

# Toxoplasmosis in immunocompetent Saudi women: Correlation with vitamin D

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## Abstract

**Objective:** *Toxoplasma gondii* (*T. gondii*) is a life-threatening parasite particularly infecting the immunocompromised women. Deficiency of vitamin D is well reported in several infectious disorders. This study was undertaken to investigate a correlation of vitamin D deficiency with the onset of *T. gondii* infection in immunocompetent women from the central of Saudi Arabia.

**Methods:** Blood samples were collected from 304 Saudi women from the Qassim region of Saudi Arabia. Specific immunoassays were used to determine the levels of *T. gondii* immunoglobulin G and vitamin D. The SPSS and the Prism Graph Pad statistical software were used for the data analysis.

**Results:** Out of 304 women, 18.8% were found to be positive for toxoplasmosis. Interestingly, the serum levels of vitamin D in toxoplasma positive cases were found to be significantly low as compared with the levels of vitamin D in toxoplasma negative cases. Moreover, sociodemographic risk factors such as age, residence location, and consumption of fruits/vegetables were also found to be associated with vitamin D deficiency and with the seroprevalence of toxoplasmosis.

**Conclusion:** This study investigated a direct correlation of vitamin D deficiency with the severity of the toxoplasmosis in Saudi women. Therefore, it is predicted that vitamin D supplementation may provide protection against toxoplasma infection.

## Keywords

risk factors, Saudi Arabia, *Toxoplasma gondii*, vitamin D, women of childbearing age

Date received: 23 May 2021; revised: 10 August 2021; accepted: 16 August 2021

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## Introduction

*Toxoplasma gondii* (*T. gondii*) is a protozoan zoonotic parasite cause toxoplasmosis and the members of cat family *Felidae* are the definitive hosts of *T. gondii*, which serve as a reservoir of this infection.<sup>1</sup> Toxoplasmosis is present throughout the world and according to the Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA, more than 60% of the population has been infected by *T. gondii*.<sup>2</sup> Despite the limited definitive hosts, toxoplasmosis has been reported on every continent, and in terrestrial as well as aquatic environments and its prevalence varies from region to region depending on the factors such as hygiene, eating habits, cultural practices, socioeconomic status, and association with pets especially cats.<sup>3,4</sup> The remarkable resistance of the oocyst wall enables dissemination of *T. gondii* through watersheds, ecosystems, and long-term persistence in diverse foods and its mode of transmissions has been either congenital or acquired or both that include the consumption or handling of undercooked meat.<sup>4</sup> Congenital infection with *T. gondii* resulted from a primary infection that occurs primarily during pregnancy and/or reactivation of past infection in immunocompromised women.<sup>5</sup> Immunocompetent women with *T. gondii* generally asymptomatic, but remain a serious cause of morbidity and mortality.<sup>6-8</sup> Diagnosis of toxoplasmosis relies on serologic tests, polymerase chain reaction (PCR), and histological examination of infected tissue, these serological examinations efficiently and most commonly used in diagnosis as well as screening for toxoplasmosis.<sup>9,10</sup> Serological examination for human *T. gondii* immunoglobulin G (IgG) is the best measured by enzyme-linked immunosorbent assay (ELISA) method as it is cheap and easy to perform, whereas detection of toxoplasmosis thorough PCR is not common, costly, and requires proficiency.<sup>9-11</sup> Vitamin D is essential for regulation of calcium and phosphorus metabolism and most importantly it plays a role in normal regulation of the immune system.<sup>12,13</sup> Interestingly, the receptors for 1,25-dihydroxy vitamin D<sub>3</sub> are present on human peripheral blood monocytes and also on active lymphocytes, indicating its role as an immune regulator.<sup>14</sup> Studies showed that the vitamin D injection in mice increases nitric oxide production via activation of macrophages, which results in a subsequent decrease of *T. gondii* proliferation.<sup>15</sup> A few studies show an association of vitamin D deficiency with *T. gondii* infection but mainly in women of childbearing age.<sup>16</sup> In the central region of Saudi Arabia, none of the studies till date reported an association of vitamin D deficiency with *T. gondii* infection. Therefore, it is of utmost importance to determine the seroprevalence and risk factors of toxoplasmosis among the women population in this region. To the best of our knowledge, this is the first study that not only investigated an association of vitamin D deficiency with toxoplasmosis, but also determined the risk factors associated with them.

## Methods

### Study design and area

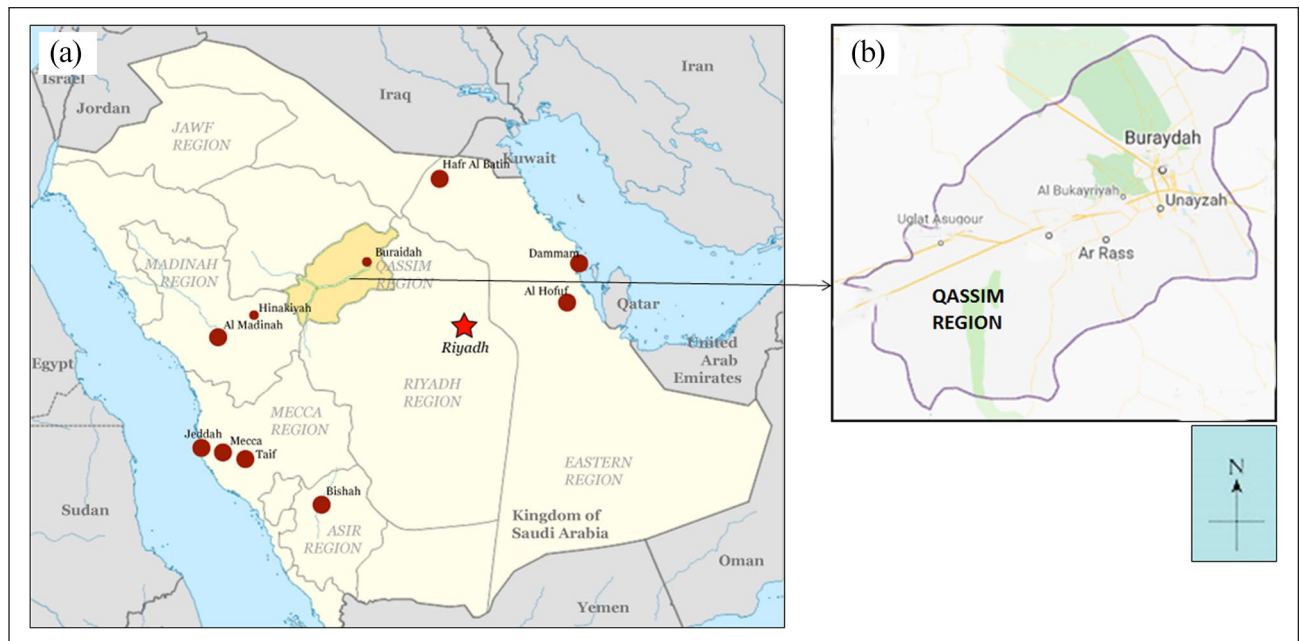
A cross-sectional study followed by the laboratory-based data collection was conducted from February 2018 to January 2019. The study was carried out at Qassim province of Saudi Arabia, which is located at the heart of the country and covers an area of 58,046 km<sup>2</sup>. The Qassim region is about ~450 km northwest of Riyadh, the capital. It is bordered by Riyadh to the south and east, by Hail city to the north, and by Madinah city to the west (Figure 1(a)). The major city of Qassim province is the capital city Buraidah, which has ~65% of the region's total population; other major cities are Unayzah, Al Bukayriyah, Ar Rass, and Uglat Asugour (Figure 1(b)). The setting of this study was the general women health facility of the Qassim University affiliated Medical Clinics, Buraidah, Qassim province, KSA. The facility has general, prenatal, reproductive, and outpatient medical care clinics, and it is where the adult women in this locality seek health care.

### Sample size and study population

The sample size required for this study was calculated according to the World Health Organization (WHO) practical manual for the determination of sample size in health studies.<sup>17</sup> Based on a 95% confidence level, a desired precision of 0.05, and an expected toxoplasmosis seroprevalence of 50%, as none of the previous study was available from the Buraidah city of Qassim province, the minimum sample size required was estimated to be 245 women having health issues. To take account of non-response rate, the sample size was inflated by 10% to get a total sample size of 269. Systematic sampling was employed to identify the women population using lists of the Qassim University affiliated Medical Clinics as a sampling frame. A total of 304 women (age = 38.0 ± 20.24 years) participated from this sampling frame. Inclusion criteria of the participants were women only of above 18 years of age, seeking to utilize facilities such as prenatal, reproductive, and outpatient medical care at the Qassim University affiliated Medical Clinics, Buraidah, Qassim province. Exclusion criteria of the participants were based on the following points: women have chronic viral diseases during 1 week prior to the study, women with cancer and diabetes, and patients with the presence of hepatic or renal disorders.

### Data and sample collection

The data were collected directly from the participants through an administration of a validated questionnaire, which included questions on the sociodemographic information such as age, marital status, monthly income, and residence.<sup>18-20</sup> The questionnaire also included questions to assess the risk factors associated with *Toxoplasma* infection



**Figure 1.** (a) Location of Qassim region in Saudi Arabia. (b) Map of Qassim region showing major cities. The diagrams shown were taken from [http://en.wikipedia.org/wiki/File: Saudi\\_Arabia\\_location\\_map.svg](http://en.wikipedia.org/wiki/File:Saudi_Arabia_location_map.svg) and figure as a whole was adopted from Rasheed et al. BMC Public Health 2019; 19(1): 384.<sup>22</sup>

such as the presence of a cat as a pet in their house, consumption of partially uncooked meat or fruit and vegetables without proper washing, drinking unpasteurized milk or water contaminated with cat feces, and exposure to garden soil without following proper hygienic techniques and hand washing. After explaining the procedure to the participating women, venous blood samples were collected and the sera were separated on the same day by centrifugation and stored at  $-20^{\circ}\text{C}$  till further analysis as described previously.<sup>21</sup>

### Serological detection of *T. gondii* infection

Participants' sera were tested for the detection of *T. gondii* antibodies using a commercial human anti-*T. gondii* IgG ELISA kit according to the instructions of the manufacturer (Institut Virion/Serion GmbH; Wurzburg, Germany). Optical density (OD) was measured at 405 nm and presented in IU/ $\mu\text{L}$ . The test results were also interpreted by calculating, for each sample, a percentage of positivity (PP) value relative to the OD of the positive control (PP Sample =  $\text{OD}_{405} \text{ Sample} / \text{OD}_{405} \text{ Positive Control} \times 100$ ). A PP value  $\geq 20$  was a priori regarded as positive (as suggested by the manufacturer), and re-evaluated after receiver operating characteristic curve analysis; PP values below 20 were considered negative.

### Measurement of vitamin D levels

Vitamin D levels were measured in the serum samples of participants by the Beckman Access 25(OH) Vitamin D

Kit Assays (catalog no. A98857) using the Beckman Coulter UniCel DxI 800 Access Immunoassay System. The measuring range of the Beckman Access total 25(OH) vitamin D in serum is 7–120 ng/mL. The reference range of the total 25(OH) D level in serum is 25–80 ng/mL.<sup>21</sup>

### Ethical consideration

Ethical approval of this study was taken from the Qassim Regional Ethical Committee, Ministry of Health, Saudi Arabia (approval no. 20180804). Written consents were taken from all participants. All participants were informed the purpose of the study and the rights of the participant, confidentiality, and the right to withdraw at any time without any obligation. Participant's anonymity has been assured by assigning each participant with a code number for the purpose of analysis only.

### Statistical analysis

Statistical analysis was performed by International Business Machines Corporation (IBM) SPSS version 21.0 and the descriptive statistics were employed to summarize the data. The statistical comparisons were further validated by GraphPad Prism-8 (San Diego, CA, USA) (one paired two-tailed *t*-test with one-way ANOVA analysis followed by Tukey's post hoc analysis or two-way ANOVA followed by Bonferroni's post hoc tests). Moreover, some data were further validated by the student's *T*-test using the GIGA online *p*-value calculator as described previously.<sup>23,24</sup>

**Table 1.** Association of sociodemographic features with the deficiency of vitamin D in seropositive cases of *Toxoplasma gondii*.

Serial number	Sociodemographic parameters	<i>T. gondii</i> seropositive cases		Vitamin D deficient cases		Seropositivity versus vitamin D deficiency
		%	p-value	%	p-value	p-value comparison
1.	Age $\geq 25$ years	75.4	0.000587	80.7	0.000112	Significantly correlated
	Age $< 25$ years	24.6		19.3		
2.	Married females	64.9	0.017737	52.6	0.348269	No correlation
	Unmarried females	35.1		47.7		
3.	Residence in rural areas	36.8	0.029964	14.0	0.000024	Significantly correlated
	Residence in urban areas	63.1		85.9		
4.	Monthly income $\geq 10,000$ SAR	56.1	0.180548	36.8	0.029964	No correlation
	Monthly income $< 10,000$ SAR	43.9		63.2		
5.	Close contact with cats	73.7	0.001039	49.1	0.440275	No correlation
	Not in contact with cats	26.3		50.9		
6.	Consumption of raw/uncooked meat	66.7	0.009063	49.1	0.440275	No correlation
	Consumption of fully cooked meat	33.3		50.9		
7.	Consumption of washed fruits/vegetables	71.9	0.001860	61.4	0.052409	Significantly correlated
	Consumption of unwashed fruits/vegetables	28.1		38.6		
8.	Consumption of unpasteurized milk	56.1	0.180548	33.3	0.009063	No correlation
	Consumption of pasteurized milk	43.8		66.7		

## Results

### Sociodemographic risk factors and the seroprevalence of *T. gondii* infection

A total of 304 women were recruited from Qassim region of Saudi Arabia. The location of Qassim in Saudi Arabia is given in Figure 1, which was adopted from Rasheed et al., 2019.<sup>22</sup> Out of 304 women tested, 245 (81.2%) were found to be negative for anti-*T. gondii*-specific IgG antibody, whereas 57 (18.8%) were found to have anti-*T. gondii*-specific IgG antibody. All positive cases were determined on the basis of concentration of *T. gondii* IgGs. Among those who tested positive, the average value for IgG levels ranged from 10 to 490 IU/ $\mu$ L. Women with positive IgG results were counseled and referred to the university clinics for further management. The sociodemographic features of the *T. gondii* positive cases were further analyzed in relation to the positivity of *T. gondii* infections, and the details are summarized in Table 1. For analyzing an age as a risk factor for the onset of *T. gondii* infection, the positive cases were divided into two age groups, the higher age group (age  $\geq 25$  years) and a lower age group (age  $< 25$  years). As shown in Table 1, 75.4% cases with higher age groups were found to be positive for *T. gondii* infection, which were significantly higher than the lower age group positive cases ( $p < 0.05$ ). The positive cases were also characterized on the basis of marital status, 64.9% the married women were found to be positive *T. gondii* infection, whereas the positivity of infection in unmarried women was only 35.1% ( $p < 0.05$ ). Moreover, we also characterized the positive cases on the basis of areas of residence, women living in urban areas were found to have

significantly more *T. gondii* infection rate as compared with the females from rural areas ( $p < 0.05$ ). Furthermore, the *T. gondii* infection rate was also analyzed on the basis of monthly incomes of the participants, for that the participants were divided in two groups, the higher income group (monthly income  $\geq 10,000$  SAR) and a lower income group (monthly income  $< 10,000$  SAR). Our analysis showed the monthly income of the participants was not affected with the positivity of *T. gondii* infection ( $p > 0.05$ ). In addition, we also characterized the positive cases on the basis of close contact with the cats as a domestic animal; women in close contact with cats were shown significantly more *T. gondii* infection rate as compared with the women away from cats ( $p < 0.05$ ). Not only have these, the positive cases were also characterized on the basis of the consumption of uncooked or cooked meat and the infection rate was found significantly more in the women consuming uncooked meat as compared to those women consuming fully cooked meat ( $p < 0.05$ ). Moreover, the infection rate of *T. gondii* was also found to be significantly associated with the consumption of unwashed fruits and vegetables ( $p < 0.05$ ), but the consumption of unpasteurized and pasteurized milk was found to be not associated with the onset of *T. gondii* infection ( $p > 0.05$ ).

### Levels of vitamin D in seropositive and seronegative cases of *T. gondii*

The levels of vitamin D were measured in the serum samples obtained from the women infected with *T. gondii* and were compared with non-infected women. The average ( $\pm$ SD) of vitamin D levels in 57 independent *T. gondii*



**Table 2.** Vitamin D levels in *T. gondii* seropositive and seronegative subjects.

	Number	Level of 25(OH) vitamin D, ng/mL (mean $\pm$ SD)	p-value
Seropositive group	57	15.5 $\pm$ 3.2	<0.05
Seronegative group	50	29.9 $\pm$ 8.1	

Vitamin D levels were measured by 25-hydroxy (OH) Vitamin D Immunoassay System (Beckman Coulter UniCel Dxl 800). The normal range of the total 25(OH) D level in women is 25–80 ng/mL.

seropositive cases was found to be  $16.2 \pm 7.5$  ng/mL, whereas the average ( $\pm$ SD) of vitamin D levels in 50 seronegative was  $29.9 \pm 8.1$  ng/mL. These novel data showed that the levels of vitamin D were significantly less in the *T. gondii* seropositive cases as compared with the levels found in non-infected seronegative women ( $p < 0.05$ ). The data are summarized in Table 2.

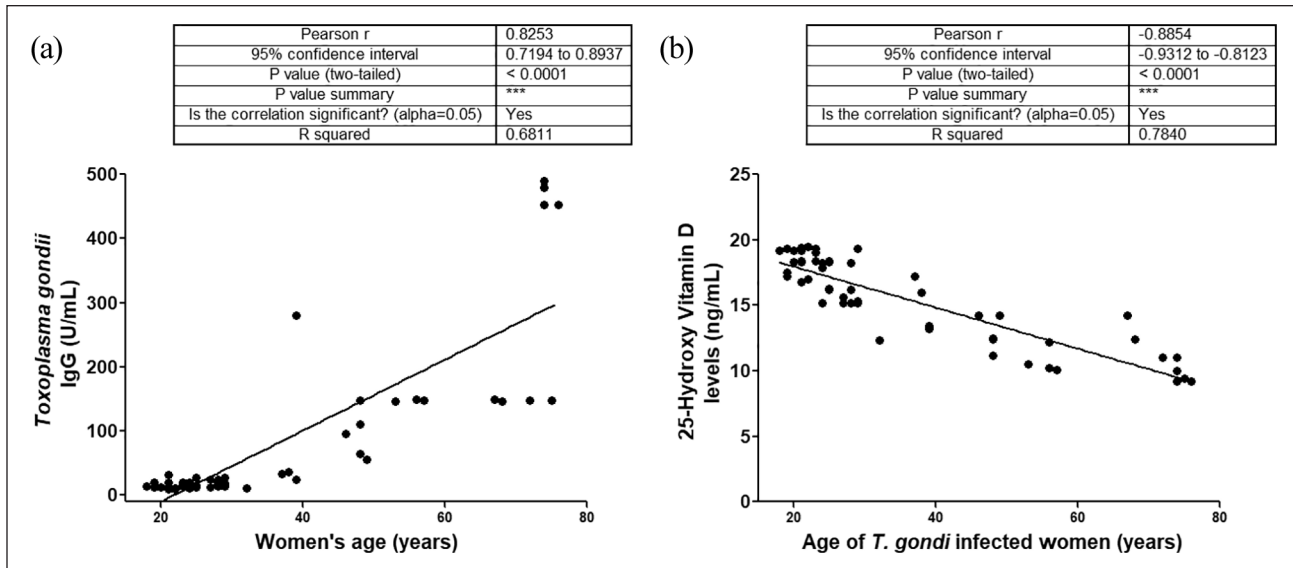
### Relation of the sociodemographic characteristics with the deficiency of vitamin D in the seropositive cases of *T. gondii*

In this study, the data also analyzed the relation of the sociodemographic risk factors with the deficiency of vitamin D in the seropositive cases of *T. gondii* and the details are summarized in Table 1. For analyzing an age as a risk factor for the onset of *T. gondii* infection, the positive cases were divided into two age groups, the higher age group (age  $\geq 25$  years) and a lower age group (age  $< 25$  years). As shown in Table 1, the 80.7% cases with higher age group were found to be vitamin D deficient, which were significantly higher than the lower age group positive cases ( $p < 0.05$ ). The positive cases were also characterized on the basis of marital status, 52.6% the married women were found to be vitamin D deficient, whereas the positivity of infection in unmarried women was found in 47.7% ( $p > 0.05$ ). Moreover, we also characterized the positive cases on the basis of areas of residence, women living in urban areas were found to have significantly more vitamin D deficient rate as compared with the women from rural areas ( $p < 0.05$ ). Furthermore, the deficiency of vitamin D was also analyzed on the basis of monthly incomes of the participants, for that the participants were divided into two groups, the higher income group (monthly income  $\geq 10,000$  SAR) and a lower income group (monthly income  $< 10,000$  SAR). Our data showed the monthly income of the participants significantly improved the status of vitamin D in the studied population ( $p < 0.05$ ). Furthermore, the vitamin D deficiency was not found to be affected with closeness of cats as a domestic animal ( $p > 0.05$ ). Not only have these, the vitamin D deficiency in the *T. gondii* positive cases were also characterized on the basis of the consumption of uncooked or cooked meat

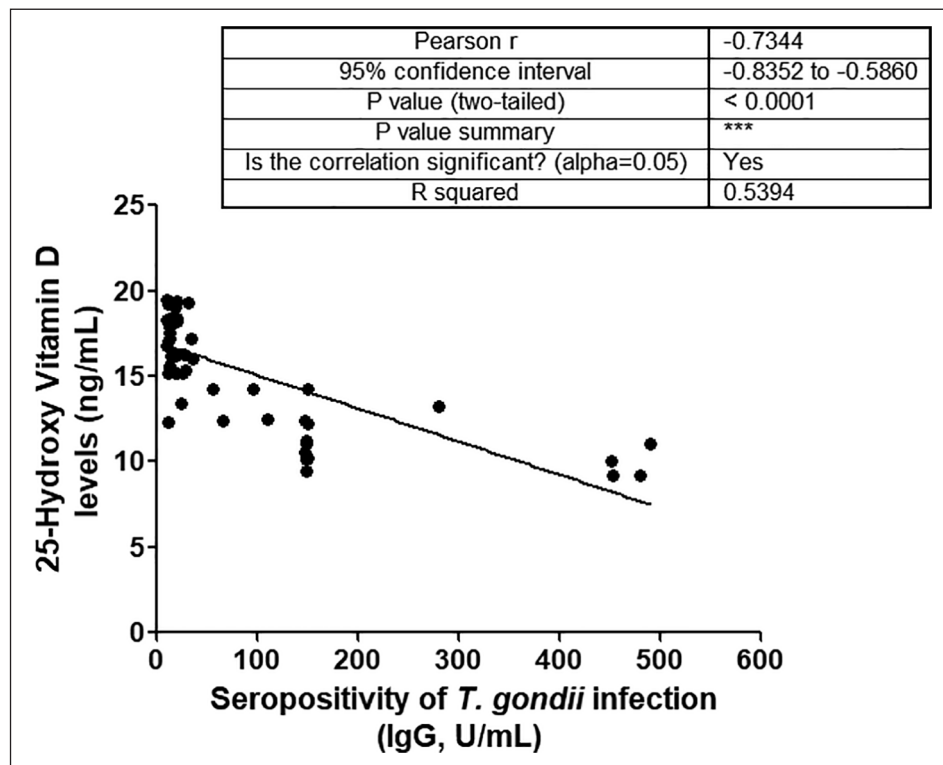
and the levels of vitamin D was found almost same in both groups ( $p > 0.05$ ). Importantly, the vitamin D deficiency was also found to be significantly associated with the consumption of unwashed fruits and vegetables ( $p < 0.05$ ), and also with the consumption of unpasteurized and pasteurized milk ( $p < 0.05$ ). Importantly, the data also analyzed the risk of the sociodemographic factors in relation to deficiency of vitamin D with the seropositivity of *T. gondii* infection. As shown in Table 1, age, residence in rural or urban areas, and consumption of unwashed or washed fruits and vegetables were significantly corrected with seropositivity as well as vitamin D deficiency in the studied women population ( $p < 0.05$ ). Whereas the other sociodemographic factors such as marital status, monthly income, contact with cats, consumption of cooked/uncooked meat, and consumption of pasteurized/unpasteurized milk were not corrected in relation to both seropositivity of *T. gondii* infection and vitamin D deficiency ( $p > 0.05$ ). The complete details are summarized in Table 1. To analyze the data on age as one of the risk factors for *T. gondii* infection and deficiency of vitamin D in depth, correlation of studied positive cases of *T. gondii* infection was studied. As shown in Figure 2(a), the age was found to be significantly corrected with seropositivity of *T. gondii* infection ( $r = 0.8253$ ;  $p < 0.0001$ ). Not only have these, the age of studied positive cases of *T. gondii* infection was also found to be significantly associated with the levels of vitamin D ( $r = -0.8854$ ;  $p < 0.0001$ ) (Figure 2(b)). To analyze the data in depth, the direct correlation of vitamin D levels in the serum with the *T. gondii* infection was studied. As shown in Figure 3, an increase in the severity of *T. gondii* infection significantly decreased the levels of vitamin D in the positive cases of *T. gondii* infection ( $r = -0.7344$ ;  $p < 0.0001$ ).

## Discussion

In this study, we determined the seroprevalence of *T. gondii* infection among the women population from the central region of Saudi Arabia. Screening of women population for seroprevalence of *T. gondii* has always provided health benefits not only for women but also for their infants.<sup>11,25</sup> In this study, only 18.8%, participated female were found to be positive for *T. gondii* infection. Prevalence of toxoplasmosis varies from region to region. Recently, a study conducted on the Moroccan women showed 43% toxoplasmosis.<sup>26</sup> In another study performed on the pregnant women population in Annaba, Algeria reported 47.8% toxoplasmosis.<sup>27</sup> Another recent study performed on women in Dhamar, Yemen, showed 21.2% seroprevalence of toxoplasmosis.<sup>28</sup> A study conducted on Pakistani women population revealed 19.4% toxoplasmosis.<sup>20</sup> Moreover, another study conducted on Lebanon women showed a comparatively high prevalence 82.6%.<sup>29</sup> Interestingly, this variation in the prevalence of toxoplasmosis has also been



**Figure 2.** Correlation of age of Saudi women with the severity of toxoplasmosis (a) and with the serum levels of vitamin D (b) in positive cases of *Toxoplasma gondii* infection.



**Figure 3.** Correlation of serum levels of vitamin D with the severity of *Toxoplasma gondii* infection.

reported in female population different regions of Saudi Arabia. As, 28.5% toxoplasmosis was reported in the Eastern region of the country,<sup>30</sup> 32.2% in Najran region,<sup>31</sup> 35% in Makkah,<sup>32</sup> 38.8% in the South-Western region,<sup>6</sup> and 38% in the capital city Riyadh.<sup>18</sup> The variation in the prevalence of toxoplasmosis in this study and the studies

performed by others may be due to the diversity in sociodemographic multiple factors among different population. To investigate the relation of sociodemographic factors with the seroprevalence of toxoplasmosis in depth, the *T. gondii* positive cases were further analyzed in relation to the sociodemographic risk factors. For considering

age as a risk factor for the onset of *T. gondii* infection, the positive cases were divided in two age groups, the higher age group (age  $\geq 25$  years) and the lower age group (age  $< 25$  years). Our findings showed that the majority of high aged women were positive for *T. gondii* infection, indicating that age plays a role in the onset of toxoplasmosis in women. Furthermore, the positive cases were also characterized on the basis of marital status, and the majority of married women were found to be positive *T. gondii* infection, this suggested that infection rate of this parasite may also associated with the marital status. In addition, the positive cases were also characterized on the basis of areas of residence, women living in urban areas were found to be more infected as compared with the women living in the rural areas. Not only have these, toxoplasmosis rate was also analyzed on the basis of monthly incomes of the participants and was found that it was not affected with the positivity of toxoplasmosis. In addition, we also characterized the positive cases on the basis of close contact with cats as a domestic animal; women in close contact with cats showed association with toxoplasmosis rate. Our data also analyzed that the consumption of cooked meat and washed fruits and vegetables may prevent the onset of this infection. Similar observations on the risk factors associated with the prevalence of toxoplasmosis have also been reported in Egypt, where high prevalence of toxoplasmosis was observed in women with the high age group and also those women from the rural area.<sup>33</sup> In addition, our findings have also been well supported by a number of studies conducted in other parts of the world such as Brazil and China.<sup>34,35</sup> As high prevalence of toxoplasmosis was also observed in the Brazilian women population with the high age group<sup>34</sup> and in the Chinese women population from rural area.<sup>35</sup>

Vitamin D is essential for the controlling proper function of immune system.<sup>36</sup> Multiple in-vitro or in-vivo studies demonstrated that the administration of vitamin D reduces the proliferation of toxoplasmosis.<sup>15</sup> To demonstrate the relation of vitamin D and the *T. gondii* infection, serum level of vitamin D was investigated in the Saudi women and was found to be significantly less in subjects positive for toxoplasmosis. These findings have also been fully supported by number of studies performed in different regions of the globe.<sup>16,37,38</sup> These studies reported an association of vitamin D deficiency with the seroprevalence of toxoplasmosis in different women populations. This might be explained by the assumption that vitamin D deficiency impairs the functionality of immune system that may put the subjects susceptible for toxoplasmosis.<sup>38,39</sup> To investigate the role of vitamin D in the prevalence of toxoplasmosis in detail, the relation of vitamin D with sociodemographic risk factors was studied in seropositive cases with *T. gondii* infection. By considering an age as a risk factor for the prevalence of *T. gondii* infection, the positive cases were analyzed at different age

group women and were found that age plays an important role in the prevalence of *T. gondii* infection, as a positive correlation between age and vitamin D deficiency was investigated in the positive cases of *T. gondii* infection. This important finding on the age of women having *T. gondii* infection was fully supported by a study conducted on the Iranian population.<sup>40</sup> Not only age, a positive correlation of vitamin D deficiency with women living in the rural or urban areas was also investigated. Seropositive women with toxoplasmosis from urban area were found to more vitamin D deficient as compared to those women from rural areas, indicating that exposure of sunlight was more in women from rural areas as compared with the urban area women. Furthermore, our findings also suggest that the consumption of washed fruits and vegetables and drinking of pasteurized milk also prevent and/or lowering the onset of toxoplasmosis. In short, a direct correlation of vitamin D deficiency with the severity of the *T. gondii* infection was investigated in this study. In addition, several sociodemographic risk factors were also investigated in relation to the positive association of vitamin D deficiency in Saudi women with toxoplasmosis. Despite extensive efforts have been made to minimize the possible shortcoming of this study, the findings of the study should be interpreted in the presence of few inevitable limitations. The most obvious limitation of the study is a single region of sample collection. It would be better that sample collection should not be confined to only one region. In addition, the cross-sectional nature of this study has not been allowed us to form a casual relationship between the outcome and predictor variables.

## Conclusion

This is the first study from the central region of Saudi Arabia investigated a direct correlation of vitamin D deficiency with the severity of the toxoplasmosis in adult women. Specifically, the study determined vitamin D deficiency was positively associated with toxoplasmosis in Saudi women. In addition, several sociodemographic risk factors such as age, residence location, and consumption of fruits/vegetables were also found to be associated with the vitamin D deficiency and the seroprevalence of toxoplasmosis. These findings may suggest that vitamin D supplementation is beneficial for women with toxoplasmosis.

## Acknowledgements

The authors acknowledged the College of Medicine, Qassim University for providing research facilities. The authors also thank Mr Casimero A Victoria for helping in experimentation.

## Author contributions

A.S., G.B.A., G.S.A., A.I.A., F.S.A., D.F.A., S.S.A., R.A., T.A., N.A.A., O.A.R., and O.F.S. carried out the experimentation and data interpretation. W.A.A. and Z.R. involved the study design,

coordination, data interpretation, and article drafting. All authors have read and approved the final article.

### Availability of data and materials

The data used in this study are available and will be provided by the corresponding author on a reasonable request.

### Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

### Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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