



Some Hormonal Assay in Toxoplasma Infected University Students

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ABSTRACT

Background: *Toxoplasma* is a parasite with a global distribution that infect both human and animal, most of human toxoplasmosis is chronic infection. Chronic toxoplasmosis can affect human behavioral and hormonal status.

Objective: *In this study the effect of toxoplasmosis on the level of certain hormones (total testosterone, estradiol (E2), follicle stimulating hormone (FSH) have been investigated.*

Methods: *One hundred and ninety serum samples from Kirkuk university students were assayed for Toxoplasma antibody (IgM,IgG), among them 40 (21.1%) samples were positive The serum of positive samples along with control group were assayed for some hormonal levels by using ELIS technique.*

Results: *From the obtained results after conducting statistical analysis, it has been found that there is a significant differences in testosterone and estradiol concentration in both infected males and females comparing with the uninfected control cohort. The concentration range of testosterone were (12.8-15.5, 1.6-3.1 ng/ml) for each of infected male and female respectively which was significantly higher than the control (6.9-8.0,0.39-0.58 ng/ml) respectively for male and female. The estradiol concentration range in infected cohort were (34.3-39.6, 184.3-197.0 pg/ml) for male and female respectively, comparing with the control (22.2-26.9, 72.8-75.5*

pg/ml) for male and female respectively. No significant differences were appeared between the FSH concentrations in infected and control cohort in both male and female. The infected males were a little taller and more depressed than uninfected one.

Conclusions: From the current study we concluded that chronic toxoplasmosis can affect some hormonal concentration in human blood which may change or influence human behavior.

Keywords : Toxoplasma, Hormone, Testosterone, Estradiol, FSH.

بعض الاختبارات الهرمونية في طلاب الجامعة المصابين بالمقوسة الكوندية

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الملخص

الخلفية المعرفية: المقوسة الكوندية هي طفيلي عالي الانتشار تصيب الانسان و الحيوان، الغالبية العظمى لاصابات الانسان هي اصابات كامنة. الاصابات المزمنة من الممكن ان تؤثر على مستويات الهرمونات و على السلوك.

الهدف: هدف هذا البحث الى دراسة تأثير الالصابة بداء القطط على مستويات الهرمونات (Testosterone, estradiol, FSH)

طريقة العمل: فحصت مائة وتسعون عينة مصل من طلاب جامعة كركوك للبحث عن الاجسام المضادة للطفيلي، بينهم ٤٠

عينة (٢١%) كانت موجبة . للعينات الموجبة مع مجموعة السيطرة تم تعيين تراكيز بعض الهرمونات

النتائج: بينت نتائج الدراسة بعد التحليل الاحصائي ارتفاع معنوي لتركيز كل من هرمون Testosterone, Estradiol في كل من الذكور و الاناث المصابين مقارنة بالاصحاء. نسبة تركيز هرمون Testosterone كان (١٢.٨-١٥.٥ نانوكرام/مل) لكل من الذكور و الاناث المصابين على التوالي و الذي كان معنويا اعلى من مجموعة السيطرة (٦.٩-٨، ٠.٣٩-٠.٥٨ نانوكرام/مل) لكل من الذكور و الاناث على التوالي. تركيز estradiol في المجموعة المصابة كان (٣٤.٣-٣٩.٦ pg/ml) لكل من الذكور و الاناث على التوالي مقارنة مع مجموعة السيطرة (٢٢.٢-٢٦.٩، ٧٥.٥-٧٢.٨ pg/ml) لكل من الذكور و الاناث على التوالي. لم يوجد فروق معنوية بين تراكيز هرمون FSH لكل من المصابين و الاصحاء. الذكور المصابين كانوا اطول بقليل و أكثر كآبة من الاصحاء.

الاستنتاج: يستنتج من الدراسة الحالية ان الاصابة بداء المقوسات الكوندية له تأثير على مستويات بعض الهرمونات في دم الانسان والتي تؤثر بدورها على تصرف وسلوكيات الانسان.

الكلمات الدالة : المقوسة الكوندية، الهرمونات، Testosterone ، estradiol ، FSH

1. INTRODUCTION

Toxoplasmosis is a disease caused by a protozoan parasite. Human and a wide range of animals are its host. The infection has a worldwide distribution. Its estimated that one-third of human population are exposed to this parasite. Human may remain infected for life and will stay asymptomatic unless immunosuppression occurs [1]. The hormone levels in a certain situations could be altered and the dissimilar effects on the immune system may induce resistance or susceptibility to different parasite attacks. The sharp elevated sex steroids could worsen toxoplasmosis; mainly through suppressing host immune-endocrine network (IEN) and progressing parasite replication [2]. Higher incidence of *Toxoplasma* encephalitis was recorded within AIDS-defining females than in males, this support that female hormones possibly predispose latent toxoplasmosis [3] and was confirmed to stimulate higher parasite load in guinea pigs. The actual dynamics stimulating latency are still unknown; however various stimuli were studied including hormonal factor [4]. In Baghdad, male total and free testosterone hormone

recorded higher significant mean in both acute and chronic toxoplasmosis person than in control subject [5]. The levels of testosterone hormone were higher in both males and females with positive toxoplasmosis than control in Al-Yarmok Teaching Hospital [6] In Al-Mahaweel healthy center in north of Babylon province pregnant women with chronic *T. gondii* infection exhibited significant increases of testosterone serum levels and significant decreased of prolactin serum levels in all trimesters, a significant increase occurred to progesterone in seropositive IgG pregnant women when compared with those of the control group during third trimester[7]. Progesterone and estrogen hormones were measured in a group of *Toxoplasma* infected Iraqi pregnant women in Baghdad, the chronic infected women had higher hormone concentration than acute one [8]. Acute and chronic toxoplasmosis males record significantly higher concentrations in both total testosterone hormone (TTH) and free testosterone hormone (FTH) than in control group. The mean concentration of FSH revealed non-significant differences between diseased and un diseased control [9]. A direct relation between *Toxoplasma* infection, cortisol and testosterone increase were observed in men and women patients referred to Sina hospital, Tehran, stress and anxiety index also increased in men and women whereas depression index increased only in men[10]. indirect support for the assumption that testosterone may be implicated in the personality and behavioral differences between *Toxoplasma*-infected and *Toxoplasma*-free subjects was provided in a study on students of the Faculty of Sciences, Charles University, Prague [11]. In the current research we aimed to find out the effect of *Toxoplasma* infection on some hormone levels and behaviors in university students because of their positive effective and significant role in building the community and the country.

2. Materials and methods

2.1. Study design: The study was carried out during the period of January till May 2015, (190) serum samples were collected from Kirkuk university students age (18-25)years for *Toxoplasma* screening, the positive samples were assayed for some hormonal status namely testosterone ,estradiol E2, follicle stimulating hormone (FSH) ,190 *Toxoplasma* negative samples were used as a control group. A personality questionnaire (depression, anxiety, and stress) also filled to each student. The tall of the students were recorded too.



2.2. Samples collection: About 5 ml of venous blood was drawn carefully and transferred into disposable tube, the specimen was left to clot then centrifuged to separate clear serum, the sera were divided in to two parts stored in separate tubes, one for *Toxoplasma* screening and the other for hormonal assay. The sera were kept at (-20°C) till used.

2.3.Toxoplasma screening: In order to investigate for serum Toxoplasma antibody, kits (BioCheck, Inc.USA) of Toxoplasma IgG and IgM Enzyme Immnoassay test were used for this purpose. The steps for detection were performed according to the kit instructions. Diluted serum was added to purified Toxoplasma antigen coated wells (12x8wells). All unbound materials were washed away by ELISA washer. HRP-conjugate was added. Excess HRP-conjugate was washed off and a solution of TMB Reagent was added. The enzyme conjugate catalytic reaction was stopped at a specific time. The intensity of the color generated is proportional to the amount of IgG or IgM-specific antibody in the sample. Optical density (OD) was read at 450nm within 15 minutes by a micro well reader compared in a parallel manner with calibrator and controls (a micro well reader Toxo antibody index of 1.00 or greater is positive and indicates the probability of current or recent toxoplasmosis).

2.4. Estimation of serum Follicle stimulating Hormone (FSH) level by ELISA Monobind Kit (USA):

2.4.1.Procedure: Microplate wells were formatted for each serum reference, control and patient specimen. 0.05 ml of the appropriate serum reference control or specimen into the assigned well were pipette. 0.1 ml of FSH-Enzyme reagent to all wells was added and incubated for 60 minutes at room temperature. The contents of the microplate were washed three times. 0.1 ml of working substrate solution was added to all wells. Incubated at room temperature for fifteen minutes. 0.05 ml of stop solution were added to each well and gently mixed for 15-20 seconds. The absorbance was read at 450nm in a microplate reader. The results should be read within 30 minutes of adding the stop solution

2.5.Estimation of serum total testosterone levels by ELISA Monobind kit (USA):

2.5.1. procedure: Ten µl of serum reference, control and patient specimen pipette to microwell. Fifty µl of working testosterone enzyme reagent were added to all wells, fifty µl of testosterone biotin reagent were also added and mixed and incubated for 60 min at room temperature. The microtiter plate washed 3 times, one hundred µl of working substrate solution were added to all



wells without shaking then incubated for 15 min. Fifty μL of stop solution were added to each well. The absorbance in each well was read at 450nm in a microplate reader.

2.6.Estimation of serum Estradiol (E2) levels by ELISA Monobind kit (USA):

2.6.1. procedure: 25 μL of the appropriate serum reference, control or specimen pipette into the assigned well. 50 μL of estradiol biotin reagent to all wells was added, incubated for 30 min at room temperature. 50 μL of Estradiol enzyme reagent was added to all wells, incubated for 90 min at room temperature. Washed 3 times.100 μL of substrate solution was added to all wells and incubated at room temperature for 20 min. 50 μL of stop solution was added to each well. Absorbance was read 450 nm. (The results should be read within 30 minutes of adding the stop solution).

2.7.Statistical analysis:

Data were analyzed by using Student's t-test manually. Results are expressed as mean \pm standard deviation (SD). Statistical significance and difference from control and test values were evaluated by Student's t-test. A probability value of $P < 0.01$ indicated a statistically significant difference (star *number refer to significance degree).

3.Results

From 190 serum samples(94male,96 female) examined for *Ttoxoplasma* antibody,40 (21.1%) samples were positive.Significant differences were revealed between the mean concentrations of testosterone and estradiol in infected and control cohort in male table 1 whereas no significant differences was detected between FSH concentrations in both infected and control cohort Table (1).

Table (1): FSH, testosterone and estradiol concentrations in infected & control male

Age group	Total No. Examined(15)		FSH concentration in (mlU/ml)M±SD		Testosterone concentration in (ng/ml)M±SD		Estradiol concentration in (pg/ml)M±SD	
	Infected	Control	Infected	Control	Infected	Control	Infected	Control
18-19	4	4	5.8 ± 0.912	4.4±1.009	12.8 ± 2.94	7.7 ± 0.643	39.6±21.260	25.3 ± 3.86
20-21	5	4	4.9 ± 0.948	3.9±1.102	14.4 ± 3.92	8.0 ± 0.736	36.5±20.675	22.2 ± 3.71
22-23	3	4	5.0 ± 1.048	4.4±1.275	15.5 ± 4.11	7.8 ± 0.821	34.3±19.984	26.9 ± 3.24
24-25	3	3	5.1 ± 1.153	5.8±1.690	14.3 ± 3.95	6.9 ± 0.548	35.1±19.574	25.7 ± 3.10
Estimated t value			1.264	1.264	19.331 ***	19.331 ***	38.087 ****	38.087 ****

Also significant differences were noted between the mean concentrations of testosterone and estradiol in infected and control cohort in female table 2. whereas no significant differences was detected between FSH concentrations in both infected and control female **Table (2)** .

Table (2): FSH, testosterone and estradiol concentrations in infected & control female

Age group	Total No. Examined (25)		FSH concentration in (mIU/ml)M±SD		Testosterone concentration in (ng/ml)M±SD		Estradiol concentration in (pg/ml)M±SD	
	Infected	Control	Infected	Control	Infected	Control	Infected	Control
18-19	6	6	17.9 ±	12.4±	1.8 ±	0.39 ±	197.0 ±	75.5 ±
			2.214	3.198	0.19	0.084	7.13	27.97
20-21	9	7	15.6 ±	16.3 ±	2.9 ±	0.55 ±	192.1 ±	73.7 ±
			2.417	3.609	0.21	0.151	7.01	26.52
22-23	7	6	16.4 ±	14.0 ±	1.6±	0.41 ±	187.6 ±	72.8 ±
			2.614	4.085	0.24	0.087	6.89	25.34
24-25	3	6	15.8 ±	15.5 ±	3.1 ±	0.58 ±	184.3 ±	72.0 ±
			2.513	4.998	0.26	0.026	5.02	25.012
Estimated t value			1.367	1.367	5.567 *	5.567 *	28.56 ****	28.56 ****

Regarding the tall of the student, the mean tall of infected males (178.6 cm) was taller with about 2.4 cm than uninfected one (176.2cm). While the females tall was not effected **Fig. (1)** .

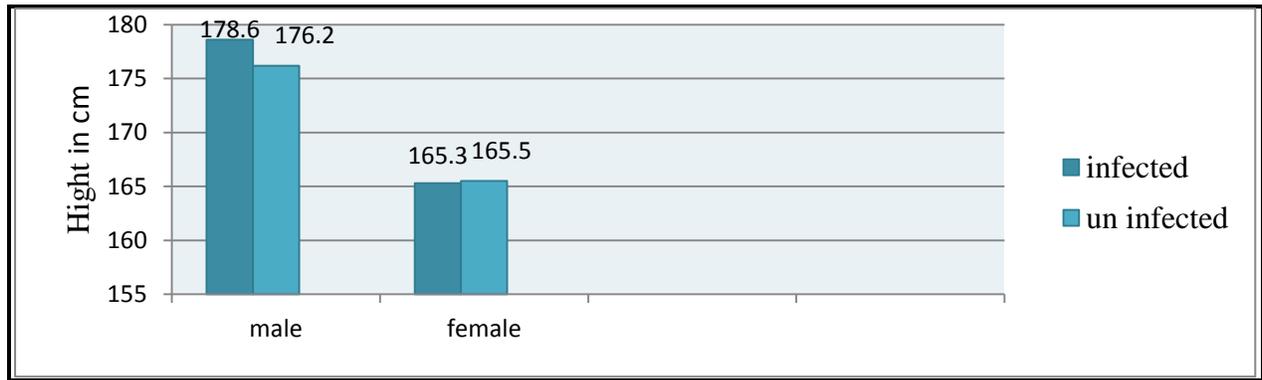


Fig. (1): Hight of infected and uninfected male and female.

About what concerning some behavior in infected and uninfected student, Fig. (2), revealed that the infected males were more jealous, suspicious, anxiety, depressed than uninfected one. Whereas the females were more jealous and selfish than uninfected one with no significant differences between the other female behavior.

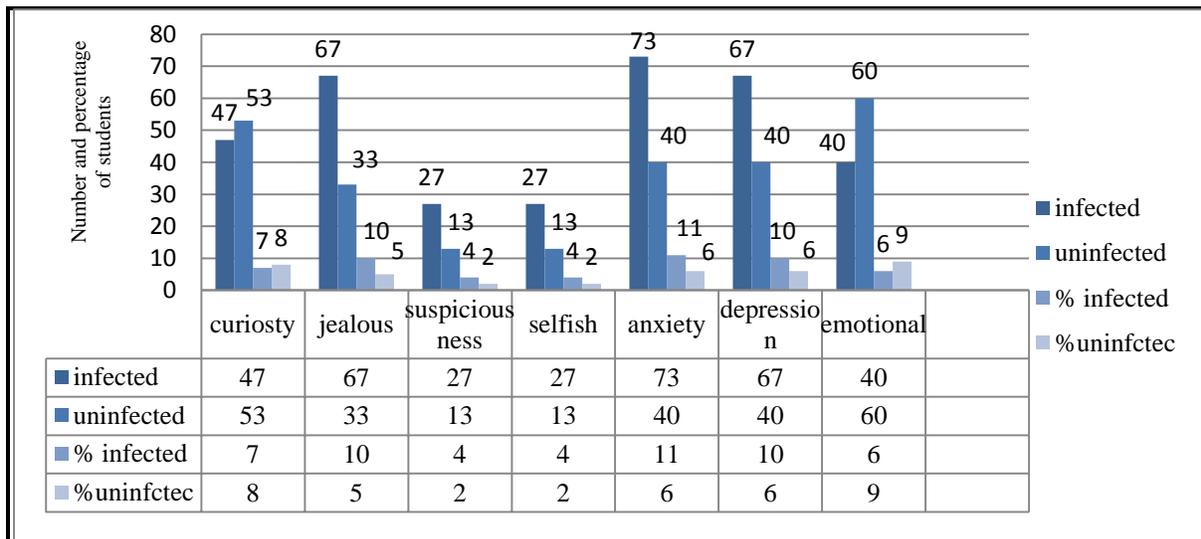


Fig. (2): Stress index of infected and uninfected male

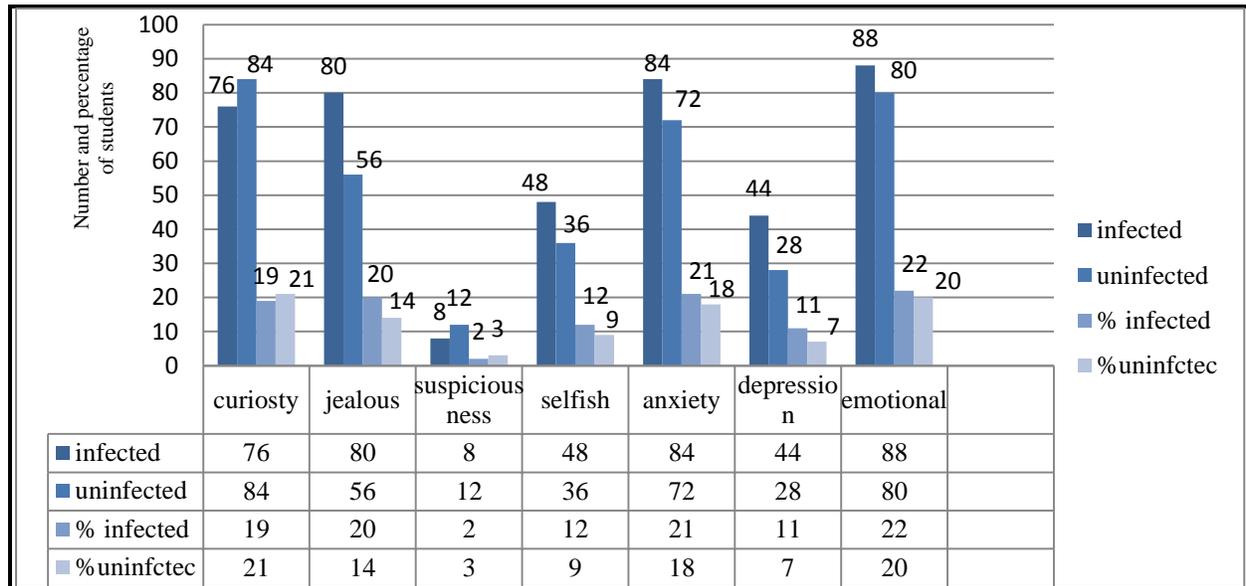


Fig. (3): Stress index of infected and uninfected female

4. Discussion

In this current research the effect of *Toxoplasma* infection on some hormonal levels in Kirkuk university students were studied. The results showed a significant effect of toxoplasmosis on testosterone and estradiol concentration in both infected males and females comparing with the uninfected control cohort. The concentration range of testosterone were 12.8-15.5, 1.6-3.1 ng/ml for each of infected male and female respectively which was significantly higher than the control (6.9-8.0, 0.39-0.58 ng/ml) respectively for male and female. In our study the estradiol concentration range in infected cohort were much higher (34.3-39.6, 184.3-197.0 pg/ml) for each of infected male and female respectively, comparing with the uninfected control (22.2-26.9, 72.8-75.5 pg/ml) for male and female respectively. No significant differences were appeared between the FSH concentrations in infected and control cohort in both male and female. Identical results of testosterone increases have been recorded in *Toxoplasma* infected (8.06, 0.72 ng/ml) male and female respectively comparing to control (4.1, 0.52 ng/ml) respectively for each gender[6]. Similarly significant ($p < 0.05$) increase in testosterone level in seropositive IgG first trimester pregnant women were noted compared to control group. Data for third trimester pregnant women showed significant differences in testosterone, progesterone and prolactin

concentrations between the seropositive and seronegative IgG pregnant women [7]. Both acute and chronic toxoplasmosis in males recorded significantly higher mean concentrations in both total testosterone hormone TTH, (12.188 ± 0.73 ng/ml), (7.837 ± 0.52 ng/ml) and free testosterone hormone FTH, (44.121 ± 1.76 pg/ml), (27.984 ± 0.94 pg/ml), in comparison to control group (6.455 ± 0.48 ng/ml) and (18.375 ± 0.78 pg/ml) respectively. The mean concentration of FSH revealed no-significant differences in both cohorts [9]. A statistically significant correlation between *Toxoplasma* infection and testosterone and cortisol increase in women and men were observed [10]. progesterone and estrogen hormones were measured in a group of *Toxoplasma* infected Iraqi pregnant women in Baghdad, the chronic infected women had higher (14.62 ng/ml, 108.02 pg/ml) hormone concentration than acute one (5.35 ng/ml, 70.66 pg/ml) respectively for each of progesterone and estrogen hormones [8]. Salivary testosterone levels was not statistically differed between *Toxoplasma*-infected and free males [12]. *Toxoplasma*-infected men had recorded a higher concentration of testosterone ,reversal to *Toxoplasma*-infected women whom recorded a lower concentration of testosterone than *Toxoplasma*-free controls [13]. The rate of testosterone synthesis was greater in testes of infected male rats with chronic toxoplasmosis comparing with control [14]. Various testosterone- related medical disorders can be attributed to latent toxoplasmosis that may also be involved in the etiology neuropsychiatric disorders [15]. In our study the mean tall of infected males (178.6 cm) was taller with about 2.4 cm than uninfected one (176.2 cm). while the females tall was not effected , also the behavior in infected was not identical to uninfected student, the infected males were more jealous, suspicious, anxiety, depressed than uninfected one. Whereas the females were more jealous and selfish than uninfected one. *Toxoplasma* infected male and female students had significantly higher extraversion and lower conscientiousness. The conscientiousness positively correlated with the duration of infection in men, suggesting that this may due to cumulative changes enhanced by latent slow toxoplasmosis , rather than acute quick infection[16]. Stress and anxiety index was increased in men and women whereas depression index increased only in men[10]. Chronic toxoplasmosis patients were taller and had lower sexual maturity age than uninfected ones [11]. Association between latent toxoplasmosis and human RhD phenotype can have an important role on psychomotor performance, personality and intelligence [17]. Latent toxoplasmosis can effect hair abundance[9], Autism spectrum disorder (ASD) [18] number of son [19], body mass, height



and personality [20]. Even anti*T. gondii* antibody titers may be associated with the risk of traffic accidents[21].It's clear from the results of our and of mentioned studies that toxoplasmosis can influence both hormonal levels and behavior of infected individual, due to the complex interaction between the parasite presence, hormonal level changes and immune response, higher level of testosterone could be responsible for the toxoplasmosis-associated changes in human and animal behavior[11, 14]. women patients with latent toxoplasmosis had increased NK-cell and monocyte counts in comparison with acute controls [22]. *Toxoplasma gondii* can elicits cellular immune response detected by increased levels of IL-12 and IFN compared with control. Not only host hormone can affect responses to infection, but parasites can both enhance production and alter hormone concentration in their hosts[6]. High levels of progesterone and estrogen and the diminished NK cell, macrophage and CD8+ may facilitate parasite survival and increase the higher possibility of latency of *Toxoplasma*[23].High levels of steroid hormones were associated with lower cellular immunity, which may contributes to the parasite survival in the body(hormones increase with weak immune system contribute to the parasite survive in body) [24].toxoplasmosis result in dopamine increase in brain which in its turn increase adrenal glands sensibility and secretion of hormones from hypothalamus hypophysis adrenal axis[25].Based on the finding of our study, the infection with *Toxoplasma* affect the concentration of some hormones in both sex and change human behavior in different ratios therefore its recommended to be periodically screened for *Toxoplasma* antibody among population especially university student because of their effective role in the community.

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