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Toxoplasmosis Seropositivity and Male Sex Hormones

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Abstract

Toxoplasmosis is a cosmopolitan disease with acute and chronic infections, caused by the obligate intracellular protozoan parasite. Toxoplasma gondii that can infect a variety of cells in almost all warm blooded animals including humans. The study aimed to determine the seroprevalence of *T. gondii* infection among males in Duhok city using ELISA (IgG and IgM).The relationship between toxoplasmosis and reproductive hormones including testosterone, free testosterone, and follicle stimulating hormone (FSH) levels and its association with male sterility were also investigated.

Keywords: Toxoplasmosis; Acute and Chronic infection; Male Sex Hormones

Introduction

Toxoplasmosis is a cosmopolitan zoonotic disease with acute and chronic infections caused by obligate intracellular protozoan parasite Toxoplasma gondii [1]. Toxoplasmosis is ubiquitous disease that can infect a variety of cells in almost all warm blooded animals including humans [2]. In immunocompetent patient, the primary infection of toxoplasmosis is asymptomatic or self-limited. Symptoms ranged from fever, malaise to cervical lymphadenopathy [2].

Toxoplasmosis is a very important reason behind bad obstetrical history (BOH) and causes serious health problems resulting in abortions and congenital disease in pregnant woman especially if the parasite is transmitted to the fetus early in pregnancy [3].

T. gondii may infect male reproductive organ tract and causing testicular damage [4]. Chronic toxoplasmosis showed an impression on men generative parameters [5].

It is also known that *T. gondii* induce changes of behavioral and neurophysiological condition in infected human and animal hosts [6,7]. Worldwide some studies have been conducted on detection of *T. gondii* infection in men particularly the effects of toxoplasmosis on male hormonal parameters [8].

Regarding Iraq and Kurdistan region, there are limited studies in this direction such as, Al-Saadii who studied (the effect of toxoplasmosis on the level of some male sex hormones in samples from National Blood Transfusion Center/Baghdad) and also Bassad *et al.* in Iraq/Thiqar who studied the (the prevalence of toxoplasmosis among male blood donors) [9,10]. Regarding to Kurdistan, In Kalar city, a study by Al-Bajalan *et al.* indicated that there is a relationship presence of an association between toxoplasmosis and secondary infertility [11]. Since the prevalence of *T. gondii* in primary infertile, secondary infertile and fertile males among 260 persons attending the hospital were 45.16%, 53.33%, and 47.37%, respectively by using ELISA tests (Table 1 and 2).

In Duhok province, there is no any study dealing with the effect of toxoplasmosis on the levels of male sex hormones and fertility. Therefore the main objective of the present study are to find out the correlation between *T. gondii* infection (acute, chronic), and the levels of serum testosterone, serum follicle stimulating hormone and fertility in a group of healthy blood donor males and those who visited the IVF center of Azadi teaching hospital.

Characteristics of subjects	Groups	N	%
Residence	Urban	125	50.4
	Rural	123	49.5
Occupation	Official	143	57.6
	Private	105	42.3
Fertility	Fertile	222	89.5
	Infertile	26	10.5
Blood groups	O+	80	32.2
	O-	7	2.8
	A+	57	22.9
	A-	5	2.0
	B+	68	27.4
	B-	4	1.6
	AB+	25	10.0
	AB-	2	0.8
Age groups	21-30	63	25.4
	31-40	105	42.3
	41-50	67	27.0
	51-60	13	5.2

Table 1: The distribution of the studied males according to their characteristics (no= 248)

Males' Characteristics	Mean	s.D
Total Testosterone (ng/ml)	6.16	1 .03
Free Testosterone (pg..ml)	14.8l	4.71
Follicle-stimulating hormone (lU/ml)	4.81	2.20
Anti-*toxoplasma* IgM	0.541	0.22
Anti-*toxoplasma* IgM	0 625	0.75

Table 2: Statistical distribution of the hormones (TTH, FTH and FSH) characteristics of males enrolled in the study using ELISA IgG and IgM Abs

In order to determine:

1- The rate of Toxoplasma infections among males in Duhok city using ELISA (IgM and IgG).

2- To find out the relationship between toxoplasmosis and blood groups and some demographic factors (Figure 1).

3- To investigate the relationship between toxoplasmosis and sex hormonal disturbances in seropositive males such as, testosterone (total, free) and follicle stimulating hormone (FSH) levels.

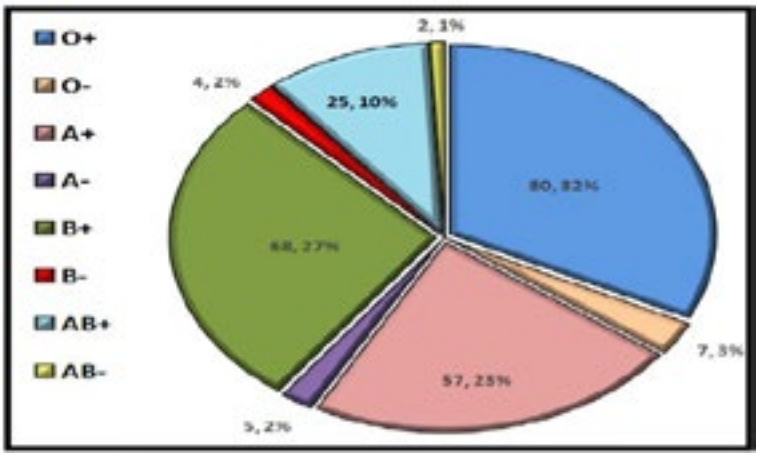

Figure 1: Blood groups distribution among males enrolled in the study.

Methods and Patients

Blood samples were collected from 248 apparently healthy males; their ages were between 21 and 60 years. They were attending the *In Vitro* Fertilization (IVF) center in Azadi Teaching hospital and the Central Blood Bank in Duhok city during the period from

November 2016 to the end of July 2017. Before collecting the samples, a questionnaire was prepared and designed which covers various details. From each donor, 5ml of blood was withdrawn by vein puncture, placed in plain tubes and centrifuged. The serum was dispensed into 1ml labeled eppendorf tubes and stored at −20 °C until serological and hormonal tests were performed.

Frequency percentage was performed to determine the prevalence of affected persons by *T. gondii* and mean standard deviation was used to understand the reproductive male hormones concentrations. One way ANOVA and chi-square tests were performed for statistical analyses. The independent t-tests and chi-square tests were performed to determine the association between toxoplasma and reproductive hormones and infertility.

Required Kits:

- The Bioactiva Toxoplasma IgG and IgM ELISA Kits were used.
- The Monobind Total & Free Testosterone and FSH ELISA were used.

Results

The mean age of the studied participants was 37.29± 8.07 years. 22.98% of male samples appeared to be affected by *T.gondii* infection. The mean concentrations of the male reproductive hormones were 6.15±1.03 ng/ml (total testosterone), 14.81±4.71pg/ml (Free Testosterone), 4.84±2.20 IU/ml (Follicle-stimulating hormone), 0.54±0.22 (IgM), and 0.62±0.75 (IgG). A significant difference was found in the samples in terms of infertility ($p<0.0001$), while p-values for testosterone ($P>0.05$), free testosterone ($P>0.05$) and follicle-stimulating hormone ($P>0.05$), were non-significant.

Conclusion

The present study showed a high rate of *T. gondii* among males in Duhok city with significant difference ($P<0.05$) in infertility between *T. gondii* seropositive and seronegative samples.

Discussion

Seropositivity of Toxoplasma gondii

Patients Characteristics	Rule of Infection (N,%)				
	Overall seropositive	22-30 Year	30-40 Year	41-50 Year	51-60 Year
Rate of Infection	57 (22.98)	14(5.6)	28(11.3)	14(5.6)	1.(0.4)
Residence	26(1 O.8)	8(3.2)	12(4.8)	5(2.0)	1(0.4)
Urban					
Rural	31(12.5)	62.4l	16(6.4}	10(4.0)	0(0.0)
Occupation Public	29(11.6)	9(3.6)	1S(6.0)	4(1.6)	1(0.4)
Public					
private	28(11.3)	3(1.2)	131S.2l	0(0.0)	0(0.0)
Fertility fertile	53(21.3	11(4.4)	27(10.8)	14(5.6)	1(0.4)
Fertile					
In fertile	4(1.6)	3(1.l)	1(0.4)	0(0.0)	0(0.0)
Blood Croups	18(7.2)	7(2.8)	9(3.6)	2(0.8}	0(0.0)
O+					
O- A+ A-	1(0.4) 9(3.6) 1(0.4)	l(0.4) 2(0.8) 0(0.0)	0(0.0) 4(1.6} 1(0.4)	0(0.0} 2(0.8} 0(0.0)	0(0.0) 0(0.0) 1(0.4)
B+	2 1(8.4)	3(1.2)	13(5.2)	S(2 .0)	0(0.0)
B-	2(0.8)	0(0.0)	1(0.4)	1(0.4)	0(0.0)
AB+	4(1.6)	1(0.4)	0(0.0)	3(1.2)	0(0.0)
AB-	1(0.4)	0(0.0)	0(0.0)	1(0.4)	0(0.0)

Table 3: The seropositivity of *T. gondii* with respect to age and other factors

According to Age: The current study showed out of 248 apparently healthy males from different ages (22-60) years enrolled in this study, 57(22.98%) were seropositive for *T. gondii* antibodies. The rate of the infection increased with the increase in the age from 5.6% in the (22-30) year's group to 11.3% in the (31-40) years group up to the age group (41-50) years at which was decreased to 5.6%, then dramatically decreased to 0.4% at the age group (51-60) years.

Various rates of seropositivity of *T. gondii* Abs using different serological tests have been reported among different age groups in previous studies performed in different parts of Kurdistan and Iraq, in some of them higher rates were reported, while in others lower rates than that reported in this study, this might be due to sample size, method of calculation, residency, age, type of test used, or other factors (Table 3).

The Distribution of Toxoplasmosis using ELISA IgG and IgM According to Blood Group

This study showed that the overall seropositivity of toxoplasmosis among different blood groups was 22.98%. Regarding blood groups, males of group B+ aged (31-40) years showed the highest rate (5.2%) followed by 3.6% in males of blood group O+ of same ages (Table 4) (Figure 2 and 3).

Age Group Yea r	lgM +			lgG +		
	N	TTH Ng/ml	ITH pg/ml	N	TTH ng/ml	FTH pg/ml
22-30	1	9.3	30.5	12	7.68	2 1.44
31-40	6	6.09	17.39	22	6.72	17.0
41-50	8	6.04	13.71	7	8.38	24.66
51-60	1	5.76	13.65	0	0	0
Total	16			41		

Table 4: The mean concentration of TTH and FTH in the sera of the studied males infected with toxoplasmosis using ELISA according to the age group

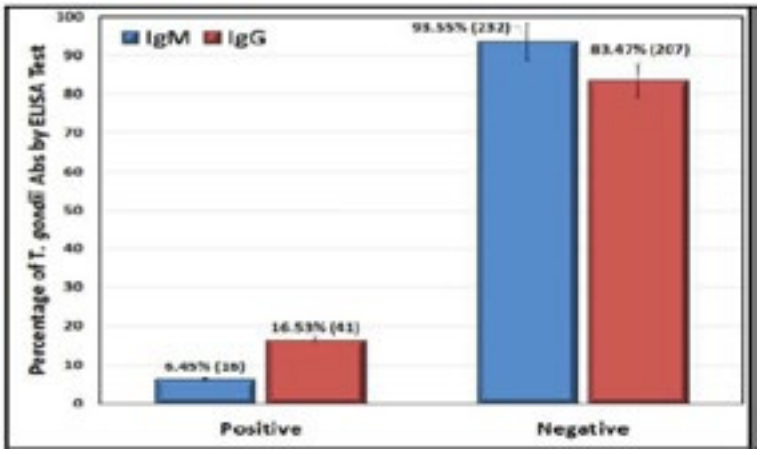

Figure 2: The distribution of the studied males according to their seropositivity of anti-*Toxoplasma* IgG and IgM antibodies using ELISA.

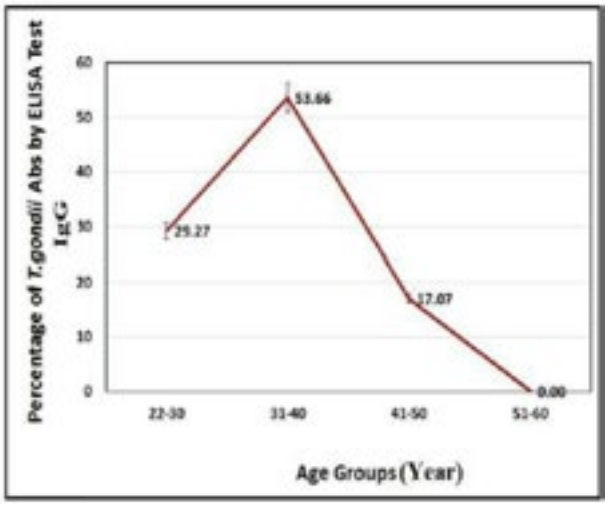

Figure (3 A): The distribution of the studied males according to seropositivity of anti-Toxoplasma IgG and age.

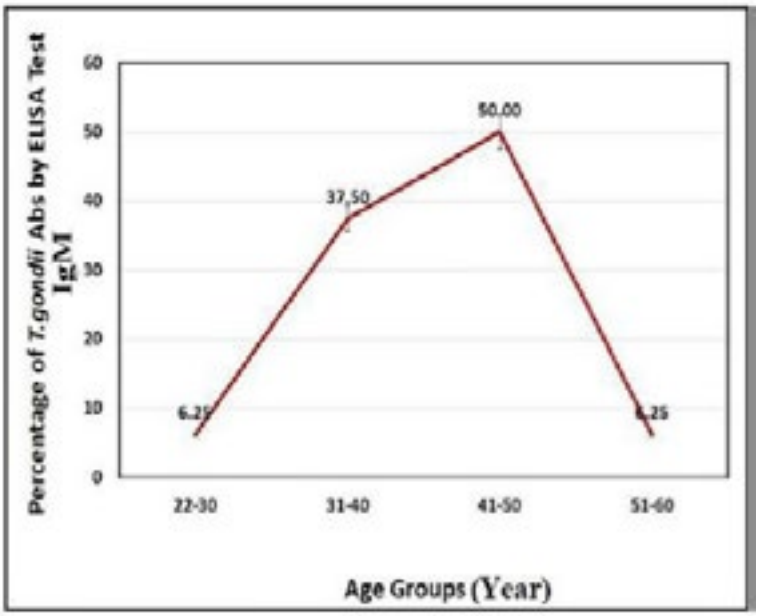

Figure (3 B): The percentage distribution of the studied males according to seropositivity of anti-Toxoplasma IgM and age.

The present results partly agree with those of Al-Kaysi et al. who found the highest percentage of seropositivity of T.Gondii [12] Abs in males of blood groups O+ and AB which were 35.8% and 38%, respectively.

Seropositivity of Toxoplasma gondii

According to Residency

Regarding residency, the results of the current study on seropositivity of *T. gondii* in rural and urban areas, showed a high percentage in rural areas. It is evident that people living in rural areas are more likely to be affected by different types of infectious microorganism such as, *T. gondii* due to more contact with animals. Regarding the age, ages from 31-40 and 41-50 years showed, higher rates of Toxoplasma Abs(6.4 and 3.6%, respectively) as compared to urban inhabitants (Table 5 and 6).

Fertility	lgM +			lgG +		
	TTH ng/ml	FTH pg/ml	N	TTH ng/ml	FTH Pg/ml	N
Fertile	6.l	13.72	13	7.27	19.5	39
Infertile	7.29	26.61	3	7.61	21.72	2
Total			16			41

Table 5: The mean concentration of TTH and FTH in the sera of the studied males infected with toxoplasmosis using ELISA according to the fertility

Age Group	N	lgM +	N	lgG +
		FSH (iu/ml)		FSH (IU/ml)
22-30	1	5.5	1 2	6.67
31-40	6	5.07	22	4.92
41-50	8	5.15	7	7.85
51-60	1	4.33		
Total	16		41	

Table 6: The relation between mean concentrations of FSH in the sera of the studied males infected with toxoplasmosis from different ages using ELISA test

Seropositivity of Toxoplasma gondii

According to Occupation: The results of the present study showed that toxoplasmosis seropositivity was different statistically with respect to occupation for difference of IgG and IgM for official and private occupation. The ages (31-40), (20-30), and (41-50) years in public sectors showed higher percentage of seropositivity (6.0%, 3.6%, and 1.6%), respectively and the age group 31-40 years in private sector (5.2%) (Table 7) (Figure 4).

Fertility	lgM +			lgG +		
	FSH (IU/ml)	N	%	FSH (IU/mI)	N	%
Fertile	4.68	13	81.25%	5.9	39	95.12%
Infertile	6.87	3	18.75%	6.54	2	4 .88%
Total		16			41	

Table 7: The mean concentration of FSH in the sera of the studied males infected with Toxoplasmosis using ELISA according to the fertility

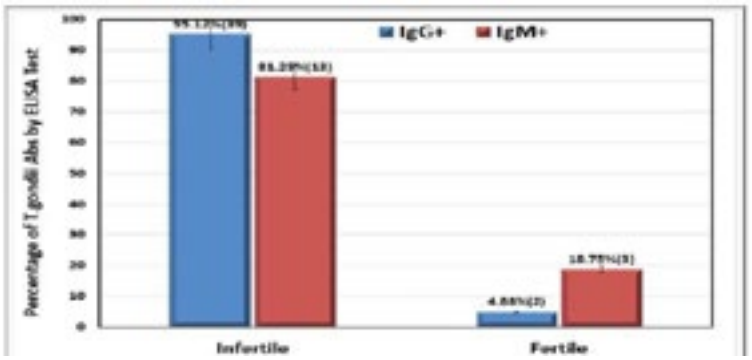

Figure 4: The distribution of the studied males according to Seropositivity of IgG and IgM Antibodies among fertile and infertile males.

Effects of Toxoplasma gondii on Humans

The current study concentrated on the effects of *T. gondii* on male reproductive hormones including testosterone, free testosterone, an FSH, as well as, their impact on male fertility. The different aspects of scrutinized characteristics of the patients are analyzed in the following sections (Table 8) (Figure 5).

Reproductive hormones			P-value (22-60 years)
Testosterone *			P>0.05
free testosterone *			P>0.05
follicle-stim ulating hormone (FSH) *			P>0.05
fertility **			**<0.0001**
Age Groups**	**Fertility N(%)**		
20-30	**Fertile**	**Infertile**	
	46 (73.0)	17 (27.0)	
3 1-40	98 (93.3)	7 (6.7)	**<0.0001**
41-50	66 (98.5)	I{1.5)	
51-60	12 (92.3)	I (7.7)	
*ANOVA one way and ** chi-squaretests was performed for statistical analyses.			

Table 8: The association of male reproductive hormones with T. gondii and fertility in men aged 22-60 years old

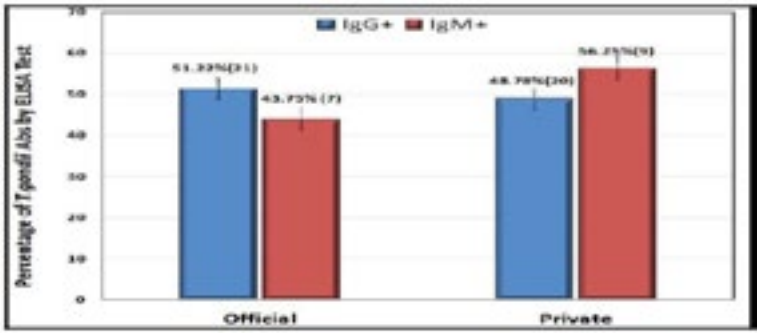

Figure 5: The distribution of the studied males according to seropositivity of toxoplasmosis and occupation.

The Relation between Toxoplasma gondii and Reproductive Hormones and Fertility in Males

The present study showed that there is a significant difference of male reproductive hormones between fertile and infertile males. Male infertility is caused by acquired or congenital urogenital abnormalities, infections in urogenital tract, increase in scrotum temperature, endocrine disturbances, hereditary abnormalities, and immunological factors [13]. Infectious agents such as bacteria, fungi, viruses and parasites are able to interfere with reproductive hormones and functions in male and female populations. The different organs of the male reproductive tract including testis, epididymis, or male accessory sex glands could be affected by infections (Table 9).

Characteristics of Males	Fertile N(%)	Infertile N(%)	P-value
Residence	114 (51.4)	11 (42.3}	0.383*
Urban			
Rural	108 (48.6)	15 (57.7)	
Occupation	125 (56.3)	18 (69.2)	0.207*
Official			
Private	97 (43.7)	8 (30.8)	
*Chi-square test was performed.			

Table 9: The association in men according to residency and occupation

The present study showed that the males infected with toxoplasmosis had the highest significant differences (P<0.001) with the mean concentration of serum FTH. Males with seropositive anti-Toxoplasma IgG antibodies and anti- Toxoplasma IgM antibodies revealed the highest mean concentration levels of 19.61pg/ml and 16.13pg/ml for FTH hormones.While TTH and FSH hormone showed non- significant difference (P>0.05) for the mean concentration between acute and chronic males infected with toxoplasmosis (Figure 6).

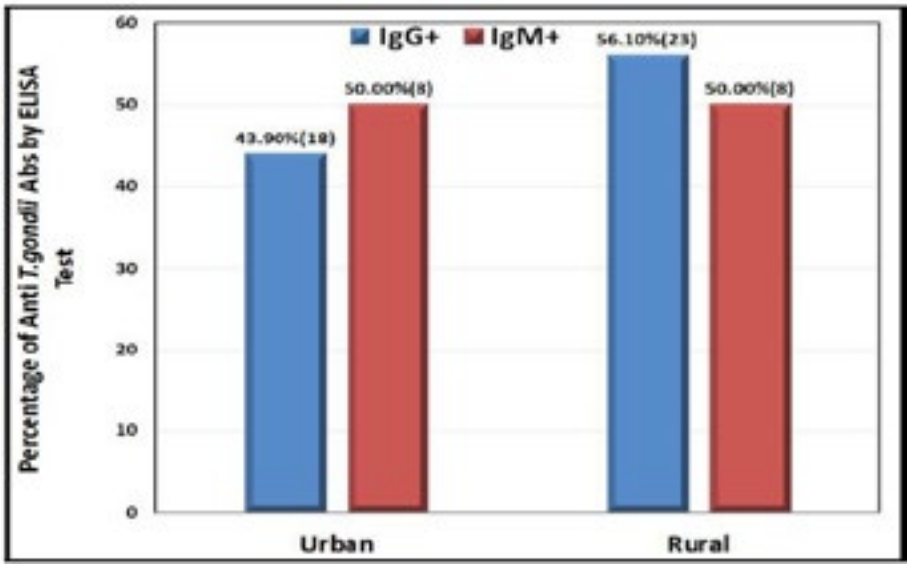

Figure 6: The distribution of Toxoplasmosis IgG and IgM Abs seropositivity according to residency.

Conclusion

From the present study, the followings are concluded:

1. The overall seropositivity rate of *T. gondii* was 22.98% in the tested sample, 6.45% with acute and 16.35% with chronic toxoplasmosis.
2. Regarding age, the highest rate (11.3%) of toxoplasmosis was among the age group (31-40) years.
3. Males with O+ and B+ blood groups showed higher percentage of infection with Toxoplasma in comparison to other blood groups.
4. Infertile males had higher percentage of both acute and chronic toxoplasmosis.
5. Official workers showed a higher rate (51.22%) of chronic toxoplasmosis than private worker (48.78%), while acute infection was higher (56.25%) in private workers than official (43.75%).
6. Rural inhabitants revealed high rates of both acute and chronic toxoplasmosis, in comparison to urban inhabitants.
7. The age group (22-30) years scored higher mean concentration of TT and FT in both acute and chronic infected males, while the age group (51-60) years showed a lower mean concentration of TT and FT in both acute and chronic infected males.

8. The TT and FT hormone levels recorded highest significant differences between fertile and infertile males. According to acute toxoplasmosis the infertile males showed a higher mean concentration of both TT and FT hormones. While fertile cases, showed lowest significant differences.
9. FSH showed higher value (6.87 and 6.54 IU/ml) and (4.68 and 5.9 IU/ml) for acute and chronic toxoplasmosis in infertile and fertile men, respectively.

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