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Molecular and Hormonal Study(Testosterone and Luteinizing hormone) among Applicants For Marriage and Blood Donors Peoples who Infected With *Toxoplasma gondii* in Babylon Province , Iraq

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Abstract:

Toxoplasma gondii is one of infectious pathogens to human and animals, it has been estimated that on third of the world population has been infected. This object aimed to detection of T.gondii by polymerase chain reaction and studies the relationship between toxoplasmosis and Testosterone hormone and Luteinizing Hormone. A total of three hundred- six blood sample were collected from applicants for marriage and blood donors, detection of parasite antibody was done by using (ELISA - IgG and ELISA- IgM). Testosterone hormone and luteinizing hormone (LH) was also detected to perform its effect during toxoplasmosis infection and detection of parasite by PCR technique 66 out of 306 blood samples were positive by using ELIZA in which20 blood samples were positive by using PCR. The percentage of infection was highest in the females for positive cases of IgG (25%) and the lowest rates were in the males for positive cases of IgG (22%) in the (37-41) age groups. there were significant difference between blood group (<0.05), the highest rates of the IgG focused in the blood group O (36.8 %)and the lowest rates of the IgG focused in the blood group B (11.1%). the results show decrease in the mean of testosterone concentration in three group(Married and have children, Married and has no children and not get married) as(6.63± 1.8, 4.45± 0.5 and 7.01 ± 2) ng/ml ,respectively, compared to control, Whereas there are increase in the means of luteinizing hormones concentration in two group married and have children and married and has no children) as (5.62±2.4 , 8.57±0.7)mIU/ml.

Keywords - Luteinizing hormone , PCR , Testosterone hormone , Toxoplasma gondii.

1. Introduction:

T. gondii is an obligate intracellular protozoan [1], it one of the most common parasites in developed countries and infect human and animals [2]. It has been estimated that on third of the world population has been infected [3].The parasite included two hosts to complete its life cycle, Cats and other members of felidae conceder the definitive hosts, while human and wide range of animals, birds and rodents act as intermediate hosts, it may cause abortion or congenital disease in its intermediate hosts [4].

The most common manifestation of acute acquired infection is lymphadenitis, fever, headache and hepatosplenomegaly [5]. The illness may resemble mononucleosis infections; central nervous system involvement is usually fatal [6].

2. Material and Method

2.1 Samples Collection

A total of three hundred six blood samples were collected from applicants for marriage and blood donor's peoples, who attend to blood banking center in Al-Musayyib Hospital during the period from September 2016 till March 2017, with age ranged between 17 and 42 year.

Five milliliter was collected as (3 ml. of serological tests and 2 ml. for DNA extraction for PCR technique). Detection of *T. gondii* antibody was done by using ELISA - IgG and ELISA- IgM. Testosterone hormone and luteinizing Hormone (LH) were also revealed to perform its effect during *T. gondii* infection and It was confirmed the presence of the parasite with polymerase chain reaction technique.

2.2 ELISA test

The bio Check Toxoplasma IgG ELISA (BC-1085) kit was done for detection of IgG antibodies [7], and The bioCheck Toxoplasma IgM ELISA (BC-1087) kit for detection of IgM antibodies [8], according to manufacture methods. The Toxoplasma IgG and IgM ELISA are choosing to estimate a patient's serologic status to *T. gondii*

2.3 Polymerase Chain Reaction Technique

2.3.1 Isolation and amplification of DNA

Lysate was prepared from two ml of blood samples as described below, only the positive samples in ELISA (IgG and IgM) was used. The primer (Bioneer –Korea) : Forward (*5'*-TTG CCG CGC CCA CAC TGA TG *-3'*) and revers (*5'*-CGC GAC ACA AGC TGC GAT AG-*3'*) was used to investigating the gene's DS29,DS30 (914bp). genetic patterns parasite *T. gondii* [9]. The following PCR cycle was used: an initial denaturation at 95C° (one minutes), denaturation at 95 C° (one minute) then annealing at 58 C° for one minute, extension at 74 C° for three minutes and final extension at 72 C° for 7minutes. Then agaros gel electrophoresis (1.5%) used to product LH analysis as [10].

2.4 Hormonal study:

2.4.1 Estimation of serum testosterone and LH levels by ELISA technique.

The kit monobind testosterone enzyme immunoassay was done according to the manufactures instructions for estimation of testosterone level in patients sera[11].also we used LH kit for estimation luteinizing hormone level in serum[12].

2.5 Statestical Analysis

The results analyzed according to scientific experiments use model was least significant differences (LSD) at the level of improbability (*P* <0.05) in addition to (Z-test) and (T-test) to demonstrate the use of significance results [13].

3. Results and Discussion

The total percentage of *Toxoplasma* among blood samples was 21.5% (66 out of 306 blood samples) and 30.3% (20 out of 66 blood samples)by using PCR. The rate of infection was high in the females for positive cases of IgG (25%) and the lowest rates were in the males for positive cases of IgG (22%), and the highest rate of acute infection in the males its (8%) than females (6.3%) and the overall percentage of infection for males and females (30%,31,2%) respectively (Table 1). This result was agreed with results of [14], who confirm that no significant difference between men and women in IgG level. and disagreed with pervious study [15] who confirm that males were high susceptible to toxoplasmosis than females, while results of [16] investigate high signification in female 61.7% higher than male 30.18%.this may be due to of probably both males or females the same susceptibility with *T. gondii* infection, according to habitation or residence area or not to follow Hygiene habits. Previous studies were targeting the effect of toxoplasmosis on females than in males fact that infection toxoplasmosis have no symptoms no significant risk to healthy males and most risk in women during pregnancy and childbirth.

As show in Table (2) the highest rates of infection revels in the (37-41) age groups for positive cases of IgG (50%) and the lowest rates of IgG (7.7%) revels in the (22-26) age groups. Whereas the highest rates was in the (22-26) age groups for positive cases of

IgM 23.1% and the lowest rates of IgM (0%,0%)was in the,(32-36),(37-41)and (42 and more) respectively and there are significantly differences (P <0.05).

The present study agreed with pervious study [17] was most frequent age group for single women was (15-19) years and with [18] who concluded that the main age of seropositive of toxoplasmosis cases were between (11-20) years. This may be due to longer risk factors related to exposure to the parasite, as the older individuals are exposed during the period in their lives long-infection more than younger ages, so their chance to be more infections as well as to the deterioration of the immune system with increasing age and get to know other chronic diseases, all lead eventually to the weakness of the administrator of the immune system to resist the nurses of various kinds. the differences in parasite strains may play an important role in the stimulation of host immune response against the parasite [19] The present result might be relevant to the different number of each infected person at each age group, also the peoples may be contact with *Toxoplasma* in childhood ,through cats connected ,soil exposure has resulted to accumulate of anti-*Toxoplasma* antibodies at different percentages within human being that lead to the chronic infection with toxoplasmosis[20].

Also Table (3) shows the highest rate of infection was among peoples not eating canned or under cooking meat for IgG (26.7%) and were higher than peoples eating canned or under cooking meat (21.6%) while(IgM) the highest rate of infection was among peoples eating canned or under cooking meat was 7.8% .

Table (1): distribution of infection with *T. gondii* according to the gender by PCR technique.

Gender	Examined No.	Total Infected No.	Positive PCR for IgG(%)	Positive PCR for IgM(%)	Total percentage of Infection
Male	50	15	11(22)	4(8*)	30.0
Female	16	5	4(25*)	1(6.3)	31.2
Total	66	20	15(22.7)	5(7.1)	30.3
Z-test	2.4 sign IgG female 0.3 IgM sign male				

Table (2): distribution of infection with *T. gondii* according to the age groups of patients by PC technique.

Age groups	Examined No.	Total Infected No.	Positive for IgG(%)	Positive for IgM(%)	Total Percentage of Infected
17-21	9	5	4(44.4)	1(11.1)	55.5*
22-26	13	4	1(7.7)	3(23.1*)	30.8
27-31	12	5	4(33.3)	1(8.3)	41.6
32-36	14	2	2(14.3)	0	14.3
37-41	6	3	3(50*)	0	50
42and more	12	1	1(8,3)	0	8.3
Total	66	20	15(22.7)	5(7.6)	30.3
LSD test	8.5 sign (37-41)IgG 5.6 sign (22-26) IgM				

Table (3): distribution of infection with T.gondii according to eating canned or under cooking meat or not of patients by PCR technique.

Eating canned meat or under cooking	Examined No.	Total Infected No.	Positive for IgG (%)	Positive for IgM (%)	Total Percentage of Infection
Yes	51	15	11(21.6)	4(7.8)	29.4
No	15	5	4(26.7)	1(6.7)	33.3
Total	66	20	15(22.7)	5(7.6)	30.3
Z- test	2.2 sign (IgG)not eating canned or under cooking meat 0.6 sign (IgM) eating canned or under cooking meat				

By using PCR technique we investigated the percentage of infection was 33.3% in patients whose not eating canned or raw meat and it higher than whose eating canned or raw meat (29.4%) ,this results was in agree with study of [21] in Manchester who found that samples of lamb sourced was positive for *toxoplasma* by PCR (61%), these samples were mainly processed meat products and therefore may have contained mixed meat derived from other animals.

The seropositivily rate of *T. gondii* was increased in France and Ethiopia, where undercooked or raw meat is regularly consumption; potential explanations include the fact that transmission through eating tissue cysts in undercooked meat is a milder mode of infection [22].

The infection in people who do not eating meat is well cooked may be due to causes other infection operations such as organ transplants or blood transfusions, or by eating contaminated vegetables with oocysts or by pollution breeding birds or lack of his pasture personal hygiene, or by mechanical transport is by the feet of insects or dirty water, milk, unpasteurized well .The possible risk of the disease transmission by consumption of infected meat must still be considered as a public health problem [23].

Table (4) shows the percentage of infection with toxoplasmosis among the patients according to blood groups. was highest rates of the IgG focused in the blood group O (36.8%)and the lowest rates of the IgG focused in the blood group B (11.1%).Whereas the highest rates of the IgM positive cases were in the blood group O (10.5%)and the lowest rates were in the blood group B (0%). where there were significant difference between blood group significantly (P <0.05) .The overall percentage of infection reveals the highest rates (47.3%)in the blood group O and the lowest rates (11.1%)in the blood group B. agree with [24] how found highest prevalence among blood group O was 281(43.6%) and the lowest prevalence with blood group AB was 27(4.2%) by IgG and IgM . and with study of [25] who found the percentage of infection in blood group O was 30.5% in women in Babylon province. The disagreement among these results and other studies may result from several factors. It is possible that the molecular variability of strains in Iraqi patients, or the use of only male patients in this study gave findings that may therefore differ from findings in other population. Or it is may be possible that AB antigens exerts a large influence on the adherence of *T.gondii* to the gastrointestinal mucosa and its contribution is evident by the high prevalence of infection by these parasites in the Iraqi population.

Table(4) : distribution of infection with *T. gondii* according to blood groups of patients by using the PCR technique.

Blood groups	Examined No.	Total Infected No.	Positive for IgG (%)	Positive for IgM (%)	Total Percentage of Infection
A	27	7	5(18.5)	2(7.4)	25.9
B	9	1	1(11.1)	0(0)	11.1
AB	11	3	2(18.2)	1(9.1)	27.3
O	19	0	7(36.8*)	2(10.5*)	47.3*
Total	66	20	15(22.7)	5(7.5)	30.3
LSD-test	8.7sign O, IgG 3.9 sign O, IgM				

Significant differences (P <0.05)

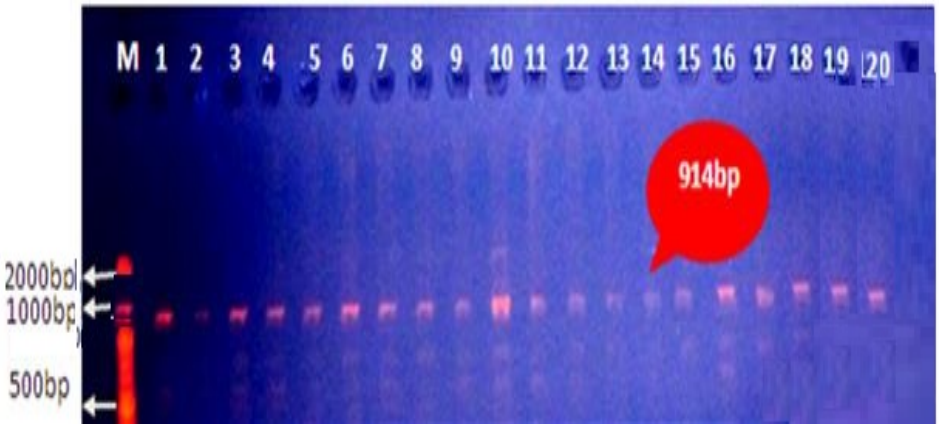

Figure (1): PCR product of DS29, DS30 (914bp) gene of *Toxoplasma gondii*, electrophoresis on 1.5% Agaros gel with Ethidium bromid. M=DNA Ladder(500-200)bp for 20 samples DNA containing the parasite, samples between(1-8)for blood donors and samples between (9-20) for applicants for marriage peoples.

Table (8): Relationship between toxoplasmosis and testosterone and luteinizing hormone in blood donor peoples according to marital status.

Marital status	Control			Infected with Toxoplasmosis		
	Control	Testosterone (mg/ml)S.D.±M	LH (mlU/ml) S.D.±M	PCR Positive	Testosterone (mg/ml)S.D.±M	LH (mlU/ml) S.D.±M
Married and have children	4	1.9±9.1	1.03±3.2	4	1.8±6.63	2.4±5.62
Married and Has no children	2	1.3±8.8	2.02±5.98	2	0.5*±4.45	0.7±8.57
Not get married	2	0.04±9.7	1.95±5.3	2	2±7.01	0.3±3.9
Total	8			8		
LSD-Test	2.3 sign not get married , Testosterone 3.1 sign married has no children, LH **S.D.=Standard deviation , M=Mean**					

*significant differences (P <0.05)

Table (9): Relationship between toxoplasmosis and testosterone and luteinizing hormones in applicants for marriage peoples according to the gender.

Gender	Control			Infected with Toxoplasmosis		
	Control	Testosterone (mg/ml)S.D.±M	LH (mlU/ml) S.D.±M	PCR Positive	Testosterone (mg/ml)S.D.±M	LH (mlU/ml) S.D.±M
Males	4	0.73±5.95	1.8±5.67	4	0.85* ±6.25	4.02±8.73
Females	8	0.31±0.54	7.06±16.63	8	0.30± 0.87	11.5* ± 23.28
Total	12			12		
LSD-Test	4.2 sign LH Female 3.3 sign male testosterone					

Table (8) Shows the relationship between the control group and infected group only blood donor patients with toxoplasmosis and some hormones(testosterone and luteinizing hormones). There are decrease in the mean of testosterone concentration in three group(Married and have children, Married and has no children and not get married) as(6.63± 1.8, 4.45± 0.5 and7.01 ± 2) ng/ml ,respectively, compared to control(1.9± 9.1, 1.3± 8.8, 0.04 ± 9.7) ng/ml. Whereas there are increase in the means of luteinizing hormones concentration in two group married and have children and married and has no children) as (5.62±2.4 , 8.57±0.7)mIU/ml, respectively but decrease in the third group (not get married) as (3.9±2.3) mIU/ml, comparison with control (1.03±3.2, 2.02± 5.98, 1.95± 5.3) mIU/ml.

The present study showed a decrease in testosterone hormone value of infected (male blood donors) for Married and they have children, Married and has no children and not married were (6.63± 1.8), (4.45± 0.5),(7.01 ± 2), respectively compared with control was(9.1±1.9) ,(8.8±1.3), (9.7±0.04),respectively.

This may be changed in testosterone concentration influences the probability of the *T. gondii* infection, because of high concentrations of testosterone are known to have immunosuppressive effects [26]. Which could result in a higher probability of acquiring *Toxoplasma* infection, also decrease of

testosterone concentration could be an adaptive response of infected mice to compensate *Toxoplasma*-induced immunosuppression and such compensation might increase the probability of the survival of infected mice after contact with various pathogens in their natural environment. It is also possible that the physiological reaction to *Toxoplasma* infection differs qualitatively between mice and humans because mice have short life comparable with the length of life in human.

While in (male and female) of applicant for marriage, the result study showed increase in testosterone hormone value, of infected was in male (6.25± 0.85) and female (0.87± 0.66), compared with control was (5.95±0.73), (0.54±0.31), respectively. Agree with study of [27] found greater testosterone synthesis in testes of infected male rats with chronic toxoplasmosis

Many studies suggest that the subjects with latent infection of the *Toxoplasma gondii* have a higher concentration of testosterone than uninfected controls, Agree with pervious study of [28] confirm that *Toxoplasma* –infected men and women have a higher concentration of testosterone was(8.0601) ng/ml in male and female was (0.7213) ng/ml than *Toxoplasma* free controls was (4.1123, 0.5249),respectively with highly significant difference .

Table (9) reveals the relationship between control group and infected only of applicants for marriage with toxoplasmosis and some hormone (testosterone and luteinizing hormone).the result were showed slightly increase in the means of the testosterone concentration in males and females in infected group(6.25± 0.85 , 0.87±0.66) ng/ml, respectively .as well as slightly increase in the means of the luteinizing hormone concentration in the males and females(8.73±4.02),(3.2821.5)mIU/ml ,respectively ,comparison with control group and there are significant differences (LSD-test) as ($\boldsymbol{P}$ <0.05) .

The present study showed increase of serum LH hormone value among Married and they have children , Married and have no children was(5.62±2.4), (8.57±0.7) mIU/ml , respectively compared with control was (3.2± 3.03) , (5.98±2.02) mIU/mlm, respectively , while not marriage decrease LH hormone value was (3.9±2.3) mIU/ml , compared with control(5.3±1.95), mIU/ml.and also applicants for marriage peoples showed increase of serum LH hormone value was in male and (8.73±4.02) (23.28±21.5) mIU/ml, respectively compared with control was (5.67±1.8),(16.63±16.06) mIU/ml, respectively.Many pervious study such as [29, 31,33,35] showed that pregnant women with toxoplasmosis its(4.49± 0.56) mIU/ml low level of LH hormone compared with high level of LH in healthy pregnant women which was (13.64 ± 13.1) mIU/ml, respectively. Other indicates disagree with current study, that infection with *Toxoplasma gondii* may not lead to an increase in LH, these agreed with the results of [30,32,34,36,37] that showed that there were not obvious changes in LH hormone in mice infected with *Toxoplasmosis*.

4. Conclusions

From the results of this study we conclude that The PCR technique more accurate from another test (ELISA technique), Peoples with blood groups O and AB were generally more exhibit to infection with toxoplasmosis. Also testosterone hormone and luteinizing hormone (LH) were affects during toxoplasmosis infection.

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