



## IMMUNOENDOCRINOLOGY ASSOCIATION OF TOXOPLASMA GONDII AND THYROID HORMONES IN IRAQI PATIENTS WITH CLINICAL MANIFESTATIONS OF TOXOPLASMOSIS

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### ABSTRACT

The aim is to determine the correlation among age, gender; thyroid hormones and anti T.gondii antibodies. Anti T.gondii antibodies; TSH, T3, T4 serum level evaluated by ELISA technique in 113 Iraqi patients. Anti T.gondii Immunoglobulines correlated with gender, T.gondii IgM inversely correlated with male gender and positively with male age groups. No significant correlation between IgM, IgG positivity and TSH levels. T.gondii IgM positive /negative correlated with T3 among females. IgM positive males have elevated T4 (0.88%) v (6.19%) for females. T.gondii specific IgM positive /negative inversely correlated with T4 among females T.gondii IgM, IgG positive responses significantly associated with gender. T3, T4 inversely correlated with anti T.gondii IgM positive response among females. Increase in T3 and T4 thyroid hormones production in toxoplasmosis, provide new clue to the complex pathogenesis of Toxoplasma-associated thyroid diseases.

**KEY WORDS:** Toxoplasma gondii; thyroid hormones; age; gender



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## INTRODUCTION

Toxoplasma gondii is an obligate intracellular parasite which infect a wide variety of avian and number of mammals as intermediate hosts and the number of cats' family as definitive hosts. Toxoplasma gondii infection stimulates both cell mediated immunity(CMI) and humoral immune response as antibody production, which includes IgM and IgG antibody<sup>1</sup>. CMI is one of the most distinctive immunological features of T.gondii infection. CMI is elicited by the parasite, resulting in host protection against rapid tachyzoite growth and consequent pathologic changes<sup>1</sup> The parasite causes a very strong response of T-helper 1(Th1) focused on Interferon gamma (IFN $\gamma$ ), Tumor Necrosis Factor alfa (TNF $\alpha$ ) and Interleukin12 (IL12), secreted by T-lymphocytes. This immune response limits the tissue extension of the parasite, and lead the parasite to convert to bradyzoite<sup>12</sup>. The macrophage, lymphocytes, natural killer (NK) cells and the cytokines are the major elements involved in immune response against T.gondii<sup>2,3</sup>. Antibodies appear to play a minor role but remain essential means for preventing reinfection by the parasite<sup>4</sup>. A hypothesis of endocrine-immune interaction based on the fact that sex hormones as example, testosterone influence the immune system. The localization of sex hormones receptors in immune cells, including lymphocytes, macrophages, granulocytes, and mast cells illustrates that there was direct connection between the endocrine and immune systems and endocrine factors can directly modulate the expression of target genes in immune cells<sup>5</sup>. Thyroid gland is an essential gland in the body of human being that produces essential hormones regulated by the hypothalamic-pituitary-thyroid axis<sup>6</sup>. The main function of thyroid gland is to secrete thyroxine to regulate basal metabolic rate. Mostly this hormone acts through nuclear receptors that are transcribed by numerous genes. These genes regulate several critical physiological functions in development and metabolism<sup>7</sup>. Thyroid disease associated with various metabolic abnormalities, due to the effect of thyroid hormones on nearly all major metabolic pathways. Thyroid hormones regulate the basal energy expenditure through their effect on protein, carbohydrate, and lipid metabolism. This might be a direct effect or an indirect effect by modification of other regulatory hormones such as insulin or catecholamine<sup>8</sup>. Male reproduction adversely affected by both thyrotoxicosis and hypothyroidism. Erectile abnormalities reported<sup>9</sup>. Thyrotoxicosis induces abnormalities in sperm motility. Hypothyroidism is associated with abnormalities in sperm morphology. In females, thyrotoxicosis and hypothyroidisms can cause menstrual disturbances. Thyrotoxicosis is associated with hypo-menorrhoea and poly-menorrhoea, while hypothyroidism associated with oligo-menorrhoea<sup>10</sup>. Thyroid dysfunction linked to reduced fertility<sup>11</sup>. The aim is to determine the anti T.gondii antibodies in Iraqi patients and possible correlation of age and gender in disease occurrence. Also to evaluate the serum levels of thyroid hormones (TSH,T3,T4) and its possible correlation with anti T.gondii antibodies.

## PATIENTS, MATERIALS AND METHODS

### Selection of patients

One hundred and thirteen patients presented with clinical manifestation of toxoplasmosis attended to private and public outpatient's clinics in Baghdad during a period from January 2015 to May 2015 selectively enrolled in this study. This study was conducted according to the principles of Helsinki declaration. Dully-filled consent form obtained from all patients participating in the study. Approval of ethical review Committee(Issue No.1/232-15,January2015),College of medicine –Diyala University-Iraq taken prior to initiation of the work.

### Methods

Five milliliters venous blood taken aseptically from one hundred and thirteen patients presented with clinical manifestation of toxoplasmosis. The samples centrifuged at 2000  $\times$  g at room temperature for five min to separate sera. Separated sera were stored at -20 C<sup>o</sup> until used for ELISA and RIA techniques.

### Detection of T.gondii antibodies

Detection of T.gondii specific IgM, and IgG antibodies were done using IgM and IgG ELISA Biokit reagents. The procedures were applied according to manufacturer's guidelines<sup>12</sup>. Toxoplasma antibodies levels using ELISA technique were taken as the level of IgM or IgG below 0.9 IU/ml considered negative, from 0.9 to 0.99 IU/ml was equivocal limit, and rechecked, while positive level was equal to 1.0 IU/ml or above.

### Thyroid function tests

#### a) Thyroid-stimulating hormone (TSH)

In current study, The DRG TSH ELISA Kit which is a solid phase enzyme-linked immunosorbent assay (ELISA)<sup>13</sup> based on the sandwich principle used to determine TSH in serum according to manufacture instruction.

#### b) Triiodothyronine (T3)

The DRG Total T3 ELISA Kit which is a solid phase enzyme-linked immunosorbent assay (ELISA),<sup>14</sup> based on the principle of competitive binding used to determine T3 in serum according to manufacture instruction.

#### c) Thyroxine (T4)

The DRG Total T4 ELISA Kit which is a solid phase enzyme-linked immunosorbent assay (ELISA)<sup>15</sup> based on the principle of competitive binding used to determine T4 in serum according to manufacture instruction.

### Statistical analysis

Data analyzed with by SPSS for window TM version 17 and Microsoft Excel for windows 2010. Pearson's chi square( $\chi^2$ ) and Pearson's Correlation coefficient used for correlation between the variables of two tests. Fisher's exact test is used to examine the significance of the association (contingency) between the two kinds of categorical classification of small sample size. Analysis of variance (ANOVA) used for determination of statistical dif-

ference between groups. The level of significant was 0.05(two tail), and the significant of correlations include 0.01 (two tail).

## RESULTS

Table (1) shown that (113) patients presented with clinical manifestation of toxoplasmosis .The mean age was (30.86 ±8.70) years, minimum (18) years and maximum (56) years. Fifty-two patients (46 %) males, with the mean age (32.75±8.43) years. The mean age of females (29.78±8.66) years; minimum (18) years; maximum (52) years. T.gondii positive IgM (37.2%) IgG positive (56.6% );both IgM+IgG positive (61.1%). As shown in table (2), Significant difference and correlation detected between gender and T.gondii specific IgM, IgG, IgM+IgG (P Value=0.001 to 0.000). Significant difference and correlation detected between males and females in T.gondii specific IgM response (P value=0.038). No significant difference or correlation detected between males and females in T.gondii specific IgG response (P value=0.092). Significant difference (P Value=0.05) without correlation detected between males and females in T.gondii specific T.gondii IgM+ IgG response(P value=0.06).As shown in table (3); The majority of IgM positive; IgG positive among male patients belong to (25-31) years age group, (7.96%), (12.39%) respectively .Among females, The majority of IgM positive; IgG positive cases at (18-24) years, (7.96%) ; (10.62%) ,followed by (32-38 ), (39-45)years . Recent infection with T.gondii not detected among males and females at(46-52),( 53-59) years .No significant difference (P value= 0.414)or correlation reported between age groups of male and female; T.gondii IgM positive; IgG positive response (P value= 0.351; P value= 0.5 ).Significant correlation reported between age groups of males and females and T.gondii IgM; IgG negative response (P value= 0.002; P value= 0.006).No significant difference(P value= 0.285) but significant correlation (P value= 0.05) reported between male age group and T.gondii specific IgM response. Among females no significant difference (P value= 0.467) or correlation reported between female age groups and T.gondii specific IgM response (P value= 0.250). Neither significant difference(P value= 0.203; P value= 502) nor correlation reported between age groups(male and female)and T.gondii specific IgG(P value= 0.543; P value= 0.101) respectively. In general, No significant difference between age groups in total *T.gondii* specific response (IgM+IgG) (P value= 0.891) as shown in table (3). As shown in table (4); the majority of IgM positive among male patients have normal serum level of TSH (10.62%) compared with (23.89%) for females. No significant difference(P value=

0.204)or correlation (P value= 0.213) reported between T.gondii IgM positive and serum TSH among males and females. Neither significant difference(P value= 0.919)nor correlation (P value= 0.921) reported between T.gondii IgM negative response and TSH among males and females. No significant difference(P value= 0.110; P value=0.598) or correlation (P value= 0.114; P value=0.605) reported between T.gondii specific IgM positive /negative and serum TSH among males and females. IgG positive males have normal level of TSH in (20.35%) compared with (32.74%) for females. No significant differences or correlation reported between T.gondii IgG positive; T.gondii IgG negative; T.gondii specific IgG positive /negative response and serum TSH among males and females. No significant difference reported between total T.gondii IgM and IgG positive and negative male and females according to TSH level (table 4). As shown in table (5); the majority of IgM positive among male patients have normal serum level of T3 (11.50%) compared with (18.58%) for females. No significant difference or correlation reported between T.gondii IgM positive; T.gondii IgM negative; T.gondii IgM positive/negative and T3 among males and females. Significant correlation reported between T.gondii IgM positive /negative response and T3 level among females. No significant difference(P value= 0.482)or correlation (P value= 0.767) reported between T.gondii IgG positive response and serum T3 between males and females. Significant difference(P value= 0.048)and correlation (P value= 0.049) reported between T.gondii IgG negative response and serum T3 between the males and females. Neither significant difference(P value= 0.325;P value= 0.750)nor correlation (P value= 0.189; P value=0.610) reported between T.gondii IgG positive /negative and T3 among males and females (table 5). Significant difference reported between total T.gondii IgM; IgG positive; T.gondii IgM; IgG negative males and females according to T3 level(table 5).As shown in table (6); the majority of IgM positive among males have normal serum level of T4 (11.50%) compared with (18.58%) for females. No significant difference or correlation reported between T.gondii IgM positive; T.gondii IgM negative; T.gondii IgM positive/negative response and T4. Significant difference (P value= 0.038), Inverse significant correlation (P value= 0.038) reported between T.gondii IgM positive /negative response and T4 level among females No significant difference or correlation reported between T.gondii specific IgG positive; T.gondii IgG negative; T.gondii IgG positive /negative response and serumT4 among males and females. No Significant difference (P value= 0.140) reported between total T.gondii specific IgM; IgG positive and negative male and females according to T4 (table 6).

**Table 1**  
**Demography of patients with clinical manifestations of Toxoplasmosis**

Parameter	Parameter	
Age (years)	Minimum	18
	Maximum	56
	Mean ± SD	30.86 ±8.70
Gender	male	52(46 %)
	female	61(54%)
Age according to gender	Male	Female
Minimum	18	18
Maximum	56	52
Mean ± SD	32.75±8.43	29.78±8.66
T.gondii specific antibodies	Parameter	No.(%)
	IgM Positive	42(37.2%)
	IgM Negative	71(62.8%)
	IgG positive	64(56.6% )
	IgG Negative	49(43.4%)
	IgG+ IgM positive	69(61.1%)
	IgG+ IgM negative	44(38.9%)
	Total	113(100%)

**Table 2**  
**Correlation and distribution of T.gondii specific antibodies according to gender of patients**

Gender	T.gondii IgM Positive No.(%)	T.gondii IgM Negative No.(%)	T.gondii IgG Positive No.(%)	T.gondii IgG Negative No.(%)	T.gondii IgM+ IgG Positive	T.gondii IgM+ IgG Negative	χ <sup>2</sup>		Pearson's correlation	
							value	P Value	value	P Value
Male	14(12.39%)	38(33.63%)	25(22.12%)	27(23.89%)	27(23.89%)	25(22.12%)	10.87 1	0.00 1	0.457	0.00 1
Female	28(24.78%)	33(29.20%)	39(34.51%)	22(19.47%)	42(37.17%)	19(16.81%)	14.42 6	0.00 0	0.486	0.00 0
<b>Total</b>	<b>42(37.17%)</b>	<b>71(62.83%)</b>	<b>64(56.64%)</b>	<b>49(43.36%)</b>	<b>69(61.06%)</b>	<b>44(38.94%)</b>				
χ <sup>2</sup>	value	4.329		2.874		3.384				
	P value	0.0374		0.090		0.05*				
Pearson's correlation	value	-0.19573		-0.159		-0.173				
	P value	0.038		0.092		0.06				

\*Fisher's Exact Test

**Table 3**  
**Correlation and distribution of *T.gondii* specific antibodies according to age of patients**

Parameter	Age group (years)	Male	Female	Total	$\chi^2$		Pearson's correlation	
					value	P value	Value	P value
T.gondii IgM positive	18-24	2(1.77%)	9(7.96%)	11(9.73%)	21.750	0.414	0.148	0.351
	25-31	9(7.96%)	5(4.42%)	14(12.39%)				
	32-38	2(1.77%)	8(7.08%)	10(8.85%)				
	39-45	1(0.88%)	6(5.31%)	7(6.19%)				
	46-52	0(0%)	0(0%)	0(0%)				
	53-59	0(0%)	0(0%)	0(0%)				
	Total	14(12.39%)	28(24.78%)	42(37.17%)				
T.gondii IgM Negative	18-24	4(3.54%)	12(10.62%)	16(14.16%)	32.275	0.222	-0.354	0.002
	25-31	13(11.5%)	11(9.73%)	24(21.24%)				
	32-38	10(8.85%)	7(6.19%)	17(15.04%)				
	39-45	8(7.08%)	1(0.88%)	9(7.96%)				
	46-52	2(1.77%)	2(1.77%)	4(3.54%)				
	53-59	1(0.88%)	0(0%)	1(0.88%)				
	Total	38(33.63%)	33(29.20%)	71(46.48%)				
$\chi^2$	X2 value	27.434	24.910					
	P value	0.285	0.467					
Pearson's Correlation	value	0.335	-0.150					
	P value	0.05	0.250					
Parameter	Age group (years)	Male	Female	Total	$\chi^2$		Pearson's correlation	
					value	P value	Value	P value
T.gondii IgG positive	18-24	2(1.77%)	12(10.62%)	14(12.39%)	28.641	0.279	-0.077	0.546
	25-31	14(12.39%)	8(7.08%)	22(19.47%)				
	32-38	4(3.54%)	11(9.73%)	15(13.27%)				
	39-45	4(3.54%)	6(5.31%)	10(8.85%)				
	46-52	0(0%)	2(1.77%)	2(1.77%)				
	53-59	1(0.88%)	0(0%)	1(0.88%)				
	Total	25(22.12%)	39(34.51%)	64(56.64%)				
T.gondii IgG Negative	18-24	4(3.54%)	9(7.96%)	13(11.5%)	22.341	0.440	-0.388	0.006
	25-31	8(7.08%)	8(7.08%)	16(14.16%)				
	32-38	8(7.08%)	4(3.54%)	12(10.62%)				
	39-45	5(4.42%)	1(0.88%)	6(5.31%)				
	46-52	2(1.77%)	0(0%)	2(1.77%)				
	53-59	0(0%)	0(0%)	0(0%)				
	Total	27(23.89%)	22(19.47%)	49(43.36%)				
$\chi^2$	X2 value	29.467	24.302					
	P value	0.203	0.502					
Pearson's correlation	value	0.086	-0.212					
	P value	0.543	0.101					
ANOVA (between groups)	F value= 0.019 ;P value= 0.891							

**Table 4**  
**Correlation and distribution of T.gondii specific antibodies according to TSH**

Parameter	TSH	Male	Female	Total	$\chi^2$		Pearson's correlation	
					value	P value	Value	P value
<b>T.gondii IgM positive</b>	Normal 0.5-5.0 $\mu$ U/mL	12(10.62%)	27 (23.89%)	39 (34.51%)	1.615	0.204	-0.196	0.213
	Elevated >5.0 $\mu$ U/mL	0(0%)	0(0%)	0(0%)				
	Decreased <0.5 $\mu$ U/mL	0(0%)	0(0%)	0(0%)				
	Not detected	2(1.77%)	1(0.88%)	3(2.65%)				
	Total No.(%)	14(12.39%)	28(24.78%)	42(37.17%)				
<b>T.gondii IgM Negative</b>	Normal 0.5-5.0 $\mu$ U/mL	37(32.74%)	32(28.32%)	69(61.06%)	0.010	0.919	0.012	0.921
	Elevated >5.0 $\mu$ U/mL	0(0%)	0(0%)	0(0%)				
	Decreased <0.5 $\mu$ U/mL	0(0%)	0(0%)	0(0%)				
	Not detected	1(0.88%)	1(0.88%)	2(1.77%)				
	Total No.(%)	38(33.63%)	33(29.20%)	71(46.48%)				
$\chi^2$	value	2.556	0.278					
	P value	0.110	0.598					
Pearson's correlation	value	-0.222	-0.068					
	P value	0.114	0.605					
Parameter	TSH	Male	Female	Total	$\chi^2$		Pearson's correlation	
					value	P value	Value	P value
<b>T.gondii IgG positive</b>	Normal 0.5-5.0 $\mu$ U/mL	23(20.35%)	37(32.74%)	60(53.1%)	0.214	0.643	-0.058	0.650
	Elevated >5.0 $\mu$ U/mL	0(0%)	0(0%)	0(0%)				
	Decreased <0.5 $\mu$ U/mL	0(0%)	0(0%)	0(0%)				
	Not detected	2(1.77%)	2(1.77%)	4(3.54%)				
	Total No.(%)	25(22.12%)	39(34.51%)	64(56.64%)				
<b>T.gondii IgG Negative</b>	Normal 0.5-5.0 $\mu$ U/mL	26(23.01%)	22(19.47%)	48(42.48%)	0.832	0.362	-0.130	0.372
	Elevated >5.0 $\mu$ U/mL	0(0%)	0(0%)	0(0%)				
	Decreased <0.5 $\mu$ U/mL	0(0%)	0(0%)	0(0%)				
	Not detected	1(0.88%)	0(0%)	1(0.88%)				
	Total No.(%)	27(23.89%)	22(19.47%)	49(43.36%)				
$\chi^2$	X2 value	0.441	0.001					
	P value	0.507	0.981					
Pearson's correlation	Pearson's correlation value	-0.092	0.003					
	P value	0.516	0.982					
<b>ANOVA (Between groups)</b>	F value= 3.377 ; P value= 0.069							

**Table 5**  
**Correlation and distribution of *T.gondii* specific antibodies according to T3**

Parameter	T3	Male	Female	Total	$\chi^2$		Pearson's correlation	
					Value	P value	Value	P value
<b>T.gondii IgM positive</b>	Normal ( 70-195 ng/dL (1.1-3.0 nmol/L)	13(11.50%)	21(18.58%)	34(30.09%)	2.011	0.366	0.207	0.188
	Elevated(>195 ng/dL (>3.0 nmol/L)	1(0.88%)	6(5.31%)	7(6.19%)				
	Decreased(<70ng/dL (<1.1nmol/L)	0(0%)	0(0%)	0(0%)				
	Not detected	0(0%)	1(0.88%)	1(0.88%)				
	Total	14(12.39%)	28(24.78%)	42(37.17%)				
<b>T.gondii IgM Negative</b>	Normal (70-195 ng/dL ) (1.1-3.0 nmol/L)	37(32.74%)	31(27.43%)	68(60.18%)	3.193	0.203	-0.024	0.845
	Elevated >195 ng/dL >3.0 nmol/L)	0(0%)	2(1.77%)	2(1.77%)				
	Decreased(<70 ng/dL (<1.1nmol/L)	0(0%)	0(0%)	0(0%)				
	Not detected	1(0.88%)	0(0%)	1(0.88%)				
	Total	38(33.63%)	33(29.20%)	71(46.48%)				
$\chi^2$	value	3.104	4.544					
	P value	0.212	0.103					
Pearson's correlation	value	0.008	-0.262					
	P value	0.957	0.041					
Parameter	T3	Male	Female	Total	$\chi^2$		Pearson's correlation	
					Value	P value	Value	P value
<b>T.gondii IgG positive</b>	Normal ( 70-195 ng/dL ) (1.1-3.0 nmol/L)	23(20.35%)	33(29.20%)	56(49.56%)	1.460	0.482	.038	0.767
	Elevated(>195 ng/dL ) (>3.0 nmol/L)	1(0.88%)	5(4.42%)	6(5.31%)				
	Decreased(<70 ng/dL ) (<1.1nmol/L)	0(0%)	0(0%)	0(0%)				
	Not detected	1(0.88%)	1(0.88%)	2(1.77%)				
	Total	25(22.12%)	39(34.51%)	64(56.64%)				
<b>T.gondii IgG Negative</b>	Normal(70-195ng/dL ) (1.1-3.0 nmol/L)	27(23.89%)	19(16.81%)	46(40.71%)	3.922	0.048	0.283	0.049
	Elevated >195 ng/dL (>3.0 nmol/L)	0(0%)	3(2.65%)	3(2.65%)				
	Decreased(<70 ng/dL (<1.1nmol/L)	0(0%)	0(0%)	0(0%)				
	Not detected	0(0%)	0(0%)	0(0%)				
	Total	27(23.89%)	22(19.47%)	49(43.36%)				
$\chi^2$	value	2.246	0.576					
	P value	0.325	0.750					
Pearson's correlation	value	-0.185	-0.067					
	P value	0.189	0.610					
<b>ANOVA (Between groups)</b>	F value= 4.011 ; P value= 0.048							

**Table 6**  
**Correlation and distribution of *T.gondii* specific antibodies according to T4**

Parameter	T4	Male	Female	Total	$\chi^2$		Pearson's correlation	
					Value	P value	Value	P value
T.gondii IgM positive	Normal(5-12 $\mu$ g/dL ) (64-155 nmol/L)	13(11.50%)	21(18.58%)	34(30.09%)	1.930	0.165	0.214	0.173
	Elevated(>12 $\mu$ g/dL ) (>155 nmol/L)	1(0.88%)	7(6.19%)	8(7.08%)				
	Decreased(<5 $\mu$ g/dL) (<64 nmol/L)	0(0%)	0(0%)	0(0%)				
	Not detected	0(0%)	0(0%)	0(0%)				
	Total	14(12.39%)	28(24.78%)	42(37.17%)				
T.gondii IgM Negative	Normal(5-12 $\mu$ g/dL ) (64-155 nmol/L)	37(32.74%)	31(27.43%)	68(60.18%)	0.513	0.474	0.085	0.481
	Elevated(>12 $\mu$ g/dL ) (>155 nmol/L)	1(0.88%)	2(1.77%)	3(2.65%)				
	Decreased(<5 $\mu$ g/dL) (<64 nmol/L)	0(0%)	0(0%)	0(0%)				
	Not detected	0(0%)	0(0%)	0(0%)				
	Total	38(33.63%)	33(29.20%)	71(46.48%)				
$\chi^2$	$\chi^2$ value	0.563	4.320					
	P value	0.453	0.038					
Pearson's correlation	value	-0.104	-0.266					
	P value	0.463	0.038					
Parameter	T4	Male	Female	Total	$\chi^2$		Pearson's correlation	
					Value	P value	Value	P value
T.gondii IgG positive	Normal(5-12 $\mu$ g/dL ) (64-155 nmol/L)	24(21.24%)	33(29.20%)	57(50.44%)	2.027	0.155	0.178	0.159
	Elevated(>12 $\mu$ g/dL ) (>155 nmol/L)	1(0.88%)	6(5.31%)	7(6.19%)				
	Decreased(<5 $\mu$ g/dL ) (<64 nmol/L)	0(0%)	0(0%)	0(0%)				
	Not detected	0(0%)	0(0%)	0(0%)				
	Total	25(22.12%)	39(34.51%)	64(56.64%)				
T.gondii IgG Negative	Normal(5-12 $\mu$ g/dL ) (64-155 nmol/L)	26(23%)	19(16.81%)	45(39.82%)	1.595	0.207	.180	0.215
	Elevated(>12 $\mu$ g/dL ) (>155 nmol/L)	1(0.88%)	3(2.65%)	4(3.54%)				
	Decreased(<5 $\mu$ g/dL ) (<64 nmol/L)	0(0%)	0(0%)	0(0%)				
	Not detected	0(0%)	0(0%)	0(0%)				
	Total	27(23.89%)	22(19.47%)	49(43.36%)				
$\chi^2$	$\chi^2$ value	0.003	0.034					
	P value	0.956	0.853					
Pearson's correlation	value	-0.008	-0.024					
	P value	0.957	0.856					
ANOVA (Between groups)	F value= 2.212 ; P value= 0.140							

## DISCUSSION

The mean age of patients in current work was (30.86  $\pm$ 8.70) years which come in line with others<sup>16,17</sup>. Males represent (46 %) and females (54 %); mean age (29.78 $\pm$ 8.66)years which come in line with<sup>18</sup>, and this could be explained by the fact that females have a higher tendency to be involved in activities or outdoor works, a like that expose them to soil. Also possibly, they are not careful about hand hygiene, which leads to increase risk of acquiring the infection. T.gondii IgM was positive in (37.2%) ;IgG positive (56.6% );both IgM+IgG positive 69(61.1%) which considered low compared with a study in waset governorate<sup>19</sup> and high compared with other local study in Ramadi<sup>20</sup>, Basra (41%)<sup>21</sup> and other area

like peninsular Malaysia, (19%) were seropositive<sup>16</sup>. Worldwide seroprevalence of the parasite measured by specific anti-Toxoplasma IgG antibodies varies between 1% and 100%<sup>22</sup>, depending on the environmental and socioeconomic conditions, including eating habits and health-related practices, general level of hygiene, host susceptibility, geographical location (geolatitude) and humidity of the soil<sup>23</sup>. This study report a significant difference and correlation between gender and T.gondii specific IgM, IgG, IgM+IgG (P Value=0.001 to 0.000) which come in concordance with other studies<sup>16,24</sup>. Significant difference and correlation detected between males and females in T.gondii specific IgM positive and negative cases (P Value=0.038). This difference may be due to the expression of specific receptors of sex-and pregnancy-associated hormones on the immune system cells that



influence the function of all immune cells and have the vital effects on immune response which are not limited to reproductive tissues but are clearly evident in the systemic immune system response<sup>16,25</sup>. For example, hormones can influence cells of the innate immune system, such as mast cells, eosinophils, macrophages, dendritic cells, and NK cells. These cells not only form the first line of defense against many organisms, but also play an important role in directing the developing adaptive immune response. These hormones also directly affect the adaptive immune response involving T cells and B cells. In current study the humoral immune response against *T.gondii* appear to be high among females and this may be associated with effect of estrogen hormone that shown to increase B-cell activity and antibody production<sup>25</sup> and to promote IL-2-driven B-cell production of IgM and IgG in spleen cells while down-regulating IgG production by normal blood B cells<sup>26</sup>. Others support our explanation, that IgG anti-Toxoplasma antibodies were significantly correlated to testosterone<sup>27</sup> and results are different according to type of Toxoplasma strain<sup>28</sup>. Neither Significant difference nor correlation detected between males and females in *T.gondii* specific IgG positive and negative cases (P Value=0.092). This might be because continuous exposure to *T.gondii* causing a state of retaining a steady level of anti-Toxoplasma IgG in serum for years. In current study a significant difference (P Value=0.05) detected between males and females in *T.gondii* specific *T.gondii* IgM+ IgG positive and negative cases. The differences in immune response among males and females against *T.gondii* reflect the possibility that nature of immune response against *T.gondii* affected by gender. Physiological differences between males and females play an important role in determining susceptibility to *T.gondii* infection. Beside the fact that sex-associated hormones can influence the immune system and response to *T.gondii*<sup>25</sup>. In current study, The majority of IgM positive; IgG positive among male patients belongs to (25-31) years, (7.96%), (12.39%) respectively. Among females, the majority of IgM positive; IgG positive cases at (18-24) years, (7.96%); (10.62%), followed by (32-38), (39-45) years respectively. These results were come in line with local study in Kirkuk, shows that the most age group infected in married women was 34-37 years old<sup>17</sup>. Other study in Kirkuk stated that, the highest rate of Toxoplasma seropositivity was among (19-35) years (38.3%)<sup>29</sup>. Current seropositivity agree with a study in Sudan<sup>30</sup>. In current study, seropositivity of IgM, IgG have no correlation with age group of male and female patients. This result come in opposites with a study in Makkah<sup>31</sup>, stated that a significant (P > 0.05) relation between Toxoplasma seropositivity rate and the female age. current study disagree with the assumption that "seroprevalence of Toxoplasma is well known to increase with age; the greater the prevalence, the earlier the rise"<sup>17</sup>. This study represents the first study in Iraq according to internet based research in scholar database about the possible effect of *T.gondii* on TSH, T3 and T4 serum levels among toxoplasmosis infected Iraqi patients. For this reason, there was no available data to compare with. In current study, the TSH at normal level in all patients.

Anti-toxoplasma IgM among males with normal TSH level represent (10.62%) compared with (23.89%) for females. Anti-toxoplasma IgG positive among male patients with normal serum level of TSH (20.35%) compared with (32.74%) for females. One of interesting results in current work, the serum level of TSH has not been affected by stage of *T.gondii* infection whether acute or chronic; Gender of patients whether male or female. The serum TSH level has been at normal without significant difference. These findings come in contrary with previous studies indicating that after experimental inoculation of *T.gondii* in a murine model, there was depression in TSH production<sup>32</sup>. The current results come in accordance with a clinical study of canine toxoplasmosis in Brazil, indicating normal serum level of TSH in all infected cases<sup>33</sup> even with subacute thyroiditis. This give an indication that during toxoplasmosis whether acute or chronic infection, the hypothalamus-pituitary axis work properly which in turn mean that hypothalamus produce essential amounts of TSH releasing hormone (TRH) which leads to production of sufficient amounts of TSH from pituitary. Regarding serum level of T3, (30.09%) of patients with positive anti toxoplasma IgM have normal T3. Compared with (6.19%) have increased serum level of T3, among them (5.31%) of females have positive IgM and increased T3 serum level with obvious significant difference (P value =0.04) among females in T3 serum level according to IgM positivity. Patients presented with positive anti-toxoplasma IgG and normal serum T3 represent (49.56%), Compared with (5.31%) have increased serum level of T3. Increase in T3 serum level was mainly among female (4.42%) compared with (1(0.88%) for males. Significant difference in T3 serum level among males and females according to anti-toxoplasma positive IgM, IgG response. Regarding serum level of T4, (30.09%) of patients with positive anti-toxoplasma IgM have normal T4. Compared with (7.08%) have increased serum level of T4, among them (6.19%) females with significant difference (P value =0.03) and inverse correlation (P value =0.03) among females in T4 level according to IgM positivity. Patients presented with positive anti-toxoplasma IgG and normal serum T4 represent (50.44%), Compared with (6.19%) have increased serum level of T4 mainly among female (5.31%) compared with (1(0.88%) males. No Significant difference in T4 level among males and females according to IgM, IgG anti toxoplasma positive response. These results come in line with a other clinical study of canine toxoplasmosis in Brazil, indicating normal serum level of T4 in 50% of infected cases, T4 was increased in (37.5%)<sup>33</sup> with significant correlation between anti-toxoplasma antibodies and serum level of T4. Decrease serum level of T4 has not been detected in all positive cases, instead in (12.5%) of positive cases in canine model have low serum level of T4<sup>33</sup>. In other study of murine toxoplasmosis, a decline in T4 level was detected<sup>32</sup> which in turn reflect the decline in T3 serum level as T4 a prohormone for T3 and the ratio of T4 to T3 released into the blood is roughly 20 to 1 in serum, which give possibility of hypothalamic dysfunction. In current study, the normal levels of T3 and T4 in IgM, IgG positive cases indicate that continuing synthesis, storage,

and release of T<sub>4</sub> is dependent on the pulsatile stimulation of the thyroid by TSH (thyrotropin). As a result of normal level of TSH in all patients in current study, T<sub>4</sub>, T<sub>3</sub> still at normal level in majority of IgM, IgG positive *T. gondii* patients. Increased serum level of T<sub>4</sub>, T<sub>3</sub> reflect the fact that *T. gondii* may initiate the development of a state of hyperthyroidism even in limited number of infected patients (6.19%-7.08%), mainly among females. This may be due to direct involvement of thyroid gland by *T. gondii*, multiplication in thyroid tissue and subsequent alteration of thyroid hormones<sup>34</sup>. Also may be due to reactivation of latent toxoplasmosis which may persist after or within 6 months of recovery from first initiation of toxoplasmosis<sup>35</sup>. *T. gondii* may cause neurological stimuli for hypothalamus-pituitary-thyroid axis for accelerating of proteolysis of the thyroglobulin, which causes re-

lease of thyroxine (T<sub>4</sub>) and triiodothyronine (T<sub>3</sub>) into the blood within 30 minutes<sup>36,37</sup>

## CONCLUSION

The current study proved that *T. gondii* IgM, IgG positive responses significantly associated with gender. T<sub>3</sub>, T<sub>4</sub> inversely correlated with anti *T. gondii* IgM positive response among females. Significant difference in T<sub>3</sub> level among males and females with IgM+IgG positive *T. gondii* in serum. Increase in serum T<sub>3</sub> and T<sub>4</sub> thyroid hormones production in toxoplasmosis (6.19%-7.08%), provide new clue to the complex pathogenesis of toxoplasma-associated thyroid diseases.

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