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Lower vitamin D level is associated with higher prevalence of Toxoplasma gondii infection – a national survey study

Running title

Vitamin D and Toxoplasma gondii

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Author Contributions

Huang JF, Wu YL and Wang MF conducted the formal statistical analysis and data management. Huang JF and Wu YL drafted the paper. Lin S and Zhu YY revised the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The author declared no conflicts of interest.

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Ethical approval

The NHANES dataset was publicly available anonymous data, therefore further ethics approval was not required. All patient records and information were anonymized prior to the analysis.

Disclosure statement

The authors have no conflicts of interest to declare.

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Data Availability Statement

Publicly available datasets were analyzed in this study. The raw data are available from National Health and Nutrition Examination Survey program (https://www.cdc.gov/nchs/nhanes/index.htm). The validation dataset was available on request.

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Abstract

Objective: Vitamin D deficiency has been found to be associated with a high susceptibility to infections. The present study was to explore the relationship between vitamin D and Toxoplasma gondii (*T. gondii*) infection based on a nationally representative database.

Methods: The study data came from the National Health and Nutrition Examination Surveys (NHANES) 2001-2004. Participants under 50 years old received both toxoplasma antibody test and serum vitamin D level test. The multivariate logistic regression and the propensity score matching methods were used to adjust potential confounders. All analysis was conducted by R 3.6.2.

Results: A total of 10613 participants were included in this study, among them 3973 (37.4%) were diagnosed with vitamin D deficiency and 2070 (19.5%) were positive in *T. gondii* antibody (seropositive group). Vitamin D deficiency was found in 42.3% of the seropositive group, compared to 36.3% in seronegative group (P<0.001). After adjusted for sex, age, body mass index, smoking history, drinking history and testing season, vitamin D deficiency was associated with higher risk of *T. gondii* infection rate (OR=1.303, 95% CI=1.136–1.495, P<0.001). This result persisted in the propensity matched cohort.

Conclusions: Low serum vitamin D level is associated with higher seroprevalence of toxoplasmosis in American population aged < 50.

Key words: Vitamin D; T. gondii; NHANES; infection

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Introduction

Zoonoses is a major challenge for public health[1]. *Toxoplasma gondii* (*T. gondii*) is an intracellular protozoan parasite that can infect nearly all mammalian cells. It is one of the common parasitic zoonoses world-wide, affecting approximately 30% of the world's population[2]. Although most of the toxoplasma infections are subclinical and benign, some may cause severe consequences, such as lymphadenopathy, hepatitis, ophthalmitis, schizophrenia and other important organ dysfunction[3-7].

Vitamin D is a steroid hormone that plays a crucial role in bone metabolism and immune regulation[8]. Vitamin D deficiency is common in general population and is associated with various health problems[9,10]. Recently vitamin D deficiency has been found to be associated with a high susceptibility to some infections, such as human papillomavirus, latent tuberculosis, COVID-19 and acquired immune deficiency syndrome [11-14]. In-vitro and in-vivo studies also demonstrated a protective role of vitamin D against *T. gondii* infection[15,16], however, the correlation between vitamin D status and toxoplasmosis in human were contradictory[17-19].

The present study was to explore the relationship between vitamin D level and *T. gondii* infection based on a nationally representative database.

Methods

Study population

The study population came from the National Health and Nutrition Examination

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Surveys (NHANES) 2001-2004, which was a periodic survey conducted by the Centers for Disease Control and Prevention of the United States and the dataset was available online. Participants with both vitamin D test and serum toxoplasma antibody test were selected in this study. This study only utilized public anonymous data; therefore, further ethics approval was not required.

Tests of toxoplasma antibody and vitamin D level

In NHANES 2001-2004, only participants aged 6-49 years received toxoplasma antibody tests, which was measured using the indirect enzyme immunoassay (EIA) method. Those test samples with results below 0 IU/mL indicated no infection in this report, while those with results greater than 0 IU/mL were considered to be positive, indicating *T. gondii* infection.

Serum 25-hydroxyvitamin D (25(OH)D) was tested in this survey population. 25(OH)D is the active form of vitamin D and it has been regarded as a biomarker for evaluation of vitamin D status[20]. Serum 25(OH)D concentration was measured by DiaSorin RIA kit (Stillwater MN). Vitamin D deficiency group was defined as the serum 25(OH)D level < 20 ng/mL[21].

All datasets used in this study and the corresponding details of the tests could be retrieved in the NHANES website (https://www.cdc.gov/nchs/nhanes/about_nhanes.htm).

Statistical analysis

Categorical variables were expressed as percentages. Continuous variables were

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expressed as means ± standard deviation (SD). The Student t-test (for variables normally distributed), the Mann-Whitney U-test (for variables non-normally distributed) and the Chi-squared test (for categorical variables) were used to compare the differences between two groups. As the risk of *T. gondii* infection may increase with age, and vitamin D level has been reported to be influenced by sex, season, race, body mass index (BMI) etc [22,23], in order to explore the relationship between serum 25(OH)D level and toxoplasma infection, we used two methods to adjust for potential confounders: the multivariate analysis and the propensity score matching (PSM). Patients were matched 1:1 based on their propensity scores, and the match tolerance value was 0.0001. All tests were two-tailed and a *P* value less than 0.05 was statistically significant. All analysis was conducted by R 3.6.2 (https://www.r-project.org/).

Results

Characteristics of study population

A total of 10613 participants underwent both vitamin D test and serum toxoplasma antibody test were included in this study (Figure 1), of whom 5153 (48.6%) were male and the average age was 22.5 ± 12.1 years old. Half of the study population (5333, 50.2%) were tested in cold season. A total of 3973 (37.4%) were diagnosed with vitamin D deficiency. More details were shown in Table 1.

Comparison of characteristics between *T. gondii* antibody seropositive group and

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seronegative group

There were 2070 (19.5%) cases positive for *T. gondii* antibody. The participants were divided into seronegative group and seropositive group accordingly. Seropositive participants were older than seronegative ones (27.3 ± 12.4 *vs*. 21.4 ± 11.8, *P*<0.001). Males were more common in seropositive group (50.8% *vs*. 48.0%, *P*=0.023). The serum 25(OH)D level was significantly lower in seropositive group (22.2 ± 8.5 *vs*. 23.5 ± 9.0, *P*<0.001). In seropositive group, there were 42.2% cases diagnosed with vitamin D deficiency, compared to 36.3% in seronegative group (*P*<0.001). Other details are shown in Table 1.

The association between *T. gondii* infection and vitamin D level in overall population

The risk of *T. gondii* infection may increase with age, and vitamin D level has been reported to be influenced by sex, season, race and BMI[22,23]. We used multivariate analysis to evaluate the correlation between *T. gondii* infection and vitamin D level. The variables with a *P* value less than 0.05 in univariate analysis were included in multivariate analysis. After adjusting for sex, age, BMI, smoking history, drinking history and testing season, serum 25(OH)D level was inversely associated with *T. gondii* infection rate (OR=0.986, 95% CI=0.980–0.992, *P*<0.001). Vitamin D deficiency was associated with higher risk of *T. gondii* infection (OR=1.219, 95% CI=1.097–1.354, *P*<0.001). (Table 2)

Comparison of characteristics between *T. gondii* antibody seropositive group and

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seronegative group in a propensity matching cohort

We used PSM to match potential confounders between seropositive and seronegative groups, including age, sex, testing season, hypertension, diabetes, smoking history and drinking history. The comparison between groups after PSM was summarized in Table 3. The difference between seropositive and seronegative groups attenuated or diminished after PSM. Compared to seronegative group, seropositive group still had significantly lower serum 25(OH)D level (22.2 ± 8.5 vs. 23.7 ± 9.1, P <0.001) and higher proportion of vitamin D deficiency (42.1% vs. 35.5%, P <0.001) after PSM. 54.3% cases were seropositive in vitamin D deficiency group compared to 47.3% in vitamin D sufficiency group (P <0.001, see Figure 2A). The risk of *T. gondii* infection was decreased with the increase of vitamin D level (see Figure 2B).

The association between *T. gondii* infection and vitamin D level in a propensity matching cohort

Multivariate logistic regression analysis was also performed after PSM to identify potential confounding factors for *T. gondii* infection. After adjusted for sex, age, BMI, smoking history, drinking history and testing season, serum 25(OH)D level was inversely associated with *T. gondii* infection rate (OR=0.982, 95% CI=0.975–0.990, P<0.001) and vitamin D deficiency was associated with higher risk of *T. gondii* infection (OR=1.299, 95% CI=1.132–1.490, P<0.001). (Table 4)

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Discussion

In this study, we found *T. gondii* antibody seropositive participants had lower serum 25(OH)D levels. Vitamin D insufficiency was significantly associated with the risk of *T. gondii* infection. This relationship sustained after adjusting potential confounding factors.

There were controversial conclusions in previous reports regarding the association of vitamin D level and seroprevalence of toxoplasmosis in human[17]. Two small sample size studies showed an association between vitamin D deficiency and the presence of the toxoplasmosis[17,18]. However, another study with three cohorts and larger sample size (n=663) did not find this relationship[19]. In present study, we used a nationally representative dataset and found a strong correlation between vitamin D deficiency and *T. gondii* infection. By considering the age as a risk factor for seroprevalence of toxoplasmosis, we used two methods to adjusted age and other confounders and found that the role of vitamin D was independent of age and other potential confounders. This result strongly supports the hypothesis that vitamin D levels may play a protective role in toxoplasmosis-associated disease.

The inversely relationship between vitamin D level and the positive rate in *T. gondii* antibody could be explained by the theory that vitamin D deficiency may impair both the innate and adaptive immune responses[24]. Several in-vitro or in-vivo studies has demonstrated the protective role of vitamin D against *T. gondii* infection. As reported by Rajapakse et al, 1,25-Dihydroxyvitamin D3 may inhibit intra cellular *T. gondii* proliferation in vivo and in vitro[15]. Moreover, 1,25-Dihydroxyvitamin D3 treatment

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induces splenocyte apoptosis and enhances sensitivity to toxoplasmosis in mice, indicating vitamin D can enhance host susceptibility to toxoplasmosis[16]. However, the benefit of vitamin D supplement against *T. gondii* infection has not yet been proven in human.

Despite extensive efforts have been made to minimize the possible confounding factors of this study, the findings of the study should be interpreted in the presence of few inevitable limitations. First, the cross-sectional nature of this study cannot establish a causal relationship between vitamin D and *T. gondii* infection. Second, the sociodemographic risk factor was not analysis in this study. Last, this study only included population aged less than 50 years old. This association in older population remains unclear.

In conclusion, the lower vitamin D level is associated with higher seroprevalence of toxoplasmosis American population aged less than 50.

Figure Legends

Figure 1 The flowchart of case selection

Figure 2 A: The seropositive rate of *T. Gondii* antibody in vitamin D deficiency and sufficiency groups; B: The risk of *T. Gondii* infection in patients with different vitamin D levels.

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