

RESEARCH ARTICLE

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# Detection of Toxoplasmosis in Association with Autoimmune Thyroid Disease During Pregnancy in Duhok, Iraq

Manal Adil Murad<sup>1\*</sup>  and Souzan Hussain Eassa<sup>2,3</sup> 

<sup>1</sup>Department of Biology, College of Science, University of Duhok, Duhok, Iraq.

<sup>2</sup>Department of Anatomy, College of Medicine, University of Duhok, Duhok, Iraq.

<sup>3</sup>BioMed Division, School of Medicine, University of Kurdistan Hewler, Erbil, Iraq.

## Abstract

*Toxoplasma gondii* is a protozoan parasite that is widely distributed in the human population and is responsible for corresponding global morbidity. Specifically, *T. gondii* causes toxoplasmosis, leading to miscarriage, stillbirth, and neural disorders. This parasite attacks different human organs and glands, such as the thyroid gland, and causes various corresponding health issues. Recently, studies have established a link between *T. gondii* and autoimmune thyroid diseases (AITD), which contributes to preterm delivery, miscarriage, low birth weight, and death. Therefore, the aim of this study was to detect the prevalence of toxoplasmosis and its association with AITD among pregnant women. A total of 180 blood samples were collected from pregnant women and examined using an enzyme-linked immunosorbent assay (ELISA). The patients were within the age range of 15–50 years old, and lived in Duhok City, Iraq; samples and clinical information was collected from August 2021 to February 2022. The corresponding blood samples were tested for anti-*Toxoplasma* IgG antibody, *Toxoplasma* IgG avidity, FT3, FT4, and TSH hormones, and TPO, Tg, and TSHR antibodies. Overall, our results showed that out of 180 pregnant women, 110 (61.1%) were seropositive for anti-*Toxoplasma* IgG antibody; specifically, 25 (22.7%) and 85 (77.3%) had recent and past infections, respectively. Approximately 54.4% (98) of the pregnant women had thyroid disorders; further, 22 (12.2%), 13 (7.2%), and 8 (4.4%) women had TPO, Tg, and TSHR antibodies, respectively. A total of 43 (23.8%) patients screened positive for AITD. Out of the 110 *Toxoplasma* IgG-positive women, 35 (31.8%) had AITD. The older women, rural residents, restaurant food consumers, and women with cat contact had relatively high infection rates. *Toxoplasma* seropositive women had more elevated autoantibodies than seronegative ones. In conclusion, this study demonstrated a high rate of toxoplasmosis and a corresponding association with thyroid hormones changes and AITD in pregnant women in Duhok, Iraq. Further, it is necessary to reduce overall infection rates through effective health and educational programs. Therefore, it is essential to measure *Toxoplasma* antibodies, screen for thyroid hormones and autoantibodies, and encourage gynecologist visits to reduce the risks to mothers and fetuses.

**Keywords:** AITD, ELISA, IgG Avidity, Pregnancy, Thyroid Hormones, Toxoplasmosis

\*Correspondence: manal.murad@uod.ac

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## INTRODUCTION

*Toxoplasma gondii* is an obligate coccidian protozoan parasite that can infect all warm blooded animals and humans, ultimately causing toxoplasmosis.<sup>1</sup> The final hosts of *T. gondii* are wild and domestic cats, while all other animals and humans act as intermediate hosts.<sup>2</sup> *T. gondii* has infected approximately one third of the world's population; therefore, effective diagnosis, prevention, and management of toxoplasmosis is required.<sup>3</sup> Specifically, *T. gondii* infection is acquired either by ingesting mature oocysts in food, water, and soil, which has been contaminated with cat feces, or consuming improperly cooked meat that contains tissue cysts.<sup>4</sup> This parasite can transmit during organ transplantation, blood transfusion, or by reactivation of latent stages.<sup>5</sup> Additionally, infections during pregnancy can cause congenital toxoplasmosis through vertical transmission from the infected mother to the fetus, ultimately resulting in miscarriage, stillbirth, hydrocephalus, microcephaly, and neurological disorders.<sup>6</sup> Currently, several methods have been developed to diagnose toxoplasmosis such as the direct detection of *T. gondii*, immunological, and molecular techniques.<sup>7</sup> The direct diagnosis of toxoplasmosis involves microscopic examination of tachyzoites or tissue cysts and strain isolation.<sup>8</sup> In clinical approaches, the most widely used method is enzyme-linked immunosorbent assay (ELISA) for the detection of IgM and IgG antibodies.<sup>9</sup> In recent decades, several molecular techniques have been developed, such as PCR, nested PCR, and real-time PCR; however, these techniques are in limited use because they require expensive equipment, complex procedures, and highly qualified technicians.<sup>10</sup> *T. gondii* can attack many organs and glands, including the thyroid gland, which releases the thyroid hormones triiodothyronine (T3), tetraiodothyronine (T4), and calcitonin, which are essential for regulating heart, brain, and bone functions alongside metabolism.<sup>11</sup>

Little is known about *T. gondii* infections in thyroid gland. Nonetheless, some recent studies have illustrated that *T. gondii* is responsible for thyroid hormone alterations, which lead to resultant changes in thyroid morphology and function; initial infection with *T. gondii*, alters T3

and T4 secretion, resulting in TSH disturbance via a dramatic increases thyroid peroxidase (TPO) levels.<sup>12</sup> In the late 1990s, Stahl and Kaneda reported that mice infected with *T. gondii* showed a decline in serum thyroxine. After determining that the thyrocytes were unimpaired, they concluded that the primary effect of *T. gondii* infection was a disturbance in the hypothalamic regulation of thyrotropin-releasing hormone; hence, the pituitary–thyroid (T4) feedback loop is secondarily affected.<sup>13</sup>

Thyroid gland abnormalities are classified as hypothyroidism and hyperthyroidism with different signs and symptoms. Hypothyroidism is a disorder in which the thyroid gland does not release enough thyroid hormones, whereas hyperthyroidism is a thyroid disorder in which the thyroid produces excessive thyroid hormones.<sup>14</sup> Consequently, hypothyroidism leads to weight gain, cold sensitivity, hair loss, and slow heart rates, whereas hyperthyroidism causes sweating, anxiety, fatigue, and weight loss.<sup>15</sup>

Autoimmune thyroid disease (AITD) occurs following a dysregulation in immune tolerance in which the body's immune system attacks the thyroid gland and hormones, causing damage and disruption to this hormone system. Specific auto antibodies are formed in AITD which targeting thyroid antigens; these antibodies are, consequently, observed in hypothyroidism and hyperthyroidism conditions.<sup>16</sup> An example of AITD is Hashimoto's thyroiditis, which is a common cause of hypothyroidism and is primarily caused by T cell–mediated autoimmune responses. Alternatively, Graves' disease is an AITD that causes hyperthyroidism through humoral autoimmunity responses. Specifically during pregnancy, AITD can cause premature delivery, abnormal neural development, and miscarriage.<sup>17</sup> The prevalence of AITD is increased by, and is associated with, various genetic and environmental factors, such as pathogens, substances, certain cytokines, sex, and other unknown factors that may contribute to its development.<sup>18</sup> Little is known about AITD; nonetheless, some recent studies have demonstrated a specific link between *T. gondii* and AITD with elevated autoantibody levels. Additionally, the molecular similarities between thyroid auto antigens and *T. gondii* pathogen

components, other autoimmune diseases, and family history are all considered to be risk factors for AITD.<sup>19</sup>

Prior studies have been conducted in Iraq to determine the prevalence of *T. gondii* infections. Corresponding studies in Duhok City, Iraq conducted by Atroshi and Mero<sup>20</sup> and Ramadhan and Sarkees<sup>21</sup> indicated *Toxoplasma* seropositivity rates as 27.7% and 44.4%, respectively. Additionally, Salih et al.<sup>22</sup> performed another study in Duhok assessing the seropositivity for *Toxoplasma* antibodies (36.3%). Additionally, another study<sup>23</sup> demonstrated that the seroprevalence of *T. gondii* infection in Kirkuk Province, Iraq, was 36.17%. Moreover, Al-Khamesi et al.<sup>24</sup> revealed that the rates of chronic and acute toxoplasmosis among pregnant women in Baghdad City, Iraq were 68.75% and 31.25%, respectively. Therefore, the aim of this study was to detect the prevalence of toxoplasmosis and its association with AITD among pregnant women. The current study is the first to address the link between *T. gondii*, thyroid hormones, and AITD in pregnant women in Duhok City, Iraq. Through this study, we aim to advise pregnant women to be aware about the various health issues surrounding toxoplasmosis, thyroid disorders, and autoimmunity in order to reduce the risks and promote early management of these diseases during pregnancy.

## MATERIALS AND METHODS

This cross-sectional study included the assessment of 180 pregnant women. These patients attended obstetrics and gynecology hospitals in Duhok City, Iraq, from August 1, 2021 until February 28, 2022; the corresponding age range was 15-50 years old. The clinical information was collected from each woman using a special informative questionnaire, including name, age, residency, educational level, number of births, pregnancy period, food habits, and contact with cats. A total of 5 mL of venous blood was obtained by vein puncture using a sterile disposable syringe, placed in a plain tube without anticoagulant, labeled, left for 20 min at room temperature to clot, and centrifuged at 3000 rpm for 10 min to obtain serum. All separated serum samples were poured into sterile 2 mL Eppendorf tubes; each

tube was labeled, named, and stored at -20°C until use.<sup>25</sup>

## Ethics of Study

Scientific and ethical approval for the study was granted by the Scientific Committee of the College of Medicine/Duhok University, and ethical approval was obtained from the Research Ethics Committee of the General Health Directorate, Duhok, Iraq. No. 13072021-7-3.

## Inclusion Criteria

All pregnant women with different periods of pregnancy were included in this study.

## Exclusion Criteria

Women who were excluded from this study included those with unknown pregnancy periods, those with other infectious diseases, and those with immunosuppressive or chronic diseases.

## Study Design

In this cross-sectional study, all pregnant women were examined using ELISA and tested for anti-*T. gondii* IgG antibody (Bioactiva Diagnostics, Germany) and *Toxoplasma* IgG avidity (Novalisa, Germany). Determination of thyroid hormones levels of free triiodothyronine (FT3; pg/mL), free thyroxine (FT4; ng/dL) and thyroid-stimulating hormone (TSH;  $\mu$ IU/mL) were measured using an AccuBind ELISA kit (Monobind, USA). Detection of TPO (IU/mL), *thyroglobulin antibodies* (Tg; IU/mL) and thyroid stimulating hormone receptor (TSHR-U/L) was conducted using the Aeskulisa ELISA technique (Germany), according to manufacturer's instructions. The optical density was measured at 450 nm with an ELISA plate reader (BioTek, USA).

## Statistical Analysis

All data were statistically analyzed using the statistical program R Studio and a chi-square test. Descriptive statistics were used to describe the data using the means, standard deviation, range for numerical variables, and frequency (n) with percentage (%) for categorical variables. The data were represented using tables, pie charts, and histograms. A P-value <0.05 was considered statistically significant.

### **Detection of Anti-Toxoplasma IgG Antibodies by ELISA**

The calibrators and serum samples were incubated in microplate wells coated with purified and inactivated *T. gondii* antigen. After incubating and washing the samples, the wells were treated with a conjugate composed of anti-human IgG antibodies labeled with horseradish peroxidase (HRP). After the second incubation and washing step, the wells were then incubated with 3,3',5,5'-tetramethylbenzidine (TMB). Finally, an acidic stop solution was added and the absorbance was read at 450nm using an ELISA microplate reader.

### **Detection of Toxoplasma IgG Avidity by ELISA**

Microtiter plates were coated with specific antigens to bind to the corresponding antibodies in the sample; these samples were then incubated and washed to remove all unbound sample material. Next, a HRP-labeled conjugate was added to the wells to bind to the captured antibodies. Then, a second washing step was conducted to remove all unbound conjugates. The immune complex formed by the bound conjugate was visualized by adding TMB. Sulfuric acid was added to stop the reaction and the absorbance was measured at 450nm using an ELISA microplate reader.

### **Detection of FT3, FT4, and TSH Hormones by ELISA**

Microplates were coated with hormone antibodies, and the enzyme reagent solution was added to each well. The wells were incubated and washed with washing buffer to remove all unbound sample material. Then, a working substrate solution was added to the wells; after a second incubation, stop solution was added, and the absorbance was read at 450 nm using an ELISA reader.

### **Detection of TPO and Tg Antibodies by ELISA**

Serum samples diluted 1:101 were incubated in the microplates coated with the specific antigen. The patient's corresponding antibodies bound to the antigen and the unbound fraction was removed in the following wash step. Then, the samples were incubated with anti-human immunoglobulins conjugated to HRP (conjugate); this conjugated antibody reacted with

the antigen-antibody complex of the samples in the wells. All unbound conjugate was washed off in the following step. Addition of TMB followed by stop solution was conducted before measuring absorbance at 450nm using an ELISA microplate reader.

### **Detection of TSHR Antibodies by ELISA**

TSHR auto antibodies (TRAb) in the serum samples, calibrators, and controls were incubated with TSH receptors coated onto ELISA plate wells for two hours. Then, the samples were discarded, leaving the TRAb, which were bound to the immobilized receptor. A human monoclonal autoantibody against TSHR that was labeled with biotin (M22-biotin) was added in a second incubation step; this antibody specifically interacted with the immobilized TSH receptors that had not been blocked by the bound TRAb. The amount of M22-biotin bound to the plate was then determined in a third incubation step by the addition of streptavidin peroxidase, which bound specifically to biotin. Excess unbound streptavidin peroxidase was discarded and TMB was added. Finally, the stop solution was added, and the absorbance was read at 450 nm using an ELISA plate reader.

## **RESULTS**

Figure 1 shows the frequency of normal thyroid and thyroid disorders among pregnant women. Out of 180 pregnant women, 82(45.6%) had normal thyroid, while 94 (52.2%) and 4 (2.2%) had hypothyroidism and hyperthyroidism, respectively.

Figure 2 shows the rates of normal thyroid and thyroid dysfunction among women with seropositive and seronegative anti-Toxoplasma IgG antibody. Out of 110 women with seropositive anti-Toxoplasma IgG antibody, 43(39.1%) had normal thyroid, 65(59.1%) and 2(1.8%) had hypothyroidism and hyperthyroidism, respectively. From 70 women seronegative anti-Toxoplasma IgG antibody, 37(52.8%) had normal thyroid, 31 (44.3%) and 2(2.9%) had hypothyroidism and hyperthyroidism, respectively. Seropositive Toxoplasma IgG antibody associated with TSH level increase. An association was obtained between toxoplasmosis and thyroid status p-value 0.04.

**Table 1.** Distribution of toxoplasmosis based on some demographic characteristics in pregnant women

Variables		n.(%)	Seropositive Toxoplasma IgG Abs. n. (%)	Seronegative Toxoplasma IgG Abs. n.(%)	P-value
Age	15-20	17(9.4 )	5(29.4)	12(70.6)	*0.02
	21-26	55( 30.6)	32(58.2)	23(41.8)	
	27-32	65(36.1 )	40(61.5)	25(38.5)	
	33-38	34(18.9 )	25(73.5)	9(26.5)	
	39-44	7( 3.9)	6(85.7)	1(14.3)	
	45-50	2(1.1 )	2( 100)	0(0.0)	
Residency	Rural	41(22.8 )	32( 78.0)	9(22.0)	*0.01
	Urban	139(77.2 )	78( 56.1)	61(43.9)	
Educational level	Illiterates	20(11.1)	17(85.0)	3(15.0)	*0.01
	Primary level	22(12.2)	15(68.2)	7(31.8)	
	Secondary level	88(48.9)	55(62.5)	33(37.5)	
	Higher education	50(27.8)	23(46.0)	27(54.0)	
Number of births	(0-3)	88(48.9)	42(47.7)	46(52.3)	*0.0007
	4-7))	70(38.9)	50(71.4)	20(28.6)	
	7<	22(12.2)	18(81.8)	4(18.2)	
Pregnancy period	1 <sup>st</sup> trimester	43( 23.9)	33( 76.7)	10(23.3)	0.05
	2 <sup>nd</sup> trimester	49( 27.2)	27(55.1 )	22(44.9)	
	3 <sup>rd</sup> trimester	88( 48.9)	50( 56.8)	38(43.2)	
Type of eating foods	Eating indoor	139(77.2 )	79( 56.8)	60(43.2)	*0.04
	Eating outdoor	20( 11.1)	16( 80.0)	4(20.0)	
	Eating indoor and outdoor		21( 11.6)	16(76.2 )	
Cat contact	Yes	31( 17.2)	25( 80.6)	6(19.4)	*0.01
	No	149(82.8 )	85( 57.0)	64(43.0)	

\*Statistically significance (p-value <0.05)

Figure 3 illustrates the percentages of TPO, Tg and TSHR antibodies among women. Out of 180 women, about 43 (23.8%) had autoantibodies, 22(12.2%), 13(7.2%) and 8(4.4%) had seropositive TPO, Tg and TSHR antibodies, respectively. From 110 women seropositive for Toxoplasma IgG antibody, 35(31.8%) had autoantibody, (17.3%) , 11 (10.0%) and 5(4.5%) seropositive TPO, Tg and TSHR antibodies respectively. While, from 70 Toxoplasma negative women, 8(11.4%) had autoantibodies classified as 3(4.3%), 2(2.9%) and 3(4.3%) had TPO, Tg and TSHR antibodies. Toxoplasma seropositive women had more elevated TPO, Tg and TSHR antibodies than seronegative ones. *T.gondii* is linked with autoimmune thyroid disease with a statistically significance (p-value 0.001).

Overall, 180 pregnant women were included in the current study; their mean age±standard deviation was 28±6.11 years

(range:15–50 years). Of these women, 110(61.1%) were diagnosed as positive for anti-*Toxoplasma* IgG antibody, 25 (22.7%) had a recent infection with a low IgG avidity and 85 (77.3%) had past infection with a high IgG avidity. Approximately 23.9% (43 women) screened positive for AITD. Table 1 shows the distribution of anti-*Toxoplasma* IgG antibody among pregnant women compared to certain demographic characteristics. The highest toxoplasmosis rates were detected among old women, rural residents, those who were illiterate, those that had multiple births, those that ate food outdoors, and those that had contact with cats. The 2 women in the 45-50-year-old group (100%) were both seropositive for anti-*Toxoplasma* IgG antibody; in contrast, low rates of infection 5(29.4%) were observed in the 15-20-year-old group, while the infection rate of women living in rural areas was 78.0% (32 women). High infection rates were detected in women who were

illiterate (17 women; 85.0%) compared to the lowest rate of infection, which was observed in those in the higher education group (23 women; 46.0%). Women with multiple children showed higher rates of infection than those with few children: 18(81.8%) and 42(47.7%), respectively. With regarding to eating habits, 16(80.0%) of women who ate al fresco were *Toxoplasma* IgGAb positive. Finally, the rate of seropositivity for anti-

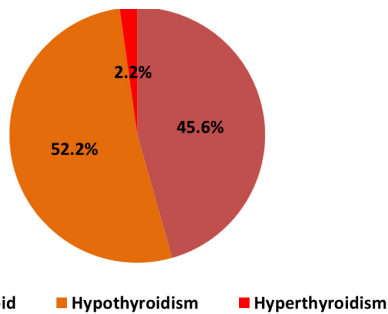
*Toxoplasma* IgG antibodies among women with cat contact was 80.6% (25 women).

Table 2 shows the analysis of FT3, FT4 and TSH hormones among pregnant women. Women with seropositive anti-*Toxoplasma* IgG antibody had more abnormal FT3, FT4 and TSH levels than IgG negative ones 45.5%,44.5% and 49.1% p-value 0.03.

**Table 2.** Frequency of FT3, FT4 and TSH hormones *Toxoplasma* IgG seropositivand seronegative women

Hormones	Cases	Seropositive <i>Toxoplasma</i> IgG Abs. n. (%)	Seronegative <i>Toxoplasma</i> IgG Abs. n.(%)	P-value
FT3	Normal	60(54.5)	42(60.0)	0.51
	Abnormal	50(45.5)	28(40.0)	
FT4	Normal	61(55.5)	42(60.0)	0.54
	Abnormal	49(44.5)	28(40.0)	
TSH	Normal	56(50.9)	47(67.1)	*0.03
	Abnormal	54(49.1)	23(32.9)	

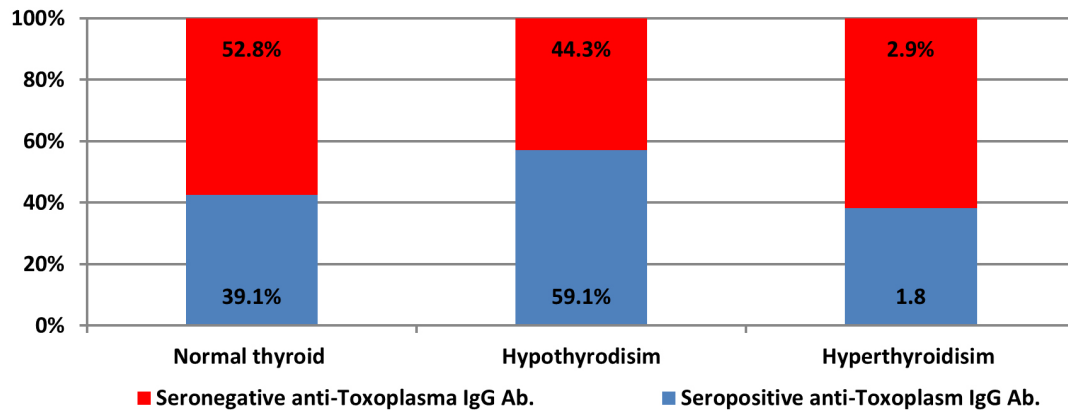
\*Statistically significance (p-value <0.05)



**Figure 1.** Frequency of thyroid status among pregnant women

## DISCUSSION

Overall, 180 pregnant women were included in this study. 110(61.1%) women were seropositive for anti-*Toxoplasma* IgG antibody; specifically, 25(22.7%) and 85(77.3%) had recent and past infections, respectively. This study was in line with study by Eisa et al.<sup>26</sup> performed in Sudan, the seroprevalance of *Toxoplasma* IgG antibody was 61.1%. Eskandarian et al.<sup>27</sup> observed similar results to our findings, the serprevalence



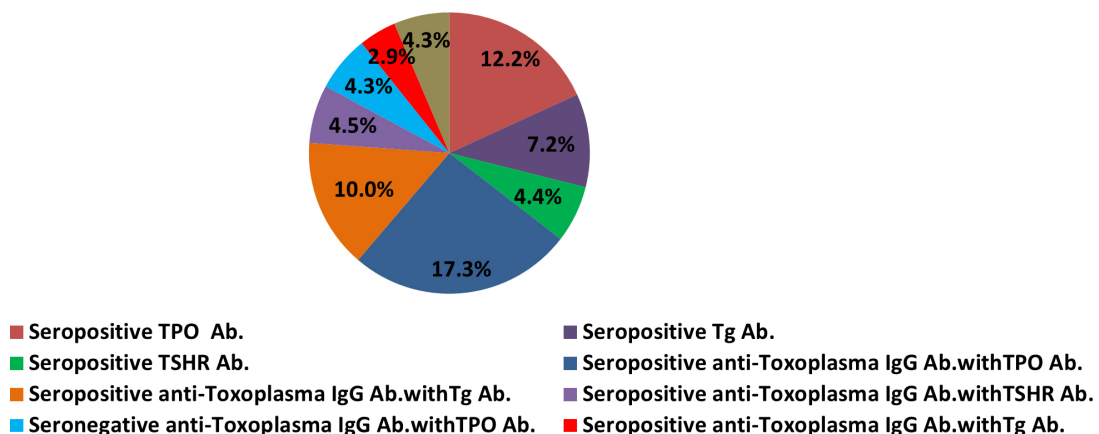
**Figure 2.** Distribution of normal thyroid and thyroid disorders among *Toxoplasma* IgG positive and negative women

of *Toxoplasma* IgG antibody in pregnant women in Iran was 62.7%. Kalantari et al.<sup>28</sup> revealed the positivity of *Toxoplasma* IgG antibody was 60.5%. The sample size, climatic similarity, nutritional habits, socioeconomic status, cat contact may play a role in this prevalence rate similarity.

The prevalence (percent socioeconomic status, and cat contact may have played a role in this prevalence rate similarity between the current study and prior studies. However, our findings disagreed with those in a study conducted by Al-Saeed et al.<sup>29</sup> in which they determined a 10% prevalence of *Toxoplasma* IgG antibodies. Additionally, Murad et al.<sup>30</sup> conducted a study in Duhok City; this study indicated that the seropositivity of *Toxoplasma* antibodies in pregnant women was 21.1%. Our results contrasted with those observed by Alvarado-Esquivel et al.<sup>31</sup>; in this study, they reported a relatively low *Toxoplasma* IgG positivity in Mexico (6.1%). The dissimilarities in the demographic characteristics, nutritional habits, socioeconomic status, and public awareness of toxoplasmosis may have led to the corresponding discrepancies observed in these studies compared to the current study. According to the phases of toxoplasmosis, the current study revealed similar findings to those observed by Saki et al.<sup>32</sup>; in particular, Saki et al.<sup>32</sup> reported that the seroprevalence rates of recent and past infections were 22.7% and 77.3%, respectively. Further, Alver et al.<sup>33</sup> showed an agreement with the current study by determining

that the prevalence of recent toxoplasmosis infections was 27.6%, while the prevalence of past infections with toxoplasmosis was 60.1%.

Cats contaminate the environment with *Toxoplasma* oocysts; additionally, there are several risk factors for toxoplasmosis, including social, cultural, and socio-demographic statuses.<sup>34</sup> In the current study, the maximum seropositivity rate for *Toxoplasma* IgG antibody was observed among the 45-50 years age group (100%), compared to the minimum *Toxoplasma* IgG positivity observed among the 15-20 years age group (29.9%). The outcome of this investigation was in accordance with a study conducted by Agorzodo et al.<sup>35</sup>, which revealed a high *Toxoplasma* seropositivity in the >45 years age group (67.0%) and a low *Toxoplasma* positivity in the <18 years age group (25.4%). Our results were comparable to those of Mousavi-Hasanzada et al.,<sup>36</sup> which found a higher seroprevalence of *T. gondii* infection in older age groups than younger age groups (53.9% and 28.7%, respectively). The current study demonstrated that the seropositivity of *T. gondii* antibody significantly increased with age ( $P < 0.05$ ). This difference in prevalence with age may be because of a higher probability of contact with oocysts, prolonged exposure to risk factors and transmission routes, and a lack of prevention and control methods for toxoplasmosis. Contrastingly, Babaie et al.<sup>37</sup> observed that *Toxoplasma* IgG seropositivity was higher in the 16-21 years age group (36.2%) than in the 32-47 years age group (35.5%). This discrepancy has been attributed to high income



**Figure 3.** Percentage analysis of positive TPO, Tg and TSHR antibodies among women.

of the older group and the younger group eating junk food that was contaminated with infective stages of *T. gondii*.

Our study indicated that the seroprevalence of *T. gondii* IgG antibody in pregnant women who lived in rural areas (78.0%) was significantly higher than those in urban areas (56.1%) ( $P < 0.05$ ). Ramadhan and Sarkees<sup>21</sup> conducted a study in Duhok, Iraq and found similar results to the current study; the seroprevalence rates of *Toxoplasma* IgG antibody in rural and urban residents were 46.7% and 43.8%, respectively. Further, Raissi et al.<sup>38</sup> demonstrated that *Toxoplasma* seropositivity in rural areas was 26.3%, whereas in urban areas it was 16.0%. Therefore, it was postulated that rural areas contain numerous stray cats that live on farms that may contaminate the environment with oocysts that can directly infect humans or can infect livestock that will be later slaughtered for human consumption; additionally, rural women have more frequent contact with soil and farming, low educational levels, and lack awareness of prevention and control strategies for toxoplasmosis. However, Al-Aqeely et al.<sup>39</sup> reported dissimilar results compared to our findings; in particular, they determined that the prevalence of toxoplasmosis was higher in pregnant women who lived in urban areas than those in rural areas: 21.0% and 15.4%, respectively. Nonetheless, this finding can be attributed to the high income of urban areas and their different eating habits, including an increase in consumption of poultry and junk food from restaurants, which have been found to be a major source of *T. gondii* transmission.

Within this study, illiterate women were determined to possess a higher *Toxoplasma* seropositivity (85.0%) than higher educated women (46.0%). Evidence from Agrozodo et al.<sup>35</sup> supported our findings; in this study, *Toxoplasma* positivity rates in illiterate and highly educated groups were determined to be 57.5% and 33.3%, respectively. Therefore, it was suggested that illiterate women were more likely to be older, live in rural areas, have contact with cats, and lack effective information about infection. In contrast, Mizani et al.<sup>40</sup> reported a higher *Toxoplasma* seropositivity in the higher education group (38.3%) than in the illiterate group (32.8%). Overall,

it was suggested that women with academic education should have more knowledge related to *Toxoplasma* biology and its corresponding prevention and control strategies. The lack of effective information about this disease, such as the route of transmission during pregnancy, and poor socioeconomic status can increase the risk of infection.

In our study, multi gravid women had a higher prevalence of *T. gondii* infection, which increased proportionally with an increasing number of children. The seroprevalence of *Toxoplasma* IgG antibodies among women with more than seven children was 81.8%, whereas women with few children had an infection rate of 47.7%. This result was in agreement with Mizani et al.<sup>40</sup> who indicated a higher prevalence of infection in the multiple births group (50.4%) than in the few-birth group (39.3%). We hypothesized that most women with multiple births are illiterate and live in rural areas. Additionally, in the present study, the seropositivity rate for *Toxoplasma* IgG antibodies in the first-trimester of pregnancy was higher than in the second and third-trimesters (76.7%, 55.1%, and 56.8%, respectively). These findings aligned with a study conducted in Egypt by Mandour et al.<sup>41</sup>; in this study, it was reported that the prevalence of infection in the first-trimester group was 68.2%, compared to third-trimester pregnancy group which was 60.2%. This difference in infection may be because most pregnant women in the first-trimester of pregnancy live in rural areas and have low educational levels regarding contact with cats. However, a study conducted in Saudi Arabia by Majid et al.<sup>42</sup> produced contrasting results to our findings; in this study, the seropositivity rate in the first and third-trimesters of pregnancy were 7.1% and 31.2%, respectively.

Data analysis within the current study demonstrated that there was a significant relationship between *Toxoplasma* seropositivity and food habits ( $p < 0.05$ ). High *Toxoplasma* seropositivity was observed among the group that ate outdoor compared to the group that ate indoor (80.0% and 56.8%, respectively). Similar results were observed by Raissi et al.<sup>38</sup>; in particular, the *Toxoplasma* positivity in the groups that ate outdoor or indoor were 35.7% and 20.7%, respectively. Further, the corresponding results in



a study conducted by Kolbekova et al.<sup>43</sup> supported these findings. Overall, we postulated that women who consume food outdoors may be infected with *T. gondii* as they lack information regarding toxoplasmosis transmission. Additionally, meat and vegetables are major sources of Iraqi meals; thus, the consumption of under cooked meat and unwashed vegetables leads to the transmission of parasites.

Our results demonstrated that *Toxoplasma* seropositivity was higher in women who had contact with cats (80.6%) than in those who did not have contact with cats (57.0%). This finding aligned with another study conducted by Al-Atroshi and Mero<sup>20</sup> in Duhok, Iraq; *Toxoplasma* IgG seropositivity among women who had contact with cats (30.8%) contrasted with those who did not have contact with cats (25.7%). Additionally, a study conducted by Babaie et al.<sup>37</sup> found similar findings; specifically, the seroprevalence of toxoplasmosis in women who had contact with cats and those who did not have contact with cats was 37.9% and 34.2%, respectively. Overall, most women who had contact with cats were from rural areas with poor socioeconomic status and hygiene. However, the results of the current study contradicted with a study conducted in Ethiopia by Fenta<sup>44</sup>; this alternative study indicated that a higher seropositivity of *Toxoplasma* was present among women who did not have contact with cats compared to those that did (82.7% and 80.0%, respectively).

In the current study, we determined that there were more pregnant women with thyroid disorders than healthy thyroid function; this aligned with Valizadah et al.,<sup>45</sup> in which *T. gondii* was associated with changes in thyroid hormones and TSH disturbance. In the current study, the highest rates of thyroid diseases were reported in women with seropositive *Toxoplasma* antibodies, rather than seronegative ones. This finding was similar to those in studies conducted by Wu et al.<sup>15</sup> and Alkhamesi<sup>24</sup>. In the current study, we reported an association between *T. gondii* with thyroid disorders and AITD; Alkhamesi et al.<sup>24</sup> corroborated our findings that *T. gondii* is associated with thyroid disorders, showing a significant decrease in T3 and T4 levels and an increase in TSH levels among seropositive

*Toxoplasma* women. During pregnancy, thyroid disorders pose risks to the mother and fetus, which may lead to miscarriage, preterm delivery, and fetal death. Raissi et al.<sup>38</sup> reported in consistent results compared to our findings; they observed a seropositive *Toxoplasma* prevalence of 21.4% among individuals with thyroid disorders and did not find any correlation between *T. gondii* infection and thyroid dysfunction.

In the current study, out of 110 seropositive *Toxoplasma* women, 35(31.8%) had AITD, aligning with a study conducted by Kankova et al.<sup>46</sup>; specifically, Kankova et al.<sup>46</sup> reported that 27.1% of 127 AITD women were positive for toxoplasmosis, *T. gondii* had an effect on thyroid production, and changes in thyroid hormone levels with highly elevated TPO antibodies were found among *Toxoplasma* IgG positive women compared to *Toxoplasma* seronegative women. Additionally, Valizada et al.<sup>45</sup> tested 1248 pregnant women and determined that acute and latent toxoplasmosis (LT) was observed in 3.4% and 29.6% of the women, respectively; the overall frequency of thyroid diseases was 18.8%, whereas, approximately 27.9% of patients with LT had thyroid diseases. Further, 13.8% of pregnant women with LT only had AITD; a significant correlation and high elevation of TPO antibodies was also found among the seropositive *Toxoplasma* IgG antibody group compared to those in the *Toxoplasma* seronegative group. These findings can likely be attributed to the antigenic similarity of *Toxoplasma* and TPO leads to cross-reactivity in the immune system, potentially causing AITD. In contrast to Tozzoli et al.<sup>47</sup>, who determined that 65.5% of seropositive *Toxoplasma* women had AITD, only 31.8% of 110 *Toxoplasma* IgG women were positive for AITD in the current study. Nonetheless, Tozzoli et al.<sup>47</sup> instead analyzed 120 AITD patients from different areas of Italy, rather than Duhok, Iraq. Our study indicated that pregnant women primarily possess forms of Hashimoto's thyroiditis and few were identified as positive for Graves' disease. However, differing results were obtained by Shapira et al.<sup>19</sup>; these differences included the number of participating women, toxoplasmosis rate, number of patients with AITD, use of tests, and geographical areas. Overall, we demonstrated that *T. gondii* is associated with thyroid disorders

as a result of autoimmunity, with corresponding abnormal levels of thyroid hormones observed in TPO-, Tg-, TSHR-, and *T. gondii*-positive women. AITD can cause miscarriage, preterm delivery, and low birth weight. Nonetheless, this study had some limitations, including the small number of patients with hypothyroidism and hyperthyroidism. Additionally, there were difficulties in the follow-up of the pregnant women and their fetuses, which would otherwise help further the understanding of the mechanisms by which *Toxoplasma* influences AITD.

## CONCLUSION

Overall, pregnant women should be encouraged to practice good hygiene through preventive and control methods to reduce the risk of toxoplasmosis. The current study indicated the importance for screening for *Toxoplasma* antibodies and IgG avidity among pregnant women to distinguish between recent and past infection. Additionally, there is a requirement in the measurement of thyroid hormones and autoantibodies to provide early therapies for pregnant women. This study provides novel information to improve the understanding of AITD pathogenesis. Nonetheless, further studies are required to completely understand the link between toxoplasmosis and AITD, and to focus on the molecular mimicry observed between thyroid components and *Toxoplasma* antigens.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHORS' CONTRIBUTION

Both authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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None.

## DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

## ETHICS STATEMENT

This study was approved by Scientific Committee of the College of Medicine/Duhok University, and ethical approval was obtained from the Research Ethics Committee of the General Health Directorate, Duhok, Iraq. No. 13072021-7-3.

## INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

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