deviation, and were compared with the Student t-test (for normally distributed variables) or Mann-Whitney U-test (for non-normally distributed variables). The risk of *T. gondii* infection may increase with age; moreover, vitamin D levels have been reported to be influenced by sex, season, race and body mass index (BMI) [23,24]. To fully adjust for potential confounders, we used two methods to investigate the relationship between serum 25(OH)D levels and *T. gondii* infection: multivariate analysis and propensity score matching (PSM). Patients were matched 1:1 according to their propensity scores, and the match tolerance value was set at 0.0001. All tests were two-tailed, and a P value less than 0.05 was considered statistically significant. All analyses were conducted in R 3.6.2 (https://www.r-project.org/).

RESULTS

Characteristics of the study population

A total of 10613 participants who underwent both serum vitamin D testing and serum *T. gondii* IgG antibody testing were included in this study (Fig 1). A total of 5153 (48.6%) participants were male, and the average age was

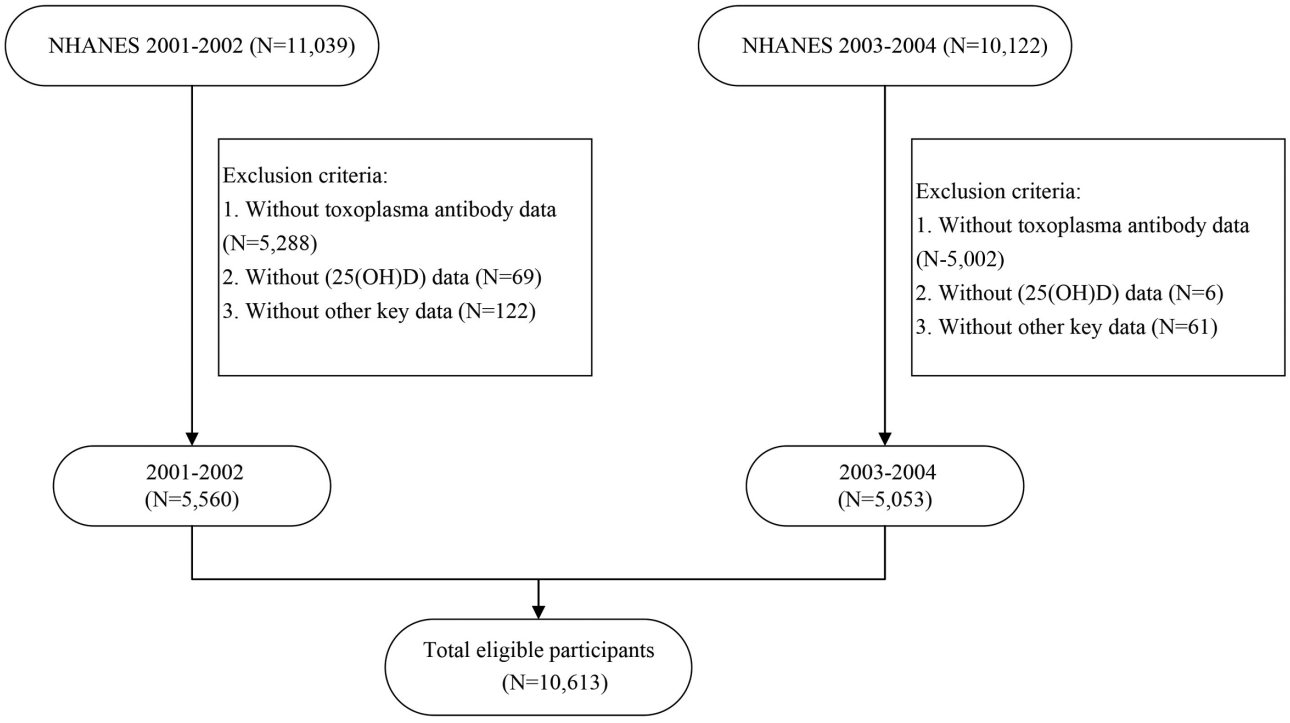

FIGURE 1 | Flowchart of case selection.

22.5 ± 12.1 years. Approximately half the participants (5333, 50.2%) received blood testing in a cold season. A total of 3973 (37.4%) participants were diagnosed with vitamin D deficiency. More details are shown in Table 1.

Comparison between the *T. gondii* IgG seropositive and seronegative groups

A total of 2070 (19.5%) participants were positive for *T. gondii* IgG antibody. The study cohort was then divided into a seronegative group and seropositive group according to the results. The seropositive participants were significantly older than the seronegative participants (27.3 ± 12.4 *vs.* 21.4 ± 11.8, $P<0.001$). The proportion of males was higher in the seropositive group (50.8% *vs.* 48.0%, $P=0.023$). The serum 25(OH)D levels were significantly lower in the seropositive group than the seronegative group (22.2 ± 8.5 *vs.* 23.5 ± 9.0, $P<0.001$). A total of 42.2% participants had vitamin D deficiency in the seropositive group, compared with 36.3% in the seronegative group ($P<0.001$). More details are shown in Table 1.

Association between *T. gondii* infection and vitamin D levels in the entire population

The risk of *T. gondii* infection increases with age, and vitamin D levels are influenced by age, sex, season, race and BMI [23,24]; thus, these factors may be major confounders in the assessment of the association between vitamin D levels and the risk of *T. gondii* infection. We used multivariate analysis to adjust these confounding factors. The variables with a *P* value less than 0.05 in univariate analysis were candidates for multivariate analysis. After adjustment for sex, age, BMI, smoking history, drinking history and testing season, serum 25(OH) D levels were found to be inversely associated with the risk of *T. gondii* infection (OR=0.986, 95% CI=0.980–0.992, $P<0.001$). Vitamin D deficiency was associated with a higher risk of *T. gondii* infection (OR=1.219, 95% CI=1.097–1.354, $P<0.001$, Table 2). In the subgroup analysis, the effects of vitamin D status on *T. gondii* infection were predominantly found in participants 40–50 years of age (S1 Table).

TABLE 1 | Baseline characteristics of the study population.

Variables	Total (n=10613)	Seronegative (n=8543)	Seropositive (n=2070)	*P* value
Male, n (%)	5153 (48.6)	4101 (48.0)	1052 (50.8)	0.023
Age (years)	22.5 ± 12.1	21.4 ± 11.8	27.3 ± 12.4	<0.001
Age group, n (%)				<0.001
6–14	3367 (31.7)	3018 (35.3)	349 (16.9)	
15–20	2741 (25.8)	2247 (26.3)	494 (23.9)	
21–30	1655 (15.6)	1286 (15.1)	369 (17.8)	
31–40	1492 (14.1)	1060 (12.4)	432 (20.9)	
41–49	1358 (12.8)	932 (10.9)	426 (20.6)	
Season, n (%)				<0.001
Cold season	5333 (50.2)	4114 (48.2)	1219 (58.9)	
Warm season	5280 (49.8)	4429 (51.8)	851 (41.1)	
Race, n (%)				<0.001
Mexican American	2950 (27.8)	2272 (26.6)	678 (32.8)	
Other Hispanic	423 (4.0)	284 (3.3)	139 (6.7)	
Non-Hispanic White	3839 (36.2)	3234 (37.9)	605 (29.2)	
Non-Hispanic Black	2969 (28)	2417 (28.3)	552 (26.7)	
Other	432 (4.1)	336 (3.9)	96 (4.6)	
Hypertension, n (%)	921 (8.7)	649 (7.6)	272 (13.1)	<0.001
Diabetes, n (%)	264 (2.5)	186 (2.2)	78 (3.8)	<0.001
Cigarette consumption >100, n (%)	2093 (19.7)	1532 (17.9)	561 (27.1)	<0.001
Overdrinking, n (%)	1190 (11.2)	859 (10.1)	331 (16)	<0.001
25(OH)D level (ng/mL)	23.2 ± 8.9	23.5 ± 9.0	22.2 ± 8.5	<0.001
Vitamin D deficiency, n (%)	3973 (37.4)	3100 (36.3)	873 (42.2)	<0.001

TABLE 2 | Multivariate regression analysis of *T. gondii* infection.

Variables	OR	95% CI	*P* value
Vitamin D deficiency	1.219	1.097–1.354	<0.001
Male	1.150	1.041–1.270	0.006
Age (years)	1.040	1.035–1.046	<0.001
Race	0.916	0.879–0.955	<0.001
Warm season	0.672	0.605–0.746	<0.001
Hypertension	1.085	0.919–1.282	0.334
Diabetes	0.994	0.748–1.321	0.967
Smoking history	0.929	0.81–1.065	0.291
Drinking history	1.171	1.005–1.365	0.044
BMI ≥25 (kg/m^2)	0.923	0.826–1.032	0.160

Comparison between the *T. gondii* IgG antibody seropositive and seronegative groups in a propensity matching cohort

We used propensity scores to match the following potential confounders between the seropositive group and seronegative group: age, sex, testing season, hypertension, diabetes, smoking history and drinking history. The comparison between groups after PSM (summarized in Table 3) indicated that the differences in most variables between groups were attenuated or diminished after matching. Compared with the seronegative group, the seropositive group still had a significantly lower serum 25(OH)D level (22.2 ± 8.5 vs. 23.7 ± 9.1, P <0.001) and a higher proportion of vitamin D deficiency (42.1% vs. 35.5%, P <0.001) after PSM. A total of 54.3% participants in the vitamin D deficiency group were seropositive for *T. gondii* IgG antibody, compared with 47.3% in the vitamin D sufficiency group (P <0.001, Fig 2A). The risk of *T. gondii* infection declined with the increase in vitamin D levels (Fig 2B).

TABLE 3 | Comparison between the *T. gondii* antibody seronegative and seropositive populations after propensity score matching.

Variables	Seronegative (n=1918)	Seropositive (n=1918)	*P* value
Male, n (%)	1015 (52.9)	962 (50.2)	0.093
Age (year)	25.5 ± 12.9	26.3 ± 12.2	0.041
Season, n (%)			0.743
Cold season	1138 (59.3)	1127 (58.8)	
Warm season	780 (40.7)	791 (41.2)	
Race, n (%)			0.148
Mexican American	667 (34.8)	624 (32.5)	
Other Hispanic	89 (4.6)	106 (5.5)	
Non-Hispanic White	616 (32.1)	587 (30.6)	
Non-Hispanic Black	467 (24.3)	523 (27.3)	
Other	79 (4.1)	78 (4.1)	
Hypertension, n (%)	182 (9.5)	219 (11.4)	0.057
Diabetes, n (%)	36 (1.9)	36 (1.9)	1.000
Cigarette consumption >100, n (%)	527 (27.5)	484 (25.2)	0.124
Overdrinking, n (%)	294 (15.3)	266 (13.9)	0.217
25(OH)D level (ng/mL)	23.7 ± 9.1	22.2 ± 8.5	<0.001
Vitamin D deficiency, n (%)	680 (35.5)	808 (42.1)	<0.001

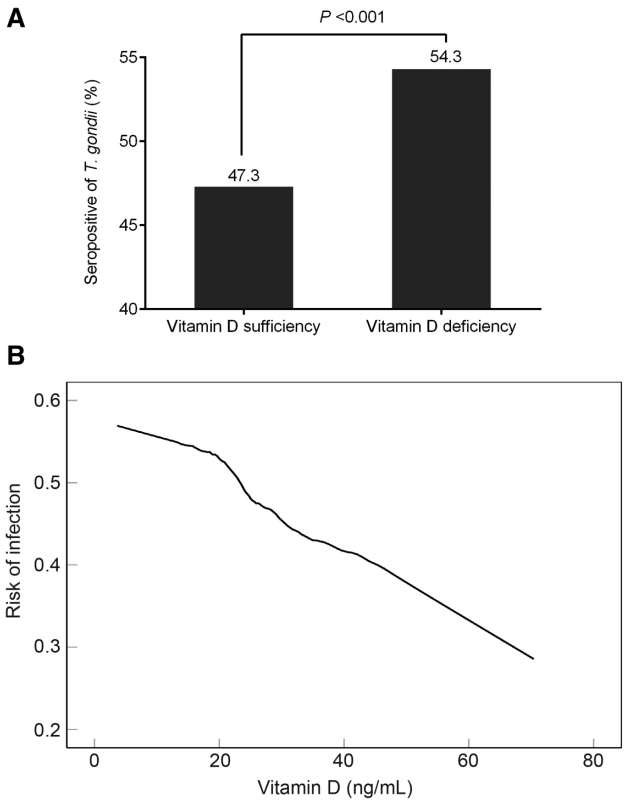

FIGURE 2 | A: *T. gondii* antibody seropositivity rates in the vitamin D deficient and sufficient groups; B: Loess plot of the prevalence of *T. gondii* antibody seropositivity (risk of infection) as a function of serum 25-hydroxyvitamin D level.

TABLE 4 | Multivariate regression analysis of *T. gondii* infection in the propensity score matching cohort.

Variables	OR	95% CI	*P* value
Vitamin D deficiency	1.299	1.132–1.490	<0.001
Male	0.936	0.823–1.065	0.315
Age (years)	1.006	1.000–1.013	0.051
Race	1.011	0.960–1.066	0.669
Warm season	1.064	0.928–1.220	0.372
Hypertension	1.157	0.924–1.449	0.204
Diabetes	0.802	0.495–1.300	0.372
Smoking history	0.829	0.695–0.989	0.038
Drinking history	0.923	0.756–1.128	0.435
BMI ≥25 (kg/m^2)	1.042	0.904–1.200	0.573

Association between *T. gondii* infection and vitamin D levels in a propensity matching cohort

Multivariate logistic regression analysis was performed in the propensity matching cohort. After adjustment for sex, age, BMI, smoking history, drinking history and testing season, serum 25(OH)D levels remained inversely associated with the risk of *T. gondii* infection (OR=0.982, 95% CI=0.975–0.990, $P<0.001$), and vitamin D deficiency was associated with a higher risk of *T. gondii* infection (OR=1.299, 95% CI=1.132–1.490, $P<0.001$) (Table 4).

DISCUSSION

In this study, we found that individuals who were positive for *T. gondii* IgG antibody had lower serum 25(OH)D levels. Vitamin D insufficiency was significantly associated with the risk of *T. gondii* infection. This effect persisted after adjustment for potential confounding factors.

The conclusions in studies of the association between vitamin D levels and *T. gondii* antibody seroprevalence have been controversial [18]. Two studies with small sample sizes have reported that vitamin D deficiency increases the risk of *T. gondii* infection [18,19]. However, another study with three cohorts and a larger sample size (n=663) has rejected this hypothesis [20]. In the present study, we used a nationally representative dataset and found a strong association between vitamin D deficiency and the risk of *T. gondii* infection. We used two methods to fully adjust for confounders, and found that the effects of vitamin D status were independent of age and other potential confounders. The results strongly support the hypothesis that vitamin D levels may play a protective role against *T. gondii* infection.

The inverse relationship between low vitamin D levels and high *T. gondii* seroprevalence may be explained by vitamin D deficiency impairing both the innate and adaptive immune responses [25]. As reported by Rajapakse et al., 1,25-dihydroxyvitamin D3 may inhibit intracellular *T. gondii* proliferation *in vivo* and *in vitro* [16]. However, the benefit of vitamin D supplementation against *T. gondii* infection has not yet been demonstrated in humans, and further investigation is needed.

Beyond a direct protective effect of vitamin D, our results may be explained by factors that confound the estimation of the relationship between vitamin D level and *T. gondii* infection. As reported by previous studies, patients with metabolic syndromes tend to have lower vitamin D levels [8,26]. Moreover, *T. gondii* exposure is elevated in women with obesity and low dietary quality [27]. Inadequate diet and higher metabolic risk may link lower vitamin D levels to a higher risk of foodborne toxoplasmosis.

Despite extensive efforts to minimize the possible influence of confounders, this study has several inevitable limitations. First, owing to the cross-sectional nature of the study, we were unable to establish a causal relationship between low vitamin D levels and *T. gondii* infection. A further prospective study would be needed to verify this finding. Second, sociodemographic risk factors were not analyzed in this study. A recent study using the same dataset as our study has found that *T. gondii* exposure is associated with unhealthful diets in women with low income [27]. Sociodemographic factors might also have played a role in our findings. Third, we were unable to analyze the use of vitamin D supplementation in this study, because corresponding data were not available in the original database. However, because 25(OH)D is the active form of vitamin D in the human body, serum 25(OH)D may represent the real vitamin D status regardless of the use of vitamin D supplements. Finally, this study included people younger than 50 years. Thus, associations in the older population remain unclear.

In conclusion, lower vitamin D levels are associated with a higher seroprevalence of *T. gondii* in the United States population less than 50 years of age.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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