Journal of Education for Pure Science- University of Thi-Qar
Vol.9, No.2 (June, 2019)
Website: jceps.utq.edu.iq Email: jceps@eps.utq.edu.iq

DOI: http://doi.org/10.32792/utq.jceps.09.02.16

Study The Relationship Between Some Sex Hormones and Toxoplasmosis Among Infertile Men Patients in Thi-Qar Province

Jabbar H. Fezaa , Mohannad Abd-Al Razzaq Gati[1], Bassad A. Al-Aboody[2]

[1,2] Department of Biology, College of Science, University of Thi-Qar, Thi-Qar, Iraq.

Received 22/01/2019, Accepted 12/03/2019, Published 02/06/2019

Abstract:
Toxoplasma gondii is the causal agent of Toxoplasmosis which infects a large proportion of the world's population, but clinically uncommonly causes significant disease. The present study was performed to estimate the relationship between toxoplasmosis and some sex hormones (testosterone hormone , follicle stimulating hormone (FSH) and luteinizing hormone) and also to estimate the prevalence of toxoplasmosis in infertility men. 280 samples of both infertile patients(180) and controls(100) had been tested by ELISA technique to detect anti-*Toxoplasma* Abs (IgG and IgM).The levels of sex hormones were also tested in men sera by ELISA test. The results of the present study in both infertile patients and healthy controls showed presence of anti-*Toxoplasma* IgG Abs in 40 out of 180 (22.23%) and 16 out of 100 (16%) respectively. While the result of ELISA- IgM test was (0 %) in both subjects .The results of sex hormones appeared the level of testosterone hormone was lowest in infertile patients infected with toxoplasmosis compared to higher level of hormone in non infected infertile patients and control groups.

Keywords: Toxoplasmosis, Sex hormones, infertility.

1.Introduction

Toxoplasmosis is a zoonotic disease caused by the protozoan parasite called *Toxoplasma gondii*, an obligate intracellular parasite capable of infected all warm-blood animals, including mammals and birds (Steven *et al.,* 2008). Human may remain infected for life and will stay asymptomatic unless immunosuppression occurs (Hill *et al*., 2005).

Birds and all mammals, including humans are intermediate hosts, whereas Felidae (cats) are intermediate and definitive host, they are the only animals that pass oocyst in their feces. Ingestion of oocyst from fecally contaminated hands, water or food also sheep and goat meats are important infection sources for toxoplasmosis (Sevgili *et al.*, 2005). Primary infection of toxoplasmosis in immunocompetent patients is usually asymptomatic or associated with self limited symptoms such as fever, malaise, and cervical lymphodenopathy.The infection acquired during pregnancy is frequently associated with transmission of *T.gondii* to the fetus, resulting in congenital disease. In immunocompromised patients, *T.gondii* causes severe manifestation, including spleenomegaly, chorioretinitis, pneumonitis, encephalitis, multisystem

organs failure, and even death (Montoya and Liesenfeld, 2004). In patients infected with human immunodeficiency virus (HIV), more than 90% of *Toxoplasma* encephalitis cases involve reactivation of a latent infection (Luft and Chua, 2000), this variability is related to various factors such as, age, sociocultural and nutritional habits, contact with domestic cats, climatic and geographical conditions (Barbosa *et al.*, 2009).

Infertility is defined as the inability to conceive naturally after one year of regular unprotected intercourse. Most of the time, infertility is some degree of subfertility in which 1 in 7 couples need specialist help to conceive. Both males and females are equally responsible for the causes. Most of the infertile couples have one of these three major causes including a male factor, ovulatory dysfunction, or tubal-peritoneal disease (Taylor ,2003). The role of *T. gondii* infection on human infertility is not investigated, but several experimental studies have shown that *T. gondii* infection plays a deleterious role in reproduction function of male and female mice. In male mice, acute toxoplasmosis can affect testes, vas deferens epididymis, prostate and thalamus and causes adverse damages (Sun L, *et al* .,2008;Abdoli *et al* ., 2012)

Reports indicate that *T.gondii* can change personality factors in men and women, it is likely that sexual hormone changes can play an important certain role in relation with *Toxoplasma* (Flegr *et al*., 2008). Androgen such as testosterone which is circulating hormone in blood stream , its production occur in Leyding cells in testicles. Testosterone play essential role in development of male characteristic, it promotes the development of secondary sexual characteristic in men , such as facial hair growth in axillae , the larynx cartilage growth ,prostate growth and development in men and depth of men sound (malkin *et al* ., 2010).

Follicle stimulating hormone (FSH)and luteinizing Hormone (LH) they are glycoproteins releasing from pituitary gland and transported by blood stream to its sites action (testes) . the releasing of FSH , as well as LH hormones is stimulating by gonadotropin-releasing hormone (GnRH). LH hormone stimulate Leyding cells to secretion testosterone hormone. .Different disorders occur when the level of FSH is high such as premature menopause , gonadal dysgenesis , poor ovarian reserve and testicular failure ,while low level of FSH lead to obesity, polycystic overian syndrome, infertility and gonadotropin deficiency (Boepple , 2008).

2. Materials and Methods:

2.1 Sample collection:

One hundred and eighty samples of blood had been collected at period from October 2017 to Jaunary 2018 from male patients with infertility with age range (20-60) years are coming to the Hussien Hospital (Infertility Center) of Thi-Qar province and one hundred samples of blood had been collected from apparently healthy persons as control. five ml of blood were collected from redial vein of each person by using disposable syringes. 2ml from blood to calculate blood groups and also to calculate blood parameters then the remaining blood was placed in gel tubes and allowed to clot at room temperature, then centrifuged at 3000 round per minute (rpm) for 10 minutes and sera were dispensed into 4 eppendrof tubes, and stored at -20 ° C .

2.2 Detection of *T. gondii*

This assay was performed according to manufacturer´s procedure using the commercial kits (Foresight, USA) for the detection of anti-*Toxoplasma* IgG and IgM antibodies. The results were read by ELISA reader.

2.3 Estimation of serum testosterone Hormone Level by ELISA technique:

The monobind testosterone enzyme immunoassay test kit (5325-300) was used.The quantitative determination of testosterone concentration in human sera by microplate enzyme immunoassay.

A dose response curve is used to ascertain the concentration of Testosterone in unknown specimens.

- The absorbance obtained from the printout of the microplate reader was recorded.
- The absorbance for each duplicate serum reference versus the corresponding Free Testosterone concentration in pg/ml on linear graph paper was ploted.
- The best –fit curve through the plotted points was drew.
- To determine the concentration of Testosterone for an unknown, the average absorbance of the duplicates for each unknown was located on the vertical axis of the graph, and read the concentration (in pg/ml) from the horizontal axis of the graph .Figure (1)

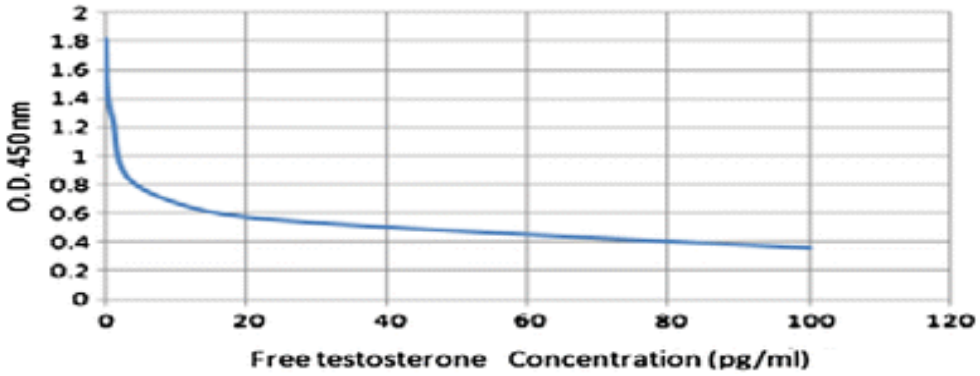

Figure (1) : Standard curve of testosterone concentration

2.4 Estimation of serum Follicle Stimulating Hormone (FSH) Levels by ELISA technique:

The FSH EIA Test Kit is a solid phase enzyme immunoassay based on a sandwich principle for the quantitative detection of FSH in human serum.

- Calculate the Mean Absorbance of each Calibrator, then plot them on the Y-axis against their concentration on the X-axis on a linear graph paper and draw the calibration curve. Draw the best-fitted line through data points and zero point to obtain a standard curve.
- Obtain quantitative specimen results of concentrations expressed in mIU/mL from their absorbance by using the calibration curve.

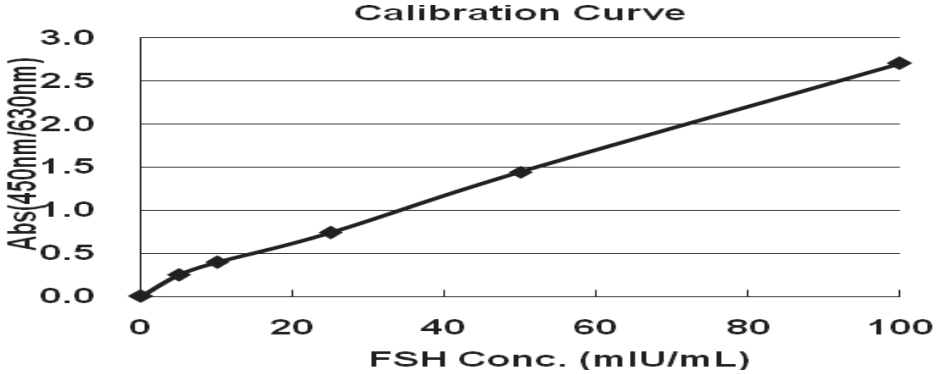

Figure (2) :Standard curve of FSH concentration

2.5 Estimation of serum luteinizing Hormone (LH) Levels by ELISA technique :

The LH EIA Test Kit was used .The quantitative determination of luteinizing concentration in human sera by microplate enzyme immunoassay.

The same and result calculation of The FSH EIA Test Kit was adopted in The LH EIA Test Kit .

2.6 Statistical analysis

Data was analyzed using statistical analysis system Statistical Package for Social Sciences (**SPSS**) to investigate the effect of different factors in *T . gondii* infection . Chi-square (X^2) was used to compare between discrete independent variables distribution in present studied groups. least significant difference (LSD) test was used to compare between means in this study.

3. Results

The result of the present study in both infertile patients and healthy control showed presence of anti-*Toxoplasma* IgG Abs in 40 out of 180 (22.23%) and 16 out of 100 (16%) respectively. While the result of ELISA- IgM test is (0 %) in both subjects (infertile patients and controls), table (1) explain the details. However, the results indicate a significant differences ($p \leq 0.05$) between infertile patients and controls in the seropositivity of anti-*Toxoplasma* Abs.

The measurement level of sex hormones in the present study showed the mean of testosterone hormone concentration was lowest in infertile patients infected with toxoplasmosis compared to higher mean of hormone concentration in non infected infertile patients and control groups, however the result showed no significant difference($P \leq 0.05$) between all groups . The mean of follicle stimulating hormone (FSH) concentration revealed significant increased ($P \leq 0.05$) in infected infertile patients group (6.80 ± 1.57 pg/ml) compared with the lowest mean of (FSH) concentration healthy control infected with toxoplasmosis (5.04±1.88 pg/ml). While no significant differences ($p \leq 0.05$) between other the results. While there are no significant difference ($p \leq 0.05$) in mean of luteinizing hormone (LH) concentration in infertile patients and healthy control groups , although the mean of hormone concentration was increased in infected infertile patients group compared with other the results, table (2) explain the details.

Table 1: Seroprevalence of anti-*Toxoplasma* Abs in infertil patients and apparently healthy controls using ELISA

Number of samples	ELISA test	
	IgG Positive (%)	IgM Positive (%)
Infertile patients (180)	40 (22.23%)	(0 %)
Control(apparently healthy) (100)	16 (16%)	(0 %)
Total	56 (20 %)	(0 %)
Statistics	X2=1.556, Df=1, P≤0.90	No statistics are computed

Table 2: The sex hormones level in infertile patients and apparently healthy controls groups infected and not infected with toxoplasmosis

Parameter / Groups	Mean of Testosterone	Mean of FSH	Mean of LH
Infertile(+ve)	2.67 ± 0.27[a]	6.80 ± 1.51[a]	5.09 ± 1.76[a]
Infertile(-ve)	3.28 ± 0.45[a]	6.08 ± 1.56[ab]	4.43 ± 0.39[a]
Control(+ve)	3.16 ± 0.46[a]	5.04 ± 1.88[b]	4.79 ± 1.03[a]
Control(-ve)	2.92 ± 0.09[a]	5.75 ± 0.85[ab]	4.58 ± 1.05[a]
L.S.D	0.692	1.251	1.085

4.Discussion

Out of 180 of infertile patients only (22.23%) were had chronic toxoplasmosis characterized by the presence of positive IgG antibodies, which was more than acute toxoplasmosis characterized by the presence of positive IgM antibodies were (0%).The result were in-line with the results obtained by Al-Ghezy (2012), Al-Abudy (2014) and Al-Mosawai (2014).

The present results showed no significant differences ($P \leq 0.05$) with the mean of serum testosterone hormone concentration in both groups (infertile patients and controls), although the mean of hormone concentration was decreased in infected infertile patients group compared with other the results .This results were similar to Dvorakova-Hortova *et al*., (2014) who observed no significant difference of testosterone hormone level in mice urine before and after infection with toxoplasmosis.The decrease of hormone may be related to the stress of the illness or injury with itself, accompanying factor such as medication, malnutrition, weight loss and fever accentuate the decrease in gonadal function. while this result was in disagreement with previous result by Flegr *et al*.,(2008) who founded that *Toxoplasma* infected men had a higher concentration of testosterone than *Toxoplasma* free controls. Also our result disagreed with Abbasian (2011) showed significant correlation between *Toxoplasma* infection and testosterone increase in men. High concentration of testosterone have immuno suppressive effects characterized by lower cellular immunity (Roberts *et al* . , 2001; Schuster and Schaub, 2001).Thus, the most explanation of the observed high testosterone-toxoplasmosis association is a higher risk of *Toxoplasma* infection in subject with higher levels of testosterone and therefore a weaker immunity. The mean of follicle stimulating hormone (FSH) concentration revealed significant increased ($P \leq 0.05$) in infected infertile patients group compared with the lowest mean of (FSH) concentration in healthy controls. This result was agree with other study such as Boepple (2008) demonstrated a significant increase in mean of FSH concentration ($p \leq 0.05$) and LH ($p \leq 0.05$) levels and significant ($p \leq 0.01$) decreases of testosterone level in *Toxoplasma* infected men patients when compared with the control group, and he explained this increase to impaired feedback of anterior pituitary. Also this result agreed with Andersson *et al*.,(2004) they found in their study that the fertile men had lower FSH levels than infertile men infected with toxoplasmosis , due to the correlation of some sex hormones with the immune response; i.e. high concentration of sex hormones correlate with low immune response, which may lead to increase susceptibility to parasitic infection . A hypogonadism involves failure of the testes to respond to FSH and LH, when primary hypogonadism affects testosterone production, testosterone is insufficient to inhibit production of FSH and LH here FSH and LH levels are elevated (AlWachi, 2008). While this result was disagreement with previous result by Makker *et al*.,(2009) they found that *Toxoplasma* infected men had equal concentration of FSH with *Toxoplasma* free controls.

On the other hand,The results revealed that there are no significant difference ($p \leq 0.05$) in mean concentration of luteinizing hormone (LH) in both groups (infertile patients and healthy control groups) . This results were similar to the results of Al Warid SH *et al.,* (2012) they found that *Toxoplasma* infection may not lead to an increase in LH level in infected patients . While this result was disagree with Khan *et al* .,(2005) they demonstrated the elevated levels FSH and LH with decreased levels of testosterone in the *Toxoplasma* infected men. The explain of this may be related to the differences in the ethnic group, environmental condition and nutritional factors which may cause the difference in susceptibility to infection and in the concentration of sex hormones.

Conclusions:

The conclusions of this study include that :

1. There are significant differences between toxoplasmosis and infertile patients ,there is a relationship between toxoplasmosis and risk factors.
2. There are high values of anti-Toxoplasma Abs (IgG) in comparison with IgM through out testing by ELISA technique.
3. There are no significant differences in the level of testosterone hormone concentration in both infertile patients and healthy control groups.
4. There is increasing in the level of FSH hormone concentration in the infertile patient infected with toxoplasmosis as compared with non infected patient and control group.
5. There are no significant differences in the level of LH hormone concentration in both infertile patients and healthy control groups.

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